



Exploring the Wide World of Meat



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Animal Welfare and Environment

**EFFECT OF CHASSIS VIBRATION DURING ROAD TRANSPORT ON
CATTLE WELFARE AND MEAT QUALITY**

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Key Words: cattle transport, vibration, heart rate, meat quality

Introduction

Road transport in a vehicle is thought to be harmful for animal welfare. In fact, there is a lack of data on animal stress evaluation during transport. In this study, the effect of chassis vibration on cattle welfare was evaluated continuously by heart rate monitoring in normally used vehicles during transport. To do that, cattle were loaded, transported, unloaded, lairaged and slaughtered following the commercial practice in Finland.

Objectives

The objective was to evaluate the effect of chassis vibration during road transport on cattle welfare and meat quality. For animal welfare evaluation, the correlation between chassis vibration and heart rate was analysed in a moving vehicle. Meat quality was analysed after slaughter.

Methodology

Six short (under 2 hours), six medium (under 8 hours) and six long distance (under 14 hours) transports were made by the usual vehicles: one lorry for short and three road trains for medium and long distances. In all 486 cattle were transported.

Before transport, a vibration data logger was installed with two clamps onto the right-hand frame of the chassis of the vehicle just below the floor of the transport box. Vibration was measured with an accelerometer that measured the vertical acceleration in the range of 0 to 10 G (frequency 3 – 40 Hz, Noreltek Oy, Finland). The location of the logger was in the middle of the longitudinal axis of the transport box. The logger was programmed to store acceleration values higher than 0.1 G every two seconds during the estimated transport time (2 – 14 hours). After unloading, the logger was removed and data was stored in a computer for further analysis. The stored data was then entered in an Excel table by time intervals of two minutes.

In this study, heart rate monitors were installed in 117 sample animals at the farm. Heart rates were monitored for one day at the farm, during transport and overnight lairage up to stunning, in all for about 40 hours (beats/min, Polar Vantage NV, Finland; Honkavaara et al. 1999). The monitors were removed after stunning before debleeding. Data from farm up to unloading was received for 47 sample animals. The collected data on heart rates and vibration was combined and processed for regression analysis.

In addition, stress levels of the above 117 cattle were evaluated by blood samples collected by jugular venepuncture into heparinised tubes on the farm one day before loading and after transport during unloading. Blood was analysed for creatine kinase (UV method, Nordic Enzyme Committee, Honkavaara et al. 1999 and 2003).

Carcass bruising were evaluated during classification 45 min post mortem (none, slight or severe including their location; Honkavaara et al. 2003). Post mortem meat quality of the 107 sample animals was analysed by pH of the M. longissimus dorsi on the 11th rib 24 hours pm, and by tenderness (Warner Brazler shear force, 8 days pm). Conventional statistical methods were used to calculate means and standard deviations.

Results & Discussion

In this study, the mean chassis vibration measured as vertical acceleration values from moving vehicles was in most cases below 2.0 G, higher values indicated an uncomfortable journey in three or four axle vehicles which can load from 14 to 20 adult cattle. This 2.0 G value is based on our earlier results with long distance transport vehicles. In practice, these accelerometer values indicate differences in road quality .

It was found that chassis vibration was higher during collection than in a full loaded vehicle. This resulted from the "bumpy" small roads near farms compared to the smooth main roads after collection up to the slaughterhouse as indicated by the values for the vehicles: the short distance lorry had the highest average chassis vibration (G-load of 60 – 96 G/hour), followed by the medium distance road train (G-load of 46 G/hour) and the long distance road train (G-load of 22 – 30 G/hour; Table 1). All vehicles had air suspension, only the front axle of the short distance lorry did not have air suspension. Moreover, chassis vibration was lower in winter than in summer, perhaps due to the snow cover of the small roads near the farms.

Postural stability of untied cattle is important for animal welfare during transport. In order to minimise aggressive behaviour of loaded animals and carcass damages resulting from animal movement during transport, single- and two-animals pens are used in Finland.

The correlation coefficient between heart rate and G-value was calculated for the 47 sample animals whose heart rate was measured. If it was significant, a simple regression equation was calculated between heart rate (dependent variable) and chassis vibration (independent variable). Ten significant correlations between heart rate and vibration were discovered in the 47 sample animals. This showed that in most cases (79 %) vibration had

no effect on heart rate during transport. Furthermore, animals which had a positive correlation between heart rate and vibration produced normal meat quality. However, one DFD bull had a negative correlation between heart rate and chassis vibration (Tables 1 and 2).

In Table 2, bull (2) differed from the others by having the highest increase in CK activity from farm to unloading (from 118 to 3655 U/l), and it developed DFD meat (ultimate pH value of 6.57 24 h pm). Moreover, that bull had slight bruising of the back. In this work, the occurrence of carcasses without any damages was highest after long transports, lower after medium and lowest after short transports. The rates of occurrence of no, slight and severe bruising were 66.4, 26.5 and 7.1 %, respectively (465 cattle). The most common damage type was slight perianal (severity/location).

The *M. longissimus dorsi* of bulls (1) and (5) and heifer (2) was a little tough. However, they were of normal tenderness as their shear force values were 8.6, 7.4 and 6.6 kg/cm², respectively. Very tender meat has a shear force value of 5.8 kg/cm² or lower, normal meat values range from 5.8 to 8.7 kg/cm², and tough meat exceeds 8.7 kg/cm² (Augustini and Spindler 2000).

Conclusions

The measured accelerometer values indicate road quality differences between animal collection and transport of the full loaded vehicle to the abattoir. Chassis vibration was lower in winter than in summer, perhaps due to the snow cover of the small roads near the farms. In most cases chassis vibration had no significant effect on the heart rate of cattle. That suggests that from the animal welfare and meat quality point of view, external factors like animal handling at the farm, during loading, transport, unloading and lairage are more significant than chassis vibration during transport.

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Tables and Figures

Table 1. Regression equations and significance of the correlation between heart rate and chassis vibration in moving vehicles.

Animal	Transport time hours	Mean heart rate bpm	Total G-load G	G-load/h G/h	Regression equation Heart rate = a • G value + b
Heifer (1)	0.7	169	64.2	95.5	11.2 • G value + 128.0 *
Bull (1)	1.3	129	76.6	59.9	21.6 • G value + 73.0 ***
Bull (2)	7.7	111	353.8	45.8	-12.3 • G value + 133.9 ***
Bull (3)	10.1	87	250.6	25.1	11.9 • G value + 78.0 ***
Bull (4)	9.8	102	215.7	22.1	6.5 • G value + 95.4 *
Bull (5)	11.6	86	333.5	28.7	3.9 • G value + 79.5 **
Bull (6)	12.6	90	378.4	30.0	6.0 • G value + 82.1 *
Heifer (2)	12.6	82	378.4	30.0	6.4 • G value + 75.0 **
Bull (7)	12.6	93	378.4	30.0	11.6 • G value + 78.5 ***
Bull (8)	11.6	81	333.5	28.7	4.5 • G value + 76.4 *

Significance of the correlation * P<0.05, ** P<0.01 or *** P<0.001.

Table 2. Blood serum creatine kinase, CK activity and meat quality of the studied animals.

Animal	Transport time hours	CK activity, U/l			pH value 24 h pm	Shear force kg / cm ²	Carcass brusings
		Farm	Unloading	Change %			
Heifer (1)	0.7	102	268	163	5.59	2.7	None
Bull (1)	1.3	62	91	47	5.65	8.6	None
Bull (2)	7.7	118	3655	2998	6.57	–	Slight back
Bull (3)	10.1	113	319	182	5.56	5.9	None
Bull (4)	9.8	113	371	228	5.58	3.1	None
Bull (5)	11.6	122	150	23	5.62	7.4	None
Bull (6)	12.6	63	110	75	5.57	5.6	Slight perianal
Heifer (2)	12.6	86	172	100	5.58	6.6	None
Bull (7)	12.6	182	295	62	5.66	–	None
Bull (8)	11.6	94	138	47	5.60	–	None
Mean (n = 117)		112	268	139	5.57	5.5	–

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EFFECT OF TRANSPORT ON PORK QUALITY

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Key Words: pork, transport, handling, meat quality.

Introduction

There are some stressor factors during pre-slaughter handling which, depending on their length of time or severity, may affect pork quality. Pig transport from the farm to the slaughterhouse has been considered a major factor related to welfare. Therefore, according to the vehicle (fixed or mobile floor) and the way the animals are moved (electrical goads or pig boards), it can be observed that poor handling, fear and effort, may reduced meat quality. The difficulties while loading and unloading the animals at farms and abattoirs, which do not have ramps with appropriate slope (more than 15 °), increase the stress level due to, fear shown by the animals being moved through sloping places. Another effect that impairs the animal handling is the low height between the vehicle decks which prevents people with boards, from better accommodating the animals in the compartments. The use of vehicles with mobile floor or hydraulic elevator leads to an easy loading and unloading, minimizing the animal efforts and the need of suitable ramps in the farms. It also allows the replacement of electrical goads by boards. This practice proves to be a method to improve animal welfare and reduce economical losses resulted from poor handling. Good pre-slaughter handling conditions are of great importance to meet the welfare international requirements. Therefore, proposals to investigate the stress level on pork quality are highly important to improve the competitiveness requested by the international market.

Objectives

The aim of this work was to evaluate the influence of transport vehicles (fixed and mobile floor) and the system to move the pigs (board and/or electrical goads) on pork quality (pH, color, water holding capacity and drip loss).

Methodology

Animals. Four hundred and eighty pigs from a commercial farm located in southern part of Brazil were used in the experiment. The animals had the same genetic line, sex and slaughter age and they were allocated in four groups (n = 120).

Treatments. Groups 1 and 2, animals were transported in vehicles which met the requirements of animal welfare (mobile floor, water sprinkling system, excrement collection). Groups 3 and 4, animals were transported in conventional vehicle (fixed floor, adjustable ramp, without water sprinkling system). Two handling systems were applied in this experiment, boards (G1 and G3) and electrical goads (G2 and G4).

Slaughter. The pigs were transported at night for about 215 km. The stock density in the truck was 0.5m²/100Kg and the slaughter was performed in July, 2004 at a commercial abattoir. Water was available to the animals during the fasting period in the farm (12h) and lairage time (4h). The animals were electrically stunned and submitted to commercial slaughter procedures normally applied by the company.

Measurements. Rate of pH decline in Semimembranosus muscle measured at 4, 6, 8, 12 and 24h after bleeding; color evaluation in Longissimus dorsi at 24h postmortem using Minolta colorimeter (CR300); water-holding capacity (WHC) in Semimembranosus evaluated at 24h postmortem according to the pressing method described by Hoffmann et al., (1982) and drip loss in Longissimus dorsi using the methodology reported by Honikel, (1998). The statistic design was randomized blocks and means were submitted to 5% significance on the Tukey test (Statistic Analysis System, 2000).

Results & Discussion

The statistic results from the quality traits evaluated on this work are shown in Table 1.

Considering the pH values (4, 6, 8, 12 and 24 h *postmortem*), (L*, a*, b*) color, drip loss and WHC, there was no significant difference in relation to pigs transported in different vehicles and moving system. Guise & Penny (1989), Barton Gade & Christensen (1998) and Warriss *et al.* (1998) evaluated other forms of stress originated from pig transport, and no statistic difference was found on meat physic-chemical traits. According to Warriss, *et al.* (1998) animals can be recovered from transport stress using a proper rest time and handling in the slaughter house. However, for Grandin (1998), the transport stress influences the pork quality, and the use of vehicles aiming at providing better animal welfare conditions are of great importance.

The color (L* values, 50.30 to 51.35) and drip loss (6.42 to 7.30%) obtained in this experiment are quite similar to those found by Warriss *et al.* (1998). According to the classification proposed by Kauffman *et al.* (1993) and Van Laack *et al.* (1994) the samples can be classified from reddish-pink, soft, exudative (RSE, L* values 52 to 58 and drip loss > 5%) to reddish-pink, firm, non-exudative (RFN, L* values 50 to 52 and drip loss < 5%).

Water holding capacity values (0.33 to 0.37) obtained in this work falls in a range considered RSE (WHC values, 0.30 to 0.40) meat according to the Hoffmann *et al.*, (1982).

Conclusions

The treatments which were evaluated on this experiment were not enough to provoke alterations on pork meat quality.

The meat qualities traits evaluated in this experiment suggested that genotype or any other stressor factor which along with the transport had its contribution to classify the meat between RFN and RSE.

It is necessary to point out that the use of methods to improve animal welfare through the reduction of stress and discomfort along transport is crucial, since it contributes to decrease economical losses from an inadequate pre-slaughter handling, as well as it offers support to the already established requirements from the import countries of Brazilian pork meat.

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Table 1. Means and standard deviations for pH, color (L*,a*, b*), drip loss (DL) and water-holding capacity (WHC) parameters in pig muscles under different means of transportation and handling system

Analyses	Muscle	G 1	G 2	G 3	G 4
pH _{4h}	<i>Semimenbranosus</i>	6,25±0,15	6,25±0,16	6,26±0,17	6,20±0,20
pH _{6h}	<i>Semimenbranosus</i>	6,21±0,13	6,29±0,11	6,25±0,14	6,22±0,16
pH _{8h}	<i>Semimenbranosus</i>	6,11±0,16	6,11±0,15	6,11±0,14	6,10±0,20
pH _{12h}	<i>Semimenbranosus</i>	6,09±0,11	6,06±0,18	5,97±0,14	5,91±0,11
pH _{24h}	<i>Semimenbranosus</i>	5,68±0,16	5,81±0,21	5,83±0,16	5,68±0,14
L*	<i>Longissimus dorsi</i>	51,35±1,54	50,30±2,49	51,28±1,80	50,91±1,95
a*	<i>Longissimus dorsi</i>	14,17±0,88	15,83±3,66	14,35±0,87	14,65±0,86
b*	<i>Longissimus dorsi</i>	1,97±0,42	2,24±1,83	2,25±0,94	1,88±0,64
DL (%)	<i>Longissimus dorsi</i>	6,42±0,89	7,30±1,51	6,73±1,67	6,93±1,14
WHC	<i>Semimenbranosus</i>	0,37±0,12	0,34±0,09	0,33±0,059	0,34±0,08

G1. Vehicle with mobile floor and handling system with boards;

G2. Vehicle with mobile floor and the use of electrical goads;

G3. Vehicle with fixed floor and handling system with boards;

G4. Vehicle with fixed floor and the use of electrical goads.

ADDITION OF LIQUID FLAVOR VITAMIN D₃ IN DRINKING WATER BEFORE CHICKEN TRANSPORT AND ITS EFFECTS ON THE STRESS, CARCASS AND MEAT QUALITY

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Key Words: Chicken, vitamin D3, carcass quality, meat quality.

Introduction

Brazilian chicken meat chain is growing in an expressive way in the last few years. Concerning to the international market in the year 2004 Brazil became the leadership reaching 2.4 million tons, approximately 16% of the total chicken meat produced in Brazil and it is the fourth in meat consumption (35kg/per person/year, AVEWORLD, 2005).

The poultry industry in Brazil is also driving attention on the application of animal welfare principles in the farm and during the pre-slaughter operations aiming to reduce the economic losses as well as to fulfill the requirements from imported countries (INDUSTRIAL POULTRY KEEPING, 2004). Considering these investments made by the chicken industry on the slaughter systems the downgrading of the chicken carcass on the Brazilian abattoir is still high varying from 5 to 15%.

A new feed additive developed in Canada has shown potential to overcome these losses by reducing the agitation of the animals during the pre-slaughter operations. This product is commercially market as a liquid flavor vitamin D3 and it is recommended to add in the drink water 5 to 7 days before the animal transport.

Objectives

The object of this study is to evaluate the addition of liquid flavor vitamin D3 in the drink water of chickens during seven days before slaughtering and its effects on carcass quality (bruise, broken bones and skin damage) and the meat (pH, color, water holding capacity and tenderness).

Methodology

Animals. A total of 13.000 chickens divided in two groups (control, n=6.500 and test - liquid flavor vitamin D3, n=6.500) constituted of one genetic line (Hybro) broadly marketed in Brazil was used in this experiment.

Slaughter. The fasting period adopted in this experiment was 7 hours. Chickens were transported during 1 hour in commercially haulier at stock density of ...kg/ m². The resting period at the abattoir varied from 0.5 to 1 hour and chickens from each group was electric stunned (1140 Hz and 50V), bled, eviscerated and cooled in a chiller.

Measurements. Three hundred chilled carcasses were evaluated according to the USDA (1992) grading systems in three classes A, B and C taking into account the presence of the bruises, broken bones, skin damage.

The *pectoralis major* was removed from carcass and pH measurements at 1h and 24h (ultimate) was carried out.

Instrumental color of *pectoralis major* was measured using Minolta Chromameter and the CIE Lab L*, a* and b* co-ordinates were recorded at 1h and 24h postmortem.

Water Holding Capacity (WHC) of *pectoralis major* was determined according to the methodology described for NAKAMURA & KATOH (1985)

Warner Blatzler shear force (WBS) of 1.27x1.27x2.54 cm core samples sheared perpendicular to the fiber direction in three steaks of *pectoralis major* were determined with a TAXT2i Texture Analyzer (full scale load 5kg/crosshead speed 200 mm/min) attached to a Warner Blatzler accessory, as the methodology proposal for FRONING & UIJTTEENBOOGAART (1988).

The statistical analysis was carried out using SAS (SAS Inst. Inc., Cary, NC, 1993). Carcass quality, pH and other meat quality characteristics (instrumental color, water holding capacity and tenderness) results were assessed by application of chi-square, repeated variance analysis and analysis of variance, respectively.

Results and Discussion

The statistical results of chicken grading according to the USDA standards are presented in Table 1. Liquid flavor vitamin D3 treatment (group test) was significantly better since the majority of the carcass (77.66%, p = 0.03) presented less damage (class A). This represents a good profit to the chicken abattoir because these carcasses normally are driving to the export market. However, the carcasses from control group, mainly class C, were downgraded significantly (51.23%, p = 0.03). Normally these carcasses are boned and sold in the internal market or it can be used as a raw material for further processed meat product.

Comparing the pH values obtained during postmortem hours evaluated (Figure 1) it can be said that the treatments studied did not differ significantly (p>0.05). The ultimate pH values reached a range considered as normal meat (5.7 to 5.8) according to Olivo et al., (2001).

The data obtained by CIE L* a* b* system for the *pectoralis major* muscle indicate that the brightness (L*), redness (a*) and yellowness (b*) measured at 24 h *post mortem* were influenced significantly (p<0.05) by the treatments studied. The liquid flavor vitamin D3 (group test) resulted higher L* and a* values as it can be seen in Figure 2. In this experiment the variations in the color composition as far as brightness (L*) values are concerned (50.25 to 51.75) falls into the range considered as normal meat (50 to 52) according to the classification proposed by Olivo et al., (2001).

The statistical analysis of water holding capacity and tenderness results revealed no significant difference (p>0.05) for the treatments studied.

Conclusions

The liquid flavor vitamin D3 has proved its efficiency in this trial since reduced carcass damaged and had no negative influence on meat quality characteristics. It is necessary to point out that the less agitation of the birds observed in the farm mostly during the catching for transport contributed to this fact positively.

When it is considered the production of the chicken meat in Brazil (8.668.000 tones of meat) the use of liquid flavor vitamin D3 will provided more chickens carcasses (15.48%) which could be exported. This fact combined to the increasing number of the countries to which Brazil is exporting (130 countries) will ensure the success of the Brazilian chicken market.

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Table 1. Number and percentage of the animals graded in class A, B and C according to the treatments Control (1) and Liquid flavor vitamin D3 (2).

Treatments	Classes			Total
	A	B	C	
1	120 62.18%	29 15.03%	44 22.80%	193 100%
2	153 77.66%	21 10.66%	23 11.68%	197 100%

P = 0.03, chi square

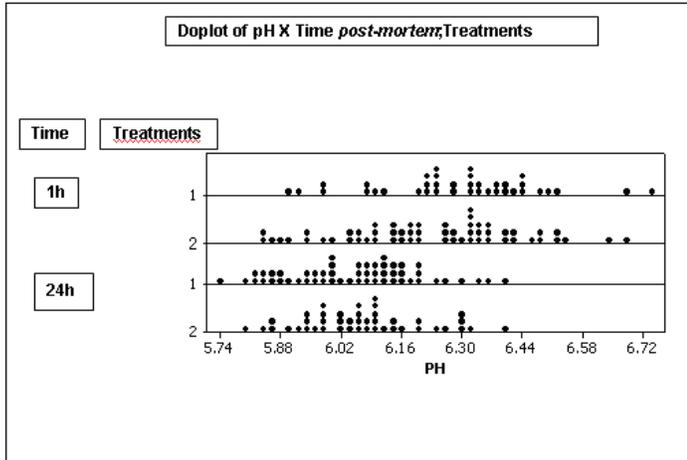


Figure 1. Change in pH values in *pectoralis major* according to the treatments control (1) and liquid flavor vitamin D3 (2)

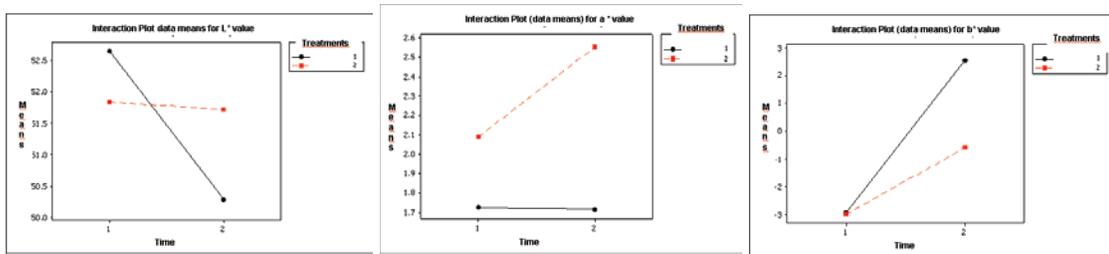


Figure 2. Effect of the treatments control (1) and liquid flavor vitamin D3 (2) on initial and final CIE color values of *pectoralis major*.

**COMPARISON OF TWO PROCEDURES FOR PIG MOVING
ON PRESLAUGHTER HANDLING AND THEIR STRESS EFFECT
ON MEAT QUALITY**

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Key Words: Pigs, stress, animal behavior, pre-slaughter handling.

Introduction

There are many factors contributing to increase animal stress during the pre-slaughter handling, which could modify drastically their meat quality, particularly in pork. One of those is an inadequate animal moving or conduction using electric prods that may result in abnormal undesirable muscle conditions. A lack of regulations associated with the easy manipulation of this particular instrument is inducing their intensive use into abattoirs plants in detriment of animal welfare. The electric prods can cause an increasing of physiological response that modifies the pig behavior (Brundige *et al.* 1998) with consequences in meat quality. High level of pre-slaughter stress causes an increase of glycolytic rate, interference in muscle glycogen reserve and pH dropped, which combined to the body temperature near physiological state reduce water retention. The substitution of electric prod by board for pig moving could be an efficient system for solving handling problems and improving pork quality.

Objectives

The aim of the present experiment was to compare the effect of two types of pig moving (conduction) at pre-slaughter handling in relation to the levels of stress and on meat quality.

Methodology

Animal. A total of 120 pigs with similar age, sex and genotype were used. All animals came from the same farm, 250km away from the slaughter plant. The pigs were divided in two groups (n=60) to submit to loading-unloading for settling in abattoir using

two apparatus for moving: G1 (board) and G2 (electric prod). The total fasting period (farm, transport and lairage) was 18 hours.

Stress evaluation. During exsanguinations plasma samples (n=120) were collected and frozen in liquid nitrogen for analysis of physiological indicators of animal stress. The total plasma cortisol concentration was quantified by means of RIA (Coat-A-Count Cortisol Kit, Diagnostic Products Corporation-DPC, Los Angeles, USA) and lactate levels by spectrometric methods (Lactat PAP enzyme, Farbtest, Rolf Greiner Biochemica, Flacht, Germany).

Meat quality evaluation. Meat quality characteristics were evaluated in 35 pigs from each treatment. The carcass pH was carried out at 4, 6, 8 and 24 hours of *post mortem* in *Semimembranosus* (SM) muscle using a pH meter (INGOLD-WTW). The color by CIE L*, a*, and b* values were determined with a Minolta chroma colorimeter (Mod. CR300) and a muscle light reflectance by Hennessy probe (HGP). The water holding capacity was determined following the methodology described by (Hoffmann *et al.*, 1982) and drip loss was evaluated according to the Honikel (1988) method.

Molecular analysis for gene *hal* characterization. A total of 24 pigs from each treatment were used to determine the gene *hal*. The Genomic DNA was extracted from muscular fiber using equipment Ryboliser that causes cellular damage by friction with the glass pearls releasing genetic material (Ludtke *et al.*, 2001). After DNA purifying it was submitted to PCR reactions using specific *primers* for amplifying DNA fragments with 81 base pairs of *hal* gene (Fujii *et al.*, 1991). The resulting PCR reactions were digested by a restriction endonuclease enzyme *Cfol* (GIBCO-BRL) followed by agarose gel electrophoresis in accordance with Sambrook *et al.* (1989) and modifying introduced by Bastos (1998).

Statistic analysis. The data for meat quality and physiological parameters of stress were analyzed by least squares analysis of variance and correlation using the general linear model (GLM) and CORR (Pearson) with the Statistical Analysis System (SAS, version 6.12).

Results & Discussion

There were significant differences ($P < 0.05$) between G1 and G2 groups (Table 1) for the muscular pH results at 4, 6, and 8 hours *post mortem*. G2 group presented lower pH values in relation to G1. This pH decline suggest that a higher glycolitic rate in the muscle had happened which was also reported by Fernandez & Tornenberg (1991). The coercion for moving pigs induces stress and consequentially PSE meat is a potential risk.

It was verified that the color and light reflectance values had no significant differences ($P > 0.05$) between G1 and G2. Similar results in color and postmortem pH were observed by Van der Wal *et al.* (1999) and D'Souza *et al.* (1998). The treatment studied had also no significant differences ($P > 0.05$) in relation to the water holding capacity (WHC) (Table 1).

Applying what was proposed by Warner *et al.* (1997) for average values of color, exudation loss and pH on treatments (G1 and G2) those sample meats could be classified as reddish, soft and exsudative (RSE) or red, firm and normal (RFN). So, it was observed significant difference ($P < 0.05$) in the incidence of PSE percentage between G1 (13.64%) and G2 (23.53%), while had no difference for meat classified as RSE. An electric prod

removing in processing plants reduced PSE (80 to 33%) and by controlling current on insensitivity equipment (Faucitano *et al.* 1998).

The PSE or RSE condition has no association with the *hal* gene because the genotype frequencies among fiber samples confirm a lack of recessive homozygote ($nn=0\%$) and low frequency of heterozygote ($Nn=14.3\%$). There were significant differences ($p<0,001$) for cortisol and lactate values (Table 2) between G1 and G2 groups. The electric prod raised cortisol and lactate concentrations which could be associated with physic and psychological stress in animals. Brown *et al.* (1998) and Warriss *et al.* (1994) have reported similar values for swine slaughtered in high stress system ($17.02\mu\text{g}\cdot 100\text{mL}^{-1}$ and $139.8\text{ mg}\cdot 100\text{mL}^{-1}$) and lower stress ($7.62\mu\text{g}\cdot 100\text{mL}^{-1}$ and $63.5\text{mg}\cdot 100\text{mL}^{-1}$).

Conclusions

The quality traits results found in this experiment were not affected ($p>0.05$) by treatments studied.

There is a need to handle pigs faster in abattoirs and this prone may not be matched by improvements in handling facilities and practices. The replacement of electric prods by boards in pre-slaughter handling improved animal stress and meat quality in the conditions applied in the present experiment work.

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Table 1. Quality characteristics evaluated in *Longissimus dorsi* (LD); *Semimembranosus* (SM) pig muscles according to the pre-slaughter handling.

Quality characteristics	pre-slaughter handling ^a		Significance
	Lower stress G1	Higher stress G2	
pH (4 h)–SM	6,58 0,22 ^b	6,36 0,3 ^c	0,0008 ***
pH (6h)–SM	6,39 0,18 ^d	6,10 0,26 ^e	0,0001***
pH (8h)–SM	6,08 0,19 ^f	5,83 0,21 ^g	0,0001***
pH (24h)–SM	5,54 0,14 ^a	5,60 0,14 ^a	0,206
Color L* – LD	48,62 1,90	49,70 4,7	0,209
Color a* – LD	0,41 0,43	0,69 1,01	0,384
Color b*– LD	7,12 0,60	7,32 1,38	0,989
Internal Reflectance (45min.) – LD	30,62 3,25	30,83 3,18	0,862
Internal reflectance (24h) – LD	70,12 11,39	72,39 11,93	0,278
Drip loss (%) – LD	7,12 3,04	8,39 2,62	0,204
WHC (cm ²) – SM	0,37 0,07	0,35 0,07	0,220
PSE (%) – LD	13,64	23,53	0,009**

Pre-slaughter handling^a; Lower stress: board; Higher stress: electric prod.

***p<0,001; **p<0,01; *p<0,05;

PSE – L* > 50, drip loss > 5% e pH_u < 6,0.

Table 2- Biochemistry Parameters in pig plasma according to the pre-slaughter handling applied.

	Pre-slaughter handling ^a		Significance
	lower stress	higher stress	
Cortisol (g/dL)	10,76 5,76	16,32 5,50	0,0004***
Lactate (mg/dL)	80,26 41,38	189,214 72,06	0,0001***

Pre-slaughter handling^a; lower stress – board; higher stress – electric prod.

***p<0,001.

Consumer Topics

**DOES VARIATION BETWEEN MUSCLES IN SENSORY TRAITS PRECLUDE
CARCASS GRADING AS A USEFUL TOOL FOR CONSUMERS?**

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Key Words: Beef grading, muscles, consumers, palatability

Introduction

While the original purpose of carcass grading or description systems may have been to sort carcasses into groups of like appearance or composition they are often assumed to also convey meaningful statements in regard to palatability levels as assessed by consumers. Presumably few consumers would believe that all carcass portions have equal palatability but there appears to be an underlying assumption that the cuts have some form of reliable palatability relationship. A 'good cook' or astute consumer is presumed to understand this basic relationship along with cut by cut cooking effects in order to produce beef meals of a consistent expected quality.

A sizeable proportion of meat science literature is devoted to studies of the *m.longissimus lumborum* (LD) muscle with corresponding detailed knowledge of other muscles or positions within muscles sparse in comparison. In both commercial grading and many scientific studies the underlying assumption is that the striploin cut (LD) is a suitable reference point for describing carcasses in total and also their component cuts or muscles. Both subjective and objective approaches assess the LD as a base for sorting carcasses into groups, assumed to correspond to palatability thresholds or ranges.

This paper challenges these assumptions using Meat Standards Australia (MSA) findings. These findings are based on extensive consumer testing of muscles subject to a wide range of animal variables, processing treatments and multiple cooking methods. It is contended that while relationships between muscles can be defined, they differ widely in response to a number of factors which interact, creating considerably different relative outcomes. To more accurately assess palatability the causative relationships or available grading inputs need to be weighted or combined differently on a muscle by muscle basis.

While extensive consumer testing can quantify these relationships and produce useful consumer predictions (Watson *et al.* (2005)) this is an expensive process and does not provide direct explanation of mechanisms involved. If the mechanisms were better understood it might be possible to more effectively predict individual muscle consumer outcomes, reducing the need for extensive consumer testing.

There is a need for more comprehensive study of muscles other than the LD to categorise compositional and mechanistic effects which could be effectively used to predict consumer satisfaction from each portion of any beef carcass.

Objectives

The objective of this study was to examine the degree to which a predicted consumer score for the anterior portion of the LD muscle remained constant as a ratio to those for other muscles over a range of production, processing and cooking scenarios.

Methodology

The MSA 2004 prediction model was used to predict consumer based MQ4 scores for muscles from a range of carcasses. The MQ4 score is a composite score created by weighting and combining consumer scores for tenderness, flavour, juiciness and overall satisfaction. The prediction process is described by Watson (2005) and development of the MQ4 score consumer testing methodology further described in Watson *et al.* (2005). The model provides a useful prediction of consumer responses to over 35,000 cuts tested within the MSA program over a ten year period utilising five cooking methods and involving in excess of 65,000 consumers.

The model was run for a series of alternative inputs and ratios calculated between the anterior grilled striploin (*m.longissimus lumborum*) MQ4 score of each carcass relative to the MQ4 score of other muscles. Muscles reported are *mm. adductor femoris* (AF), the cranial and distal portions of the *biceps femoris* (*syn. Gluteobiceps*) (BFC and BFD), *gluteus medius*, divided between the “eye” (GME) and “D” (GMD) positions, *infraspinatus* (IF), *psoas major* (PM), *rectus femoris* (RF), *semimembranosus* (SM), *semitendinosus* (ST), *serratus ventralis cervicis* (SV), *spinalis dorsi* (SD), *triceps brachii caput longum* (TB), and *vastus lateralis* (VL). These muscles were selected to represent major commercial cuts.

An achilles hung, non-implanted steer carcass of 0% *bos indicus* content, 250kg carcass weight, 150 ossification, 250 marbling with an ultimate pH of 5.5 @ 3.0°C with all cuts aged seven days post slaughter was selected as a base example. The ratio of predicted MQ4 scores for a range of cuts and cooking method combinations from this carcass are presented in Table 1.

Table 1. Ratio of cut by cook MQ4 scores for selected muscles (Base carcass)

MUSCLE	Code	Grill	Roast	Stir Fry	Thin Slice	Slow Cook
<i>m.longissimus lumborum</i>	LD	100	101	104	105	na
<i>m.spinalis dorsi</i>	SD	136	118	135	128	na
<i>m.psoas major</i>	PM	136	134	140	130	na
<i>m.infraspinatus</i>	IF	113	109	118	123	na
<i>m.triceps brachii caput longum</i>	TB	96	102	104	106	107
<i>m.gluteus medius ("D")</i>	GMD	90	105	99	109	95
<i>m.gluteus medius (eye)</i>	GME	95	109	108	107	na
<i>m.biceps femoris (cap)</i>	BFC	104	na	118	119	na
<i>m.biceps femoris (distal)</i>	BFD	na	71	75	98	103
<i>m.rectus femoris</i>	RF	83	106	97	103	84
<i>m.vastus lateralis</i>	VL	65	85	79	91	93
<i>m.semitendinosus</i>	ST	77	84	80	85	88
<i>m.adductor femoris</i>	AF	71	na	91	95	89
<i>m.semimembranosus</i>	SM	62	77	77	100	93
<i>m.serratus ventralis cervicis</i>	SV	95	97	98	104	118

Subsequent predictions were run for carcasses with amended *bos indicus* content, differing ossification, sex and marbling scores, implanted with hormone growth promotants (HGP), suspended by the aitch bone and aged for differing periods. Ratios were recalculated for the selected muscles within each carcass specification in relation to the grilled LD, the score for which was set to 100 in each case.

Results & Discussion

Results are discussed in relation to the efficacy of an anterior grilled striploin MQ4 score as a basis for grading other commercially significant carcass muscles. It is assumed that the objective of grading is to provide an accurate estimate of consumer satisfaction in relation to the final cooked product.

As shown in Table 1 the MQ4 ratios vary extensively between muscles, between cooking methods and for position within some muscles. While there is little ratio difference between cooking methods for the LD extreme differences are evident in the VL, BFD and SM and moderate differences in the GMD, GME, RF, AF and SV. The consumer is predicted to obtain a superior result by roasting the GMD, GME, RF, VL and SM in comparison to grilling. The SV records its best result slow cooked in contrast to the SM which is best thin sliced. Within muscle position differences are also predicted for the GMD versus GME and for the BFC in comparison to the BFD.

Koohmaraie *et al* (2002) have stated that beef tenderness can be explained by the cumulative effects of sarcomere length, connective tissue toughness and proteolysis in individual muscles. It would be advantageous to reduce the requirement for extensive consumer testing by applying more detailed knowledge of the relative importance, action and interaction of these three factors on an individual muscle or muscle by position basis. If the ratios displayed between the grilled LD and other muscle by cook combinations were consistent then it follows that detailed experimental results from the LD could be used to predict the balance of the carcass. This reflects the base assumption of carcass grading.

However, as demonstrated by Table 2, this assumption is seriously flawed. The calculated ratios (for grills only), displayed for the base and 7 alternative carcasses, differ widely. As an example the SD MQ4 score has a ratio of 136 to the LD in the base carcass but ranges from 112 in carcass C to 171 in carcass A due to the calculated model interactions. While the SD ratios are similar for the base and carcass B, and while carcasses D and F, and E and G are similar to each other the SD ratios differ widely between pairs.

Table 2. The ratio of predicted MQ4 scores (Grill) for selected muscles from a range of carcasses.

** AT=achilles tendon, TX=obturator foramen

MODEL INPUTS		CARCASS INPUTS							
		Base	A	B	C	D	E	F	G
% <i>bos indicus</i>		0	100	0	0	60	35	0	60
Sex		M	F	M	M	M	F	M	M
HGP implant		No	No	Yes	No	No	No	No	Yes
Carcass Wt (Kg)		250	250	380	280	290	250	380	290
HANG **		AT	AT	AT	TX	TX	AT	AT	AT
Ossification		150	120	170	120	170	500	190	190
Marbling		250	200	330	350	330	200	500	300
Rib fat (mm)		5	5	5	5	5	5	15	5
pHU		5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Loin temp °C		3	3	3	3	3	3	3	3
Days aged		7	7	14	21	14	28	14	21
MUSCLES	CODE	RATIO TO LD MQ4							
<i>m.longissimus lumborum</i>	LD	100	100	100	100	100	100	100	100
<i>m.spinalis dorsi</i>	SD	136	171	134	112	124	147	121	143
<i>m.psoas major</i>	PM	136	162	134	114	125	144	122	142
<i>m.infraspinatus</i>	IF	113	142	110	91	99	120	100	114
<i>m.triceps brachii caput longum</i>	TB	96	114	95	84	85	87	87	95
<i>m.gluteus medius ("D")</i>	GMD	90	121	89	90	95	92	82	95
<i>m.gluteus medius (eye)</i>	GME	95	128	94	94	100	98	87	101
<i>m.biceps femoris (cap)</i>	BFC	104	139	101	96	102	106	94	109
<i>m.rectus femoris</i>	RF	83	104	83	79	84	77	77	85
<i>m.vastus lateralis</i>	VL	65	80	66	65	67	56	61	66
<i>m.semitendinosus</i>	ST	77	98	79	72	71	63	72	80
<i>m.adductor femoris</i>	AF	71	88	70	72	78	70	68	74
<i>m.semimembranosus</i>	SM	62	77	62	65	70	60	60	65
<i>m.serratus ventralis cervicis</i>	SV	95	117	96	83	86	97	89	97

Alternative muscles display further range differences and the pairings described above also differ. While the base carcass and B share equal ratios between their LD and RF, and similar ratios for most muscles, the RF ratios differ widely between pairs D and F and E and G which were previously paired by their SD ratios.

This pattern of irregular association is seen across the range of cuts within each carcass and reflects the differential effect of various model inputs on predicted consumer scores for each muscle. Input differences, shown in the upper portion of Table 2, were from 0 to 100% *bos indicus* content, male versus female, implanted (HGP) or not, achilles tendon (AT) or obturator foramen (TX) suspension and a range of carcass weights, ossification and marbling scores, together with variation in ageing. Within each of the carcasses any change to the ageing period would produce further substantial changes in ratios as muscles age at different rates and ageing further interacts with carcass suspension. (Watson. 2005)

While sarcomere length, connective tissue toughness and proteolysis might explain all tenderness differences, the large ratio differences within the example carcasses in table 2 suggest that if this is so these causative factors are in turn affected differentially by mechanical effects including carcass suspension, by biological effects such as sex, HGP implant use and ossification level, and by directly observed muscle characteristics such as marbling and pH. Consequently relative muscle palatability is not fixed, but varies widely and individually in response to other influences. These influences may act by modifying sarcomere length, connective tissue toughness and proteolysis individually or by interaction.

The MSA prediction model is effective due to its development from extensive consumer testing. This is an expensive process if a large number of potential individual muscle effects and interactions must be tested to develop predictions. The ideal approach is to combine knowledge of muscle composition, characteristics and causative mechanisms to accurately predict the performance of all commercially important muscles without the requirement of blanket consumer testing. At present the literature is dominated by LD data which does not adequately relate to other muscles. More research is required on alternative muscles to build an adequate knowledge base to facilitate eating quality prediction.

In recent studies National Cattlemen's Beef Association (2000) presented data on a number of chuck and round muscles while Rhee *et al.* (2004) have reported extensive data on major muscles which will assist in developing the required knowledge. Further studies and collaboration are needed to estimate the relative impact of sarcomere length, connective tissue toughness and proteolysis in individual muscles and to more adequately describe relationships between these factors and the mechanical or biological mechanisms which modify their influence.

A suitable prediction regime developed from improved data might provide a basis for estimation of consumer satisfaction and allow consumer testing to be used as a validation tool rather than as a means of obtaining primary data.

Conclusions

The analysis conducted demonstrates that it is not possible to provide meaningful estimates of consumer satisfaction for a range of cuts from a simple LD relationship. The relationship between muscles varies extensively depending on a range of inputs including the period of ageing, carcass suspension, cooking method, % bos indicus, marbling, ossification, sex, carcass weight and use of hormonal growth promotants.

The implication is that for grading systems to be useful to consumers they need to independently estimate specific muscle results. This challenges the notion of grading a carcass as a single unit. While a common set of grading inputs may be appropriate they need to be applied differentially by muscle to adequately estimate eating quality. This implies a need to modify thinking from grading carcasses to grading individual cuts.

A related caution is raised regarding attempts to interpolate objective LD based experimental results to other carcass muscles. Further studies and collaboration are needed to estimate the relative impact of sarcomere length, connective tissue toughness and proteolysis in individual muscles and to more adequately describe relationships

between these factors and the mechanical or biological mechanisms which modify their influence.

A suitable prediction regime developed from improved data might provide a basis for estimation of consumer satisfaction and allow consumer testing to be used as a validation tool rather than as a means of obtaining primary data.

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TRACEABILITY AS A NEW APPROACH TO MEAT PRODUCTS SAFETY MONITORING IN RUSSIA

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In the past five years a great change from the economy of decline to the economy of recovery has been occurring in Russia. In general, the situation is changing to better, and now one can say that it is a sustained trend. Nevertheless, the situation in such an important sector of national economy of Russia as agriculture and especially in production of animal husbandry products remains complicated.

In 2000 – 2003 sufficiently large growth of production volumes of the main meat products was achieved, and this was done without significant modernization of production, with stable volumes of processing of cattle and poultry, mainly owing to more deep, complex use of raw materials, rational utilization of secondary materials, reduction of losses using intensive refrigeration technologies, implementing new advanced resources-saving technologies. During this period, production of meat and by-products of the first grade increased by 45%, sausage products – 71%, meat semi-finished products – 2.3 times. This is connected, among other things, with the large volumes of imported meat raw materials, increase of demand in meat and meat products of the Russian population. The structure of nutrition of Russian population is presented in Fig. 1.

The more dynamic development of the market for meat and meat products in Russia is constrained first of all by shortages of meat raw materials. Meat industry enterprises work under the conditions of deficiency of domestic animal raw materials. A minor growth of domestic raw material resources along with significant volumes of imported raw materials has led to some increase in production of meat and meat products in recent years. However, in spite of positive trends in the meat industry development in these past years, the level of dependence from the import in this segment of the food market remains sufficiently high: in 2003 the share of import in forming of meat and meat products resources in Russia was 30.2%, though this figure is lower than that for 2002 (34%) (Fig. 2).

The unavoidable entry of Russia into the World Trade Organization in the near future, in the opinion of meat producers, is connected with many problems in the agroindustrial complex of the country. The active participation in the international trade requires that all the restrictions on the access of foreign foods to the interior market should be removed and customs barriers eliminated.

Along with this, the concern about the safety of foods was never as high as now. Recent crises in food production in Europe cast doubt in the conscious of consumers and gave rise to distrust with regards to the products supplied to the market. During these years mass media paid much attention to such problems as BSE, avian influenza, Listeriosis, dioxins, pesticides, etc.

Great changes in society and trade taking part during last 30 years necessitated more integrated approach to safety of foods. The integrated approach comprises all the components that can have impact on safety of foods on each level of the food chain “from farm to fork”. This approach demands the interaction of all the participants of food chain from feeds producer to individual consumer.

Safety of foods became the main priority for the chain of meat supply. However, it should be noted that it is difficult to separate such notions as safety and quality. Depending on the degree of environment pollution with one or other types of contaminants, they can lead to ecological stress, or ecological crisis with the result of violation of all the cycle of production of safe and quality products.

In our institute we have developed the concept of production of guaranteed safe meat products that contains a complex system of provision of quality and safety of protein products based on the use of hurdle technologies, predictive microbiology, critical control points, principles of production management, system of traceability of safety and quality of the product along the whole technological chain of its production, transportation and selling. All that the soil contains goes to plants, all that plants contain goes to the organism of the animal and then to products of animal husbandry and then to foods.

Under the conditions of increased anthropogenic contamination of the biosphere, production of ecologically pure animal products is a great challenge. The range of chemicals used in agriculture and animal husbandry is very large and contains a lot of preparations which can have a threat to health of people. The content of xenobiotics of chemical and biological origin in foods is the main threat to human organism. The group of xenobiotics of chemical nature contains toxic elements (lead, cadmium, mercury, arsenic), pesticides, radioactive isotopes, etc. According to FAO/WHO, toxic elements have pronounced carcinogenic and mutagenic effect and are of the first priority in the list of chemical substances, dangerous for the environment and health of people.

How it occurs, how the chemical composition of the product undergoes modifications and what is the effect of all this on the safety of the products? To answer these questions combined efforts are required, therefore, at the present time we are more actively cooperating with the related agricultural Institutes to create the common system of monitoring. It should be noted that introduction of the system of quality managing at an enterprise is a continuous and complicated process involving all the services, all personnel. It is not limited to drawing up documentation and doing the activities imitating the order. Our approaches to solving this problem implicate deep penetration into the occurring processes. To introduce the quality managing system it is necessary to teach specialists of the working group and persons responsible for operative control, correction of technological documentation. The system of traceability being effective by its impact and cost, as distinguished from the simple identification of the general commodity group, is able to indicate the any problem with regards to food safety, related to specific geographical origin, equipment for primary and technological processing, the chain of supply, to the farm or even to the specific animal.

The necessity of introduction of the system of traceability in Russia is connected with requirements of the federal laws “About technical regulation”, “About quality and safety of food products”. The main task of the law “About technical regulation” – elimination of technical barriers and production of safe products. The law is aimed at production of safe

products – the State declares it, and producers themselves must provide proper quality in their competition for preferences of the consumer.

GNU V.M. Gorbatov Meat Research Institute of the Russian Academy of Agriculture carries out work to get the enterprises prepared to certification in the systems ISO HACCP. The organ on certification HACCP- MEAT is accredited in the Institute. And this field of activity as well as efficient use of all the components of technical regulation is the pledge of successful solution of many important problems in this country, the problem of production safety among them.

A certified system of quality and safety management is the proof of fulfillment of producers' obligations in satisfying the requirements of the consumer and achieving the desired quality. But it is impossible to raise the quality without changing relation to the notion of the quality per se on all the levels. The calls to increase the quality won't be realized, if the executives of different levels don't consider the quality as a style of life. Of no small importance is the fact that during introduction of the systems of quality ensuring a change in the psychology of the workers of the enterprise occurs. They realize of importance of stable quality and begin to understand what should be the management of the modern organization, ensuring best results of its activities. Therefore, the investments pay back quickly, because at the enterprise appears a harmonious system, allowing not only to guarantee quality and safety of the products, but also to optimize production due to detection and reduction of unjustified expenses.

According to European legislation, traceability is not only the possibility to trace back movement, location and origin of foods, feeds, animals and components of animal origin designed of supposed for use as food products, on all the stages of production, processing and distribution.

From the point of view of management of informational processes, introduction of traceability system in the production chain of meat products requires systematic combination of the physical flow of raw materials, semi-finished products and prepared products with the informational flow from all the involved partners. To guarantee continuous informational flow every participant of the production chain should exchange certain data necessary for organization of the system of traceability, with the next participant of the chain, thus presenting the opportunity to further use all the principles of traceability. The ability to obtain data quickly and exactly along the whole production chain is the basis for creating the system of traceability. To do this it is necessary to manage the consecutive bond between the components of all what is produced, packed, stored and shipped on the whole chain of supply (step-by-step: one step up, one step down along the chain).

The most important aspect of the program of safety of food products during production of meat, fish, eggs, and milk is the systematic control of substances which can appear in the products as a result of use of antibiotics, hormones, pesticides, contamination of the environment or non-observance of technological conditions.

The national program of control should provide the possibility of identification and evaluation of substances, corresponding to the class of the product; introduction of corresponding national standards, harmonized with the international ones, based on the data about the residues of harmful substances, exceeding the established limits; collection of data and reports about the obtained results.

In general, the importance of the program of control for toxic substances implies not only collection of information, but also, owing to the principle of traceability – ensuring the protection of population health through the ban to direct meat raw materials, containing residues of toxic substances exceeding limit values to trade or processing.

Russian Federation needs the efficient and agreed program of control of the level of toxic substances residues, to ensure protection of health of population and to participate in the International trade of foods. Traceability as the main principle of the control program, that will prevent (in case of exceeding the established limit concentration) getting animals and primary products of animal husbandry into the food chain, will make a large contribution to production of safe products for human consumption.

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- bakery products
- meat products
- dairy products
- eggs
- fish products
- oil
- sugar
- potatoes
- vegetables
- fruits

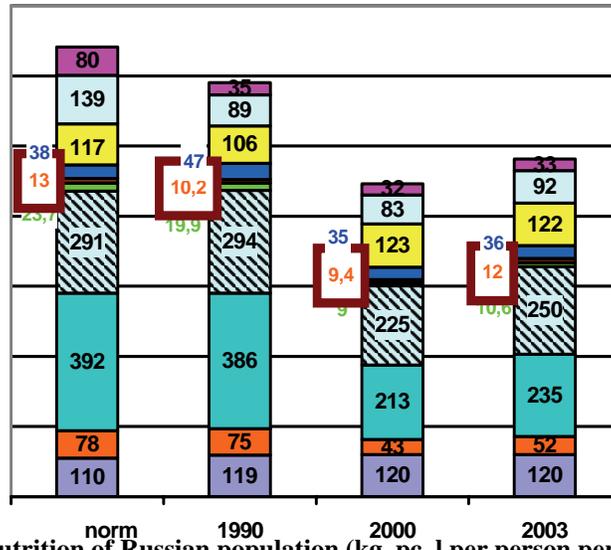


Fig. 1. Structure of nutrition of Russian population (kg, pc, l per person per year)

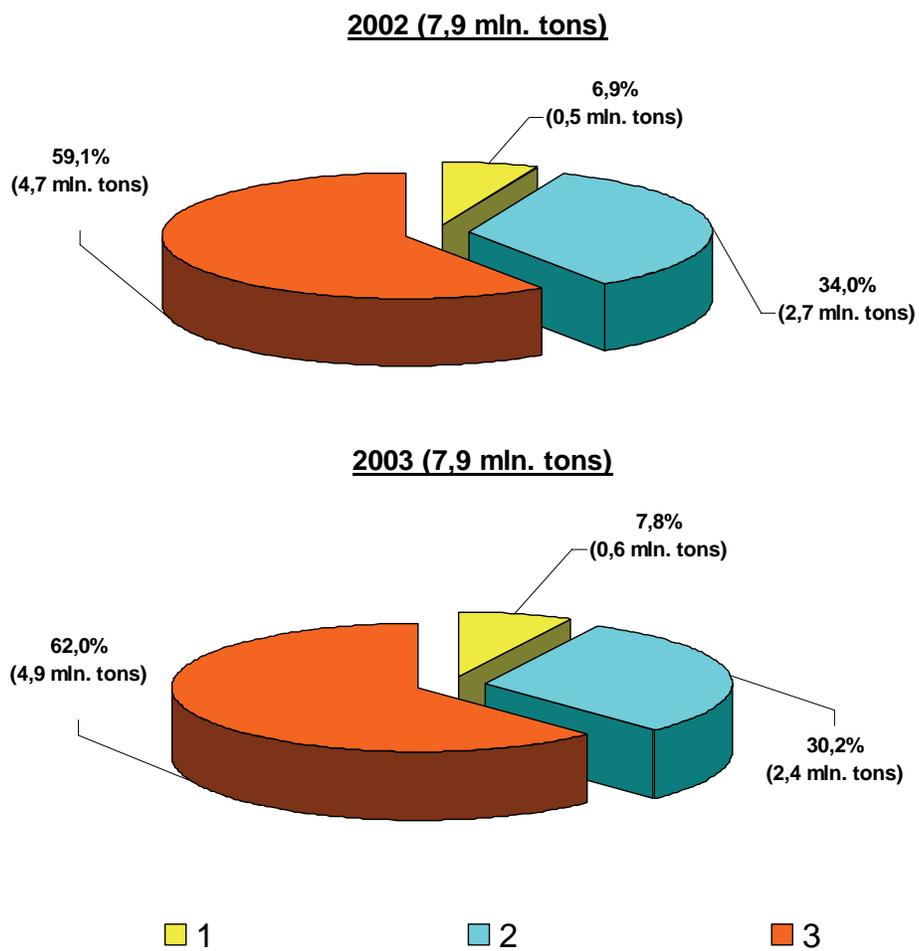


Fig. 2. Structure of formation of resources of meat and meat products in Russia in 2002 and 2003: 1 – Reserves in the beginning of the year; 2 – Import; 3 – Produced

UPGRADING AND DEVELOPMENT OF MEAT HYGIENE AND TECHNOLOGY EDUCATION IN EGYPT

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Summary

This project is designed. to enhance the quality, efficiency, relevance and outcomes of the teaching and training programs of meat hygiene and technology for the veterinary students, meat inspectors, meat hygienists and workers in Egypt. The stages of accomplishment of the project are:1) upgrading the curriculum of meat hygiene and technology, 2) preparation of textbooks, lecture notes and teaching materials, establishing up-to-date labs to help perform complex tests and investigation of quality of meat and meat products, 3) transfer of advanced teaching methodology in meat hygiene and technology, 4) setting up a system of training and re-training the specialists in meat hygiene and technology and 5) providing services to the sector of meat industry (training and inspection of meat and meat products).

Also, two important centers will be established: the modern industrial abattoir and pilot plant for meat processing. The two centers will be responsible for providing training courses for students, veterinarians , meat hygienists and workers in abattoirs and meat industry. All teaching and training programs will be kept in database system, which will be available on the internet via the project's website. The use of modern teaching technology and up-to-date labs will be available. Dissemination and sustainability will be supported by an alumni association which will spread the experience gained from the project and continue the centers' work beyond the lifetime of the project.

During the first quarter of the project (February to May, 2005), the following activities have been implemented:1) revised and newly developed curricula of the theoretical and practical meat hygiene and technology, 2) The new curricula for the undergraduate veterinary students shall be introduced at four universities in September, 2005. 3) Modern industrial abattoir and meat pilot plant are being constructed.

Keywords: Meat hygiene - K.Q of meat - Meat products - Meat technology - Industrial Abattoir - Meat pilot plant

Introduction

The science of food hygiene appertains mainly with establishment food and protect the consumers against unsafe food. The origin of food hygiene can be found already in very old societies of people living in the earth. Regarding to the establishment of food: since the beginning of the mankind food hygiene gatherers had the task of identifying the

edible food and discarding the inedible ones. Thus, they had the extra task of finding food that was also safe. In early time, edible and safe were probably synonymous when applied to food. The early Egyptians illustrated the inspection of an animal's heart at the time of slaughter and smelling of blood on an inspector's fingers to detect signs of putrefaction. The Romans were known to have confiscated foods thought to have spoiled. In twelfth-century Holland inspectors were appointed to examine the fish sold at markets (Kampelmacher, 1973).

Meat hygiene represents a major branch of the food hygiene and no one can deny its economic and public health significance in our life. On the other hand, the modern food technology is becoming more and more complicated and has always constituted special risk. Therefore, the food hygiene programs are improving for food of animal origin particularly meat, fish, poultry and their products. Any national food hygiene programme represents a dynamic process, which must keep pace with the overall national social and economic development and the newly acquired scientific knowledge. Taking into account the six components of the national food hygiene programmes (Matyas, 1989), it is obvious that they cannot be run by single service/agency. It is a multisectoral and multiprofessional undertaking in which food hygiene control plays an important role.

So, education of veterinary students and hygienists should provide a broad knowledge of a variety of matters related to food including food hygiene control and particular to hazard analysis critical control points (HACCP) (ICMSF1988, USDA, 1989, WHO, 1982), and to predictive microbiology, technological, epidemiology, nutritional and economic aspect (Matyas, 1992)

Modern Meat Technology

The development of meat technology in the last decades has markedly changed and becoming more and more complicated. Therefore veterinary food hygienists must have a good knowledge of productions and technology, preservation and transportation techniques. Modern education must take into consideration the rapid developments in food industries and also cover the hygiene and technology of modern products (Matyas, 1978). In the developed countries special risk have always appeared from mass production of meat and meat products.

Good hygienic practice are necessary prerequisites for the successful application of specific risk management strategies. In particular, the faculties should be conceived, maintained and used to prevent contamination (Codex, 2003). Meat is an important carrier agent for many diseases, consideration will always form a basic part of meat hygiene programs. In general, the meat hygiene programs and the area of meat technology require highly qualified meat hygienists, inspectors and veterinarians. The following problems are prevented or at least reduced due to application of the right food hygiene programs by well educated and trained persons:

- prevention of food borne infections and intoxications in consumer and prevention of occupational food borne illness,

- prevention of environmental pollution from meat production and processing sources, as well as from industry,
- reduction of spoilage and reduction of meat losses,
- reduction of malnutrition, contribution to the program on surveillance,
- prevention and control of zoonoses,
- control of chemical contaminants and additives and also radio nuclides,
- control of rodents-vectors of diseases,
- facilitating national and international trade.

The above results due to application of the well organized and managed meat hygiene programs by well educated and trained persons, are sufficient justifications of proposals for strengthening, developing and upgrading of meat hygiene and meat technology education and training particularly in Egypt.

Meat Hygiene and Meat Technology in Egypt

The existing conditions for slaughtering and meat handling in Egypt cause quality deterioration ; post-harvest losses of meat and food borne diseases in consumers. The achievement of national levels of meat quality, efficiency and profitability presents a difficult challenges for Egypt due to:

1. lack of effort to provide knowledge and skills in adequate hygienic slaughtering, meat cutting and handling.
2. the absence of meat preservation techniques presents a serious constraints to the development of viable meat production by resource-poor rural livestock produces.
3. growing consumer awareness of food borne diseases particularly the emerging diseases i.e. Mad Cow disease, listeriosis, E.coli O157:H7 (causing Hamburger disease).
4. workers and management in Egypt Meat Industry are generally insufficiently trained and skilled in recognizing, preventing or reducing the health and safety risks related to their work. Middle management often lacks the necessary skills for policy making and management of this aspect of their conduct of business.

Meat hygiene and meat technology education has to take into account these new and changing awareness. The importance of this topic is to provide the meat industry in abattoirs and meat factories with a highly qualified veterinarians that they are working as meat inspectors and hygienists. This target will be done through the upgrading and development of meat hygiene and meat technology education for under and post-graduate students.

The Curriculum Content of Meat Hygiene and Technology

The current situation of the course of meat hygiene and meat technology for undergraduate students in the faculty of Veterinary medicine, comprises of 6 h. (theoretical lectures) and 4h (practical) per week as illustrate in Table 1. The recommendations of the two International Congress of Food Hygiene and Human

Table 1. Curriculum Contents of Meat Hygiene and Inspection in the Veterinary Medicine of Assiut University - Egypt

Meat Hygiene and Meat Technology		
Meat Hygiene		Meat Technology Training
<p style="text-align: center;"><u>Theoretical Part</u></p> <ul style="list-style-type: none"> • Food animals • Abattoir • Methods of slaughtering • Rigor mortis • Abnormal conditions • Affection of specific parts • Bacterial, viral & parasitic diseases of food animals • Meat microbiology • Meat preservation • Chemical residues in meat • Consumer protection • Poultry hygiene • Fish hygiene • Byproducts 	<p style="text-align: center;"><u>Practical Part</u></p> <ul style="list-style-type: none"> • Comparative anatomy • Lymphatic system • Chemical examination • Abnormal conditions and yellow coloration • Detection of freshness • Inspection of fish • Inspection of poultry • Microbiological investigation • Lesions in the different organs of slaughtered animals 	<p style="text-align: center;"><u>Meat Technology</u></p> <ul style="list-style-type: none"> • Lack subjects related to the modern meat technology • Few knowledge related to the technology of traditional meat product • Few knowledge related to the modern meat preservation <p style="text-align: center;"><u>Training</u></p> <ul style="list-style-type: none"> • Few training in abattoir “mainly meat inspection” • No training in meat factories • Generally, Lack of modern and advanced training programs

Health that were held in Assiut University in 2001 and 2003 and the workshop on the evaluation of the food hygiene education in Egypt (during the first congress) focused on the following:

1. The improving and upgrading of the contents of the whole courses of food hygiene education and increasing the teaching hours of theoretical and practical per week.
2. Updating the methods of teaching of the theoretical and practical meat hygiene and technology . Developing of advanced programs for training students and veterinarians in modern abattoirs and meat factories.

From the above Table (1) and the recommendations of the two International Congress of Food Hygiene and Human Health, it became very important to revise the current curriculum of the meat hygiene to conquer all defects in it. Furthermore, advanced meat technology course and training programs should be created according to the international standards.

The contents of the meat hygiene course (theoretical ad practical), and the training policy for students, meat hygienists and workers have not been considerable developed in all faculties of Veterinary Medicine in Egypt at all levels over the last 40 years. Furthermore, teaching of modern meat technology is completely ignored. Unfortunately, most employees in the meat industry (abattoirs and meat factories) have limited education and skills training. On the other hand, due to the bad situation of the most abattoirs in Egypt, serious problems and constraints in supplying expanding populations with good quality meat and meat products can be expected.

This area of meat hygiene and meat technology entirely fits into national priorities, standards in quality control, public service development and social policy. Therefore, the academic programs must: develop curriculum that reflect market place expectations, enhance the efficiency of resource utilization, embrace new technologies that provide novel methods or information delivery, and reassess cooperative linkages among industrial with governmental organizations and Universities.

The needs of the consumer and subsequent challenges to the meat and farming industry, will be proposed as the driving force behind the changes occurring in veterinary public health. The current risks to consumers, from food borne diseases particularly the emerging diseases as Mad Cow Disease, Hamburger disease (caused by E.coli O157:H7) are highlighted. The two examples given above are evidence of the increasingly important role of meat processing in the meat sectors of Egypt

From the above knowledge's, it is necessary to focus on promoting the teaching and training program of meat hygiene and technology for students and meat hygienists (Codex, 1998) .The following requirements should be acquired:

1. Updating and introduction of new subjects of meat hygiene course according to the international standards, also the course should include an advanced and up-to-date part for meat technology..
2. Training staff members to use educational aids.
3. Training of the professionals and technicians on meat technology and inspection.
4. Organizing training courses in both meat technology and inspection for meat hygienists and workers in meat industries.
5. Upgrading of the teaching class, Lab of students.
6. Establishment of modern abattoirs and small meat processing units (pilot plant) in Assiut University. Through this pilot plant training of students, meat hygienists and the technical assistance of the project will be provided.

Conclusions

Food hygiene programmes (food control) in Egypt tends to be inadequate, due to limited resources and often poor management. Food control laboratories are frequently poorly equipped and lack suitably trained analytical staff. This is accentuated where multiple agencies are involved in food control. A lack of overall strategic direction means that limited resources are not properly utilized. Food control systems may also suffer from poorly or inadequately developed compliance policies.

Modern food control systems call for science-based and transparent decision-making process, and require access to qualified and trained personnel in disciplines such as food hygiene, technology, chemistry, biochemistry, microbiology, veterinary science, medicine, epidemiology, agricultural sciences, quality assurance, auditing and food law. Food control authorities need to better appreciate the role of science in the risk-based approach and to take advantage of scientific resources in the international community.

Recommendations

- There is no doubt that, the well organized food sections of any countries (developed or developing) are successfully implemented by well educated and trained persons.
- Well trained veterinarians, meat hygienists, meat inspectors are the basis of effective and long-lasting improvements in the level of food hygiene.
- Food hygiene should be considered as an integral part of the total food system
- Food hygiene control program is an important component of any national food hygiene program.
- Education and training must keep pace with developments and reflect the needs of the respective countries
- Primary health care concept, hazard analysis critical control points system (HACCP), the microbial food safety risk assessment, health system research (HSR), health economics and socioeconomic consequences of food borne

infections and intoxications should also become part of education and training (Caswell, 2000; Lammerding, 1997).

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PUBLIC HEALTH IMPACT OF POST-HARVEST CONTAMINATION OF BEEF CARCASSES SOLD AT BUTCHERS MEAT STALL

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Abstract

To assess the extent of microbial contamination of beef carcasses dressed by butchers at slaughter yards and retail sales, meat samples collected from beef carcasses of meat stalls at BAU Campus and Mymensingh town were examined. The higher counts obtained in thigh muscle can be attributed to possible chances of more exposure to contamination from the feet and viscera. It is observed that equipment, working tools and other meat hygiene practices such as washings of carcass with water could introduce microorganisms during slaughtering, skinning, washing and dressing of carcasses. It is interesting to note that maximum microbial load of air was found at the location where customers usually stand to purchase retail cuts of meat. The highest contaminants with microbes were obtained during peak selling period between 10 a. m. to 11 a. m. The most commonly isolated organisms were *Staphylococcus* sp. 36.12% followed by *Streptococcus* sp.15.26%, *Bacillus* sp.12.71%, *Micrococcus* sp.11.23%, *Escherichia coli* 10.28%, *Proteus* sp. 5.24%, *Pseudomonas* sp. 3.56% and *Enterobacter* sp.3.25%. The post-harvest contamination in meat samples with Coliform and Staphylococci at the present level presents potential hazard and alarm to public health. Improving slaughter hygiene and introduction of effective meat inspection programs are indispensable and urgently needed to maintain safety and wholesomeness of meat foods.

Introduction

Meat as a protein although provides an excellent source in human nutrient, but if unhygienically produced can serve as a common source of pathogens and may propagate to people via the "contamination chain". The important contamination comes from external sources during bleeding, handling and processing. The exterior of the animal harbors large numbers of diversified microorganisms from soil, water, feed, and manure, as well as its natural body surface and the intestinal contents. Knives cloths, air, hands and clothing of the workers can serve as intermediate sources of contaminants. During handling of the meat, contamination can come from carts, boxes, or other containers (Frazier and Westhoff, 1995). Special equipment such as grinders, sausage, stuffers casings, and ingredients in special products e.g. fillers and spices may add undesirable organisms in appreciable numbers. Unfortunately in Bangladesh, the consumers due to unawareness and lack of non-enforcement of legislation are the potential danger of having contaminated meat. The present study was therefore, undertaken to find out the

sources and extent of microbial contamination of beefs obtained from butcher's meat stall, which may likely to be a health hazards, when it entered into the food chain.

Materials And Methods

Preparation of meat samples and examination for bacteriological studies

The different samples for bacteriological examination were as follows:

1. Sample from air
2. Raw meat samples
3. Samples collected from carcass rinses and washes
4. *Samples from air*: To determine the evidence of bacterial pollution in the air of the meat market, petri-dishes of 110 mm diameter containing nutrient agar were kept at three different places of the sale corner, where the butchers usually prepare retail cuts and sells these to the consumers. The lids of the petri-dishes were made open at the selling period beginning from 8.00 am to 12.00 noon. The plates were exposed to the air for a period of five minutes.

The petri-dishes so exposed to the air of the meat market were later transported to the laboratory and kept in the incubator at 32°C for 24 to 48 hours. After incubation the colonies developed on the media were counted with the help of a colony counter.

1. *Raw meat samples*: Raw meat samples of beef carcasses were collected directly from the retail hanging display of butchers.

Quantities of about 500 gm of meat cut samples were collected from the different regions of the carcass, such as thigh, chest, neck, and forelimb regions. Individual sample is minced and a quantity of 50 gm was transferred into sterile containers containing 450 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender and 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from 10^{-2} – 10^{-6} were prepared according to the recommendation of International Organization for Standardization (ISO, 1995).

In another experiment, fresh meat samples were collected at different market hours. All these samples were subjected to bacteriological analysis such as determination of total viable count (TVC), total coliform count (TCC) and total staphylococcal counts (TSC). In case of TVC, TCC and TSC, Plate count agar, MacConkey agar, Staphylococcal media no. 110 were employed, respectively.

2. *Carcass rinses ad washes*: All the equipment and instruments used by butchers were kept immersed in sterile diluent, which was taken in a sterile container. These were rinsed and washed. Washing materials (10 ml) was introduced into 90 ml of the diluent, which were then considered to be 10^{-1} dilution. Serial decimal dilutions were prepared as required and transferred on Plate counts agar and MacConkey agar. The count represents per milliliter of washings.

In case of hand washing, samples were taken from two groups of butchers, one who take general hygienic measures and the other, who were indifferent to take hygienic measures. In each caseses, three samples were taken before beginning, at the peak hour and at the end of the work. The washed samples were obtained by allowing the butchers to wash their hands by rinsing in 500 ml of water.

In case of carcass washing, rinses and washes of carcasses were collected in the abattoir. The water used for rinsing and washing was municipal supplied tap water.

Isolation and identification

The isolates were identified on the basis of morphology, cultural characteristics and biochemical properties as described by the ISO, (1995); ICMSF, (1982) and Cowan, (1985).

Results And Discussion

The results as evidenced in Table 1 show the TVC, TCC and TSC of samples from beef carcass regions or sites, such as brisket, neck and thigh. The average values per gram of meat samples as found in these sites are log 5.65, log 2.66, log 3.26 and log 5.37, log 2.17, log 2.76 and log 6.31, log 3.17 and log 2.41 in brisket, neck and thigh regions, respectively.

Table 1. General viable counts of selected microbial groups contaminating meats of different regions of beef carcasses

Region of carcasses	TVC			TCC			TSC		
	Max	Min	AV	Max	Min	AV	Max	Min	AV
Brisket	6.27	5.03	5.65	3.00	2.32	2.66	3.86	2.66	3.26
Neck	5.71	5.03	5.37	2.50	1.84	2.17	3.20	2.32	2.76
Thigh	6.61	6.01	6.31	3.41	2.94	3.17	2.97	1.85	2.41

TVC: Total viable Count TCC: Total Coliform Count; TSC: Total Staphylococcal Count

* All counts are expressed in logarithms

The higher counts are obtained in thigh muscle, which is thought to be due to possible chances of more exposure to contamination from the feet and viscera. During dressing operations and inappropriate handling of beef carcasses the thigh regions had more chance to get contaminated with the vicinity of visceral cavity, as a result the organisms from these sources could have gained accesses to yield higher counts in the samples. Borse *et al.* (1998) found greater initial bacterial counts in the brisket and neck regions. Robert *et al.* (1980); Johanson *et al.* (1983); Charlebois *et al.* (1991); Perieto *et al.* (1991); Zeleke *et al.* (1994); Untermann *et al.* (1996) made similar views. They designated brisket and thigh as most contaminated sites and pointed out the indications of improper slaughter hygiene and operations and faulty meat handling and evisceration techniques.

Table 2 represents bacterial counts per ml of water of hand washings, instrument washings, carcass washings, meat from retail cuts, and table scraps rinses.

Unsanitary equipment provides a second source of contamination. Equipment become dirty when these were of poor designs and difficult to sanitize adequately. It is observed

from Table 2 that equipment, working tools and other meat hygiene practices such as washing of carcass with water can introduce microorganisms during slaughtering, skinning, washing, and dressing of carcasses.

The investigation further revealed that the working surfaces or platforms of butchers preparing and selling meat in retail markets could be abodes of microorganisms where maximum microbial density is detected. The average microbial load per ml of

Table 2. Microbial agents from different sources contaminated beef carcasses

Nature of sample	Unit measurement	TVC			TCC			TSC		
		Prior to work	Peak hour of work	End of the work	Prior to work	Peak hour of work	End of the work	Prior to work	Peak hour of work	End of the work
1. Hand washings	Per ml	5.16	5.65	5.45	4.19	3.32	2.31	3.69	3.87	3.67
2. Instruments washings	Per ml	6.00	5.65	4.03	3.67	3.41	3.70	3.89	4.25	4.29
3. Carcass washings	Per ml	5.74	–	–	4.58	–	–	3.38	–	–
4. Meat from retail cuts	Per gram	5.1	5.0	4.5	1.85	2.61	2.52	2.30	2.70	2.73
5. Table scraps rinses	Per ml	4.29	5.10	4.50	1.51	1.61	1.44	3.89	5.23	5.61

TVC: Total Viable Count; TCC: Total Coliform Count; TSC: Total Staphylococcal Count

* All counts are expressed in logarithms

Butcher hand washings, instrument washings, carcass washings, meat from retail cuts and table scraps rinses obtained from beef carcasses were log 5.42, log 4.22, log 5.74, log 4.86 and log 4.96, respectively. The result signifies that there is gradual increase in microbial load during every operation performed at the time of slaughtering and dressing. Gracey and Collins (1992) observed that blade of knives could carry 80,000 to 40 million bacteria per blade. The hands of meat operative could carry as many as 2 million bacteria and the unclean equipment could harbor millions of microbes (Rahman *et al.*, 1979 and Lasta *et al.*, 1992). Personnel and air in the environment can contribute additional microorganisms. Personnel contribute contamination to meat either by transfer of microorganisms directly from person to product or by mishandling and practices such as cross contamination from raw to finished product.

Table 3 shows the microbial contamination of air in a butcher meat stall during different selling hours. It is interesting to note that the air during the maximum selling period at 10 am and 11.00 am had maximum contaminants with microbes.

It is clearly evidenced from the phenomena that the air in the location where customers usually stand to purchase retail cuts of meat accumulated the maximum

microbial load in comparison with other locations. This is due to the fact that the customers, who directly or indirectly transfer microorganisms from their own to the environment. Moreover, due to movement of people more dust turbulently accumulate and other contaminants in the vicinity could be potential sources of microorganisms gaining access to the air. Rahkio and Kordeala (1997) obtained similar findings. They conducted a study on the microbial contamination of air and found many transient bacteria.

Table 3. Microbial load of air in a butcher meat stall during different selling periods

Time of experiment	Colony forming units on 110 mm diameter petri-dishes exposed for 5 minutes											
	In the corner of cutting floor				Beside the chopping wooden block				Adjacent to customers standing platform			
	Plate no.			Average	Plate no.			Average	Plate no.			Average
	1	2	3		1	2	3		1	2	3	
8.00 am	370	420	330	373	300	360	450	370	350	430	370	383
9.00 am	450	560	500	503	350	390	320	353	500	470	380	450
10.00 am	420	500	405	441	520	750	610	626	900	720	931	850
11.00 am	300	280	310	296	400	390	350	380	600	505	630	578
12.00 noon	250	284	330	288	360	370	350	360	105	140	70	105

Distribution of selected bacteria present (%) in beef carcass, carcass washings, butchers hand washings, instrument washings, table scraps rinses and air are presented in Table 4. It is found that *Staphylococcus* sp. (36.12%), *Staphylococcus* sp. (15.26%), *Bacillus* sp. (12.71%), *Micrococcus* sp. (11.23%), *Escherichia coli* (10.28%), *Proteus* sp. (5.24%), *Pseudomonas* sp. (3.56%), and *Enterobacter* sp. (3.25%) were isolated and identified. These findings are in agreement with Rahman *et al.* (1979); Schuppel *et al.*, (1996) and Mukhopadhyay *et al.*, (1998). In these studies, Staphylococci showed the highest percentage of occurrence. The presence of high number of pathogenic Staphylococci in meat is alarming.

Table 4. Distribution of bacterial isolates obtained from meat samples

SL. No	Name of isolates	Distribution (%)
1.	<i>Staphylococcus</i> sp.	36.12
2.	<i>Streptococcus</i> sp.	15.26
3.	<i>Bacillus</i> spp.	12.71
4.	<i>Micrococcus</i> sp.	11.23
5.	<i>Escherichia</i> sp.	10.28
6.	<i>Proteus</i> sp.	5.24
7.	<i>Pseudomonas</i> sp.	3.56
8.	<i>Enterobacter</i> sp.	3.25
9.	<i>Salmonella</i> sp.	1
10.	Others	6

Next to Staphylococci, Streptococci ranked the second position. The high percentage *E. coli* indicates poor sanitary conditions during handling, slaughtering, dressing and transportation of meat. The presence of all these organisms in meat foods should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication.

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GENETIC CAUSES OF VARIATION IN BEEF TENDERNESS PRELIMINARY RESULTS

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Key Words: beef, tenderness, calpastatin, genetic variation, selection

Introduction

Eating quality is of increasingly importance to the consumer. Tenderness is the most important beef quality trait, followed by flavour and juiciness (Jeremiah, 1982, Devitt et al., 2002). However, inconsistent meat tenderness has been identified as the major cause of consumer dissatisfaction with beef worldwide (i.e. Hwang et al., 2002; Koohmaraie et al., 2002; Thompson, 2002).

Beef tenderness may be improved by various efforts along the production chain, such as avoidance of pre-slaughter stress, electrical stimulation, chilling regimes and post-mortem ageing etc. (Koohmaraie, 1996; Thompson, 2002). However, studies have shown that even when these factors are optimised, considerable individual variation in tenderness remains (Hildrum and Tornberg, 1998; Hildrum et al., 1999).

Several genetic studies of beef tenderness have reported heritabilities for Warner-Bratzler shear force tenderness in the range of 0.30 to 0.50 (Renand, 1988; Shackelford et al., 1994). This suggests that genetic factors have an important impact on beef tenderness variability. This is probably partly related to proteolytic activity of the calpain enzyme system in the muscle tissue. Shackelford et al. (1994) reported a heritability of 0.65 for calpastatin activity in muscle 24 h post mortem, and a genetic correlation of 0.50 between calpastatin activity and WB shear force. In addition, areas of the bovine genome that seems to have a clear effect on beef tenderness have been identified (Keele et al., 1999; Smith et al., 2000). Inclusion of beef quality traits in cattle breeding schemes may thus be an additional tool for more consistent beef tenderness, implying a permanent improvement at non-recurring expenses.

Objectives

The aim of the present study is to investigate the relative importance of genetic causes for variability in beef tenderness, including genetic/biochemical studies of enzymes/hormones etc. in muscle tissue that may be related to tenderness. In addition, the objective is to evaluate whether biotechnological and/or traditional quantitative genetic selection methods could be utilised to improve the tenderness of beef.

Methodology

This study (2004-2007) will finally include data from ca. 900 NRF (Norwegian Red; a dual purpose breed) bulls, sired by 50-60 NRF A.I bull sires. Data have so far been recorded on 491 bulls, progeny of 32 sires. The bulls were culled after a test for growth and conformation at a performance test station (150-300 d of age). The test regime is a part of the national breeding scheme for the NRF breed. Approx. 90% of the NRF population is included in the breeding programme through use of A.I. Additionally, this breed accounts for 90% of the beef produced in Norway. The test bulls included in this study are thus genetically related to most of the contemporary commercial slaughter cattle. The test station represents a standardised pre-slaughter environment, a clear advantage in genetic studies of tenderness.

The bulls are transported (1 h) and slaughtered in batches (6/year) at a commercial abattoir immediately after arrival. The carcasses are electrically stimulated (90V) approx. 20 min. pm. Animal identification, carcass weight and EUROP grading for carcass conformation (EURC) and fatness (EURF) are recorded. Muscle tissue samples for enzyme- and DNA analyses are collected together with a hot-boned sample of the loin (m.l.dorsi; 10th thoracic to 2nd lumbar vertebrae). The loin is immediately vacuum-packed and conditioned at 12⁰C for 10 h before ageing at 4⁰C for 7 days, followed by measurements of Warner-Bratzler shear force (WB), fresh surface colour (L*a*b) and analysis (Soxlet) of intramuscular fat (IMF).

For WB analyses, meat slices (3.5cm thick) were vacuum packed, heated in a water bath at 70⁰C for 50 min and chilled in ice water for 45 min. Slices (1cm thick) were cut twice in the fiber direction to give samples (ten replicates) of 2x1x1cm³ which were cut using a WB force device (triangular version) in an Instron Materials Testing Machine.

Samples for calpastatin activities were extracted at 24-31 hrs pm. using the heated calpastatin procedure of Shackelford et al. (1994). The muscle material was trimmed for visible fat and connective tissue and finely diced. Muscle extracts were prepared by homogenising 5g of meat in 15ml extraction buffer (100mM tris base, 10mM EDTA, 0.05% 2-mercaptoethanol (2-MCE), 20mg/l trypsin inhibitor, 6mg/l leupeptin, 2mM phenylmethylsulfonyl fluoride (PMSF), adjusted with HCl to pH 8.3) 3x30 sec at 22 000 rpm (Polytron).

The samples were centrifuged at 16 000 xg for 60 min. at 4⁰C, followed by heating in a water bath at 95⁰C for 15 min., and chilled in ice water for 15min. The samples were centrifuged at 22 000 xg for 60min at 4⁰C and dialyzed against elution buffer (40mM tris base, 0.5mM EDTA, 0.05% 2-MCE, pH 7.35) overnight at 4⁰C. The supernatant volume was recorded, and the supernatant filtered through cheesecloth. Calpastatin activity was determined using BODIPY-FL labeled casein according to Thompson et al. (2000).

Micro-array and gene-expression analyses have recently been initialised, and no results are ready for presentation. The data were analysed with SAS (SAS, 1999). The heritabilities (h^2) were estimated both with a sire model (ANOVA) and an animal model (DMU, Version 6).

Results & Discussion

Overall statistics for the data set is presented in Table 1. These test bulls are slaughtered younger than commercial bulls, resulting in poorer EUROP classification than is usual with this breed. Additionally, there were large variation in both WB and IMF, even in as young animals as represented in this study. The large range in WB is partly due to the relatively short ageing time (7 d).

Table 1. Overall mean, standard deviation and range of variation for important traits.

Trait	Mean	Std.dev.	Min.	Max.
<u>Carcass quality:</u>				
Daily gain (g/day)	1300	121	850	1678
Age at slaughter (mo.)	12.8	0.8	11.2	16.0
Carcass weight (kg)	231	23	163	341
EURC	O-		P+	O+
EURF	2		1+	3
<u>Meat quality:</u>				
WB-shear (N)	65.4	19.2	32.5	136.1
Calpastatin (Units/g)	2.58	0.64	1.32	3.59
IMF (%)	1.16	0.5	0.3	5.70

The results from the statistical analyses with the sire model are presented in Table 2. The heritability (h^2) for WB estimated on the present data set was 0.20 and 0.11 with the sire and animal model, respectively. These are lower than reported in the studies mentioned above, but correspond well with Devitt et al. (2002). The heritability for IMF (0.70) was higher than most literature estimates (i.e. Aass, 1996; Devitt and Wilton, 2001). The heritability for CALP of 0.22 was lower than reported by Shackelford et al. (1994). However, this preliminary estimate, based on a low number of bulls ($n=98$), may change considerably as the number of data in the set increases. In addition, there were some initial problems regarding the analyses of CALP due to variation in substrate concentration and activity in the standard enzyme solution. An increase in WB was positively related to an increase in CALP ($r_p=0.44$). However, a higher correlation (0.80) was observed when analyses were based on the four last batches only.

Table 2. ANOVA-table with mean sum of squares (MS) for the fixed effects, the residual (MSE), R^2 and the heritability (h^2) for WB-shear, calpastatin and IMF, respectively.

Model	Batch	Slaughter age	Pen (Batch)	Sire	Residual	R^2	h^2
WB-shear	206 148 ***	164 206 ***	27 060	34 890 **	21 717	0.52	0.20
Calpastatin	21 574 ***	2 886 **	ns.	783	602	0.89	0.22
IMF	4 798 ***	17 810 ***	ns.	7 149 ***	1 814	0.41	0.70

*** $p \leq 0.01$

** $p \leq 0.05$

The high influence of environmental factors on beef tenderness is illustrated in Table 2. In spite of the standardised handling of animals, a highly significant effect of slaughter batch was observed for all traits. This effect is related to unknown or not easily

observable factors associated with rearing temperature, feed quality, personnel, transport, the slaughter situation etc.

The overall effect of pen within batch on WB was not clearly significant ($p=0.12$). However, a pair-wise t-test revealed significant effect of pen within some of the batches, with differences of approx. 20N between pens. This suggests that factors involving the social hierarchy between bulls in the same pen may in fact have an important impact on beef tenderness.

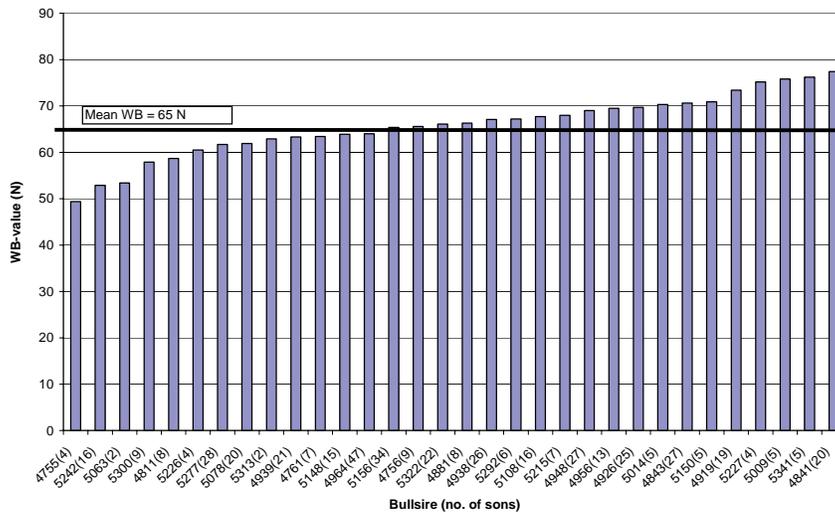


Figure 1. Adjusted mean WB shear force values for progeny groups (sons) of 32 NRF bull sires

The adjusted least square mean WB values for the progeny groups of the respective 32 bull sires are presented in Figure 1. The variation in Figure 1 reflects the genetic variation in WB between the NRF family lines represented so far. As the present data set only represents 50% of all bull sires in one generation of selection, it is reasonable to believe that the heritability (h^2) in WB will increase as progeny of new bull sires are added to the data set. The progeny of the most extreme sires (tender and tough) will be objects for more thorough biochemical and genetic studies during the project.

Table 3 illustrates the distribution of progeny from the 32 NRF bull sires in tenderness groups when the bull sires were classified as tender, average and tough, respectively, according to the sire distribution in Figure 1. As an example, this table suggests that substituting a “Tough” bull sire with a “Tender” bull sire would increase the percent of offspring with tender carcasses in the market from 15% to 30%, while the corresponding proportion of tough carcasses would decrease from approx. 40% to 20%. This corresponds well with the conclusions reported by Devitt et al. (2002).

Table 3. Distribution of progeny from the 32 NRF bull sires in tenderness groups when the bull sires were classified as tender, average and tough, respectively, according to the sire distribution described in Figure 1.

	Tender sires (n=11)	Average sires (n=10)	Tough sires (n=11)
Nb. of obs.	121	190	157
Average progeny WB (N)	59	65	70
Tender (WB ≤ 50 N; %)	31	16	15
Intermediate (WB 50-70 N; %)	52	56	42
Tough (WB ≥ 70 N; %)	17	28	43

Conclusions

The preliminary results of this study imply that genetic variation both in WB tenderness, calpastatin activity and IMF are present in the NRF breed, and that genetic factors account for a significant part of the variation observed in eating quality of beef. Thus, there seems to be sufficient genetic variation present to change the proportion of tender vs. tough beef through animal breeding, if superior sires for tenderness may be identified and utilised systematically in the population. In addition, the results are illustrative for the high influence of environmental factors on beef tenderness.

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ASSOCIATIONS BETWEEN EARLY DRIP LOSS MEASUREMENTS, GENE MARKERS, AND PURGE LOSS DEVELOPMENT OF CASE-READY PORK

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Key Words: Drip Loss, Case-ready Pork, Gene Marker Technology

Introduction

Drip loss of fresh pork is an economically important meat quality characteristic as it affects both yield and quality of the end product. Case-ready fresh meat that shows high amounts of purge loss has an unattractive appearance for the consumer, which may lead to a reduction in sales at the retail store. Furthermore, high drip loss or low water-holding capacity reduces the yield of further processed products. The genetic make-up of a pig, the entire system of live animal production and handling through initial chilling, and finally storage and handling of the meat and case-ready products all play significant roles in influencing the amount of moisture that is lost by the end product (Andersen, 2000).

It is of economic interest to know whether drip loss of case-ready products can be predicted by earlier measurements of drip loss in order to improve the genetic make-up of a pig by using selective breeding techniques. Examples of accepted early drip loss measurements are the Honikel method (Honikel, 1987) and EZ-DripLoss method (Rasmussen and Andersson, 1996), which could be used in full- and half-sib-carcass analysis for selection purposes. In addition meat quality markers can be used to directly select live pigs with the best genetic make-up for reduction of drip loss (Dekkers et al., 2001, Knap et al. 2002, Plastow et al. 2004).

Objectives

The objectives of this paper were to study the associations between standardized early drip loss measurements and daily purge loss measurements of case-ready packaged pork during a one-week period. Furthermore, the effect of several genetic markers on drip loss was quantified.

Methodology

The experimental pigs were crossbreds from four lines reared at one commercial farm. Piétrain based sires were mated to crossbred dams originating from a three line cross of Large White, Landrace and Leicoma based lines. DNA analysis showed that 213 pigs were homozygous for the stress resistant allele of the ryanodine receptor gene (NN genotype) and 161 were of the heterozygous Nn genotype. There were 191 gilts and 183

barrows included in this study. All pigs were processed at a commercial abattoir in batches of about 60 pigs during 7 different slaughter days.

At 24h post-mortem case-ready meat samples were cut from the *M. longissimus dorsi* of all 374 pigs. Purge loss was measured on each of the 7 days (CRM₁₋₇) after packaging of these loin samples. At 24h post-mortem loin samples were also cut for the reference drip loss methods as described in Honikel (1987; HM method) and Rasmussen and Andersson (1996; EZ-DripLoss (EZ-DL) method). Drip loss was measured at 24 and 48 h after preparation for the HM samples and at 48h after cutting for the EZ-DL probes. Drip loss is expressed as percentage of the initial weight.

Drip loss data (HM and EZ-DL) of a previous trial conducted at the same slaughter house were added to the total data base (1155 pigs of similar genotypes) for the association analysis between the different drip loss measurements (CRM₁₋₇, HM, and EZ-DL) and genetic markers. The ryanodine receptor genotypes were determined according to Fujii *et al.* (1991; *RYRI*; *HAL1843*TM). Genotypes were also obtained for markers in *PRKAG3* (Milan *et al.*, 2000; Ciobanu *et al.*, 2001) and *HMGAI* (Kim *et al.*, 2004). The *PRKAG3* I199V mutation is located on chromosome 15 and for the population under study the genotype frequency was II=89, IV=534, and VV=496. The *HMGAI* locus is on chromosome 7 and the *NaeI* polymorphism used is in intron 5 of the gene. Only a few pigs showed the genotype 11 of marker *HMGAI* (n=18), whereas the genotypes 12 and 22 accounted for 355 and 709 animals.

Association between DNA markers and the amount of drip loss was tested by analysis of variance using the general linear model procedure (GLM) of SAS (SAS Institute Inc., Cary, NC, USA). The following model was used for the analysis ($Y_{ijklmnop}$):

$$Y_{ijklmnop} = \mu + L_i + F_{ij} + D_k + S_l + R_m + RYRI_n + M_o + e_{ijklmnop}$$

The model included the fixed effects line (L_i), farm within line (F_{ij}), slaughter day (D_k), sex (S_l), resting time (R_m), *RYRI* genotype ($RYRI_n$) and *PRKAG3* I199V or *HMGAI*, respectively (M_o). Furthermore, drip loss obtained with the Honikel method was corrected for the sample weight.

Results & Discussion

The HM method samples taken 24h post-mortem showed a drip loss of 1.8 and 3.1% after 24 h and 48 h storage, respectively. The EZ-DL method had a drip loss of 4.7% after 48 h storage. This is in the same range as was previously reported by Otto *et al.* (2004) but in contrast to Christensen (2003) who reported 1.2% more drip loss when using the HM method compared to EZ-DripLoss technique. The 24 h and 48 h HM drip loss measurements were highly correlated (0.98), as were the 48 h measurements of both the HM and EZ-DL methods (0.89).

Increasing display times of meat in retail shops require meat of consistently good quality. Therefore, the development of purge loss was examined during a one-week period. Mean drip loss % of the case-ready meat samples (CRM) increased substantially from 1.6% at 24 hours to 5.6% during 7 days of storage (Figure 1). High variation was obtained for all drip loss measurements resulting in coefficients of variation ranging from 35.2 and 70.4 for CRM at day 7 after sampling and HM after 24 h storage, respectively.

A large variation was shown in the development of case-ready purge loss. The 25% worst purge loss samples had an average drip loss of 8.4% drip after 7 days of storage. The best 25% of the case-ready samples showed a purge loss of only 3.5% at day 7 (Figure 1). The 25% undesirable drip loss samples had especially high amounts of drip within the first days of the observation period. Correlations between the CRM₁₋₇ purge loss measurements and the HM or EZ-DL drip loss % were in the range of 0.82 to 0.90 (Table 1). This indicates that the earlier drip loss measurements carried out at the slaughterhouse could be highly informative of predicting the purge loss found in case-ready packaged pork after several days of storage at a retail store.

The use of marker-assisted selection in animal breeding is of particular interest with meat quality traits. Improvement of meat quality is difficult using conventional selection methods, since most traits of interest can only be measured after slaughter and, therefore, only information of relatives can be used for selection (Dekkers et al., 2001). The effects of the *RYRI* gene on meat quality are well known and the drip loss results in this study were as expected. Heterozygous carriers of the recessive stress susceptible allele (Nn) and homozygous stress resistant (NN) animals showed 5.8% and 3.7% drip loss with the EZ-DL method, respectively (Table 2). The *PRKAG3* I199V and *HMGAI* markers were also found to significantly influence drip loss % as measured with the EZ-DL method. The difference between the extreme genotypes within these markers was 1.0% and 1.1% drip loss, respectively (Table 2). Better meat quality of carriers of the II genotype of *PRKAG3* I199V in terms of higher loin pH was also observed by Ciobanu et al. (2001). The analysis across five commercial lines resulted in loin pH values of 5.78, 5.74 and 5.71 for genotypes II, IV and VV, respectively. Low frequencies of the *HMGAI* 11 genotype caused a high standard error. Therefore, more research has to be done to verify the effects of this particular marker. Overall the results indicate the usefulness of marker-assisted selection for reduction of drip loss in pigs.

Conclusions

Early measurements of drip loss using both the Honikel and EZ-DripLoss methods are adequate to predict purge loss in case-ready packaged pork over a one-week period. The EZ-DripLoss method is a standardised easy-to-perform method and is therefore recommended for use in selection programs of breeding animals. Finally, marker-assisted selection is shown to be a powerful tool for reducing drip loss of case-ready meat, which can be used for early selection of breeding animals.

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Tables and Figures

Table 1: Residual^a correlations^b between sample drip loss measurements^c after 24 or 48 h storage and daily measurements of case-ready pork drip loss^d

	Drip loss % of case-ready pork at day						
	1	2	3	4	5	6	7
HM ₂₄	0.86	0.88	0.87	0.86	0.85	0.85	0.85
HM ₄₈	0.82	0.87	0.87	0.88	0.87	0.87	0.87
EZ-DL ₄₈	0.87	0.90	0.89	0.89	0.88	0.88	0.87

^a Residual correlations after adjustment for the effects slaughter day, resting time, sex, *RYRI* genotype and additionally for sample weight when using bag method₄₈.

^b Significance of all estimated regression coefficients was $P < 0.001$.

^c Abbreviations: HM₂₄: Drip loss measured using Honikel method from 24 to 48 h post-mortem; HM₄₈: Drip loss measured using Honikel method from 24 to 72 h post-mortem; EZ-DL₄₈: Drip loss measured using EZ-DripLoss method from 24 to 72 h post-mortem.

^d Sampling took place at 24 h post-mortem

Table 2: Least squares means (standard errors) of several genetic marker genotypes for drip loss by using EZ-DripLoss method at 48 h after sampling

Marker	Marker genotype			P ^a
	11	12	22	
<i>RYRI</i> ^b	.	5.8 (0.13)a ^c	3.7 (0.13)b	***
<i>PRKAG3</i> I199V ^d	3.9 (0.24)a	4.7 (0.13)b	4.9 (0.13)b	***
<i>HMGAI</i>	3.6 (0.50)a	4.9 (0.15)b	4.7 (0.13)ab	*

^a * $P < 0.05$; *** $P < 0.001$.

^b *RYRI*, HAL1843TM – allele 1 is allele T or amino acid Cys, allele 2 is C or Arg.

^c Within rows, values with the same letter are not significantly different.

^d Genotypes of *PRKAG3* I199V: 11=II; 12 = IV; 22=VV.

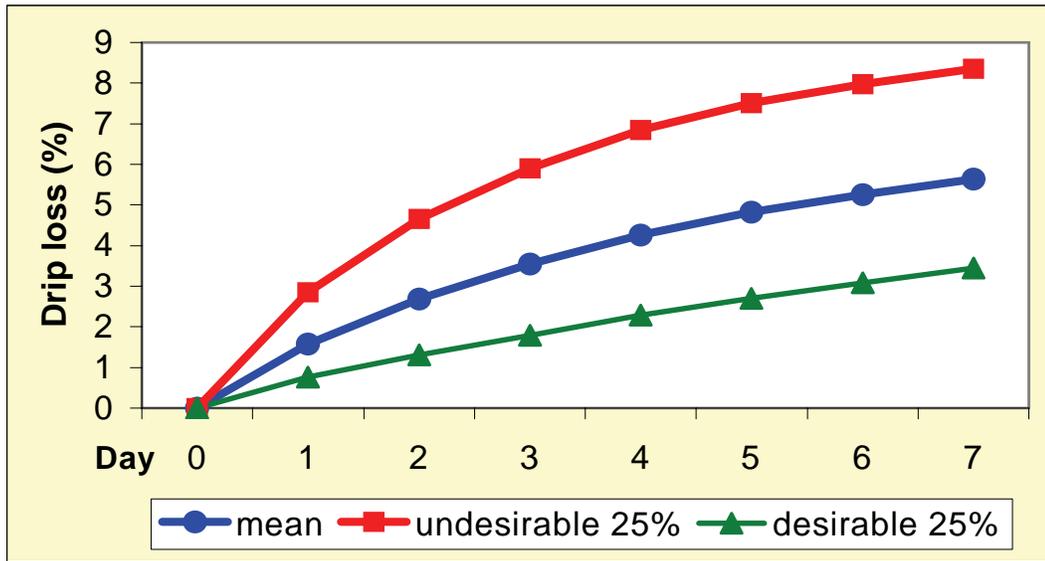


Figure 1: Development of purge loss in case-ready pork within a week (n=374)

SEX INFLUENCE ON INTRAMUSCULAR LIPID COMPOSITION AND NUTRITIONAL VALUE OF BARROSÃ-PDO VEAL

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Introduction

Estimated in about 7500 dams, Barrosã effective is one of the most important among the autochthonous cattle breeds in Portugal. Produced by more than 2000 small farmers in the Norwest region of the country, these animals show medium/low growth rate and an appreciable propensity to deposit precociously intramuscular fat. The Barrosã-PDO (Protected Denomination of Origin) veal is obtained from non weaned and non castrated calves, raised close to their mothers, suckling and feeding on pasture according to a traditional grazing system and slaughtered from 5 to 9 months old in agreement with European Union regulation (Commission Regulation n° 1263/96, EC). Barrosã-PDO veal is highly appreciated by consumers due to its unique sensory quality and the natural production system applied.

Meat supplies high-quality and readily digested protein and energy and represents the main source of highly available iron in the diet (Lombardi-Boccia *et al.*, 2002). However, epidemiological and clinical studies have suggested that its consumption is related to certain diseases such as obesity, coronary heart diseases and cancer (Chizzolini *et al.*, 1999). On this concern, cholesterol level and fat composition of meat and their effects on human health have also been largely reported (Enser *et al.*, 1998; Laborde *et al.*, 2001). Although the fatty acid profile in meat from ruminants is mostly dependent on rumen hydrogenation of the dietary fatty acids, some changes in this profile could be related to the diet composition. Beef from grass fed animals has been reported to contain higher proportions of *n-3* polyunsaturated fatty acids (PUFA) and total monounsaturated fatty acids (MUFA), a more favourable *n-3/n-6* ratio and higher levels of conjugated linoleic acid (CLA) (French *et al.* 2000) than meat from concentrated fed animals. All this issues have positive biological effects on human health (Simopoulos, 1999). On the other hand, intramuscular fat contributes to organoleptic characteristics (Laborde *et al.*, 2001), being related to flavour, juiciness, tenderness and colour.

Although sex-related differences in fatty acid composition of beef cattle have been widely reported for older animals (Zembayashi *et al.*, 1995; Malau-Aduli *et al.*, 1998; Laborde *et al.*, 2001; Deland *et al.*, 2001), the information about sex influence in calf fat composition is still scarce.

Objectives

The objective of the present study was to evaluate the influence of sex on the amount and composition of intramuscular lipid and nutritional value of Barrosã-PDO veal.

Methodology

Barrosã calves were reared on pasture with their dams, according to PDO specifications until slaughter at 7.4 ± 0.9 months of age (carcass weight: 99.4 ± 16.1 kg). One day after slaughter, about 200 grams of *Longissimus dorsi* (L4-L6) and *Supra spinatus* muscles were excised and stored at -20°C until analysis.

Intramuscular total lipids (ITL) were extracted from duplicate 20 g samples of muscle, trimmed of visible adipose and connective tissues as described by Folch *et al.* (1957). Separation into neutral lipids (NL) and phospholipids (PL) was performed according to Juaneda & Rocquelin (1985). Lipid extracts were esterified with KOH (2N) in methanol (ISO 5509, 2000) and resulting fatty acid (FA) methyl esters were analysed by gas-liquid chromatography, using a HRGC 5160, Mega series from Carlo Erba instruments, equipped with a flame ionisation detector and a 60 m long DB 23 capillary column. Identification of FA was based on comparison of retention times with standard FA mixtures (Supelco and Nuchek GLC reference standard FAME mixture) and confirmed by GC-MS (Saturn 2200, Varian, Walnut Creek, CA, USA). FA were expressed as weight percentage.

Muscle cholesterol content (mg/g muscle) was quantified according to Roseiro *et al.* (2002), using a HPLC (Spectra-Physics Model Spectra 100) set at 206 nm and equipped with a Spherisorb S5W silica cartridge, $5\mu\text{m}$, 4.0×125 mm (Waters PSS 845549). The mobile phase was hexane/isopropanol (97:3) at a flow rate of 1.0 mL/min.

α -Tocopherol (mg/100 g muscle) was determined according to European Standard-EN 12822 with minor modifications, using a HPLC (Spectra-Physics, model Spectra 100) equipped with a Spherisorb S5W silica cartridge (Waters PSS 845549), set at 292 nm. The mobile phase was hexane/1,4-dioxane (99:1) at a flow rate of 0.8 mL/min. Recovery of α -tocopherol from meat was determined by the addition of an internal standard to samples before saponification.

Heme iron was quantified following the analytical procedure of Lombardi-Boccia (2002). Briefly, samples were analysed for heme pigment content according to Hornsey (1956) and the heme iron concentration was calculated from the standard curve as follows: $\text{heme iron (mg/100g)} = \text{hematin content (mg/100g)} \times \text{AW/MW}$, where AW is the atomic weight of iron and MW the molecular weight of hematin.

Protein content (g/100g) was performed using the Bicinchonic acid protein assay kit (Sigma, Saint Louis, USA).

The influence of sex on the results was evaluated in Ld and Ss muscles by analysis of variance (ANOVA) according to a general linear model procedure of SAS (2000). Analysis of means was performed by the LSD test for 95% of probability.

Results & Discussion

The effect of sex on intramuscular total lipids (ITL), neutral lipids (NL), polar lipids (PL), cholesterol, α -tocopherol, heme iron and protein contents in Ld and Ss muscles are depicted in Table 1. Although results were not affected by sex on both muscles, males and females had higher ITL, NL and heme iron mean values in Ld and Ss muscles, respectively. Irrespective of muscle considered, males showed higher cholesterol and PL and lower protein contents than females ($P>0.05$). Cholesterol level found in the present study was similar to that reported by Prates *et al* (2005a) for Ld muscle. In contrast, α -tocopherol content was lower. This difference could be attributed to diet, slaughter season effects and also to the size of the analysed populations.

The amount of intramuscular lipid fractions is referred to be dependent on the metabolic properties of muscle, with those presenting a more oxidative profile having higher amounts of total and neutral lipids, phospholipids and cholesterol than the predominantly glycolytic ones (Alasnier *et al.* 1996). Our results did not totally agree with those findings since Ss muscle, with much more pronounced oxidative status than Ld muscle (Roseiro *et al.*, 2004), showed lower total and neutral lipid contents.

Iron plays an essential role in many metabolic processes including oxygen transport, oxidative metabolism and cellular growth (Lynch, 1997). Beef consumption improves iron status in the body both by supplying highly available heme iron and by improving absorption of the dietary nonheme iron (Cook and Mosen, 1976). Heme iron content in Barrosã-PDO veal ranged from 1.35 to 1.76 mg/100 g which is considerably higher than the value reported by Lombardi-Boccia for veal (0.71 mg/100 g). Based on consumption of a 100 g steak, Barrosã-PDO veal provides 15-20 % of daily iron recommended requirements for adults (Guéguen, 2004).

Working on Semitendinosus muscle of Limousin males slaughtered between 6 and 12 months of age, Jurie *et al.* (1995) observed that protein content remained close to 18 g/100 g. A similar muscle protein level was observed in the present study, except in Ld muscle from female calves which presented a higher content (20.93 g/100 g).

In both sexes and muscles, the most abundant FA was C18:1 *cis*-9, followed by C16:0 and C18:0, which together comprised more than 70% of total FA in NL fraction. The same trend was obtained by Webb *et al.* (1998) in triacylglycerol fraction of *Longissimus thoracis* from Belgian Blue breed. In PL, the most abundant FA were C18:1 *cis*-9, C16:0 and C18:2 *n*-6, representing together more than 50% of this fraction for both sexes and muscles.

Regarding NL fraction from Ld muscle, females contained higher C16:1 *cis*, C17:1, C18:1 *cis*-9 and total MUFA contents than males which in turn, showed higher C18:0 and total SFA contents (Table 2). Zembayashi *et al.* (1995) also found differences among sexes in NL from Ld muscle, with heifers presenting higher C18:1 and total MUFA and lower total SFA proportions than steers.

Concerning PL composition of Ld muscle, females presented higher *anteiso*-C17:0 and C18:2 *cis*-9, *trans*-11 and lower C18:2 *n*-6 contents than males.

Differences between sexes in NL composition of Ss muscle were significant for *anteiso*-C15:0, C15:0, C15:1, C18:0, C20:0 and total SFA with higher contents in males, whereas females showed higher C16:1 *cis*, C17:1, C18:1 *cis*-9 and total MUFA and h/H (hypocholesterolaemic/hypercholesterolaemic ratio) levels than males (Table 3).

Concerning PL of Ss muscle, sex variations were limited to C20:3 *n*-3, C24:1 and C22:6 *n*-3 FA.

Terrel *et al.* (1968) reported that sex effects in cattle were associated with the NL fraction rather than the PL fraction FA. The same tendency was observed in the present study, with sex mostly affecting the NL fraction of both muscles. In contrast Malau-aduli (1998) reported extensive sex differences in PL composition among yearlings and postulate that these variations were due to hormonal differences between steers and heifers. According to those authors, steers had more C14:0, C16:1, C18:0, C18:1 *n*-9, C18:1 *n*-7, C22:1 and total MUFA than heifers whereas the latter shown more C18:2, C20:4, C20:5, total PUFA, *n*-3 PUFA, *n*-6 PUFA and a higher P/S (polyunsaturated fatty acids/saturated fatty acids) than steers.

A minimum value of 0.45 and a maximum value of 4.0 for P/S and *n*-6/*n*-3 PUFA, respectively, have been recommended in dietary fat for humans (Department of health, 1994). The indexes P/S and *n*-6/*n*-3 PUFA were similar between females and males on ITL of Ld (0.35 vs. 0.34 and 3.94 vs. 4.09, respectively) and Ss muscle (0.52 vs. 0.56 and 3.46 vs. 3.92, respectively) (data not shown). The P/S value in Ld muscle was not in line with nutritional demands, due to its higher NL content, which has a lower P/S value than PL. Males showed a higher *n*-6/*n*-3 PUFA proportion than females for both muscles, probably due to higher C18:2 *n*-6 content. The h/H index, considered nowadays a better approach to evaluate the nutritional value of dietary fat, was higher in females than in males (2.28 vs. 2.25 and 1.91 vs. 1.83, in Ss and Ld muscle, respectively). These values were slightly lower than those reported by Prates *et al.* (2005b) for Ld muscle (T1-T3) from Carnalentejana-PDO beef slaughtered at autumn and at spring (2.03 and 2.38, respectively).

Conclusions

Sex had a significant effect on the nutritional value of intramuscular fat, particularly on NL FA composition. In general, females presented higher levels of C18:1 *cis*-9, total MUFA and h/H and lower *n*-6/*n*-3 PUFA content than males. These results suggest that meat from females is more nutritionally desirable than meat from males.

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Table 1. Effects of sex on intramuscular total lipids (ITL, g/100g), neutral lipids (NL, g/100g), polar lipids (PL, g/100g), cholesterol (mg/g), α -tocopherol (mg/100g), heme iron (mg/100g) and protein contents (g/100g) in *Longissimus dorsi* and *Supra spinatus* muscles of Barrosã calves

Trait	Ld				Ss			
	Female	Male	SE	P	Female	Male	SE	P
n	46	46			46	46		
ITL	2.94	3.01	0.16	ns	2.38	2.15	0.11	ns
NL	2.40	2.46	0.17	ns	1.57	1.40	0.10	ns
PL	0.66	0.65	0.01	ns	0.73	0.75	0.02	ns
Cholesterol	0.50	0.54	0.02	ns	0.55	0.58	0.02	ns
α -Tocopherol	0.23	0.23	0.02	ns	0.25	0.25	0.02	ns
Heme Iron	1.35	1.43	0.05	ns	1.76	1.65	0.05	ns
Protein	20.93	17.98	1.61	ns	18.62	18.52	0.99	ns

ns = not statistically significant ($P > 0.05$)

SE = standard error of the analysis of variance

Table 2. Effect of sex on fatty acid composition (w/w %) of neutral lipids and polar lipids in *Longissimus dorsi* muscle

Fatty acids (%)	Neutral lipids				Polar lipids			
	Female	Male	SE	P	Female	Male	SE	P
n	20	20			20	20		
C12:0	0.22	0.24	0.01	ns	0.03	0.04	0.01	ns
C14:0	5.11	5.17	0.22	ns	0.52	0.42	0.06	ns
C14:1	0.87	0.78	0.06	ns	0.08	0.06	0.01	ns
<i>iso</i> -C15:0	0.22	0.21	0.01	ns	0.06	0.03	0.01	ns
<i>anteiso</i> -C15:0	0.24	0.29	0.01	ns	0.06	0.05	0.01	ns
C15:0	0.66	0.71	0.03	ns	0.26	0.25	0.01	ns
C15:1	0.25	0.26	0.01	ns	0.13	0.13	0.03	ns
C16:0	24.20	24.79	0.32	ns	19.77	18.95	0.44	ns
C16:1 <i>cis</i>	4.00	3.57	0.13	*	1.18	0.99	0.07	ns
<i>iso</i> -C17:0	0.72	0.72	0.01	ns	0.54	0.51	0.03	ns
<i>anteiso</i> -C17:0	0.64	0.63	0.01	ns	0.21	0.16	0.01	*
C17:0	1.01	1.06	0.03	ns	0.51	0.51	0.03	ns
C17:1	0.82	0.74	0.01	*	0.55	0.57	0.03	ns
C18:0	12.77	14.54	0.42	**	10.06	11.09	0.40	ns
C18:1 <i>trans</i>	0.53	0.44	0.21	ns	0.33	0.39	0.06	ns
C18:1 <i>cis-9</i>	41.44	39.47	0.64	*	19.69	19.22	0.66	ns
C18:1 <i>cis-11</i>	0.00	0.03	0.03	ns	2.09	2.28	0.18	ns
C18:2 <i>n-6</i>	2.55	2.79	0.18	ns	16.25	17.97	0.50	*
C18:2 <i>cis-9, trans-11</i>	0.90	0.80	0.04	ns	0.37	0.29	0.02	*
C18:3 <i>n-6</i>	0.12	0.09	0.03	ns	0.16	0.12	0.03	ns
C18:3 <i>n-3</i>	0.58	0.62	0.03	ns	2.33	2.36	0.16	ns
C20:0	0.11	0.13	0.01	ns	0.12	0.13	0.03	ns
C20:1	0.12	0.10	0.01	ns	0.08	0.10	0.01	ns
C20:3 <i>n-6</i>	0.06	0.05	0.01	ns	0.39	0.43	0.04	ns
C20:3 <i>n-3</i>	0.07	0.08	0.03	ns	1.73	1.69	0.08	ns
C20:4 <i>n-6</i>	0.20	0.19	0.06	ns	10.64	9.54	0.64	ns
C20:5 <i>n-3</i>	0.12	0.07	0.03	ns	5.31	4.79	0.31	ns
C22:4 <i>n-6</i>	0.09	0.09	0.03	ns	0.01	0.01	0.00	ns
C24:1	0.13	0.09	0.03	ns	3.12	3.27	0.18	ns
C22:6 <i>n-3</i>	0.01	0.01	0.01	ns	0.96	0.86	0.08	ns
SFA	46.04	48.60	0.57	**	32.72	32.67	0.68	ns
MUFA	48.17	45.50	0.59	**	27.42	27.11	0.64	ns
PUFA	4.73	4.80	0.29	ns	38.55	38.46	1.15	ns
P/S	0.10	0.10	0.01	ns	1.20	1.20	0.06	ns
<i>n-6</i> PUFA	3.14	3.21	0.24	ns	27.53	28.15	1.00	ns
<i>n-3</i> PUFA	0.79	0.79	0.07	ns	10.66	10.02	0.47	ns
<i>n-6/n-3</i> PUFA	4.09	4.22	0.25	ns	2.66	2.98	0.18	ns
h/H	1.54	1.45	0.04	ns	2.90	3.00	0.24	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

n-3 PUFA = C18:3 *n-3* + C20:3 *n-3* + C20:5 *n-3* + C22:6 *n-3*

n-6 PUFA = C18:2 *n-6* + C18:3 *n-6* + C20:3 *n-6* + C20:4 *n-6* + C22:4 *n-6*

h/H = hypocholesterolaemic/hypercholesterolaemic ratio = [(sum of C18:1 *cis-9*, C18:2 *n-6*, C18:3 *n-6*, C18:3 *n-3*, C20:3 *n-6*, C20:3 *n-3*, C20:4 *n-6*, C20:5 *n-3*, C22:4 *n-6* and C22:6 *n-3*)/(sum of C12:0, C14:0 and C16:0)]

ns = not statistically significant; * = P<0.05; ** = P<0.01; *** = P<0.001

SE = standard error of the analysis of variance

Table 3. Effect of sex on fatty acid composition (w/w %) of neutral lipids and polar lipids in *Supra spinatus* muscle

Fatty acids (%)	Neutral lipids				Polar lipids			
	Female	Male	SE	S	Female	Male	SE	S
n	20	20			20	20		
C12:0	0.18	0.21	0.01	ns	0.03	0.02	0.01	ns
C14:0	3.99	4.48	0.25	ns	0.41	0.40	0.01	ns
C14:1	0.79	0.67	0.06	ns	0.03	0.04	0.01	ns
<i>iso</i> -C15:0	0.18	0.18	0.01	ns	0.05	0.05	0.00	ns
<i>anteiso</i> -C15:0	0.20	0.26	0.01	**	0.05	0.05	0.01	ns
C15:0	0.50	0.61	0.03	*	0.22	0.23	0.01	ns
C15:1	0.19	0.24	0.01	*	0.12	0.11	0.01	ns
C16:0	22.15	22.83	0.39	ns	17.11	17.56	0.44	ns
C16:1 <i>cis</i>	4.04	3.28	0.14	**	0.95	0.96	0.04	ns
<i>iso</i> -C17:0	0.72	0.68	0.03	ns	0.47	0.47	0.01	ns
<i>anteiso</i> -C17:0	0.60	0.60	0.01	ns	0.19	0.17	0.01	ns
C17:0	0.91	1.01	0.06	ns	0.47	0.47	0.01	ns
C17:1	0.80	0.72	0.02	*	0.53	0.54	0.04	ns
C18:0	12.58	15.72	0.47	***	10.94	11.28	0.44	ns
C18:1 <i>trans</i>	0.59	0.56	0.22	ns	0.36	0.43	0.06	ns
C18:1 <i>cis</i> -9	42.23	38.60	0.75	**	18.82	19.20	0.59	ns
C18:1 <i>cis</i> -11	0.18	0.03	0.07	ns	2.17	2.30	0.10	ns
C18:2 <i>n</i> -6	3.68	4.07	0.28	ns	17.36	18.40	0.56	ns
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.85	0.75	0.06	ns	0.38	0.38	0.01	ns
C18:3 <i>n</i> -6	0.11	0.09	0.03	ns	0.12	0.10	0.01	ns
C18:3 <i>n</i> -3	0.69	0.71	0.06	ns	2.10	2.18	0.13	ns
C20:0	0.07	0.11	0.01	**	0.12	0.12	0.01	ns
C20:1	0.08	0.09	0.01	ns	0.11	0.08	0.01	ns
C20:3 <i>n</i> -6	0.07	0.09	0.01	ns	0.53	0.46	0.06	ns
C20:3 <i>n</i> -3	0.15	0.16	0.03	ns	2.07	1.86	0.06	*
C20:4 <i>n</i> -6	0.75	0.82	0.19	ns	12.15	11.11	0.52	ns
C20:5 <i>n</i> -3	0.32	0.33	0.07	ns	5.17	4.70	0.33	ns
C22:4 <i>n</i> -6	0.23	0.24	0.07	ns	0.01	0.01	0.00	ns
C24:1	0.38	0.36	0.08	ns	3.63	3.21	0.13	*
C22:6 <i>n</i> -3	0.07	0.09	0.03	ns	1.12	0.79	0.07	**
SFA	42.39	46.83	0.56	***	30.69	31.30	0.47	ns
MUFA	49.61	44.60	0.67	***	26.84	26.98	0.60	ns
PUFA	6.83	7.37	0.61	ns	41.41	40.38	0.80	ns
P/S	0.16	0.16	0.03	ns	1.36	1.30	0.04	ns
<i>n</i> -6 PUFA	4.75	5.31	0.49	ns	30.22	30.15	0.77	ns
<i>n</i> -3 PUFA	1.25	1.32	0.47	ns	10.81	9.84	0.42	ns
<i>n</i> -6/ <i>n</i> -3 PUFA	3.92	4.29	0.25	ns	2.87	3.22	0.17	ns
h/H	1.85	1.66	0.06	*	3.46	3.35	0.10	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

n-3 PUFA = C18:3 *n*-3 + C20:3 *n*-3 + C20:5 *n*-3 + C22:6 *n*-3

n-6 PUFA = C18:2 *n*-6 + C18:3 *n*-6 + C20:3 *n*-6 + C20:4 *n*-6 + C22:4 *n*-6

h/H = hypocholesterolaemic/hypercholesterolaemic ratio = [(sum of C18:1 *cis*-9, C18:2 *n*-6, C18:3 *n*-6, C18:3 *n*-3, C20:3 *n*-6, C20:3 *n*-3, C20:4 *n*-6, C20:5 *n*-3, C22:4 *n*-6 and C22:6 *n*-3)/(sum of C12:0, C14:0 and C16:0)]

ns = not statistically significant; * = P<0.05; ** = P<0.01; *** = P<0.001

SE = standard error of the analysis of variance

**EFFECTIVENESS OF A TOTAL QUALITY MANAGEMENT (TQM) SYSTEM
AT PRODUCING BEEF STEAKS WITH EXCEPTIONAL PALATABILITY
(PACCP APPROACH) COMPARED TO OTHER METHODS OF IMPROVING
BEEF PALATABILITY**

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Key Words: Beef, Palatability, Enhancement

Introduction

The palatability of beef is one of the most important attributes in determining customer satisfaction. Beef consumers are willing to pay higher prices for more palatable beef than for less palatable beef (Platter et al., 2005). The palatability of beef offered to consumers is quite variable (Brooks et al., 2000), and this variation probably contributes to some customer dissatisfaction.

One method proposed by researchers for improving the consistency of beef palatability is a Total Quality Management (TQM) approach, sometimes referred to as 'Palatability Assurance Critical Control Points' or PACCP approach (Tatum et al., 1999; Thompson et al., 1999). Because factors in every aspect of beef production from genetics through cooking can impact beef palatability, it has been suggested that a TQM approach would produce the most consistently palatable beef.

Other approaches to improving beef palatability and reducing palatability variation include the 'sorting palatability' approach (grading) and the 'enhancing palatability postmortem' approach (enhancing). In the U.S., Beef carcasses are grouped according to expected palatability through the use of the USDA Quality Grading system (USDA, 1997). Carcasses that have the highest expected palatability receive the highest quality grade, USDA Prime. Enhancing beef with water, salt and phosphate can improve palatability and reduce palatability variation (Vote et al., 2000). Indeed, enhanced beef is becoming more popular in the marketplace. A patented method of pH enhancement of beef products developed by Freezing Machines, Inc. of Dakota Dunes, SD uses ammonium hydroxide to increase the pH of meat (Hand et al., 2005; the technology is the subject of various issued and pending patents, and is validated to reduce or eliminate potential pathogens in meat products processed using the technology; other patent pending technology also includes dissolved carbon dioxide in the injection process). Ammonium hydroxide is a GRAS ("Generally recognized as safe" according to U.S. Food and Drug Administration) food additive.

In the quest for beef with exceptional palatability, the best approach is unknown. Which approach would yield the most consistent palatable beef: the TQM approach, the grading approach, or the enhancing approach?

Objectives

Hypothesis: A Total Quality Management (TQM) system designed to produce palatable beef steaks is more effective at creating exceptional beef eating experiences than other methods of improving beef palatability.

Objective: Determine if a Total Quality Management (TQM) system for producing beef steaks with exceptional palatability was superior to other methods of improving beef palatability.

Methodology

A Total Quality Management (TQM) system for producing beef steaks with exceptional palatability, also called a PACCP approach, was developed. All 'critical control points' in the PACCP system resulted from an extensive review of scientific literature. The PACCP system included 12 'critical control points' throughout the entire production process from animal genetics to postmortem meat handling: 1. Half-blood Jersey cows, 2. Half-blood Angus cows, 3. Marbling EPD in top 1% for maternal Angus sire, 4. Known-tender terminal sire, 5. Castration of males at birth, 6. No hormonal implants, 7. Minimum 150 d on high-corn diet, 8. Maximum 20 months age at harvest, 9. Maximum four h from feedlot to slaughter, 10. Pre-rigor skeletal separation at 12th/13th rib, 11. Delayed chilling for two h, and 12. Postmortem aging for 21 d. All carcasses (n = 11) produced through the PACCP system in a given time frame were used in this study with no sorting or culling based on carcass evaluation or gender. Consumer panels were conducted using longissimus lumborum (LL) and semitendinosus (ST) steaks to compare PACCP beef to average U.S. beef (AVG), Certified Angus Beef USDA Prime beef (PRIME), salt/phosphate-enhanced USDA Select beef (SPE), and pH-enhanced USDA Select beef (PHE). The AVG beef was obtained from non-enhanced beef at retail stores in California, Kentucky, South Dakota, and Texas (four stores per state). The PRIME beef was obtained as boxed beef and aged at least 14 d. The SPE beef was injected to 111% of green weight with a target of 0.4% sodium tripolyphosphate and 0.3% salt in the final product. The PHE beef was injected with a patent pending solution of water, ammonium hydroxide, carbon oxide, and salt.

Consumer panelists were recruited from the Brookings, SD area using fliers and newspaper and radio advertising. A minimum age of 23 years was enforced to minimize the number of college students in the panelist pool. Two hundred and four consumers participated in the study over 11 different panel times. Steaks were cooked on gas grills, turning every 2.5 min, to a target internal temperature of 66 degrees C. Immediately following cooking, steaks were cut into uniform 1.3 by 2.5 cm samples using a sample sizing guide, placed into styrofoam bowls with holes punched in the bottom to allow juices to drain, covered with aluminum foil, and held in a 60 degree C warming oven until served. Panels were conducted in booths preventing panelist interaction. Prior to the start of the panel, panelists were given brief instructions about panel procedures and were asked to sign a notice of informed consent. All samples were served under red lights to limit differences in visual appearance. One sample of each treatment combination was served in a random order to the panel. The first sample was always a longissimus steak obtained from the SDSU Meat Lab and was used as a warm-up sample to prevent first-

sample bias; this data was not included in the analysis of data nor were any conclusions drawn from that sample. Samples were coded with a random code to blind consumers to treatment combinations. Consumer panelists rated steaks on 10-point scales (10 = like extremely, 1 = dislike extremely) for ‘overall like’, ‘like of tenderness’, and ‘like of flavor’.

Results & Discussion

Carcass trait means for PACCP carcasses were 332 kg hot weight, 1.0 cm fat thickness, and Modest 29 marbling score (data not presented in tabular form). These carcass characteristics are typical of average U.S. carcasses except that PACCP carcasses had higher-than-average marbling scores (McKenna et al., 2002).

A wide range of consumer demographics were represented in the consumer panel (Table 1). A higher-than-normal proportion of young (23 to 29) consumers were sampled.

Longissimus lumborum steaks from all treatments except SPE rated higher for ‘overall like’ than AVG LL steaks (Table 2). pH-enhanced LL steaks had higher ‘overall like’ ratings than SPE and AVG LL steaks. There was no difference in LL ‘overall like’ ratings between PACCP and PRIME ($P > 0.05$), indicating that the TQM approach produced LL steaks similar in palatability to the top grade from the sorting approach. It is important to note that PACCP approach utilized 100% of carcasses while the sorting approach utilized only the top 2% of carcasses (McKenna et al., 2002). There was no difference in LL ‘overall like’ ratings between PACCP and PHE ($P > 0.05$). Because PHE was USDA Select beef, did not incur the added production expenses of PACCP beef, and had added solution, it is likely much less expensive to produce PHE beef than PACCP beef. Therefore, PHE resulted in beef equal in palatability to PACCP beef at presumably much lower production costs.

Longissimus lumborum steaks from PACCP rated higher for ‘like of tenderness’ compared to all other treatments (Table 2). pH-enhanced LL steaks rated higher for ‘like of tenderness’ than AVG and SPE LL steaks. pH-enhanced LL steaks rated the highest of all treatments for ‘like of flavor’. Longissimus lumborum steaks from PRIME and SPE rated higher than AVG LL steaks for ‘like of flavor’.

pH-enhanced ST steaks rated higher for ‘overall like’ than all other ST steaks except PACCP ST steaks (Table 3). Ratings on ST steaks for ‘like of tenderness’ were highest for PHE and PACCP compared to other treatments. Semitendinosus steaks from PRIME rated the lowest for ‘overall like’ and ‘like of tenderness’ of all treatments. Semitendinosus steaks that were enhanced by any method (PHE and SPE) rated higher than AVG ST steaks for ‘like of flavor’.

Conclusions

The Total Quality Management (TQM) approach resulted in beef that had superior palatability to average U.S. beef and USDA Prime beef; however, pH enhancement, likely a less costly approach, produced consumer ratings equal to TQM. Therefore, in the pursuit of consistently palatable beef, ‘enhancing palatability postmortem’ may be a more effective and less expensive method than either ‘managing palatability’ or ‘sorting palatability’.

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Tables and Figures

Table 1. Demographic profile of 204 consumer panelists.

	Number of consumers	% of consumers
<u>Age</u>		
23 to 29	87	43
30 to 39	23	11
40 to 49	29	14
50 to 59	26	13
60 and older	38	19
<u>Household Annual Income</u>		
Under \$20,000	66	33
\$20,000 to \$39,000	70	35
\$40,000 and higher	64	32
<u>Working Status</u>		
Not employed	35	17
Part-time	30	15
Full-time	89	44
Student	49	24
<u>Gender</u>		
Male	114	56
Female	89	44
<u>Times per week beef is consumed</u>		
1 to 3	60	29
4 to 5	74	36
6 or more	72	35
<u>Times per month steak is consumed</u>		
0 to 2	80	39
3 to 4	65	32
5 or more	61	30

Table 2. Mean ratings (10 = like extremely, 1 = dislike extremely) from consumer panel (n = 204 consumers) for longissimus lumborum steaks (means within a column lacking a common letter differ, P < 0.05).

	Overall Like	Like of Tenderness	Like of Flavor
U.S. Average	6.85c	7.31cd	6.48c
PACCP	7.31ab	8.09a	6.79bc
USDA Prime	7.25ab	7.58bc	6.91b
Salt/Phosphate-Enhanced	6.98bc	7.03d	6.98b
pH-Enhanced	7.48a	7.70b	7.50a
SEM	0.12	0.13	0.13
p	0.0027	< 0.0001	< 0.0001

Table 3. Mean ratings (10 = like extremely, 1 = dislike extremely) from consumer panel (n = 204 consumers) for semitendinosus steaks (means within a column lacking a common letter differ, P < 0.05).

	Overall Like	Like of Tenderness	Like of Flavor
U.S. Average	5.43c	5.18b	5.48bc
PACCP	5.99ab	5.94a	5.74ab
USDA Prime	5.08d	4.43c	5.33c
Salt/Phosphate-Enhanced	5.72bc	5.28b	5.97a
pH-Enhanced	6.19a	6.26a	6.05a
SEM	0.12	0.13	0.13
p	<0.0001	< 0.0001	< 0.0001

CHARACTERISTICS OF MEAT TEXTURE OF SEVERAL EUROPEAN CATTLE BREEDS

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Key Words: beef, ageing, texture, compression

Introduction

The present study is part of an ongoing EU project with animals from 15 different breeds representing the genetic diversity of European cattle. This project will undertake a rigorous comparison meat quality from this range of cattle breeds with animals raised under similar management conditions in order to minimise environmental variation and to identify and define genetically determined component of that variation.

In particular, meat tenderness is the most important cause of consumer dissatisfaction and any improvement would increase the value of the final product (Brooks *et al.*, 2000).

Objectives

The main objective was to determine the influence of breed and ageing time on the instrumental textural characteristics of beef meat, related to the myofibrillar and connective components.

Methodology

Four hundred and thirty six young entire males from 15 different European cattle breeds: Jersey, South Devon, Aberdeen Angus and Highland from United Kingdom; Holstein, Danish Red Cattle and Simmental from Denmark; Asturiana de los Valles, Casina, Avileña and Pirenaica from Spain; Piemontese and Marchigiana from Italy and

Limousin and Charolais from France. All animals were fed *ad libitum* with a standardised diet comprising high barley (about 80%), soya (9%) and chopped straw (10%) with minerals and vitamins. Energy density was approximately of 12.5 kJ/kg dry matter.

All the welfare regulations were taken into account when handling the animals. The bulls were slaughtered at the nearest EU licensed abattoir, to minimise the effect of the transport stress on meat quality. Slaughter was about 14-16 months of age. Stunning was by captive bolt pistol. Carcasses were chilled at 4° C for 24 hours. After this time the *m. longissimus thoracis* (between 8th and 13th ribs) was removed from the left side of carcass and stored at 3° C until 48 hours. Later on a section was subdivided in slices, vacuum packaged and frozen; another section was aged for 10 days, cut, vacuum packaged and frozen. Samples were frozen at -18° C, until instrumental analysis. To analyse the texture in raw meat the loin samples were sliced into 4 cm thick steaks.

Before instrumental analysis meat was thawed, in their vacuum bags, in tap water for 4 hours until reaching an internal temperature of 17-19° C. Samples, 1 cm² in cross-section were cut with muscle fibres parallel to the longitudinal axis of the sample. Samples were analysed using a modified compression device that avoids transversal elongation of the sample (Lepetit & Culioli, 1994). Stress at 20% (C20) and 80% (C80) of maximum compression were assessed using an Instron 4301 machine. Lepetit and Culioli (1994) observed that low compression values (C20) were related to the resistance of the myofibrils to deformation (compression) and it is known that higher stress rates (C80) are mainly related to the connective tissue components (Lepetit and Culioli, 1994).

Statistical analysis was performed using SPSS 11.5 software. An ANOVA procedure was carried out with breed as unique effect, within ageing time.

Results & Discussion

Global results are shown in Table 1.

Myofibrillar component (C20)

Breed was a very important factor on C20 texture measurements. With a short ageing (48 h.) breeds ranged from 5.45 N/cm² to 9.77 N/cm². Rustic breeds (Highland, Avileña, Casina) or dual purpose breeds (Simmental) had the highest C20 values whilst dairy breeds (Holstein and Danish Red Cattle) had the lowest values. Double-muscled breeds gave intermediate values. These results apparently contradict the two opposite ideas that double-muscled animals have tough myofibres (Clinquart, Hornick, Van Eenaeme and Istasse, 1998) and that animals with high muscle development have faster protein degradation and higher ageing rates due to a predominance of fast contracting white fibres (Ouali, 1990) Therefore the observed results are likely to be a compromise between two different biological mechanisms.

With 10 days ageing values in C20 were lower. The C20 variable reflected a progressive tenderization of the meat as ageing advanced confirming that myofibrils are the components that change the most during ageing (Lepetit, Salé and Ouali, 1986; Tornberg, 1996). Asturiana de los Valles (double muscled breed) had the lowest value (3.84 N/cm²) and Marchigiana, Simmental and Aberdeen Angus had the highest values (5.48 N/cm², 5.15 N/cm² and 4.49 N/cm² respectively).

Connective component (C80)

C80 values for breeds ranged from 30.68 N/cm² to 46.29 N/cm² (48 h. ageing) and from 31.21 N/cm² to 52.52 N/cm² (10 days ageing). C80 values were significantly different between breeds with a short ageing (48 h.), higher in dairy breeds (Holstein and Danish Red Cattle) and rustic breeds (Highland, Casina) and lower in specialised beef breeds (Asturiana de los Valles, Piemontese, Pirenaica and Limousin).

Holstein, Danish Red Cattle (dairy breeds) had the highest values in C80 with 10 days ageing whilst some specialised meat breeds (Piemontese, Limousin and Asturiana de los Valles) had the lowest values. This agrees with the general view that double-muscling is associated with lower collagen content in muscles (for review, see Culioli, 1999).

Ageing did not affect C80 values. Differences between ageing times ranged from 0.1% to 16.3%. These results agree with those of Eikelenboon, Barbier, Hoving-Bolink, Smulders and Culioli (1998) who reported that connective tissue did not change during ageing.

Conclusions

We can conclude that breed is an important factor on meat tenderness in beef. Differences between breeds in myofibrillar texture were reduced with ageing times. With 10 days ageing Asturiana de los Valles, Limousin, Piemontese, South Devon and Pirenaica breeds showed lower texture values than other breeds.

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Tables and Figures

Table 1. Texture determinations on *longissimus thoracis* muscle aged 48 hours and 10 days in 15 European cattle breeds: means and (standard deviation)

	JER	SD	AA	HIG	HOLS	RED	SIMM	ASV	CA	AV	PI	PIE	MARC	LIM	CHAR
<i>N</i>	31	27	30	29	29	29	20	30	31	30	31	30	28	31	30
Carcass weight (kg)	189.68	346.92	335.72	245.12	319.97	318.72	344.35	348.74	244.69	328.91	371.45	335.86	307.52	360.68	386.64
48 hours of ageing															
C20 (N/cm ²)	7.18 _{cde} (2.17)	7.01 _{cde} (1.95)	7.31 _{cde} (1.98)	9.77 ^a (2.51)	5.45 ^f (2.02)	6.25 ^{ef} (1.94)	8.29 _{abc} (2.52)	6.96 _{cde} (2.78)	8.07 _{abc} (4.21)	8.37 _{abc} (3.30)	6.63 _{def} (2.45)	6.48 _{def} (2.72)	9.37 ^{ab} (3.44)	6.45 _{def} (1.84)	7.43 _{cde} (3.07)
C80 (N/cm ²)	37.58 _{cd} (8.95)	35.46 _{de} (9.13)	39.89 _{cd} (6.58)	42.27 _{abc} (12.30)	43.50 _{ab} (7.35)	46.29 ^a (6.76)	40.22 _{bc} (8.27)	30.68 ^f (6.99)	42.35 _{abc} (10.57)	37.76 _{cd} (6.44)	31.28 _{ef} (6.35)	29.81 ^f (6.37)	42.87 _{ab} (8.85)	31.83 _{ef} (5.06)	39.40 _{bcd} (7.96)
10 days of ageing															
C20 (N/cm ²)	4.21 _{bc} (0.76)	4.06 _{bc} (0.50)	4.49 ^b (0.84)	4.35 _{bc} (0.79)	4.03 _{bc} (0.47)	4.40 _{bc} (0.66)	5.15 ^a (1.81)	3.84 ^c (0.39)	4.22 _{bc} (0.60)	4.20 _{bc} (0.84)	4.03 _{bc} (0.46)	4.13 _{bc} (0.81)	5.48 ^a (2.54)	4.00 _{bc} (0.42)	4.26 _{bc} (0.74)
C80 (N/cm ²)	39.58 _{bcd} (8.15)	35.21 _{def} (9.03)	39.93 _{bc} (6.61)	36.45 _{cde} (6.64)	49.52 ^a (8.99)	52.52 ^a (11.35)	40.01 _{bc} (7.63)	32.57 _{ef} (9.17)	39.03 _{bcd} (6.52)	41.56 _b (6.19)	36.38 _{cde} (8.16)	27.83 _g (6.17)	41.82 _b (7.12)	31.21 _{fg} (3.71)	43.25 _b (8.12)

Different letters in the same row mean significant differences (at least $p \leq 0.05$)

C20: Stress at 20% of maximum compression; C80; stress at 80% of maximum compression.

JER: Jersey; **SD:** South Devon; **AA:** Aberdeen Angus; **HIG:** Highland; **HOLS:** Holstein; **RED:** Danish red Cattle; **SIMM:** Simmental; **ASV:** Asturiana de los Valles; **CAS:** Casina; **AV:** Avileña; **PI:** Pirenaica; **PIE:** Piemontese; **MARC:** Marchigiana; **LIM:** Limousin; **CHAR:** Charolaise.

THE ADDITION OF RACTOPAMINE TO THE FEED OF LIGHT AND HEAVY SWINE AND ITS IMPACTS ON MEAT QUANTITATIVE CHARACTERISTICS

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Key Words: ractopamine, swine, meat quantity.

Introduction

In the last years, the search for better efficiency in meat production has been the most important challenge faced by swine producers and the scientific community, leading to the application of technological innovations in animal production. Reproduction, health care, management, genetic selection and the use of nutrient repartitioners in swine feed have been evaluated.

With the approval of ractopamine hydrochloride as a feed additive by the Brazilian Ministry of Agriculture and Food Supply, swine producers have an excellent alternative to increase meat production and benefit the swine production chain.

Several studies have demonstrated that ractopamine hydrochloride increases the amount of muscle, depresses fat deposition, besides increasing weight gain and improving feed conversion without interfering with the pork quality. In this context, detailed studies on the effect of ractopamine on meat quantity should be carried out in our country to prove the benefits of this type of feed additive on the swine herd.

Objectives

The aim of the present work is to evaluate the effect of different levels of ractopamine (5 and 10mg/kg) on some slaughter traits like live and carcass weight, lean meat and fat percentage in the carcass and meat quantity in some main cuts.

Methodology

Animals. A total of 80 animals were randomly divided into the eight experimental groups: 4 control groups with different finishing feed consumption periods and 4 experimental groups with different ractopamine and lysine levels. 40 females with an initial weight of 68 kg (light animals) - T1 (n=10); T2 (n=10); T5 (n=10); T7 (n=10), and 40 females with an initial weight of 88 kg (heavy animals) - T3 (n=10); T4 (n=10); T6 (n=10); T8 (n=10) were used in this experiment. The animals came from the same farm and same genetic origin.

Evaluation station building. The building consists of 80 individual pens with semi-automatic feeders, nipple drinkers and an area of 2.50 m²/animal.

Slaughter. The light and heavy animals were kept in the station the period necessary to reach 100 and 120 kg, respectively. Then they were slaughtered in a commercial abattoir. The chilled carcasses were transported to the Meat Technology Center and prepared for boning purposes according to the methodology described by WALSTRA & MERKUS, 1996.

Results & Discussion

Tables 1 and 2 contain the statistical results of the meat quantitative evaluations from the light and heavy animals, respectively.

In Table 1 it can be seen a significant increase ($p < 0.05$) in the meat quantity of the ham, loin and shoulder as the level of ractopamine increases. This additional amount of meat in these cuts represents an economical advantage for the pork industry since both markets, fresh and further processed products, are reached simultaneously. Increases in meat amounts considered as expressive were found in ham (1.88kg and 2.51kg), loin (0.92kg and 0.77kg), belly (0.96kg and 0.42kg) and shoulder (0.75kg and 0.87kg) by adding 5mg/kg and 10mg/kg ractopamine, respectively. The present study showed an average increase in live weight (4.53kg and 2.44kg), and percentage of lean meat (3.57% and 4.17%) with the addition of 5mg/kg and 10mg/kg ractopamine to the feed, respectively. The high advantage of ractopamine was evident in relation to the percentage of lean meat present in the carcass.

The effect of ractopamine on the meat amount in cuts from heavy animals is presented in Table 2. The statistical results showed the same tendency than for light animals, the meat quantity in ham, loin and shoulder was significant higher as the level of ractopamine increased. An average increase in the meat of cuts with high commercial value as ham (1.98kg) and shoulder (0.76kg) was found by adding 10mg/kg ractopamine to the feed. The percentage of lean meat (3.26%) increased significant ($p < 0.05$) with the addition of 10mg/kg ractopamine to the feed.

The results of the present study corroborate with Zagury et al., (2001) that reported a significant increment ($p < 0.05$) in the percentage of meat in the groups of animals that received ractopamine. In another experiment Zagury (2002) concluded that the addition of 5mg/kg of ractopamine resulted a profit of 3,31kg in the weight of slaughter; 2,82kg in the weight of carcass; 0,75% in the percentage of lean meat; 2,12mm in the backfat depth and 2,98kg in the total meat considering the main cuts.

Conclusions

The addition of 5 or 10 mg/kg ractopamine to the feed contributes to increase the amount of meat and decrease fat in both light and heavy animals. Thus, the percentage of lean meat, the weights of the ham, shoulder, loin and belly had been benefited with the ractopamine. This fact has an important technological meaning for the processing of cooked ham (ham), salami (shoulder) and bacon (belly), as the higher yield obtained in the industrial production of these products assures their economical.

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Table 1. Mean weight values of live animals, meat quantity in the main cuts, fat and percentage of lean meat in light animals.

	T1	T2	T5	T7
Live weight (Kg)	96,30 ^b ± 5,01	101,82 ^a ± 6,19	100,83 ^{ab} ± 4,70	98,74 ^{ab} ± 5,01
Ham (Kg)	12,93 ^b ± 1,43	14,62 ^a ± 0,74	14,81 ^a ± 1,81	15,44 ^a ± 1,17
Loin (Kg)	7,27 ^b ± 0,69	7,27 ^b ± 0,61	8,19 ^a ± 0,90	8,04 ^a ± 0,73
Belly (Kg)	4,54 ^b ± 0,88	5,02 ^{ab} ± 0,57	5,50 ^a ± 0,43	4,95 ^{ab} ± 0,33
Shoulder (Kg)	5,59 ^b ± 0,65	6,02 ^{ab} ± 0,23	6,34 ^a ± 0,61	6,46 ^a ± 0,67
Fat (Kg)	15,77 ^a ± 1,14	16,63 ^b ± 1,23	14,61 ^a ± 1,06	14,30 ^a ± 1,02
Lean meat (%)	55,41 ^c ± 2,40	56,46 ^{bc} ± 2,27	58,98 ^{ab} ± 2,62	59,58 ^a ± 1,88

Lines with different letters indicate significant differences ($p < 0,05$) between treatments.

Mean ± standard deviation.

T1: basic finishing feed during 28 days.

T2: basic finishing feed during 32 days.

T5: feed with addition of 5mg/Kg ractopamine and 1,15% lysine during 28 days.

T7: feed with addition of 10mg/Kg ractopamine and 1,15% lysine during 28 days.

Table 2. Mean weight values of live animals, meat quantity in the main cuts, fat and percentage of lean meat in heavy animals.

	T3	T4	T6	T8
Live weight (Kg)	120,32 ^a ± 7,64	124,35 ^a ± 7,70	124,16 ^a ± 3,92	121,81 ^a ± 8,45
Ham (Kg)	16,36 ^b ± 1,63	16,78 ^{ab} ± 1,24	17,58 ^{ab} ± 1,55	18,34 ^a ± 1,42
Loin (Kg)	8,52 ^b ± 0,70	9,13 ^{ab} ± 1,01	9,63 ^a ± 0,77	9,60 ^a ± 0,68
Belly (Kg)	5,82 ^a ± 0,92	6,31 ^a ± 0,74	6,45 ^a ± 0,84	6,50 ^a ± 0,65
Shoulder (Kg)	6,70 ^b ± 0,65	7,23 ^{ab} ± 0,55	7,33 ^{ab} ± 0,38	7,46 ^a ± 0,72
Fat (Kg)	21,19 ^a ± 1,58	20,01 ^a ± 1,45	20,24 ^a ± 1,51	18,97 ^a ± 1,41
Lean meat (%)	55,49 ^b ± 2,97	55,49 ^b ± 2,05	57,17 ^{ab} ± 1,96	58,75 ^a ± 1,79

Lines with different letters indicate significant differences ($p < 0,05$) between treatments.

Mean ± standard deviation.

T3: basic finishing feed during 48 days.

T4: basic finishing feed during 52 days.

T6: feed with addition of 5mg/Kg ractopamine and 1,04% lysine during 28 days.

T8: feed with addition of 10mg/Kg ractopamine and 1,04% lysine during 28 days.

**EFFECT OF RACTOPAMINE FEEDING LEVEL ON GROWTH
PERFORMANCE, CARCASS COMPOSITION, FATTY ACID PROFILE, AND
LOIN QUALITY MEASUREMENTS**

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Key Words: Fatty Acid Profile, Pork, Ractopamine, Quality

Objectives

The focus of this experiment was to determine the effect of ractopamine (RAC) and feeding level on growth performance as well as its subsequent effects on carcass composition, fatty acid profile of the clear plate and belly, and loin quality measurements.

Methodology

Barrows (n = 75) and gilts (n = 75) were randomly allotted to one of three dietary treatments resulting in a randomized complete block design with location as a blocking factor and the pen as the experimental unit for growth performance, backfat depth, muscle depth, and hot carcass weight. The dietary treatments (as-fed basis) were: 1) Control diet containing no RAC; 2) RAC fed at 5 ppm; and 3) RAC fed at 10 ppm. Pigs, within a gender, were randomly assigned to treatment (5 pigs per pen and 10 pens per treatment). All diets were formulated to contain 1.2% lysine and fed for a five-week period. Pigs were acclimated for 5 days prior to initiation of treatments. Feed allocation was recorded on a daily basis. Pigs were weighed and feed was weighed back when diets were changed, in order to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) by feeding period. At the end of the 33 d feeding period, backfat depth and loin eye area (LEA) were measured on all pigs using real-time ultrasound (Aloka 500; Corometrics Medical Systems, Wallingford, CT). Two pigs were randomly selected per pen at allotment and blood samples were obtained from the jugular vein at d 0, 7, 14, 21, 27, and 33. Pigs were not fasted before blood samples were obtained. Plasma was obtained by centrifugation (1600 x g) of blood samples at 5°C for 30 min. Plasma was stored at -20°C until analysis of urea nitrogen concentrations.

At the conclusion of the five week period all pigs were tattooed for slaughter and the two pigs closest to the average pen weight were identified for subsequent carcass and muscle quality evaluation. At the time of slaughter hot carcass weight and Fat-o-Meter measures of fat depth and muscle depth were collected. Percent carcass yield was

calculated using off-test weight and HCW. Fat was sampled from the clear plate of 60 carcasses and they were broken into three parts; ham with foot on, loin and belly, and shoulder, placed into combos and transported by truck to the University of Illinois for dissection and muscle quality evaluation. Taste panels were conducted utilizing a 15 cm anchored, unstructured line scale for tenderness, juiciness, and off-flavor. Warner-Bratzler shear force value is the average of 4 cores per chop.

Statistical analyses of the data were performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Dietary treatment, gender, and interaction were examined to determine their effects on growth and carcass characteristics. Sequence of measurement was included as a covariate for the analysis of Minolta Color and session was included as a fixed effect for taste panel measures. Pen was the experimental unit for growth performance, hot carcass weight, fat depth muscle depth, loin area, and fatty acid composition. Animal was considered the experimental unit for carcass composition and muscle quality data.

Results & Discussion

Pigs fed RAC displayed greater ($P < 0.05$) live weights each week with the exception of initial body weights at allotment when compared to control pigs as can be seen in Table 1. They also had increased ($P < 0.05$) average daily gains (ADG) over the course of the 5-week study while average daily feed intake (FI) did not differ ($P > 0.05$) (Table 2). Hot carcass weight (HCW) dressing percent (percent yield) increased ($P < 0.05$) with the addition of RAC while loin eye area was significantly larger ($P < 0.05$) only at the 5ppm level (Table 3). Fat depth over the loin did not differ between treatments while loin depth was greatest ($P < 0.05$) for the 5 ppm level, followed by the 10 ppm, and lastly the control treatment.

Feeding RAC had no effects ($P > 0.10$) on fatty acid profile and calculated IV of belly fat, but had an effect ($P < 0.05$) on linoleic acid in clearplate fat. The percentage of linoleic acid in clearplate fat from pigs fed 10 ppm RAC (18.6) was on average 7% higher (17.4) than pigs fed control diet and 5 ppm RAC. This resulted in a 3% increase in IV, 8% increase in polyunsaturated fatty acid and 8.5% decrease in the ratio of monounsaturated to polyunsaturated fatty acids from pigs consuming 10 ppm RAC compared to pigs consuming control and 5 ppm RAC diets. No significant difference ($P > 0.05$) was detected between pigs fed control diet and 5 ppm RAC concerning any fatty acid measurements (Tables 4 and 5).

Carcass cutting yields, expressed as a % of HCW, revealed minimal differences between control diet and pigs fed RAC. The tenderloin was increased ($P < 0.05$) as a % of HCW by the addition of RAC to the diet, but no other significant differences were noted (Table 6). Loin Quality measurements (Table 7) were not affected by feeding RAC with the exception of Minolta a^* which was lowered by both levels of RAC and b^* with was lower for 10 ppm ($P < 0.05$). Trained sensory panel and Warner-Bratzler shear force also did not reveal any significant differences between treatments ($P > 0.05$).

Conclusions

The addition of RAC to the diet resulted in a favorable growth response yielding heavier carcasses ultimately resulting in more lean pork than the control diet. There was however, no major difference in percent yield of lean cuts from the carcass. Feeding 10 ppm of RAC increased the enrichment of linoleic acid in clearplate fat, but there was no overall effect of RAC on the fatty acid profile of belly fat of finishing pigs and thus should be no effect on belly fat quality.

Tables and Figures

Table 1. Effect of ractopamine feeding level on BW and growth performance by week.

	Control	5 ppm	10 ppm	SEM
Pens, n	10	10	10	--
Pigs, n	50	50	50	--
Initial BW, kg	76.2	76.7	77.0	0.30
<i>Week 1, 7 d</i>				
Pigs, n	50	50	50	--
BW at 7 d, kg	81.7 ^b	83.0 ^a	83.5 ^a	0.40
ADG, kg	0.79	0.90	0.94	0.06
Feed Intake, kg	2.56	2.71	2.73	0.06
G:F	0.31	0.33	0.35	0.02
<i>Week 2, 7 d</i>				
Pigs, n	49	50	50	--
BW at 14 d, kg	89.3 ^b	92.0 ^a	91.9 ^a	.60
ADG, kg	1.09 ^b	1.29 ^a	1.20 ^{a,b}	0.06
Feed Intake, kg	3.00	3.10	3.08	0.07
G:F	0.36	0.42	0.39	0.02
<i>Week 3, 7 d</i>				
Pigs, n	48	50	50	--
BW at 21 d, kg	96.9 ^b	99.9 ^a	100.3 ^a	0.70
ADG, kg	1.08	1.13	1.19	0.07
Feed Intake, kg	3.09	3.13	3.15	0.08
G:F	0.35	0.36	0.38	0.02
<i>Week 4, 6 d</i>				
Pigs, n	48	50	50	--
BW at 27 d, kg	103.7 ^b	107.4 ^a	107.7 ^a	0.80
ADG, kg	1.14	1.25	1.24	0.05
Feed Intake, kg	3.08	3.19	3.27	0.08
G:F	0.37	0.39	0.38	0.02

<i>Week 5, 6 d</i>				
Pigs, n	48	50	50	--
BW at 33 d, kg	107.0 ^b	110.5 ^a	111.2 ^a	0.90
ADG, kg	0.56	0.52	0.59	0.07
Feed Intake, kg	3.15	3.20	3.28	0.09
G:F	0.18	0.16	0.18	0.02

^{a, b} Within a row, means lacking a common superscript differ ($p < 0.05$)

Table 2. Effect of ractopamine feeding level on growth performance in finishing pigs by time period.

	Control	5 ppm	10 ppm	SEM
Pens, n	10	10	10	--
<i>Week 1 to 2, 14 d</i>				
Pigs, n	49	50	50	--
ADG, kg	0.94 ^b	1.09 ^a	1.07 ^a	0.04
Feed Intake, kg	2.78	2.91	2.90	0.06
G:F	0.34	0.38	0.37	0.01
<i>Week 1 to 3, 21 d</i>				
Pigs, n	48	50	50	--
ADG, kg	0.98 ^b	1.10 ^a	1.11 ^a	0.03
Feed Intake, kg	2.88	2.98	2.98	0.06
G:F	0.35 ^b	0.37 ^a	0.37 ^a	0.01
<i>Week 1 to 4, 27 d</i>				
Pigs, n	48	50	50	--
ADG, kg	1.02 ^b	1.14 ^a	1.14 ^a	0.02
Feed Intake, kg	2.93	3.02	3.05	0.06
G:F	0.35 ^b	0.38 ^a	0.37 ^a	0.01
<i>Week 1 to 5, 33 d</i>				
Pigs, n	48	50	50	--
ADG, kg	0.93 ^b	1.02 ^a	1.04 ^a	0.03
Feed Intake, kg	2.97	3.06	3.09	0.07
G:F	0.32	0.34	0.34	0.01

^{a, b} Within a row, means lacking a common superscript differ ($p < 0.05$)

Table 3. Effect of ractopamine feeding level on carcass weight and composition.

	Control	5 ppm	10 ppm	SEM
Pigs	48	50	50	--
Pens	10	10	10	--
RTU fat depth, mm	15.9	14.8	15.0	0.5
RTU loin area, cm ²	41.5 ^b	44.7 ^a	43.1 ^{a,b}	0.8
Pigs	45	46	46	--
Pens	10	10	10	--
Hot carcass wt., kg	78.9 ^b	83.2 ^a	83.8 ^a	0.7
Percent yield	73.8 ^b	75.3 ^a	75.4 ^a	0.4
FOM fat depth, mm	16.4	16.1	15.8	0.5
FOM loin depth, mm	55.8 ^c	61.1 ^a	58.5 ^b	0.9

^{a, b} Within a row, means lacking a common superscript differ ($p < 0.05$)

Table 4. Effect of ractopamine feeding program on fatty acid profiles of porcine clear plate fat ¹

Fatty Acid (% weight)	Control	5 ppm	10 ppm	SEM
C14:0	1.65	1.65	1.70	0.05
C16:0	23.40	23.50	23.38	0.22
C16:1	2.97	2.92	3.01	0.06
C18:0 ²	11.14	10.75	10.41	0.21
C18:1t	0.52	0.50	0.52	0.02
C18:1c	38.06	37.79	37.19	0.36
C18:2 ²	17.24 ^a	17.56 ^a	18.54 ^b	0.34
C18:3 ²	1.05	1.10	1.21	0.07
C20:1 ²	0.17	0.18	0.17	0.01
Other ⁴	3.81	4.06	3.89	0.21
Iodine value ^{2,3}	68.74 ^a	69.12 ^a	70.72 ^b	0.52
Saturated	36.18	35.90	35.46	0.26
Monounsaturated	41.55	41.21	40.72	0.35
Polyunsaturated ²	18.41 ^a	18.83 ^a	19.93 ^b	0.37
Mono/Poly ratio	2.27 ^a	2.24 ^a	2.06 ^b	0.06
US/S ratio	1.67	1.68	1.72	0.02

¹ Each mean represents 10 pens with 2-3 pigs. Pen was used as the experimental unit in statistical analysis.

^{a, b} Within a row, means lacking a common superscript differ ($p < 0.05$)

² Treatment by gender interaction ($p < 0.05$). See attached interaction graphs.

³ Calculated as $IV = C16:1(0.95) + C18:1(0.86) + C20:1(0.785) + C18:2(1.732) + C18:3(2.616)$.

⁴ Other fatty acids (e.g., C10:0, C12:0, C20:3 and C20:4) of low concentration.

Table 5. Effect of ractopamine feeding level on fatty acid profiles of porcine belly fat ¹

Fatty Acid (% weight)	Control	5 ppm	10 ppm	SEM
C14:0	1.65	1.71	1.76	0.06
C16:0 ²	23.03	23.43	22.93	0.33
C16:1	3.24	3.22	3.15	0.08
C18:0	10.18	10.42	10.19	0.23
C18:1t ²	0.51	0.46	0.50	0.01
C18:1c	39.80	39.01	39.56	0.47
C18:2	16.80	16.43	17.09	0.41
C18:3 ω 6	0.72	0.73	0.75	0.03
C18:3 ω 9	0.17	0.17	0.17	0.01
Other ⁴	3.91	4.43	3.89	0.25
Iodine value ^{2,3}	68.86	67.59	69.20	0.62
Saturated ²	34.86	35.56	34.89	0.47
Monounsaturated	43.71	42.85	43.39	0.46
Polyunsaturated	17.52	17.16	17.84	0.43
Mono/Poly ratio	2.51	2.53	2.45	0.08
US/S ratio	1.76	1.69	1.76	0.02

¹ Each means represented 10 pens with 2-3 pigs. Pen was used as the experimental unit in statistical analysis.

² Treatment by gender interaction ($p < 0.05$). See attached interaction graphs.

³ Calculated as IV = C16:1(0.95)+C18:1(0.86)+C20:1(0.785)+C18:2(1.732)+C18:3(2.616).

⁴ Other fatty acids (e.g., C10:0, C12:0, C20:3 and C20:4) of low concentration.

Table 6. Effect of ractopamine feeding level on loin quality.

	Control	5 ppm	10 ppm	SEM
Pigs, n	20	20	20	--
Ultimate pH	5.57	5.60	5.62	0.02
Color score	3.4	3.3	3.4	0.10
Marbling score	2.2	2.0	2.2	0.10
Firmness score	3.2	3.2	3.4	0.10
Minolta L*	47.4	47.7	47.2	0.50
Minolta a*	6.0 ^a	5.2 ^b	4.8 ^b	0.30
Minolta b*	2.4 ^a	2.0 ^{ab}	1.7 ^b	0.20
Drip loss, %	2.6	2.5	2.4	0.20
Taste panel tenderness score	7.3	7.2	7.5	0.30
Taste panel juiciness score	7.6	7.1	6.8	0.30
Taste panel off-flavor score	0.09	0.01	0.02	0.03
Cooking loss, %	24.4 ^{ab}	21.3 ^a	24.5 ^b	1.10
Warner-Bratzler shear force, kg	3.1	3.1	3.5	0.20
Proximate analysis moisture, %	75.3	75.3	75.3	0.10
Proximate analysis fat, %	1.7	1.5	1.4	0.10

^{a, b} Within a row, means lacking a common superscript differ ($p < 0.05$)

Table 7. Effect of ractopamine feeding level on ham, loin, and belly composition as a % of Hot Carcass Weight.

	Control	5 ppm	10 ppm	SEM
Pigs, n	18	18	18	--
Whole Ham, %	24.6	24.4	24.9	0.20
Skinned Ham, %	20.6	20.4	20.8	0.20
Inside, %	3.7	3.7	3.7	0.10
Outside, %	5.2	5.3	5.4	0.10
Knuckle, %	2.9	2.9	3.0	0.10
Lt Butt, %	0.57	0.6	0.66	0.03
Pigs, n	19	19	19	--
Whole Loin, %	27.2	27.4	27.3	0.30
Trimmed Loin, %	22	22.2	22.1	0.30
Canadian Back, %	7.2	7.5	7.5	0.10
Tenderloin, %	0.98 ^b	1.11 ^a	1.08 ^a	0.03
Sirloin, %	1.49	1.64	1.62	0.06
Pigs, n	19	19	19	--
Untrimmed Belly, %	15.2	15.5	15.6	0.2
Trimmed Belly, %	11.8	12.0	12.1	0.2
Spareribs, %	3.8	3.6	3.7	0.1
PA moisture (Belly), %	52.6	54.4	53.9	10
PA fat (Belly), %	30.7	27.8	28.4	1.3

^{a, b} Within a row, means lacking a common superscript differ ($p < 0.05$)

**THE EFFECT OF SUPPLEMENTING PAYLEAN AND LIPOIC ACID TO A
COMPLETE SWINE FINISHING DIET FOR THE LAST 31 DAYS OF
PRODUCTION ON PORK LONGISSIMUS SHELF LIFE**

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Introduction

Paylean is the commercial name of ractopamine hydrochloride; an orally active growth promotant that effectively shuttles energy away from fat deposition to muscle accretion. Currently, Paylean is approved (United States Food and Drug Administration) for supplementation between 41 to 109 kg of body weight. Past research has indicated that Paylean has limited impact on pork quality (Apple et al., 2004).

Lipoic acid is a naturally occurring compound that is found in all body tissues, with muscle having the highest concentrations (Rentfrow et al., 2004). This antioxidant has been described as universal due to its ability to be both fat and water soluble. In addition, lipoic acid has the ability to regenerate fat soluble vitamins (E and C) which scavenge the free radicals that cause lipid oxidation.

Color is the main quality parameter that consumers use to gauge desirability when buying pork. Vitamin E is an antioxidant that has been shown to increase the shelf life of beef and pork when supplemented to normal finishing diets. It is conceivable that lipoic acid supplementation could accomplish this goal as well. There is limited research regarding Paylean fed with or without an antioxidant on the shelf life of pork.

Objectives

The objectives of this research were to characterize the affect of Paylean and lipoic acid supplemented to a complete swine finishing diet on pork Longissimus shelf life.

Materials and Methods

Twenty-four crossbred barrows (Hampshire x Yorkshire) were ultrasounded, weighed, and blocked into treatments (n = 6/ treatment) stratifying to percent muscle. The pigs were given ab libitum access to water and a corn-soybean meal diet that contained 1.2% total lysine. The animals were penned (14.9 m²) according to treatment with three feeders and two nipple waters per pen. The lysine level was increased to achieve the full benefits of Paylean to maximize muscle growth. The treatment delivery system used 40 g of nursery pig diet that was moistened with water into a dough then supplemented with either 18 mg of Paylean (PAY), 300 mg of lipoic acid (LA), or a combination of both (COMBO). Control pigs received only dough balls. The dough balls were hand fed twice

daily (0800 and 1800h) for 31 d. Prior to treatments, pigs were given 14 d to acclimate to the basal diet and trained to eat dough balls.

Pigs were harvested according to humane harvesting practices at the University of Missouri abattoir on d 32. Following a 24 h chill (4° C), the left Longissimus muscle was removed and one, 2.54 cm chop was removed posterior of the 10th/11th rib interface, placed on a styrofoam tray and over wrapped with a oxygen permeable polyvinyl chloride film (PVC). Chops were placed in a retail display case (2° C) with constant cool white fluorescent lighting (1300 lux). Daily objective color measurements (L*, a*, and b* values) were recorded for 8 d using a Hunter Lab Miniscan XE Plus colorimeter (D65/10°) calibrated to a black and white tile over wrapped with PVC to allow calibration to account for retail tray over wrap. The calibration technique allowed for color data to be recorded without removing the over wrap. A small mark was made on each package to ensure that objective color measurements were taken from the same location each day. Hue angle ($\arctan(b^*/a^*) \times 57.3$) and chroma ($\sqrt{a^{*2} + b^{*2}}$) was calculated from daily a* and b* values.

The Mixed model procedure of SAS (SAS Inst. Inc, Cary, NC) was used to used to analyze the affects of objective color measurement x days of retail display. When the F-test was significant (P < 0.05), treatment means were separated by a Bonferonni adjustment.

Results and Discussion

Paylean, LA, and COMBO had no effect (P = 0.09) on the L* values (lightness) during the eight days of retail display (Table 1). Past research has indicated that Paylean and lipoic acid have no affect on L* values (Berg et al., 2003; Apple et al., 2004).

Table 1. The L* values (lightness) for days of retail display x treatment.

Treatment	0	1	2	3	4	5	6	7	SEM
CON	50.98	52.37	52.17	51.74	51.87	52.09	52.37	52.54	1.333
PAY	51.12	53.37	52.55	52.30	52.61	51.97	53.08	53.45	1.333
LA	51.49	53.72	52.89	52.62	53.14	53.03	53.42	53.56	1.333
COMBO	54.11	56.27	55.36	55.05	55.55	55.71	56.22	57.30	1.333

CON = control; PAY = paylean; LA = lipoic acid; COMBO = paylean + lipoic acid.
The L* value is an indication of lightness; 100 = white; 0 = black.

Although differences (P > 0.0001) were presented on d 0, 1, and 4 for b* values (yellowness), no consistent trend was observed throughout retail display (Table 2).

Table 2. The b* values (yellowness) for days of retail display x treatment.

Treatment	0	1	2	3	4	5	6	7	SEM
CON	14.93 ^d	17.76 ^a	18.31 ^a	18.43 ^a	18.10 ^a	17.52 ^b	17.42 ^b	17.37 ^b	0.332
PAY	14.25 ^e	16.47 ^c	18.08 ^a	18.01 ^a	17.67 ^a	17.23 ^b	17.08 ^b	16.90 ^b	0.332
LA	14.62 ^d	16.51 ^c	17.90 ^a	17.82 ^a	17.39 ^b	17.05 ^b	16.94 ^b	16.88 ^b	0.332
COMBO	15.30 ^d	16.67 ^b	17.92 ^a	17.85 ^a	17.35 ^b	17.03 ^b	17.07 ^b	16.91 ^b	0.332

^{a, b, c, d, e}. Means in a column that do not have common superscripts differ by $P < 0.05$.
 CON = control; PAY = paylean; LA = lipoic acid; COMBO = paylean + lipoic acid.
 The b* value is a measure of yellowness (higher value, more yellow).

After 2 and 4 days of retail display the COMBO and PAY had lower a* values ($P < 0.0001$; Table 3) and higher hue angle values ($P < 0.0001$; Table 4), respectively. Past research has shown that Paylean supplementation lowers a* and b* values (Apple et al., 2004). This is in agreement with the current research. The hue angle is a calculation of true red, where 0 equals true red. Although there is limited research on the affects of Paylean on hue angle, it is conceivable that lower a* and b* values would produce higher hue angle values. Berg et al. (2003) showed that lipoic acid supplementation has no affect on a* and b* values.

Table 3. The a* values (redness) for days of retail display x treatment.

Treatment	0	1	2	3	4	5	6	7	SEM
CON	9.170 ^b	12.48 ^a	12.28 ^a	11.92 ^a	10.91 ^a	9.810 ^b	9.450 ^b	8.770 ^b	0.798
PAY	7.870 ^c	11.32 ^a	11.66 ^a	10.98 ^a	9.940 ^b	9.070 ^b	8.640 ^b	7.850 ^c	0.798
LA	8.690 ^b	11.47 ^a	12.14 ^a	11.63 ^a	10.61 ^a	9.920 ^b	9.500 ^b	8.870 ^b	0.798
COMBO	10.14 ^b	10.58 ^a	10.33 ^b	9.630 ^b	8.310 ^b	7.290 ^c	5.710 ^c	5.920 ^c	0.798

^{a, b, c, d, e}. Means in a column that do not have common superscripts differ by $P < 0.05$.
 CON = control; PAY = paylean; LA = lipoic acid; COMBO = paylean + lipoic acid
 The a* value is a measure of redness (higher value, more red).

Table 4. The hue angle (true red) calculation for days of retail display x treatment.

Treatment	0	1	2	3	4	5	6	7	SEM
CON	58.36 ^c	54.97 ^c	56.24 ^c	57.29 ^c	59.13 ^c	60.98 ^b	61.72 ^b	63.40 ^b	1.997
PAY	61.10 ^b	55.53 ^c	57.22 ^c	58.66 ^c	60.68 ^b	62.26 ^b	63.17 ^b	65.05 ^a	1.997
LA	59.38 ^b	55.23 ^c	55.96 ^c	57.02 ^c	58.76 ^c	59.99 ^b	60.94 ^b	62.44 ^b	1.997
COMBO	57.49 ^c	57.74 ^c	60.44 ^b	62.09 ^b	64.87 ^a	67.29 ^a	67.80 ^a	71.04 ^a	1.997

^{a, b, c, d, e}. Means in a column that do not have common superscripts differ by $P < 0.05$.
 CON = control; PAY = paylean; LA = lipoic acid; COMBO = paylean + lipoic acid.
 The hue angle calculation is a measure of true red (the lower the value, the closer to true red).

Chroma is a measurement of how vivid or saturated a color appears, with a higher value indicating a more saturated color. After 5 days of retail display, the COMBO treatment had lower chroma values ($P < 0.0001$) and at day 7 the COMBO and PAY had lower values ($P < 0.0001$) than CON and LA. Apple et al. (2004) indicated that Paylean had no affect on chroma calculations. There is limited research on the effect of lipoic acid supplementation on the pork Longissimus. Rentfrow et al. (2004) showed that lipoic acid supplemented to a beef finishing ration has no affect on chroma calculations

Table 4. The chroma (color vividness) calculations for days of retail display x treatment.

Treatment	0	1	2	3	4	5	6	7	SEM
CON	17.53 ^d	21.72 ^a	22.09 ^a	22.01 ^a	21.22 ^a	20.18 ^b	19.93 ^b	19.59 ^b	0.535
PAY	16.29 ^e	19.99 ^b	21.52 ^a	21.10 ^a	20.28 ^b	19.49 ^b	19.16 ^b	18.65 ^c	0.535
LA	17.03 ^d	20.10 ^b	21.65 ^a	21.30 ^a	20.41 ^b	19.77 ^b	19.47 ^b	19.12 ^b	0.535
COMBO	18.59 ^c	19.78 ^b	20.77 ^a	20.39 ^b	19.36 ^b	18.66 ^c	18.63 ^c	18.06 ^c	0.535

^{a, b, c, d, e.} Means in a column that do not have common superscripts differ by $P < 0.05$.

CON = control; PAY = paylean; LA = lipoic acid; COMBO = paylean + lipoic acid.

The chroma calculation is a measure of how vivid a color appears (higher the value, the more vivid appearance).

Conclusion

These data indicate that the supplementation of Paylean fed with or without the antioxidant lipoic acid produced less desirable a*, hue angle, and chroma values after 7 days of retail display.

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**EFFECTS OF RACTOPAMINE HCL (OPTAFLEXX®) ON SENSORY
PROPERTIES
OF BEEF**

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Introduction

Consumer demand for a consistent and acceptable eating experience has led the beef industry to invest significant resources to provide consumers with a predictable, high quality product from a sensory and palatability perspective. Considerable efforts have been made to measure and quantify changes in meat sensory properties and palatability; specifically, flavor, juiciness and tenderness. The importance of these quality and sensory attributes was confirmed by results of the 1991, 1995 and 2000 National Beef Quality Audits and the 1990 and 1999 National Beef Tenderness Surveys.

The ultimate determination of consumer satisfaction is the consumer's eating experience. Consumers consider flavor, juiciness and tenderness as they evaluate beef palatability, with tenderness as one of the most important attributes. Researchers have spent years trying to identify a reliable and objective tool to use under controlled conditions to predict and evaluate meat tenderness. To date, no mechanical process has been identified, that can be used commercially, which consistently indicates a specific degree of tenderness.

Since the measurement of consumer satisfaction and eating experience is a personal preference and a decision influenced by a complex interaction of numerous factors, the test of consumer preferences is difficult to replicate in the laboratory. Many times definitive consumer preferences are difficult to ascertain even through the use of large uncontrolled consumer panel evaluations. As an alternative, trained sensory panels and Warner-Bratzler shear force tests are typically used to provide an indication of meat palatability when the use of large uncontrolled consumer panel evaluations is impractical.

Objectives

The objective was to determine the effects of feeding various levels of ractopamine HCl, (Optaflexx®) on beef sensory properties and Warner-Bratzler shear force in boneless strip loin steaks.

Methodology

Sample Preparation and Attributes Measured

Boneless strip loin steaks were collected from carcasses from control and treated animals. Optaflexx treatments were 0.0, 9.1, 18.2 and 27.3 g/ton (100% DM), which provided approximately 0, 100, 200 and 300 mg/hd/d, respectively. For additional information on trial procedures for assessing live performance and carcass variables, refer to Elanco Animal Health Optaflexx FOI, NADA 141-221.

Trained sensory panel evaluation and Warner-Bratzler shear force measurement of beef strip loin steaks (*longissimus* muscle) were conducted at Iowa State University, Department of Food Science and Human Nutrition. Samples were obtained from a representative group of carcasses (n = 90 per treatment) from five steer and four heifer trials. Evaluations were conducted on 2.54 cm thick steaks cooked to a medium degree of doneness (71°C). Additional parameters measured included raw weight, cooked weight, cooking loss, and ultimate muscle pH.

Preparation and evaluation of samples were conducted with procedures described in the *Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat* (American Meat Science Association, 1995). Training of sensory evaluation panelists enabled the panelists to detect incremental differences in attributes more discreetly than the average consumer. Trained panelists (n=10) used a 150 mm line scale to critically evaluate the sensory traits of juiciness, initial tenderness, sustained tenderness, beef flavor, and off flavor.

Statistics

Data from all trials were pooled and statistically analyzed using a mixed model analysis (PROC MIXED, SAS). Trial heterogeneity was tested using a residual and random component. Depending on the results of these tests, either a weighted mixed model analysis was conducted for traits showing trial heterogeneity or an un-weighted mixed model analysis was conducted for all traits without trial heterogeneity.

Results & Discussion

Cooking Loss and Ultimate pH of Fresh Muscle

Results for cooking loss and ultimate pH of fresh muscle are shown in Table 1. No differences ($P > 0.05$) were observed for cooking loss percentage and ultimate pH between control and treated steaks.

Trained Sensory Panel Traits

The effects of ractopamine (Optaflexx®) on sensory variables are shown in Table 1. Using a 150 mm line scale, panelists were trained to evaluate samples for initial tenderness, juiciness and sustained tenderness, as well as beef flavor. Values in the range

of approximately 70 mm to 85 mm were considered to be only slightly juicy, slightly tender and having slight beef flavor. Samples exhibiting the characteristics of being moderately juicy, moderately tender, and having moderate beef flavor were judged to fall in the range of approximately 85 mm to 120 mm. Values above 120 mm to the maximum of 150 mm were considered to be very juicy, very tender and having intense beef flavor.

Values for all sensory traits of all treatments were within the range of 89 mm to 112 mm, considered to be moderately juicy, moderately tender and moderate in beef flavor and were judged to be in the mid range of acceptability for each trait.

Panelists were trained to evaluate off flavors and identify samples without off flavors with a zero (0 = none) and intense off flavors with values as high as 150 (intense). A minimal number of samples exhibited off flavors resulting in very low scores (<1) for off flavor.

Understanding the meaning of the results of the trained sensory panels and the relationship to the preferences of the consumer can be somewhat subjective. In the opinion of the principal investigator in this study, a shift of 10 to 15% (15 mm to 22 mm change on the line scale) would be required for the consumer to detect a difference in a sensory trait. In this study, no sensory trait was changed by more than 7 mm compared to controls. When evaluating beef steaks, highly trained sensory panelists are conditioned to distinguish between minute differences in sensory traits that may be statistically different but have no practical meaning to the consumer.

No differences ($P > 0.05$) were detected for juiciness, flavor and off flavor for any of the Optaflexx treatments. No differences ($P > 0.38$) were detected for initial and sustained tenderness at the 100 or 200 mg/hd/d Optaflexx feeding level. Initial and sustained tenderness values were lower at the 300 mg/hd/d level ($P < 0.05$) compared to controls. However, these changes (5.7 to 6.6 mm, respectively) were below what would be considered to be the threshold of consumer detection (15 to 22 mm).

Warner-Bratzler Shear Force

Warner-Bratzler shear force results for this study are presented in Table 1. For all treatments values were 3.95 kg or less, considered acceptably tender and well within the normal variation observed in today's meat industry. No differences were observed ($P > 0.45$) when comparing the controls to the 100 mg/hd/d and 200 mg/hd/d levels. The Warner-Bratzler shear force for the 300 mg/hd/d level was increased ($P < 0.05$) compared to controls.

The results of the Warner-Bratzler shear force measurements on *longissimus* muscle were well within the normal range of shear forces observed in the meat industry as reported in the scientific literature and considered acceptably tender for cooked strip loin steaks. Small increases in Warner-Bratzler shear force values may be expected in longissimus dorsi samples from cattle treated with ractopamine, since the treated cattle had a larger ribeye area, due to hypertrophy similar to pigs (Aalhus et al., 1992; Solomon et al., 1991). Therefore, size of the muscle fiber is increased most likely by increasing the number of myofibrils or diameter of myofibers. A logical conclusion is that muscles with slightly larger muscle fibers may require slightly more shear force to shear the larger myofibrils and muscle fibers. Increased myofibrils would potentially have an impact upon both subjective and objective measurements.

Star Probe Compression Force (STRPB)

Star probe compression force test results for this study are presented in Table 1. No differences ($P > 0.12$) were detected in STRPB for the 10 ppm and 20 ppm concentrations of ractopamine compared to controls. STRPB was increased ($P = 0.0007$) for the 30 ppm concentration of ractopamine compared to controls. Increased star probe values may be expected in longissimus dorsi samples from cattle treated with ractopamine since the treated cattle had a larger ribeye area, most likely due to hypertrophy similar to pigs (Aalhus et al., 1992; Solomon et al., 1991). Therefore, size of the muscle fiber is increased, containing more protein, which in turn increases the diameter of myofibrils. Muscles with slightly larger muscle fibers and denser myofibrils may inevitably have slightly higher STRPB compression force values.

Interpretation of Warner-Bratzler Shear Force Tests, Threshold Values for Shear Force, Sensory Panel Results and Classifications of Tenderness

Understanding and interpreting the meaning of the Warner-Bratzler shear force, trained sensory panels and the relationships of each method to the preferences of the consumer is not an exact science and can be somewhat subjective. To date, no mechanical process has been identified that consistently indicates degree of tenderness or exactly predicts the preferences of consumers.

While investigating the relationship between Warner-Bratzler shear force and trained sensory panels evaluation of tenderness, Bourne (1982) reported correlation coefficients (r) of -0.001 to -0.942 with many researchers reporting correlations in the range of $r = -0.5$ to -0.7 . This means that about 25% to 50% of the variability in trained sensory panel tenderness ratings can be explained by the use of Warner-Bratzler shear force, with the remainder attributable to other factors.

Since the review by Bourne (1982), numerous research reports (McKeith et al., 1985; Harris and Shorthose, 1988; Shackelford et al., 1991; Miller et al., 1995; Shackelford et al., 1997; Shackelford et al., 1999; Wheeler et al. 1999; Miller et al. 2001) have concurred with the review, citing many factors that impact meat juiciness, flavor and tenderness and ultimately consumer satisfaction. Published research reports emphasize that factors such as cooking method, gender, chronological and physiological age, connective tissue, stress, meat color, feed ingredients, implant program, post mortem handling of carcasses or meat, electrical stimulation and mechanical stimulation all have considerable effects on the tenderness, flavor and juiciness of meat. Of the factors affecting meat palatability, cooking method and degree of doneness has been identified as major contributors leading to a less than desirable eating experience (Cross et al., 1976; Wheeler et al., 1999).

Efforts by researchers (Miller et al, 1995; Huffman et al, 1996, Boleman et al. 1997; Shackelford et al. 1991, 1997, 1999; Wheeler et al., 1999; Bruns et al. 2000; and Miller et al. 2001) have attempted to measure and describe definitive thresholds or breakpoints in meat tenderness as measured by Warner-Bratzler shear force tests in order to classify meat for tenderness. Instead of trying to classify meat as either tender or tough, their approach groups the tenderness assessment of cooked steaks into 3 primary categories: **1)** tender, **2)** acceptable or intermediate and **3)** unacceptable or tough.

Table 2 summarizes the various threshold values for Warner-Bratzler shear force reported by the various authors.

Use of a three category classification tends to segregate meat into the following three groupings:

1. **Tender** - with a shear force in the range of 3.6 to 4.1 kg or less. Typically the *psoas* muscle from the loin otherwise known as the tenderloin or filet mignon and the chuck muscle called the *infraspinatus*, more recently referred to as the flat iron steak.
2. **Acceptable or Intermediate** – with a shear force ranging from 3.6 to 4.1 kg up to 4.5 to 5.9 kg (differing depending on individual researchers and reports). Typically muscles such as the *longissimus* and *gluteus* of the strip loin and top sirloin, respectively.
3. **Unacceptable or Tough** – with a shear force of approximately 5.4 to 5.9 kg or greater. Typically brisket or roasts from the chuck and round requiring moist heat cooking methods.

Interpretation of the Practical Significance of Changes in Warner-Bratzler Shear Force

As one might expect, a practical or meaningful change in Warner-Bratzler shear force that might be detectable to the consumer is an area of debate. Miller et al. (1995) found that a change in shear force of about 1 kg was necessary for detection by consumers in restaurants and about 0.5 kg being noticed by the consumer when eating at home. Huffman et al. (1996) found that a shear force change of about 1 kg or more is necessary in order for the consumer to find a noticeable difference between steaks.

Practical Use of Information Obtained from Trained Sensory Panels and Warner-Bratzler Shear Force Tests Conducted under a Standard Protocol and Common Institution.

Wheeler et al. (1997) reported a high repeatability of about 85% between the results of a trained sensory panel and Warner-Bratzler shear force and considered both methods reliable as long as both are conducted at the same institution. In such cases, panelists are trained to differentiate between samples with a known shear force obtained from complimentary samples cooked by the same methods. Wheeler et al. (1997) recommended use of a standard protocol to achieve meaningful results from sensory evaluation tests.

Following recommendations of experts in the areas of meat science and sensory evaluation, steaks from cattle fed Optaflexx were evaluated for sensory traits and Warner-Bratzler shear force using a standard protocol at a single institution.

Understanding the relationships between the meat sensory evaluation results obtained from trained sensory panel and Warner-Bratzler shear force has proven to be a challenge and area of debate for researchers over many years. There are many references that demonstrate very low to very high relationships between trained sensory panel results and Warner-Bratzler shear force tests. The interpretation of the results from Warner-Bratzler shear force and trained sensory panel test results is an area filled with many differing opinions and opportunity for discussion or debate.

Conclusions

After a thorough review of the sensory data collected from strip loin steaks obtained from cattle fed ractopamine, (Optaflexx®), it was concluded by the US FDA CVM, that no differences in palatability as defined by juiciness, flavor and tenderness would be detected by the consumer.

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Tables and Figures

Table 1. Effects of Optaflexx on Sensory Variables and Warner-Bratzler Shear of Strip Loin Steaks^a

Variables	Optaflexx, mg/hd/d				SE
	0	100	200	300	
No. Samples	90	90	90	90	
Cooking Loss, % ^b	21.5	21.5	21.0	21.8	0.13
Ultimate pH of Fresh Muscle	5.56	5.54	5.56	5.56	0.01
Juiciness ^c	104.6	104.5	106.0	103.3	1.6
Initial Tenderness ^d	111.7	110.7	111.5	106.0*	1.8
Sustained Tenderness ^d	101.8	100.5	100.3	95.2*	1.8
Flavor ^e	90.3	89.0	90.5	88.7	1.7
Off Flavor ^e	0.252	0.222	0.156	0.157	0.098
Warner-Bratzler shear force, kg	3.54	3.49	3.62	3.95*	0.16
Star probe compression force, kg	5.03	5.17	5.25	5.56*	0.21

^a Least squares means

^b Cooking Loss = (raw weight – cooked weight) / raw weight) * 100

^c Juiciness evaluation: 0 = not juicy, 150 = very juicy

^d Tenderness evaluation: 0 = not tender , 150 = very tender

^e Flavor / Off Flavor evaluation: 0 = none, 150 = intense

* P ≤ .05 compared to controls

Table 2. Independent Research Reports of Threshold Values for Warner-Bratzler Shear Force of Primarily Cooked Strip Loin Steaks and Classification of Tenderness^a

Reference / Author	Year	Tenderness Classification		
		Tender	Acceptable or Intermediate	Unacceptable or Tough
		Threshold kg of Warner-Bratzler Shear Force		
Shackelford et al.	1991	<3.9	≤4.6	>4.6
Miller et al. ^b	1995		<4.3	
Huffman et al.	1996	<4.1	≤4.5	>4.5
Boleman et al.	1997	<3.6	<5.4	>5.9
Shackelford et al.	1997		≤6.0	>6.0
Shackelford et al.	1999		≤5.0	>5.0
Wheeler et al.	1999		≤5.0	>5.0
Bruns et al.	2000	<3.5	3.6-4.9	>5.0
Miller et al.	2001	<3.0	3.0-4.6	>4.6

^a threshold values are from steaks cooked to a medium degree of doneness (approximately 71⁰ C)

^b a mixture of steaks from the chuck, rib, loin and round were utilized in this study

THE EFFECTS OF WHOLE COTTONSEED OR FUZZPELLET® (PELLETED WHOLE COTTONSEED) ON RETAIL SHELF LIFE AND WARNER-BRATZLER SHEAR FORCE OF BEEF STRIPLOIN STEAKS

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Key Words: Beef, TBA, lean color, tenderness

Introduction

When new meat products are developed or new practices are explored that could ultimately effect meat products, it is imperative that research scientist address potential problems that could affect the value and acceptability of the consumer's product. Because of the need to find balance between case longevity and meat palatability, a thorough and scientific approach is needed that specifically addresses the objectives of the cooperators. Thus, this project was designed to evaluate the effect of whole cottonseed or FuzZpellet® on retail shelf life and tenderness of beef steaks.

Objectives

To determine the effect feeding FuzZpellet®, Vitamin E and whole cottonseed on retail shelf life, oxidative rancidity and shear force of beef steaks.

Methodology

Experimental Diets. Treatment diets were: **STD** = standard 90% concentrate steam flaked corn based finishing diet; **WSC** = whole cottonseed, the finishing diet with all the cottonseed meal and added fat, and a portion of steam-flaked corn, replaced with 15% whole cottonseed; and **FUZZ** = a finishing diet similar to the WCS, except that FuzZpellet, a pelleted whole cottonseed product, was used in place of whole cottonseed (Buckeye Technologies, Inc., Memphis, TN). All diets were formulated to be isonitrogenous and to provide equal percentages of added fat and neutral detergent fiber (NDF) from "roughage" (NDF from alfalfa and cottonseed hulls for STD and NDF from whole cottonseed for WCS and FUZZ). Vitamins, minerals, Rumensin (30 g/ton DM basis), and Tylan (10 g/ton DM basis) were provided by a premix included at 2.5% of the dietary DM. The STD diet contained additional vitamin E to provide approximately 900 to 1,000 IU/steer daily, which was provided in the form of a ground corn-based premix (formulated at 0.25% of the dietary DM to supply an additional 93.6 IU/kg of DM) (Galyean et al., 2004).

Meat Collection. The cattle were transported to a harvesting facility (Excel Corporation, Plainview, TX). Trained personnel from Texas Tech University individually

identified each of the carcasses. Four cattle from each of the 30 pens were selected to represent each treatment and feeding block totaling 120 carcasses. However, one carcass tag was lost during carcass chilling and only 119 of the carcasses maintained identification. Beef loin, strip loin, boneless subprimals were obtained from one side of each carcass, vacuum packaged, and transported to the Texas Tech Meat Science Laboratory, Lubbock. Upon arrival to Texas Tech Meat Science Laboratory, the strip loins were stored at 2 °C for a 14 day period. Vacuum packages were checked daily for leaks and repackaged, as needed.

Strip loin Processing. On day 14, five 2.54 cm steaks were cut from each strip loin and were randomly assigned to either proximate analysis, Warner-Bratzler shear analysis, or 0, 3, and 5 day retail display periods. Warner-Bratzler shear steaks and day 0 steaks were individually vacuum packaged and stored at -10 °C until further analysis. Day 3 and day 5 steaks were placed on black Styrofoam trays and covered with polyvinyl chloride film (PVC). The steaks were then placed in a coffin-style retail case for their respective days. All steaks were subjected to 24 hour exposure of retail display lighting, and temperatures for each case were documented.

Warner-Bratzler Shear Force. The strip loin steaks for Warner-Bratzler Shear Force analysis (WBSF) were thawed for 18-24 hours at 2 °C. The internal temperature was obtained for each steak before and after cooking in the geometric center of the steak. Steaks were prepared on a Magi-Grill belt grill (MagiKitchen, Inc., Quakertown, PA) that was pre-heated for 15 minutes. Steaks were cooked to an internal temperature of 70 °C (\pm 2 °C). Cooked steaks were covered with Saran® film and allowed to cool for 24 hours at 2 °C before coring. Six, 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation and sheared once, perpendicular to the muscle fibers, on a United Testing Machine (United Calibration Corporation, Huntington Beach, California). Degree of doneness was recorded for each steak according to Beef Steak Color Guide (AMSA, 1995).

Color Evaluation. Strip loin steaks allocated for 5 d retail display were evaluated daily by a trained panel, consisting of a least six members, for lean color, fat color, percent discoloration, and overall acceptability according to AMSA (1991) color guidelines. Also, Commission Internationale de l'Eclairage (CIE) L* (muscle lightness), a* (muscle redness), and b* (muscle yellowness) values were determined daily, through the overwrap, for all steaks allocated for the 5 d retail display period from two random readings per steak using a Minolta Spectrophotometer (Minolta Camera Co., LTD, Osaka, Japan). L*, a*, b* values were also obtained approximately 1.5 hours after the carcasses went through the bloom chain and were graded at Excel- Plainview on the day of meat collection.

Oxidative Rancidity. Oxidative rancidity was measured using Thiobarbituric Acid (TBA) analysis according to Assay of lipid oxidation in muscle samples (Buege and Aust, 1978, Methods in Enzymology 52:302). Samples were read at 531 nm against a Tetraethoxypropane (TEP) standard curve containing all the reagents (less the sample) on a spectrophotometer (Beckman Coulter Inc., Fullerton, CA). Standard curves were constructed each day that TBA analysis was performed. The standard curve had to have an R² value of 0.975 or greater before samples were run. All samples were run in

duplicate. Coefficients of variation (CV) values were calculated for the duplicates and were considered acceptable when calculated at $\leq 10.00\%$.

Statistical Analyses. Thiobarbituric Acid analysis (TBA) and color evaluation were analyzed as a completely randomized design with repeated measures using PROC MIXED (SAS Inst. Inc., Cary, NC). Animal was the experimental unit, Steak ID was the repeated measure, and the residual was used to test for treatment effect. For TBA analysis, percent fat was included in the model as a covariate. Warner-Bratzler Shear Force data were analyzed as a completely randomized design using PROC GLM (SAS Inst. Inc., Cary, NC). Animal was the experimental unit and the residual was used to test for treatment effect. Least squares means were used to separate means when significant F-test for treatment was noticed.

Results & Discussion

Warner-Bratzler Shear Force. WBSF values are presented in Table 1. Dietary treatment had no effect on WBSF ($P = 0.2925$). In agreement, Brooks and Krehbiel (2001) reported that dietary treatment, which included FuzZpellet, had no effect on the WBSF of steaks tested.

Oxidative Rancidity. Dietary treatment did not effect ($P = 0.2133$) TBA values (Table 4). In contrast, Brooks and Krehbiel (2001) found that control steaks had significantly higher TBA values than steaks from dietary treatments that included FuzZpellet. However, steaks from STD treatment tended ($P = 0.0798$) to have lower TBA values than WCS treatment. Data analysis showed that day of retail display had an effect on TBA values ($P < 0.001$). An increase in TBA values was observed between day 0 and 3, as well as day 0 and 5 ($P < 0.001$). However, there was no difference ($P = 0.3513$) between day 3 and day 5 TBA values.

Color Evaluation. Data analysis revealed no treatment x day interaction for subjective color scores ($P = 0.9987$). Scores for lean color, fat color, percentage discoloration, and overall acceptability were analyzed for dietary treatment differences and results are presented in Table 3. Dietary treatment had an effect ($P = 0.0001$) on all color scores. FUZZ treatment resulted in higher ($P < 0.05$) lean color scores when compared to the STD and WCS treatments. Moreover, STD treatment resulted in higher ($P = 0.0206$) lean color scores than the WCS treatment. A significant dietary treatment effect was observed among fat color scores with FUZZ and STD treatments producing higher ($P < 0.05$) fat color scores when compared to the WCS treatment, while no difference ($P = 0.8395$) existed between FUZZ and STD treatments for fat color. Results of analysis of percentage discoloration scores were similar to fat color scores, showing that FUZZ and STD scores were similar, and resulted in higher ($P < 0.05$) percentage discoloration scores than WCS treatment. Finally, dietary treatment effects on overall acceptability scores showed that FUZZ and STD treatments yielded higher ($P < 0.05$) overall acceptability scores when compared to the WCS treatment, whereas no difference in overall acceptability existed between FUZZ and STD treatments ($P = 0.2019$). As expected, day of retail display had a significant effect on subjective color scores ($P < 0.001$; data not presented) showing that all scores declined as day of display increased. Because this is a natural digression and there was no dietary treatment x day interaction, data were not reported.

Color was evaluated objectively with L*, a*, b*, Chroma, and hue values (Table 2). Dietary treatment showed to have and affect on L* values (P = 0.0044) with FUZZ treatment resulting in higher L* values in comparison with STD (P = 0.0482) and WCS (P = 0.0011) treatments. However, no differences existed for L* between STD and WCS treatments (P = 0.1853). As a result of dietary treatment, STD a* values were higher (indicating more red and less green) than FUZZ (P = 0.0399) and WCS (P = 0.0184) treatments. However, no difference (P = 0.7523) existed between FUZZ and WCS treatments for a* values. Furthermore, the FUZZ treatment resulted in higher b* values (indicative of more blue and less yellow) than WCS treatment (P = 0.0101). Moreover, STD treatment tended (P = 0.0606) to have higher b* values than WCS treatment, while no difference existed between FUZZ and STD treatments for b* values (P = 0.4823). Chroma and hue values were calculated from the L*, a*, b* values and analyzed. Dietary treatment had no significant effect on chroma and hue values.

Conclusions

The results of the current study imply that FuzZpellet may replace whole cottonseed in finishing diets of cattle in order to increase consumer perception of overall acceptability of steak color over extended periods of time in a retail display case. FuzZpellet also exhibits the ability to produce more desirable lean colors and higher L* values than standard and whole cottonseed diets. Even though it is also implied that there is little significant difference between the feeding of a standard finishing diet and a diet containing FuzZpellet on the effects of retail case life or consumer perception of acceptability, it should be noted that the standard diet was supplemented with a substantial amount of Vitamin E. It may be implied that the additional Vitamin E accounted for the lack of difference between the two diets and that FuzZpellet could prove to be a more cost effective alternative to the direct addition of Vitamin E to cattle finishing diets.

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Tables and Figures

Table 1. Least squares means of Warner-Bratzler shear force (kg) values by treatment

Treatment ^a					
FUZZ	STD	WCS	SEM ^b	n ^c	P-value
2.39	2.30	2.42	0.06	119	0.2925

^aTreatments were: FUZZ = finishing diet with 15% of the dietary dry matter (DM) as FuzZpellet cottonseed replacing all the roughage, added fat, and natural protein in the diet; STD = Standard 90% concentrate finishing diet; WCS = finishing diet with 15% of the dietary DM as whole cottonseed replacing all the roughage, added fat, and natural protein in the diet.

^bSEM = Standard error of mean.

^cn = Number of observations.

Table 2. Least squares means of L* a* b*, Chroma, and hue values by treatment

Treatment ^a						
Color Value	FUZZ	STD	WCS	SEM ^b	n ^c	P-value
L*	46.37 ^x	45.96 ^y	45.69 ^y	0.14	119	0.0044
a*	13.86 ^y	14.14 ^x	13.82 ^y	0.10	119	0.0379
b*	3.20 ^x	3.09 ^x	2.80 ^y	0.03	119	0.0288
Chroma	14.36	14.59	14.26	0.05	119	0.0522
hue	37.35	39.56	40.61	4.45	119	0.8689

^aTreatments were: FUZZ = finishing diet with 15% of the dietary dry matter (DM) as FuzZpellet cottonseed replacing all the roughage, added fat, and natural protein in the diet; STD = Standard 90% concentrate finishing diet; WCS = finishing diet with 15% of the dietary DM as whole cottonseed replacing all the roughage, added fat, and natural protein in the diet.

^bSEM = Standard error of mean.

^cn = Number of observations.

^{x,y}Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3. Least squares means of subjective color by treatment as evaluated by trained sensory panel

Trait	Treatment ^a			SEM ^b	n ^c	P-value
	FUZZ	STD	WCS			
Lean Color	5.31 ^x	5.12 ^y	4.90 ^z	0.07	119	0.0001
Fat Color	6.01 ^x	6.02 ^x	5.78 ^y	0.05	119	0.0004
Percentage Discoloration	7.23 ^x	7.19 ^x	7.02 ^y	0.04	119	0.0016
Overall Acceptability	5.13 ^x	5.01 ^x	4.66 ^y	0.07	119	<0.001

^aTreatments were: FUZZ = finishing diet with 15% of the dietary dry matter (DM) as FuzZpellet cottonseed replacing all the roughage, added fat, and natural protein in the diet; STD = Standard 90% concentrate finishing diet; WCS = finishing diet with 15% of the dietary DM as whole cottonseed replacing all the roughage, added fat, and natural protein in the diet.

^bSEM = Standard error of mean.

^cn = Number of observations.

^{x,y,z} Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 4. Least squares means of Thiobarbituric Acid values (TBA) by treatment

Treatment ^a					
FUZZ	STD	WCS	SEM ^b	n ^c	P-value
0.88	0.84	0.92	0.03	119	0.2133

^aTreatments were: FUZZ = finishing diet with 15% of the dietary dry matter (DM) as FuzZpellet cottonseed replacing all the roughage, added fat, and natural protein in the diet; STD = Standard 90% concentrate finishing diet; WCS = finishing diet with 15% of the dietary DM as whole cottonseed replacing all the roughage, added fat, and natural protein in the diet.

^bSEM = Standard error of mean.

^cn = Number of observations.

GROWTH IMPLANTS' EFFECT ON TENDERNESS AND PROTEIN DEGRADATION

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Key Words: growth implants, beef, protein degradation

Introduction

Growth implants are routinely used to improve efficiency of meat production by improving red meat yield. Increased feed efficiency and increased *longissimus* muscle area have all been reported with the use of hormonal implants. One problem with this is that there is also a reduction in marbling and therefore fewer numbers of animals grading Choice (Roeber et al., 2000; Platter et al., 2003). Furthermore, these researchers both reported reduced sensory preference for steaks from animals that had been implanted when compared to steaks from animals that had never been implanted.

To accomplish muscle growth, protein accretion must exceed protein breakdown. The enzyme system that is thought to be responsible for controlling protein accretion and breakdown is the calpain system (Boehm et al., 2000). The two parts of the calpain system, μ - and m-calpain, breakdown proteins while the inhibitor, calpastatin, blocks calpain actions. Endogenous growth hormones including testosterone increase the activity of calpastatin and thus increase muscle growth. Uses of exogenous growth hormones such as testosterone in implants increase growth and therefore affect the calpastatin activity. Implant use also increases the proliferation and fusion of satellite cells to increase DNA for protein synthesis (Johnson et al., 1998).

Tenderness of beef has been associated with protein degradation postmortem (Paterson & Parrish, 1986; Huff-Lonergan, 1996). Postmortem protein degradation starts with the calpain system breaking down the cytoskeletal proteins (titin, nebulin, desmin) along with troponin T (Boehm et al., 1998). Some researchers have tried to relate calpain activity to tenderness but have been unsuccessful. The activity of calpastatin at 24 hr, however, has been shown to explain roughly 40% of the tenderness variation in steaks (Whipple et al., 1990; Shackelford et al., 1994).

Recently concern has been raised about aggressive implant strategies reducing tenderness of steaks from treated steers. Roeber and co-workers (2000) reported that steaks from British steers treated with an estradiol benzoate (24 mg) and trenbolone acetate (120 mg) implant followed by no implant had significantly higher Warner-Bratzler shear values than steaks from steers that were never implanted. Furthermore, consumer panelists found steaks from implanted steers were less tender and juicy than steaks from non-implanted steers. Platter et al. (2003) reported that the closer the implant strategy was applied to slaughter that the shear values were more likely to be affected (implanting during the backgrounding phase significantly increased WBS values when

compared to non-implanted animals) however this change was not seen in consumer satisfaction.

Many researchers have reported reduced marbling and increased ribeye area with the use of implants on British based cattle. Platter et al., (2003) utilized steers with various genetic backgrounds, but did not analyze the project to determine if there was a compounding effect on tenderness when implants were administered to animals with a genetic propensity for greater growth. Late maturing, heavily muscled animals already have a larger rate of protein accretion with reduced degradation than earlier maturing light muscled animals. The addition of growth implants may increase the rate of growth and may compound any tenderness problems that may be created by growth implants.

Objectives

The objective of this experiment was to evaluate the effect of growth implants on the rate, extent, and manner of postmortem degradation of protein as well as tenderness of beef cattle.

Methodology

Steers of British (n=14) and Continental (n=17) breed descent were assigned to either implant or nonimplant treatment. A combination implant containing estradiol benzoate (24 mg) and trenbolone acetate (120 mg) was used. After steers and heifers had been on feed for 120 days, they were shipped to a commercial processing facility (8 h travel with 12 h rest) and harvested following normal industry procedures. After 24 h at 4°C loin sections (7.62 cm) were removed from each carcass. The loin sections were cut into one 3.3 cm steak for tenderness analysis. Small (10g) samples were vacuum packaged and aged for 7, 14 and 21 days for evaluation of protein degradation.

Steaks were thawed at 4°C for 24 hours. Each steak was weighed before and after cooking to determine cook loss. Eight to ten samples (1.27 x 1.27 x 2.54 cm) for shear force evaluation were removed from each steak parallel to the fiber direction. Samples were sheared once perpendicular to the fiber direction with a TMS 30 Food Texturometer fitted with a Warner-Bratzler shear attachment. The average of the samples sheared was used for statistical analysis.

Purified myofibrils were prepared by the procedure of Boles et al., (1992). After purification, myofibrils were washed twice by re-suspension in 10 volumes (wt/vol; of the original ground muscle) of 5 mM Tris-HCl, pH 8.0, and centrifuged at 3,050 x g for 10 min at 4°C (Wang, 1982). The resulting pellet was then re-suspended in four volumes (wt/vol) of 5 mM Tris-HCl, pH 8.0, and protein content was determined by a modified biuret procedure conducted in the presence of Tris (Robson et al., 1968). For preparation of samples for SDS-PAGE analysis, 1 mL of the protein solution (5.2 mg/mL) was added to 0.2 mL of tracking dye solution (5% [wt/vol SDS, 50% wt/vol sucrose, .05% [wt/vol bromophenol blue, and 50 mM 2-N-morpholinoethanesulfonic acid, pH 6.5) and 0.1 mL of 2-mercaptoethanol and heated at 50°C for 20 min (Paterson and Parrish, 1986).

The SDS-PAGE was performed according to the procedure of Laemmli (1970) with modifications to accommodate separation of proteins with widely different molecular weights. The amount of protein loaded onto each lane of a slab gel (mm) was 15µg. Gels

containing a 4% (wt/vol) acrylamide stacking gel over a 10% (wt/vol) acrylamide separating gel were prepared from a stock solution of 30% (wt/vol) acrylamide (37: 1 ; acrylamide:N,N'-bis-methylene acrylamide), 0.375 M Tris-Ha, pH 8.0, 0.1% (wt/vol) SDS, and 2 mM EDTA. Changes in large-molecular- mass proteins such as titin were monitored by using a 5% (wt/vol) acrylamide (100:1 acrylamide N,N'-bis-methylene acrylamide) separating gel containing 0.375 M Tris-HCl, pH 8.0, 0.1% (wt/vol) SDS, and 2 mM EDTA, without a stacking gel. Gels were run at 35 mA on BioRad Mini Protean gels (BioRad, San Francisco, CA). Gels were stained overnight in 0.1% (wt/vol) Coomassie brilliant blue R-250 (Sigma Chemical), 10% (vol/vol) glacial acetic acid, 50% (vol/vol) methanol and then were destained in two changes of the same solution, excluding the Coomassie brilliant blue. A molecular weight protein standard (Sigma Chemical) containing rabbit myosin heavy chains (~205 kDa), β -galactosidase (~116 kDa), phosphorylase b (~97 kDa), BSA (~66 kDa, egg albumin (~45 kDa), and carbonic anhydrase (~29 kDa) was used in the 10% gels to aid in identification of proteins.

Individual animals were used as the experimental unit in both studies. The GLM procedure of SAS was used to analyze carcass and tenderness data. Planned comparisons between implant strategy (implant versus no implant) and genetic classifications (high retail product versus high marbling) or implant strategy, sex and growth potential were done.

Results & Discussion

Growth implants used in Continental steers significantly affected shear force values. When implants were used shear force values were significantly higher ($P < 0.01$) for steaks that were from non-implanted steers (Table 1). Implants significantly increased the shear force values of steaks (77.5N with vs. 66.7N without). Continental steers also had a significantly higher shear force value (77.5N) than British steers (65.7N).

Protein degradation was uniform among all samples (Fig. 1). The samples on gels the in Figure one were from Continental cattle with and without implants and Figure 2 shows cytoskeletal (5% gels) protein breakdown of meat from British cattle, with and without implants. No matter what the breed or implant strategy the degradation of proteins was similar. There was no difference in the appearance of the 30,000 dalton component between tough and tender steaks from implanted and non-implanted steers. MacBride and Parrish (1977) reported a relationship between the appearance of a 30,000 dalton component and the tenderness of steaks. Furthermore no difference in the appearance of titin and nebulin (Fig 2) suggest that there were no differences in the breakdown of cytoskeletal proteins. Huff-Lonergan et al. (1995) reported that the rate of conversion of titin from T1 to T2 was slower in less tender samples than in tender samples

Conclusions

Evaluation of protein degradation on SDS-PAGE showed no effect of growth implants on the rate, extent, or manner of protein degradation in either breed or treatment group. No difference in protein degradation suggests no major differences in protein breakdown or calpastatin activity at these times postmortem. Therefore, more information

is needed to establish the cause of the different tenderness values observed with the use of implants.

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Table 1: Effect of breed type and growth implants on tenderness.

		Shear force, N
Breed type ^a	Continental	77.5
	British	65.7
	P – value	<0.01
Implant	With	77.5
	Without	66.7
	P – value	<0.01
Breed × Implant	Continental with	85.3
	British with	68.6
	Continental without	69.6
	British without	62.8
	P – value	0.26

^aBreed types were characterized by what the sire was known to be. Continental descent cattle were from Simmental sires whereas British descent cattle were from Angus and Hereford sires.

Figure 1: Ten percent SDS-PAGE of isolated myofibrils from normal and implanted steers at 7, 14, and 21 days. 15µg of total protein was loaded onto each lane. Number to the left indicates the protein molecular weights in kDa. Sample 515 was a Continental implanted, 593 was a Continental non-implanted steer, 629 was a Continental implanted steer, and 607 was a Continental non-implanted steer.

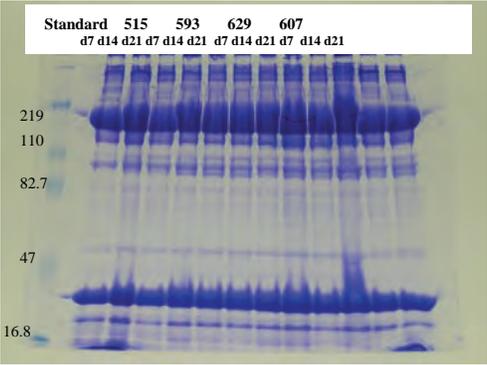
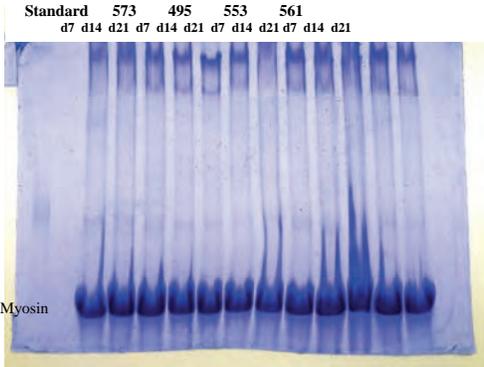


Figure 2: Five percent SDS-PAGE of isolated myofibrils from normal and implanted steers at 7, 14, and 21 days. 15µg of total protein was loaded onto each lane. Sample 571 was a British non-implanted steer, 495 was a British non-implanted steer, 553 was a British implanted steer, and 561 was a British implanted steer.



**SUPPLEMENTATION OF FED STEERS WITH A BIOVANCE
TECHNOLOGIES, INC. ANIONIC COMPOUND TO IMPROVE BEEF
TENDERNESS**

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Key Words: Meat Quality, Beef Tenderness, Dietary Cation-Anion Balance

Introduction

Calcium has been known to improve postmortem tenderness by the means of calcium-activated proteases (calpains) that deteriorate myofibrillar structure (Koochmarai, 1992). Recent research has been investigating methods to achieve a higher concentration of Ca^{+2} in postmortem muscles. One method of increasing calcium is by injection of CaCl_2 solution into beef cuts to enhance meat tenderness (Wheeler et al., 1993).

Altering serum Ca^{+2} concentrations prior to harvest has been hypothesized to result in increased intracellular muscle Ca^{+2} content, leading to improved tenderness in postmortem muscle. Nutritional supplementation of products that effect absorption and circulation of Ca are currently being investigated. Although scientific consensus has not been reached on the topic (Scanga et al., 2001), several studies (eg., Karges et al., 2001; Montgomery et al., 2002) have shown that oral supplementation of feedlot cattle with supranutritional levels of Vitamin D₃ during the late stages of finishing, just prior to harvest, may improve beef tenderness. Feeding dairy cows a negative “Dietary Cation-Anion Balance” (DCAB) diet during the pre-partum period to induce mild metabolic acidosis increases plasma Ca^{+2} concentrations around parturition (Block, 1984) to reduce the incidence of milk fever.

Objective

The objective of the two studies was to test and demonstrate the concept of improving beef tenderness by treating fed cattle with a negative DCAB diet achieved through the supplementation of a Biovance Technologies, Inc. anionic compound.

Methodology

Cattle Selection

Trial 1—British and Continental cross steers (N=120) on feed at the Colorado State University Agricultural, Research, Development, and Education Center were divided into

two treatment groups. Steers were blocked by weight in ten head pens, and pens were randomly assigned to a treatment group. Treatments consisted of supplementation (n=62 head), and negative controls (n=58 head), with genetics as a block effect. The supplement was incorporated into a total mixed ration (TMR) for the following inclusion rate: (a) days 1 and 2 = 0.11 kg/hg/d; (b) days 3 and 4 = 0.23 kg/hg/d; (c) days 5 and 6 = 0.34 kg/h/d; and (d) days 7 through 14 = 0.46 kg/h/d to obtain a negative DCAB. Feedlot performance data were collected over the 14 day trial.

Trial 2—Three large pens of *Bos taurus* and *Bos indicus* cross steers (N=410), located at a commercial feedlot, were identified 113 days prior to harvest. Steers were of similar source and biological type within each pen. Allocation of steers to treatment groups occurred by dividing each pen into two by means of alternating animals as they were processed, for a total of 6 pens (3 treatment pens; n=206 head, 3 negative control pens; n=204 head). At the time of processing, individual ear tags were inserted and breed scores (Species: *Bos taurus* or *Bos indicus*) were recorded by Colorado State University (CSU) personnel. Supplementation with the anionic compound occurred via a TMR fed three times daily for the following inclusion rate: (a) day 1 = 0.037 kg/hd/d; (b) day 2 = 0.11 kg/hd/d; (c) days 3 and 4 = 0.23 kg/hd/d; (d) days 5 and 6 = 0.34 kg/hd/d; and (e) days 7 through 14 = 0.46 kg/hd/d. *Carcass Selection*

All cattle for both trials were harvested at a commercial facility, and individual identities were maintained via tag transfer and recording of plant carcass numbers. Following a 36-hour chilling period, personnel of CSU determined actual and adjusted preliminary yield grade, marbling score, and percentages of kidney, pelvic and heart fat, as well as recorded hot carcass weight, and USDA Yield and Quality Grade. Ribeye areas for carcasses, from trial 1, were measured using a Computer Vision System (CVS) manufactured by Research Management Systems USA, with trial 2 ribeye areas being measured by CSU personnel.

Trial 1—Carcass data were collected on all 120 steers. Sixty carcasses were randomly selected for Warner-Bratzler shear force (WBSF) data prior to USDA grading, balancing for genetic effect. Strip loins (*Longissimus* muscle) from the left side of 59 out of 60 carcasses (30 treatment, 29 control) were collected during in-plant fabrication. Strip loin samples were shipped to the CSU Meats laboratory for vacuum-packaging and aging. Steaks were aged for 3, 7, 14, 21, and 28 days postmortem, after which all steaks were subjected to WBSF evaluation. All steak samples were chilled (never frozen), cut 2.54 cm in thickness from the anterior end of the strip loin, and evaluated for shear force characteristics.

Trial 2—One hundred and four carcasses were randomly selected for carcass and WBSF data following USDA grading, balancing the number of Choice vs. Select, and *Bos taurus* vs. *Bos indicus* cattle for each treatment group. The strip loin from the left side of each carcass was collected during in-plant fabrication. Two strip loins were not obtained resulting in 50 strip loins from negative control carcasses in comparison to 52 strip loins from supplemented carcasses. Strip loin samples were shipped to the CSU Meats laboratory for portioning, vacuum-packaging and aging. Steaks were cut from the anterior end of each strip loin and were aged for 7 and 14 days, respectively, frozen, and subsequently subject to WBSF evaluation. Frozen steaks were faced to a thickness of 2.54 cm.

Warner-Bratzler Shear Force

In trials 1 and 2, strip loin steaks were cooked on an electric conveyor grill (model TBG-60, Magikitch'n, Quakertown, PA). After cooking, each steak was allowed to equilibrate to room temperature (22°C). Core samples (1.27 cm in diameter; 5 to 9 cores/steak) were removed from each steak parallel to the muscle fiber orientation and sheared perpendicular to the fiber, using an Instron testing machine fitted with a Warner-Bratzler Shear head. Measurements of peak shear force were recorded and averaged to obtain a single shear force value for each steak.

Trial 1—Strip loin steaks were cooked to a target internal temperature of 70°C. Each steak was cooked at a constant time of 6 minutes and 35 seconds at a setting of 162.8°C for the top and bottom heating platens. Peak internal temperature measurements were recorded for each steak using a hand held thermometer (model HH21 thermometer; Omega Engineering, Inc., Stanford, CT).

Trial 2—WBSF values were determined for steaks using the procedures of AMSA (1995). Strip loin steaks were tempered for 48 hours at approximately 2°C, and cooked to a target internal temperature of 72°C. The steaks were cooked at a constant time of 6 minutes and 35 seconds at a setting of 176°C for the top and bottom heating platens. Peak internal temperature measurements were recorded for each steak using a hand held Type K thermocouple (model 39658-K, Atkins Technical, Gainesville, FL).

Data Analysis

In the two trials, Mixed Model procedures of SAS (SAS Inc., Cary, N.C.) were used to analyze feedlot performance, urine pH, and carcass and WBSF data.

Trial 1—Least squares means for feedlot performance, urine pH, and carcass data were computed. In the model, treatment was a fixed effect, and initial BW was included as a covariate to equalize initial variability in starting weight. Data from WBSF was tested with a repeated measures analysis that included postmortem aging time, treatment, and breed, the treatment by age interaction, and the treatment by breed interaction. Postmortem aging time was treated as a repeated measurement.

Trial 2—Least squares means for carcass data were computed. Data from WBSF was tested with a repeated measures analysis that included postmortem aging time, treatment, breed, USDA Quality Grade, the treatment by age interaction, the treatment by breed interaction, and the treatment by USDA Quality Grade interaction. Postmortem aging time was treated as a repeated measurement.

Results & Discussion

Warner-Bratzler Shear Force

Trial 1—As expected, length of postmortem aging time influenced ($P<0.001$) WBSF values (Table 1). Shear force values decreased from d 7 through 28. An initial analysis of shear force values included aging periods of 3, 7, 14, 21, and 28 in the model, and resulted in no differences between the supplemented steers and controls. However, results from the analysis including d 3 in the model were very sensitive to the influences of tenderness at d 3 of aging; very little difference was observed in mean WBSF values

from carcass of supplemented steers versus carcasses of control steers at d 3, but a noticeable difference in WBSF occurred between carcasses of treated versus un-treated steers at other postmortem aging times. Therefore, further analyses were conducted in which WBSF values for steaks aged only 3 d were omitted; this evaluation revealed an overall treatment effect ($P=0.03$) in which supplemented steers generated steaks having lower shear force values than did control steers (Table 2).

Trial 2—WBSF values were significantly different ($P<0.05$) across USDA Quality Grades, but no treatment or breed effect were seen ($P>0.05$). Least squares means were numerically higher for supplemented steers (3.84 kg) versus the controls (3.62 kg), opposite of what was observed in trial 1. Due to the discrepancy between trials 1 and 2 in regards to treatment effect, investigation of ration samples occurred. Ration analysis from trial 2 indicated that a negative DCAB was not achieved and therefore did not result in improved postmortem tenderness.

Feedlot Performance

Trial 1—Effects of supplementation of an anionic compound on feedlot performance and urine pH are shown in Table 3. Average daily feed intake were similar across treatments. Urine pH for the supplemented steers was lower ($P<0.05$) than urine pH for negative control steers.

Carcass Characteristics

Trial 1—Least squares means for carcass characteristics (Table 4) indicated that preliminary yield grade of supplemented cattle and, consequently, adjusted preliminary yield grades, were higher ($P<0.05$) than those of controls. All other carcass characteristics were similar ($P>0.05$) across treatments.

Trial 2—Carcass traits for Choice and Select cattle are displayed in Table 5. There were no significant differences ($P>0.05$) between treatment groups when comparing carcass traits.

Conclusions

From the results of the two trials, when a negative Dietary Cation-Anion Balance is achieved, supplementation with a Biovance Technologies, Inc. anionic compound improves postmortem beef tenderness without adverse effects on live animal or carcass performance. Currently, a third trial is being conducted to confirm the results of these studies.

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Tables

Table 1. Effects of aging on Warner-Bratzler Shear Force Values for aging periods 3, 7, 14, 21, and 28 days postmortem for treatments and overall sample population in trial 1

	3 day	7 day	14 day	21 day	28 day
Supplemented					
WBSF, kg	7.52±0.26 ^a	4.34±0.26 ^b	3.93±0.26 ^{bc}	3.30±0.26 ^{cd}	3.10±0.26 ^d
Control WBSF, kg	7.55±0.27 ^a	4.59±0.26 ^b	4.23±0.26 ^{bc}	3.60±0.26 ^{cd}	3.40±0.26 ^d
Overall WBSF, kg	7.53±0.19 ^a	4.46±0.19 ^b	4.08±0.19 ^b	3.45±0.19 ^c	3.25±0.18 ^c

^{a, b, c, d} Means, within row, lacking a common superscript letters, differ ($P < 0.05$)

Table 2. Effects of supplementing a Biovance Technologies, Inc. anionic compound on Warner-Bratzler Shear Force Values

	Supplemented	Negative Control	SEM	<i>P</i>
Trial 1 WBSF, kg	3.64 ^b	3.99 ^a	0.1570	0.0293
Trial 2 WBSF, kg	3.84	3.62	0.1410	0.1126

^{a, b} Means, within row, lacking a common superscript letters, differ ($P < 0.05$)

Table 3. Effects of Biovance Technologies, Inc. anionic compound on final BW, dry matter intake, and urine pH for Trial 1

	Supplemented	Negative Control	SEM	<i>P</i>
Final BW, kg	523.9	526.0	4.03	0.74
ADF Intake ^a , kg DM/d	9.61	9.21	0.38	0.47
Urine pH	5.4 ^c	6.3 ^b	0.17	0.005

Initial BW was used as a covariate.

Urine pH was sampled from one head per pen.

^a Average Daily Feed Intake

^{b,c} Means, within row, lacking a common superscript letters, differ ($P < 0.05$)

Table 4. Least squares means of carcass characteristics for both treatment groups in Trial 1

	Supplemented	Negative Control	SEM	<i>P</i>
HCW ^a , kg	322.1	325.2	4.0	0.609
Dressing %	60.8	60.9	0.005	0.880
PYG ^b	3.2 ^e	3.0 ^f	0.078	0.031
Adjusted PYG ^b	3.4 ^e	3.2 ^f	0.064	0.028
KPH ^c , %	2.5	2.3	0.059	0.171
Ribeye Area, cm ²	82.0	80.5	0.903	0.299
Final Yield Grade	3.0	2.9	0.114	0.323
Marbling Score ^d	412	382	9.0	0.053

Initial BW was used as a covariate.

^a Hot Carcass Weight

^b Preliminary Yield Grade

^c Kidney, Pelvic, and Heart Fat

^d Traces = 200, Slight = 300, Small = 400, Modest = 500, and Moderate = 600

^{e,f} Means, within row, lacking a common superscript letters, differ ($P < 0.05$)

Table 5. Least squares means of Choice and Select carcass characteristics for both treatment groups in Trial 2

	USDA Choice				USDA Select			
	Supplemented	Negative Control	SEM	<i>P</i>	Supplemented	Negative Control	SEM	<i>P</i>
HCW ^a , kg	352	358	10.9775	0.5854	353	351	9.8980	0.8
PYG ^b	3.2	3.2	0.1239	0.9736	3.0	3.0	0.0917	0.7
Adjusted PYG ^b	3.3	3.4	0.1061	0.8802	3.2	3.2	0.0862	0.6
KPH ^c , %	2.2	2.2	0.0823	0.7069	2.0	2.1	0.0784	0.6
Ribeye Area, cm ²	82.4	82.1	2.9884	0.9270	86.6	86.9	2.9860	0.9
Final Yield Grade	3.2	3.2	0.1931	0.7032	2.8	2.7	0.1933	0.7
Marbling Score ^d	441	435	10.4966	0.5233	338	347	4.7197	0.0

^aHot Carcass Weight

^bPreliminary Yield Grade

^cKidney, Pelvic, and Heart Fat

^dTraces = 200, Slight = 300, Small = 400, Modest = 500, and Moderate = 600

Means, within row, lacking a common superscript letters, differ ($P < 0.05$)

**FEEDING FIELD PEAS TO MARKET PIGS HAD ONLY MINIMAL EFFECTS
ON CARCASS COMPOSITION, MEAT QUALITY, OR COOKED PORK
PALATABILITY**

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Key Words: Carcass quality, Field peas, Palatability, Pigs

Introduction

Field peas (*Pisum sativum* L.) production in the Midwestern United States is rapidly increasing and field peas have great potential for use as an alternative feedstuff in swine diets (Stein et al., 2004). Even though field peas are not commonly used in swine diets in the United States, field peas are utilized in other countries (Stefanyshyn-Cote, et al, 1998). Utilization of field peas could be economically beneficial to swine producers due to the high nutritional quality of pea protein (Stein et al., 2004). Field peas may be a potential substitute for corn and soybean meal in diets for pigs. However, the effects of field pea inclusion in swine diets on carcass composition, meat quality, and cooked pork palatability are yet to be researched and reported.

Objectives

To determine if field peas fed at various levels to growing-finishing pigs affect pig performance, carcass composition, meat quality, or cooked pork palatability.

Methodology

Pigs and Diet

Forty-eight pigs (initial BW: 22.7 ± 1.41 kg) were fed using a three phase program during the grower and finisher periods (34, 36, and 46 d, respectively for each phase). Pigs were randomly assigned to one of three dietary treatments (n = 16 pigs per treatment): 1. Control diet consisting of corn, soybean meal and no field peas; 2. Field Pea 1 diet consisting of corn, soybean meal, and 36% field peas in all three phases; 3. Field Pea 2 diet consisting of corn and 66% field peas in phase 1, 48% field peas in phase 2, and 36% field peas in phase 3. Pigs were individually weighed at the end of each phase and at the end of the entire trial. Feed intakes were recorded and gain:feed was determined for each pen. Following the third phase, all pigs were harvested at the SDSU

meat laboratory using common slaughter procedures. Pigs had an average final live weight of 123.6 ± 1.06 kg.

Carcass traits

pH was measured in the longissimus 24 h after exsanguination using a pH star (Model 5000 SFK tech., Herlev, Denmark) equipped with a puncture-type combination pH electrode (LoT406-M6-DXK-S7/25, Mettler-Toledo, GmbH, Urdorf, Switzerland). The left side of each carcass was ribbed between the 10th and 11th ribs at 24 h postmortem. Loin eye area, loin eye depth and fat thickness were measured at the 10th rib. In addition, subjective color and marbling scores were obtained according to the National Pork Producers Council Quality Standards (NPPC, 1999). L* color values for the longissimus were also measured using a Minolta Chroma Meter CR-310 (Minolta Corp., Ramsey, NJ) set at D65 illuminant. Finally, an area, large enough for the Minolta Chroma Meter aperture to fit in, just cranial of the 10th rib, was skinned in order to obtain L*, a*, and b* color values for the 2nd layer of fat, counting from the skin inward, using a Minolta Chroma Meter CR-310 (Minolta Corp., Ramsey, NJ) set at D65 illuminant.

Sample fabrication

At 48 h postmortem, the loins were removed from the left side of each carcass. The loins were made boneless and the 3rd through 10th rib section was vacuum packaged, aged for 10 d and used for shear force analysis. Starting at the 11th rib location and continuing towards the caudal end, one chop was removed for drip loss and the remainder of the loin was vacuum packaged for purge loss. Loin trimmings were pooled by treatment, ground, and formed into 115 g patties using a hand patty form.

Drip loss

As previously described, a 2.5-cm thick chop was removed from the 11th rib location of the loin. The chop was then weighed to the nearest 0.01 g, placed on a white Styrofoam tray and retail wrapped. Next, the chop placed at an approximate 30-degree angle and placed in a 1.4°C cooler for 48 h. After 48 h, the chop was removed from the package and weighed again to the nearest 0.01 g. Drip loss was determined as a percentage of initial weight.

Purge loss

Once the drip loss chop was removed, the remainder of the loin, from the 11th rib location to the caudal end, was weighed to the nearest 0.01 lb, vacuum packaged, and stored at 1.4°C for 7 days. After 7 days, the loin was removed from the vacuum package bag and allowed to drip for 15 min. After the 15 min drip time, the loin was weighed to the nearest 0.01 lb. Purge loss was determined as a percentage of initial weight.

Warner-Bratzler shear force

As previously described, the 3rd through 10th rib section of the loin was vacuum packaged, aged for 10 d after exsanguination and frozen. Two, 2.5-cm thick, chops were removed from the caudal end and allowed to thaw for 24 h at 1.4°C before cooking. Chops were cooked in an impingement oven (Lincoln Foodservice Products, Inc., Ft. Wayne, IN) set at 190°C for 13.5 min. The chops were weighed raw (prior to cooking) and again after cooking to the nearest 0.01 g. Cooking loss was determined and expressed as a percentage of initial raw weight. After the chops cooled to room temperature, three 1.27-cm diameter cores were taken from each chop (six cores per loin) parallel to the muscle fiber orientation. Peak shear force was measured, once on each core, using a Warner-Bratzler shear force machine.

Cooked Pork Palatability

A trained sensory panel evaluated cooked longissimus chops for tenderness, juiciness, flavor intensity, and off-flavors, and cooked ground pork patties for texture, juiciness, flavor intensity, and off-flavors. Briefly, a panel was recruited and trained to evaluate pork loin chops and ground pork patties according to guidelines set by AMSA (1995). Pork loin chops were cooked on a clamshell-style grill to a target internal temperature of 71°C, cut into 1.3 x 2.5 cm cubes using a template, and placed into a Styrofoam bowl with holes in the bottom to allow meat juice to drain away from the sample. Samples were held in a 50°C warming oven until served. Panelists received samples identified by code in segregated sensory booths under red lights. To evaluate ground pork palatability, patties were cooked to a target internal temperature of 71°C. Cooked patties were sliced into six pie-shaped portions and placed into Styrofoam bowls with holes in the bottom to allow meat juice to drain away from the sample. Samples were held in a 50°C warming oven until served. Panelists received samples identified by code in segregated sensory booths under red lights.

Results & Discussion

Growth Performance. There were no differences in pig performance during any of the three phases (Table 1.). Likewise, for the entire experimental period, ADG and average G:F did not differ among treatment groups. When considering live performance, Field peas appear to be an acceptable substitute for corn and soybeans in swine grower/finisher diets.

Carcass Composition and Meat Quality. Carcass composition and lean quality are reported in Table 2. Lean color score was higher/darker ($P < 0.05$) for Field Pea 2 diet than for the control and Field Pea 1 diet. Drip loss was lower ($P < 0.05$) for Field Pea 2 than for control and Field Pea 1. No other carcass composition or meat quality traits were affected by treatment.

Meat Palatability Traits. Trained panelists indicated patties from Field Pea 2 carcasses had a higher ($P < 0.05$) incidence of “stale” off-flavors than patties from Control and Field Pea 1 carcasses ($P < 0.05$). All other sensory traits were similar for all treatments ($P > 0.05$).

Conclusions

Feeding field peas to market pigs had no effect live performance, carcass composition and only minimal effects on meat quality and palatability. Therefore, field peas may replace all the soybean meal and a portion of the corn in diets for growing and finishing pigs.

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Tables and Figures

Table 1. Performance data for growing/finishing pigs fed diet various levels of field peas (Con = 0% field peas, FP1 = 36% field peas in all three phases, FP2 = 66% field peas in phase 1, 48% field peas in phase 2, and 36% field peas in phase 3).

	Treatment			SEM	P
	CON	FP1	FP2		
Initial weight, kg	22.9	22.7	22.7	0.45	0.50
Slaughter weight, kg	123.6	122.0	125.3	4.24	0.59
Phase 1 - grower					
ADFI, kg	1.86	1.85	1.83	0.078	0.93
ADG, kg	0.800	0.811	0.814	0.028	0.89
G/F	0.430	0.440	0.446	0.0077	0.38
Phase 2 – finishing 1					
ADFI, kg	2.87	2.81	2.99	0.230	0.28
ADG, kg	0.926	0.956	0.995	0.056	0.26
G/F	0.325	0.341	0.333	0.0098	0.31
Phase 3 – finishing 2					
ADFI, kg	3.33	3.03	3.45	0.164	0.11
ADG, kg	0.886	0.822	0.863	0.042	0.57
G/F	0.269	0.272	0.254	0.0118	0.53
Overall					
ADFI, kg	2.74	2.60	2.82	0.147	0.13
ADG, kg	0.871	0.860	0.889	0.035	0.60
G/F	0.319	0.331	0.317	0.0087	0.38

Table 2. Influence of feeding field peas to growing/finishing pigs on carcass composition and meat quality (Con = 0% field peas, FP1 = 36% field peas in all three phases, FP2 = 66% field peas in phase 1, 48% field peas in phase 2, and 36% field peas in phase 3).

	Treatment			SEM	P <
	CON	FP1	FP2		
HCW, kg	92.5	90.3	93.5	3.09	0.42
Loineye Depth, cm	6.17	5.92	6.07	0.192	0.26
Loineye Area, sq. cm	46.1	44.5	46.3	2.40	0.37
10th rib fat, mm	23.2	24.0	24.1	4.01	0.81
Percent Muscle	51.7	51.0	51.2	2.40	0.68
L*	58.6	58.1	56.0	0.83	0.07
Subjective Marbling	1.06	1.06	1.03	0.123	0.97
Subjective Color (scale 1-5)	2.41x	2.72x	3.22y	0.156	0.003
pH	5.42	5.41	5.44	0.038	0.37
Fat color, L*	80.0	80.6	80.3	0.49	0.52
Fat color, a*	5.87	5.60	5.79	0.307	0.71
Fat color, b*	5.93	5.90	5.82	0.336	0.95
Drip loss, %	3.39x	2.51xy	1.95y	0.351	0.03
Purge loss, %	2.18	1.84	1.82	0.355	0.55

Table 3. Meat quality characteristics of pork loin chops and ground pork from pigs fed field peas (Con = 0% field peas, FP1 = 36% field peas in all three phases, FP2 = 66% field peas in phase 1, 48% field peas in phase 2, and 36% field peas in phase 3).

	Treatment			SEM	P
	CON	FP1	FP2		
Cooking Loss, %	19.85	19.80	20.27	0.92	0.90
Warner-Bratzler Shear Force, kg	3.54	3.90	3.86	0.206	0.42
Chop panel (8-point scale)					
Tenderness	5.50	5.57	5.47	0.253	0.92
Juiciness	5.30	5.46	5.27	0.159	0.65
Flavor	5.38	5.26	5.26	0.151	0.72
Patty panel (8 point scale)					
Texture	5.96	5.82	5.79	0.161	0.72
Tenderness					
Juiciness	5.59	5.55	5.48	0.195	0.93
Flavor	5.11	5.30	5.25	0.140	0.60
Off Flavors, %					
Piggy	0.089	0.070	0.088	0.0305	0.89
Rancid	0.070	0.088	0.018	0.0235	0.11
Stale	0.035 _x	0.000 _x	0.106 _y	0.0246	0.02
Other	0.070	0.108	0.052	0.0337	0.52
Total Off Flavors	0.27	0.27	0.27	0.059	0.99

**EFFECT OF PIG GENOTYPE AND MAGNESIUM – TRYPTOPHAN
SUPPLEMENTED DIETS ON THE MEAT SENSORIAL ATTRIBUTES AND
TEXTURAL PROPERTIES**

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Key Words: genotype, natural tranquillizers, pork, texture, sensory

Introduction

From the animal welfare point of view, the handling of animals before slaughter is one of the most important causes of stress in pigs. Together with genetics, the *antemortem* treatment has an important influence on the final meat quality obtained.

A supplementation of magnesium (Mg) or tryptophan (Trp) in the diet of pigs may reduce the effect of stress by reducing the concentration of cortisol and catecholamines in plasma (Kietzmann and Jablonski, 1985), or by enhancing the synthesis of brain serotonin (Leathwood, 1987; Peeters et al., 2004), respectively.

Several authors have analyzed the effect of Mg-supplemented diets (D'Souza et al., 1998, 2000; Swigert et al., 2004) or Trp-supplemented diets (Adeola and Ball, 1992; Henry et al., 1996) on pork meat quality (color, water holding capacity). Short-term supplemented diets (2-5 days) were found effective (D'Souza et al., 1998; Frederick et al., 2004; Hamilton et al., 2002). The improvement of meat quality by the use of Mg-supplemented diets seems to be related with the stress-susceptibility of the pigs (Apple et al., 2000; Caine et al., 2000) and also with the degree of stress suffered by the animals before slaughter (Souza et al., 2000; Geesink et al., 2004; Font i Furnols et al., 2004).

Tryptophan is a precursor of skatole, one of the compounds responsible for the boar taint problem in pork (Vold, 1970; Walstra and Maarse, 1970). Thus, it is interesting to study the effect of Trp-supplemented diets on the sensory characteristics of meat.

In the present work, the effect of supplementation of the diet of pigs from two halothane genotypes with Mg and Trp on the sensorial attributes and on the meat texture is presented.

Objectives

The aim of this study was to evaluate the effect of the supplementation of the diet with Mg and Trp on the sensorial attributes and on the meat textural properties of pigs from two different genotypes with respect to the halothane gene (NN, negative; nn, positive).

Methodology

Animals, diets and pre-slaughter treatment: Seventy-one entire male pigs (36 NN and 35 nn) from Pietrain, Landrace and Large White lines (average live weight: 108.51 ± 8.32 kg) were used. They were housed individually and fed the same diet until 5 days before slaughter, when three diet groups were established for each genotype: control group, with no supplement; Mg + Trp group, same diet supplemented with 1.2 g elemental Mg and 8 g L-Trp per kg; Trp group, same diet supplemented with 8 g L-Trp per kg. The animals were submitted to the following *antemortem* conditions: they were mixed in the truck according to diet and genotype, and transported for one hour from the farm to the experimental abattoir on secondary roads; the lairage time was about 30 min for the first animal slaughtered. They were stunned with 90% CO₂ and slaughtered. The animals from the 6 groups (2 genotypes x 3 diets) were slaughtered alternatively.

Sampling: *Longissimus* muscle samples were obtained at 24 h *postmortem*. Samples for sensorial analysis were taken at the last rib level, and those for texture measurements at the $\frac{3}{4}$ rib level from the last rib. They were vacuum-packed in aluminium bags and kept at -20°C until analysis.

Sensorial analysis

1. Selection and training of the assessors and elaboration of the descriptive profile. Assessors were selected for their sensitivity to androstenone and skatole. This was done by conducting a direct olfactory test on vials containing standard androstenone or skatole. A total of 10 panelists were selected and participated in the training sessions and in the elaboration of the descriptive profile, according to Font i Furnols et al. (2000) with some modifications. The descriptive profile included the following attributes:
 - *Odor:* androstenone, skatole, sweet, pig.
 - *Flavor:* androstenone, skatole, sweet, metal, pig, sour.
 - *Texture:* hardness.
 - The panelists evaluated the intensity perceived of each attribute on a 10 cm-long non-structured lineal scale, anchored at 0.5 cm of the extremes, with the terms 'none' and 'very high'.
2. *Experimental design:* Ten sessions were carried out. In each session the panelists evaluated odor, texture and flavor of 6 samples, one of each genotype*diet.
 - The samples were presented to the panelists in a monadic way. Each assessor received the samples in a different order, designed to avoid the first sample and carry-over effect (Macfie et al., 1989). The evaluations were performed in a standardized sampling room (ISO 8589, 1988).
3. *Sample preparation:* The loin samples were thawed at $4^{\circ}\text{C}/24\text{h}$, and then cut into slices of 1.5 cm thickness. Each slice was cut into 4 pieces 1.5 cm wide; each piece contained subcutaneous fat (Agerhem and Dijksterhuis, 1997). These pieces were placed in closed test-tubes (10 cm long with an inner diameter of 2 cm) and heated in a preheated oven at 175°C until they reached an internal temperature of 75°C . Then they were served to the panelists.

Textural analysis (Warner-Bratzler test): The loins (1.5 cm thickness) were thawed at 4°C/24 h in their vacuum-packed plastic bags and then cooked in an oven at 110°C, until they reached an internal temperature of 75°C (25 min). They were cooled down at room temperature (20±2°C) before the analysis. The samples were obtained by cutting 6 to 8 rectangles of 1.5 x 1.5 cm of cross section, obliquely (45°) to the muscle fiber direction (Guerrero and Guàrdia, 1999; Poste et al., 1993). They were cut using a Warner-Bratzler shear blade with a triangular slot cutting edge in a Universal testing machine - MTS Alliance RT/5. The samples were cut at a crosshead speed of 2 mm/s. The average value for each sample was recorded (mean of 6 to 8 replicates).

Maximum shear force (N) (Moller, 1980), shear firmness (N/cm) (Brady and Hunecke, 1985) and the total work performed to cut the sample (Kg.cm) were recorded.

Statistical analysis: The GLM procedure of SAS software (SAS, 1999) was used. For the sensory analysis genotype, diet and the interaction between them (if significant) were considered as fixed effects. Panelist and session within panelist were considered as blocking variables. For the texture analysis genotype, diet, and the interaction between them (if significant) were considered as fixed effects. When the cooking day was significant it was included in the model as a blocking variable. Significant differences between least squares means were obtained after applying the Tukey test.

Results & Discussion

Table 1 shows the least squares means and root mean squared error of the scores of sensorial attributes evaluated by the trained panelists. Some differences were found between genotypes as well as between diets. Loins from halothane positive animals presented significantly lower androstenone and skatole odor and flavor than halothane negative animals, suggesting that meat from nn pigs could present less boar taint problems. Halothane positive pigs also presented higher sweet odor. As expected, hardness was evaluated higher in nn than in NN pigs, since PSE meat is less tender than normal meat. Moelich et al. (2003) find significant differences in juiciness, but not in tenderness according to the halothane genotype. With respect to the diet, the Mg + Trp group showed lower androstenone odor than the control group, and lower skatole flavor than both the control and the Trp groups. The group of diet did not affect hardness.

One of the objectives of this study was to ascertain if the Mg and/or Trp-supplemented diets would affect meat texture. No significant differences between genotypes or diets (Table 2) were found in any of the texture parameters measured by the Warner-Bratzler test. With respect to the genotype, this result was in accordance with De Smet et al. (1995, 1996), who report no differences due to halothane susceptibility. In contrast, Moelich et al. (2003) find higher shear force values for nn than for NN animals. In the case of diets, the lack of differences could be due to the high drip losses found in the three groups, which even though they were significantly lower in the supplemented diets, they were still high (7.34%) (Panella et al., 2004).

Conclusions

Supplementation of (Mg + Trp) or Trp in the diet of pigs from the two different genotypes did not affect the boar taint perception assessed by the panel test, whereas,

from the two genotypes, the nn pigs could present less boar taint problems. The hardness evaluated by the panelists was higher in meat from the nn genotype. The instrumental texture properties of the meat (Warner-Bratzler test) did not vary between genotypes or diets.

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Tables

Table 1. Least squares means and root mean squared error (RMSE) of sensorial attributes evaluated by a panel test in the loin of pigs from the two genotypes fed the three groups of diets

	Genotype		Group of Diet*			RMSE	
	NN	nn	Sig	Control	Mg + Trp		Trp
<i>ODOR</i>							
Androstenone	2.36	1.82	**	2.44 ^a	1.71 ^b	2.12 ^{ab}	1.88
Skatole	3.77	2.78	***	3.31	3.02	3.51	2.07
Sweet	1.84	2.12	*	1.95	2.09	1.89	1.11
Pig	3.91	4.11	ns	3.78	3.82	4.43	1.29
<i>TEXTURE</i>							
Hardness	3.75	4.96	***	4.44	4.45	4.19	1.62
<i>FLAVOR</i>							
Androstenone	0.94	0.70	*	0.96	0.73	0.76	1.08
Skatole	2.20	1.38	***	1.97 ^a	1.41 ^b	1.98 ^a	1.69
Sweet	1.86	1.81	ns	1.75	1.9	1.85	0.98
Metallic	1.85	2.06	ns	1.98	2.0	1.88	1.12
Pig	2.94	2.99	ns	2.89	2.77	3.23	1.26
Sour	0.18	1.49	ns	0.13	2.02	0.35	1.33

* Different superscripts within a row indicated significant differences between groups of diet (P<0.05); ns: non significant.

Table 2. Least squares means and root mean squared error (RMSE) of textural properties (Warner-Bratzler test) of the loin of pigs from the two genotypes fed the three groups of diets

	Genotype			Group of Diet			Sig.	RSME
	NN	nn	Sig.	Control	Mg + Trp	Trp		
Maximum Shear Force (N)	50.30	50.48	ns	52.39	51.65	47.13	ns	9.53
Shear firmness (N/cm)	70.83	75.99	ns	75.42	74.00	70.81	ns	14.12
Total Work (kg.cm)	6.38	6.39	ns	6.84	6.34	5.97	ns	1.18

ns: non significant.

NOVEL DATA ON DIETARY SUPPLEMENT OF RAW POTATO STARCH AND BOAR TAIN IN PUBERTAL MALE PIGS

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Key Words: Androstenone, Indole, Skatole, Raw Potato Starch

Introduction

Castration of male piglets is widely practiced in most European countries to prevent boar taint, an unpleasant odour in meat from some entire male pigs that occurs when they reach sexual maturity. Nowadays, there is a growing interest in use of entire male pigs instead of castrated because of improved animal welfare and superior carcass characteristics. However, if entire male pigs are to be used for pork production, the taint has to be eliminated to avoid consumer dissatisfaction.

Skatole (3-methylindole) and androstenone (5 α -androst-16-en-3-one) are the major contributors to boar taint. Indole may also contribute to the taint, but to a lesser degree because of relatively weak odour and weak lipophilic properties. Both skatole and indole are produced from the amino acid tryptophan in the large intestine by bacteria, and feeding system affects their levels in entire male pigs (Van Oeckel et al., 1998) and castrates (Van Heugten and Van Kempen, 2002; Willig et al., 2005).

Androstenone is synthesized in the testes and mainly affected by puberty stage and individual ability of the pig to produce this steroid (Bonneau, 1998). In the regulation of androstenone levels, dietary factors are less important unless they affect puberty (Øverland et al., 1995). Studies investigating nutritional effects on boar taint, have demonstrated that specific ingredients in the diet are not involved in the regulation of androstenone levels (Whittington et al., 2004; Zamaratskaia et al., 2004).

Objectives

The aim of the present study was to evaluate the effect of live weight at slaughter and dietary supplement of raw potato starch on the levels of skatole, indole and androstenone in fat and plasma from entire male pigs. This study was the second replicate of a large project on effects of diet, raising system and live weight at slaughter on boar taint and aggression level. The results of the first replicate are presented elsewhere (Zamaratskaia et al. 2004).

Methodology

A total of 100 entire male pigs of a crossbred (Swedish Landrace sires x Swedish Yorkshire dams) were included in the study. The pigs were slaughtered at the average live weight (LW) of either 90 or 115 kg. All pigs slaughtered at 90 kg LW (n=28), and half of the pigs slaughtered at 115 kg LW (n=36), were fed commercial diet. The remaining pigs (n=36) were additionally fed 0.6 kg of raw potato starch (RPS, Lyckeby Culminar AB) per pig and day for two weeks prior to slaughter. Skatole, indole (HPLC) and androstenone (ELISA) were measured in plasma, collected the day before slaughter, and adipose tissue (HPLC for all) collected at slaughter. Androstenone levels in plasma were measured by ELISA with and without extraction with ethyl acetate.

The fixed effects of live weight (90 or 115 kg LW) and diet (with or without RPS) were evaluated using Procedure Mixed (Statistical Analysis System, version 8.2, SAS Institute, Cary, NC, USA). The effects were evaluated separately for plasma and fat levels of investigated substance. Effects of individual pig nested within dam and sire (for plasma model), or dam and sire (for fat model) were included as random factors.

Results & Discussion

The diet with RPS induced a significant reduction in skatole levels in fat and plasma ($P < 0.001$), but indole levels remained unchanged ($P > 0.05$) (Table 1). This reduction in skatole levels was expected since RPS is not fully digestible in the upper gastrointestinal and undergoes bacterial fermentation in the large intestine affecting the microflora and intestinal functions (Jensen et al., 1995, Claus et al., 2003). However, the precise mechanism of dietary impact on skatole levels is still unclear. Claus et al. (2003) explained RPS impact on skatole levels by the inhibition of cell apoptosis and thus reduction in tryptophan availability for skatole production. However, if skatole reduction in the pigs fed RPS is due to decreased tryptophan availability, indole reduction could also be expected. Thus, the involvement of other factors in the mechanism of RPS action in the large intestine should be considered. Firstly, diet may dramatically affect intestinal transit time and, therefore, the absorption of skatole from intestinal walls (Jensen et al., 1995). Secondly, tryptophan biotransformations in the gut are pH dependent (Jensen et al., 1995), and altered skatole/indole ratio in the present work might be due to a shift in the synthesis because of lower pH. Interestingly, Willig et al. (2005) reported lower indole concentrations in faeces when pigs were fed RPS, while Rideout et al. (2004) demonstrated no significant changes in indole levels in faeces after feeding chicory inulin. The differences in the response between skatole and indole to dietary changes require further investigations, in which qualitative and quantitative evaluations of intestinal microflora should be included. It is likely that diet affects indolic compounds through the alterations in the gut flora and pH, and potential changes in tryptophan availability are less important.

As expected, the diet with RPS did not affect androstenone levels in fat ($P > 0.05$) (Table 1). As mentioned above, androstenone levels primarily depend on maturity stage (Bonneau, 1998), and diet does not affect the levels (Whittington et al., 2004; Zamaratskaia et al., 2004a). However, the effect of RPS on plasma androstenone levels differed depending on the method used to measure androstenone. Even if the correlation

coefficient between these two methods was high ($r=0.70$), androstenone values obtained by direct ELISA were much greater compared to those obtained after including the extraction step. This difference might be because most androstenone in plasma exists in sulphated form (Sinclair et al, 2005). It is likely, that ELISA without extraction procedure measured total androstenone (free and conjugates), whereas extraction separates free and sulphated forms, and obtained final values reflect free androstenone concentrations in plasma. Androstenone levels measured by direct ELISA (without extraction procedure) were not affected by diet. Conversely, the levels measured by ELISA including extraction step were significantly lower in the pigs fed RPS compared to those in the pigs fed only commercial diet. This was unexpected and difficult to explain. Generally, in the discussion about androstenone metabolism, only hepatic metabolism is considered. However, androstenone metabolism might occur in other tissues, e.g. the intestine. The presence of phase II enzymes, including sulphotransferase, in the intestine has been demonstrated (Falany, 1997), and these enzymes can be either up-regulated or inhibited by components of the diet. We speculate that dietary changes accelerate intestinal sulphation of androstenone through the activation of the enzyme sulphotrasferase. Thus, the ratio free/conjugated androstenone was changed, whereas the levels of total androstenone remained constant. This is an important finding. Upon sulphation, sulphostrasferase substrates become polar and thus suitable for rapid excretion. We suggest that manipulation of intestinal enzymes by dietary changes can increase androstenone sulphation and thus reduce fat levels of androstenone. Interestingly, androstenone levels in fat in the present study slightly decreased after feeding RPS (0.56 vs. 0.81 $\mu\text{g/g}$), although this decrease did not reach statistical significance. These results about a possible impact of RPS on androstenone levels in plasma have never been shown before, but probably more than 14 days of RPS feeding is needed to reach a significant reduction in fat. More research is certainly needed to investigate the dietary impact on androstenone metabolism and excretion.

Slaughter at lower weight (90 kg) did not affect the levels of skatole, indole and androstenone in plasma, and skatole and indole in fat (Table 1). An age-related increase in the levels of those compounds was demonstrated in our previous studies (Babol et al., 2004 and Zamaratskaia et al., 2004). It was suggested that the increase in skatole levels might be due to decreased activities of enzymes CYP2A6 and CYP2E1 in older pigs (Zamaratskaia et al., 2005). In the present study, however, the activities of CYP2A6 and CYP2E1, measured in the liver from the same animals, did not differ between pigs slaughtered at 90 and 115 kg (unpublished). This might explain the absence of age-related increase in the levels of indolic compounds. Additionally, an increase in skatole levels often coincides with onset of puberty (Zamaratskaia et al., 2004), and the levels of circulating endogenous steroids, particularly androstenone, are implicated in the increase in skatole levels (Doran et al., 2002). Given that plasma androstenone levels in the present study did not vary with live weight, no variations in skatole levels could be expected. Increased androstenone levels in fat in the pigs at 115 kg suggest that androstenone accumulation in fat does not ultimately depend on plasma levels. This suggestion is supported by conflicting results from the correlation analysis between androstenone levels in fat and plasma obtained in different studies. Sinclair et al. (2001) explained this observation by the ability of androstenone to accumulate differently in the

fat with different composition. However, the nature of this effect is not clear and should be further studied.

Conclusions

Dietary supplement of raw potato starch significantly reduced skatole levels in fat and plasma, but indole levels remained unchanged. The levels of free androstenone in plasma were significantly lower in pigs fed RPS although the levels of total androstenone were not affected by diet. Androstenone levels in fat were slightly lower in pigs fed RPS; however, this decrease did not reach statistical significance. Therefore, dietary supplement of raw potato starch can be used to manipulate skatole and perhaps also androstenone levels in entire male pigs. The effects of potato starch on the levels of boar taint compounds need to be further investigated.

Slaughter at lower weight (90 kg vs. 115 kg) did not reduce the levels of skatole, indole and androstenone in plasma, and skatole and indole levels in fat, but fat androstenone levels were significantly lower in pigs at 90 kg.

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Table 1. Least-squares means and 95% confidence intervals (within brackets) of skatole, androstenone and indole levels from entire male pigs at two slaughter weights and diets

Site of measurements	Compound	Slaughter weight /diet			P-value
		90kg	115kg no RPS	115kg + RPS	
Plasma, ng/mL	Skatole	4.80 ^a (3.43-6.70)	3.88 ^a (2.68-5.62)	0.74 ^b (0.51-1.07)	0.001
	Indole	1.44 (1.03-2.01)	1.67 (1.17-2.39)	1.69 (1.18-2.43)	0.223
	Androstenone (direct ELISA)	15.01 (11.78-19.12)	16.10 (11.97-21.66)	17.98 (13.41-24.12)	0.253
	Androstenone (ELISA with extraction step)	4.02 ^a (3.02-5.36)	4.52 ^a (3.12-6.56)	2.09 ^b (1.45-3.02)	0.001
Fat, µg/g	Skatole	0.06 ^a (0.04-0.11)	0.05 ^a (0.03-0.09)	0.01 ^b (0.01-0.01)	0.001
	Indole	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.682
	Androstenone	0.40 ^a (0.21-0.78)	0.81 ^b (0.43-1.51)	0.56 ^{ab} (0.30-1.04)	0.072

Data are presented after back-transformation. Least-squares means with different superscripts differ ($p < 0.05$).

EFFECT OF EMULSION IVERMECTIN WITH VITAMINS A, D₃, E INJECTION ON COMMERCIAL BRAZILIAN MIXED CROSSED BREED BEEF MEAT QUALITY

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Key Words: lipid oxidation, vitamins A, D₃, E, parasiticide, texture, *Longissimus dorsi*

Introduction

Brazil, in 2003, was ranked as the first beef exporter country in the world comprising of 195 mi herds being around 140 mi of *Bos indicus* breed representing an important segment for the country economy (IBGE, 2005). Ivermectin is a semi synthetic macro cyclic lactone insoluble in water and soluble in organic solvents and acts as endo-ecto parasiticide capable of eliminating parasites from animal gastro intestinal (Ndong et al., 2005). It acts by inhibiting parasites nerve transmission through neuron hyper polarization (Keisler, 1993). It has been routinely injected by itself and recently a commercial emulsion came out mixed with vitamins A, D₃, and E. As it is known, diet supplemented vitamins A, D₃, E possess the function of protecting animal health and subsequently enriching the meat quality. Essentially, vitamin A gives a further protection to the epithelial tissues and to increase the amount of meat marbling up to 23 mo of age (Akio et. al., 1998) and presents an antioxidant role (Morrisey et al., 1998). The use of dietary vitamin D₃ few days before slaughtering improved *longissimus* texture (Swanek et al, 1999, Montgomery et al., 2002) because of the increase of calcium ions availability to enhance calpain enzyme system activity. The role of vitamin E as a natural antioxidant has been recognized by stabilizing polyunsaturated phospholipids inhibiting lipid oxidation at cell membranes (Liu et al., 1995; Soares et al., 2003, 2004).

Objectives

The objective of this work was to investigate the effect of subcutaneous injection of emulsion ivermectin with vitamins A D₃, E on commercial Brazilian beef herds on *Longissimus dorsi* lipid oxidation and texture

Methodology

Samples

Eighteen crossed breed bovine between European, *Bos taurus*, and Nelore, *Bos indicus*, male, raised on pasture, averaged weight of app. of 350 kg were randomly divided into two lots: Control Lot (CL) (n=9) and Injected Lot (IL) (n=9). One subcutaneous 8.0 mL injection of an oily emulsion (ADE-TEC[®]) containing 80 mg of ivermectin mixed with 2.0 mi UI vitamin A, 0.56 mi UI vitamin D₃ and 400 UI vitamin E was shot exactly 52 days before slaughtering. After injection, animals were confined under a ration diet of 40 to 50 kg constituted by triticale and corn, 0.5 kg cotton seeds, 300 g of soybean meal, 300 g of oat and 140 g of minerals (1% limestone, 1% dicalcium phosphate and 98% of salt) for each animal per day. Animals were slaughtered in a commercial abattoir and the carcasses were kept under refrigeration at 6±1°C for 2 days and *Longissimus dorsi* m. was selected and sliced and packed and individually coded. Thereafter, samples were frozen at -18°C.

Texture measurement

This measurement was carried out in refrigerated 2 days stored samples and also in 90 days frozen samples and cooked in plastic bags in water bath at 85° C until the internal temperature reached the value of 75°C. Samples were cut in size of 1x2x1cm and submitted to WBS TATX-2i for texture measurement and results were expressed in Newtons.

Lipid oxidation analysis

This determination was carried out following the technique of Tarladgis et al. (1964) in 2 days post mortem samples and in frozen samples kept at -18°C after 30 and 60 days of storage

Statistical analysis

Results were submitted to STATISTICA program version 5.0 (Oklahoma, USA 1995). Student t test was employed to determine the significance level between CL and IL in relation to lipid oxidation and texture.

Results & Discussion

Meat texture

Table 1 shows the results of WBS measurement after injection of an emulsion of ivermectin mixed with vitamins A, D₃, E in a beef longissimus m. The 2 days refrigerated samples and its cooked samples were 19.6% and 13.6% more tender, respectively in relation to control samples (p≤0.05).

Table 1. Effect of a subcutaneous injection of emulsion ivermectin mixed with vitamins A, D₃, E on shear force values of *Longissimus dorsi m.* beef kept under 2 days refrigeration and its cooked sample.

Treatments	Shear force (N)	Shear force (N)
	Fresh samples	Cooked samples
Control lot	32.87 ^a ± 3.54	65.44 ^a ± 8.78
Injected lot	26.41 ^b ± 3.62	56.49 ^b ± 8.81

^{ab} Means followed by different letters on the same column are significantly different by Student t test (p≤0.05). N: Newtons

These results are the consequence of the vitamin D₃ properties to regulate calcium ions absorption therefore potentializes the calpain protease system activity as first shown by Swanek et al (1999) further corroborated by other reports (Montgomery *et al.* 2000). Calcium ions would preferentially be deposited on Z-lines thus initiating the meat sarcomere degradation hence tenderizing the meat (Whipple and Koohmaraie, 1993). Despite of being located in the tropics region there was the possibility of increasing calcium deposits within the tissues and this fact could be a health hazard to the animals (Montgomery *et al.* 2002). This was not the case since no problem regarding to the health was observed.

Lipid oxidation

Table 2 shows the results of lipid oxidation measured as TBARS (Tarladgis et al., 1964) after injection of an ivermectin emulsion mixed with vitamins A, D₃, E in a beef *longissimus m.* Analysis was carried out in 2 days post mortem refrigerated samples and in 30 and 60 days stored frozen *longissimus* samples. Results show that refrigerated meat from treated animals presented 22.6% lower lipid oxidation than control samples. Treated frozen stored samples of 30 and 60 days presented 22.6 and 30.7% lower rancidity in relation to controlled samples (p≤0.05).

Table 2 – Effect of a subcutaneous injection of emulsion ivermectin mixed with vitamins A, D₃, E on lipid oxidation measured as TBARS (mg/kg) of *Longissimus dorsi m.* beef kept under 2 days refrigeration and 30 and 60 days frozen samples.

Samples	Control lot (n=9)	Injected lot (n=9)
Fresh	0.14 ^a ± 0.03	0.11 ^b ± 0.02
30 days frozen	0.11 ^a ± 0.03	0.09 ^b ± 0.01
60 days frozen	0.15 ^a ± 0.05	0.11 ^b ± 0.03

^{ab} Means followed by different letters on the same line are significantly different by Student t test (p≤0.05).

Few reports are available with vitamin A as having an antioxidant role (Palace et al., 1999) however much is known about vitamin E. Recent reports clearly emphasizes meat rancidity inhibition by dietary α-tocopherol since it accumulates at cell membranes through its polar portion linkage to polyunsaturated phospholipids (Faustman et al.,

1989). Because of this further fatty acids stability, it has been pointed out that it inhibits the phospholipase A₂ activities thus preventing the initiation of formation of abnormalities such as PSE in pigs and poultry (Cheah et al., 1995; Olivo et al., 2001, Soares et al, 2003). By inhibiting rancidity formation, α -tocopherol and other vitamins also enhances the meat color (Hill et al., 1995), although not evaluated in this experiment. Our results indicate that ADE-TEC[®] presents beneficial effects by not allowing the growth of animal gastro intestinal parasites and also enhancing desirable meat qualities for the consumers as tenderness and lipid fraction stability.

Conclusions

The emulsion ivermectin enriched with a mixed of vitamins A, D₃, E, apart from preventing the gastrointestinal parasite infection inhibits meat lipid oxidation and improves its tenderness.

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THE EFFECTS OF FEEDING HIGHER FIBER FEED ON MEAT QUALITY CHARACTERISTICS OF *M. LONGISSIMUS DORSI*

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Key Words: Sheep, Date-palm, Byproducts, Meat quality

Introduction

In the Sultanate of Oman, the protein and energy requirements of growing livestock are obtained mainly from soybean and maize, the major ingredients in concentrate feeds. These ingredients are imported at high cost. The country does not produce enough animal feed due to an acute shortage in fresh water and limited utilization of arable land. However, there are some readily available livestock feeds in the natural range grazing and browsing land as well as agricultural by-products, especially those from date palms and prosopis pods (*Prosopis juliflora*). The byproducts offer a cheap potential animal feed resource in Oman, which if effectively utilized could improve the supply of animal feeds and increase profit for local farmers.

Meat quality has recently become an important aspect in the marketing of meat products in Oman. Therefore, it is time to produce high quality meat. This is also applicable for the whole Gulf region due to similarity in breeds and environmental conditions and consumer's habits. An efficient marketing system for the Omani meat industry needs more information on meat quality in relation to consumers.

Objectives

This study aimed to investigate the effects of replacing commercial concentrate with a concentrate made mainly from native feed resources and a roughage made from palm frond silage on meat quality characteristics of *longissimus dorsi* (LD) muscle of Omani sheep.

Methodology

Thirty-two, 6 month old Omani male sheep were used in a feeding trial for 120 days. Animals were randomly divided into four groups of eight animals each and allocated randomly to one of four experimental diets groups. Animals for the first group were fed ad libitum chopped Rhodes grass hay plus 400 g of the commercial concentrate. Animals of the second group were fed ad libitum palm frond silage plus 400 g of the commercial concentrate. Animals of the third group received 400 g of the local by-products

concentrate plus ad libitum chopped Rhodes grass hay. Animal of the fourth group received 400 g of the local by products concentrate plus ad libitum palm frond silage.

The by-product concentrate was made from 25% ground date fronds, 25% wheat bran, 20% ground prosopis pods, 15% barley and 12% dried sardines plus vitamin and mineral additives. The palm date frond silage was prepared by shredding palm fronds to approximately 1-2 cm size. A 3% urea solution was prepared by dissolving commercial fertilizer grade urea in tap water in a large sprayer's tank. Shredded palm fronds was weighed, loaded in a Reel Augie mixer, sprayed with urea solution using a sprayer and mixed thoroughly. The fronds-urea mix was then transferred into a 600-gallon plastic tank and manually pressed as hard as possible to reduce air. The tank was tightly sealed to avoid air entrance to the silage to allow anaerobic processing of fronds and urea mixture. The silage was kept in the tank for 4 weeks at the end of which it was emptied and spread for drying before feeding to animals.

Feed (Rhodes grass hay, palm frond silage, by-product and commercial concentrates) were analysed for dry matter (DM) by drying in an oven at 60⁰C until no decrease in weight occurred, and for organic matter (OM) and ash by ashing at 450⁰C for 12 h in a muffle furnace. Triplicate samples of approximately 2 g each were freeze-dried for 4 days for ether extracted (EE) by petroleum ether in a Soxhlet apparatus. Nitrogen was determined by Kjeldahl method according to the procedures of AOAC (1990). Acid detergent fiber (ADF), cell wall constituents neutral detergent fiber (NDF) were determined by the methods of Van Soest et al. (1991). Gross energy (GE) was measured using a calorimeter bomb. Analysis for all items was done in duplicates and expressed on dry matter basis.

The animals were slaughtered at the Municipality slaughterhouse in Muscat (Sultanate of Oman) according to routine slaughterhouse methods. The carcasses were kept at 1-3⁰C for 24 h and then longissimus dorsi from the lumbar (loin) region was removed from the left side of the carcasses and frozen at -20⁰C until processing. Meat quality-related measurements included ultimate pH, WB-shear force, sarcomere length; expressed juice and color (CIE L*, a*, b*) were determined. The ultimate pH was assessed in homogenates at 20-22⁰C (using a Ultra Turrax T25 homogenizer) of duplicate 1.5-2 g of muscle tissue in 10 ml of neutralized 5-mM sodium iodoacetate and the pH of the slurry measured using a Metrohm pH meter (Model No. 744) with a glass electrode. Chilled muscle samples (13 mmx13 mm cross section) for assessment of shear force by a digital Dillon Warner-Bratzler (WB) shear device after prepared from muscle samples cooked in a water bath at 70⁰C for 90 min (Purchas, 1990). Sarcomere length was determined by laser diffraction according to procedure Cross, et al. (1980/1981). Expressed juice was assessed by a filter paper method, as the total wetted area less the meat area (cm²) relatively to the weight of the sample (g). Approximately 60 min after exposing the fresh surface, CIE L*, a*, b* light reflectance coordinates of the muscle surface were measured at room temperature (25±2⁰C) using Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Japan), with a colour measuring area 1.1 cm diameter. It was calibrated using a Minolta calibration plate (L* =97.59, a* =-5.00, b* =+6.76). The L* value relates to Lightness; the a* value to Red-Green hue where a positive value relates to the red intensity; and the b* value to the Yellow-Blue where a positive value relates to yellow. The average of two measurements from each sample was recorded as the colour coordinate value of the sample.

Data were analysed using the GLM procedure within SAS (SAS, 1993), with the model containing items for the four treatments.

Results & Discussion

All experimental animals including those fed the palm by-products did not show signs of ill health throughout the trial. As g%DM, the by-product and commercial concentrates contained 17 and 19% CP; 24 and 6% ADF; 38 and 18% NDF; 12 and 7% ash; and 18 kJ/g gross energy, respectively. The date palm silage and Rhodes grass contained 3 and 7% CP; 58 and 45% ADF; 69 and 76% NDF; 12 and 8% ash; and 21 kJ/g gross energy, respectively. The chemical composition of the two concentrates and two roughages used are presented in Table 1. On dry matter basis, the local by-products concentrates and commercial concentrates contained 17 and 19% CP; 24 and 6% ADF; 38 and 18% NDF; 12 and 7% ash; and 18 and 18 KJ/g gross energy, respectively. The date frond silage and Rhodes grass contained 3 and 7% CP; 58 and 45% ADF; 69 and 76% NDF; 12 and 8% ash; and 21 and 21 KJ/g gross energy, respectively. These diets are cheap and readily available and can be used for feeding sheep.

The main factor determining the quality of meat is its pH, which is related to biochemical processes during the transformation of muscle to meat. Therefore, changes in the pH during the post-mortem period influence the meat quality characteristics. Higher ultimate pH produces dark meat color, reduces storage life and can lead to tougher meat (Chrystall & Daley, 1996). A low plane of feeding can result in chronic nutritional stress, characterized by low reserves of muscle glycogen and increased final pH values in the meat (Bray, et al., 1989). Meat ultimate pH values in the present study were not significantly ($P>0.05$) different between the four diet groups ranging between 5.77 to 5.9 (Table 2), and they were within the range for sheep reported by Carson, et al. (2001). However, they were higher than those reported by Devine, et al. (1993) and Lanza, et al. (2003) for ultimate pH values. The ultimate pH value depends on glycogen levels at slaughter. Therefore, lack of differences in ultimate pH values between the four diet groups indicates that there was no effect of the diet, on the muscle glycogen content at slaughter.

Lightness (L^*), redness (a^*) and yellowness (b^*) values of LD muscle were comparable among the four groups (Table 2). Meat color differences can occur due to a direct effect of the diet on the chemical state of the myoglobin on the surface of the meat (Purchas, 1989). Meat color is related to other factors such as ultimate pH, structure and physical state of muscle proteins, carcass fatness, age, carcass weight and proportion of intramuscular fat (Carson et al., 2001; Priolo, et al., 2001). All these parameters that were similar in the present study across the four diet groups may have collectively contributed towards producing similarity in meat color. The color of the LD muscle in the present study was lower than that reported by Devine, et al. (1993); Lanza, et al. (2003) and Carson et al. (2001) for sheep, which may be due to differences in ultimate pH. Values of ultimate pH above the isoelectric point of proteins of 5.5 result in an open structured muscle and a greater diffusing of light between the myofibrils of the muscle, which make the cut face of the meat darker (Seideman and Crouse, 1986). The lower L^* values of the meat samples from the present study are in line with the expected changes in lightness values as given by Seideman and Crouse, 1986). Moreover, increased pigmentation in

meat may be due to a higher iron content in diets rich in forages and concentrates or may be also due to age differences. The low L^* and high a^* values could be related to higher haem pigment which has been noted to increase with age (Devol et al., 1985).

Water holding capacity is the ability of meat to retain its constituent water when an extraneous force or treatment is applied to it. This property affects the retention of vitamins, minerals and salts, as well as the volume of water retained. Muscles that lose water easily are drier and lose more weight during refrigeration, storage and marketing. Water holding capacity measured as expressed juice was similar between the four diet groups. Sarcomere length was not affected by the experimental diets. Tenderness variation arises mainly through changes to the myofibrillar protein structure of muscle in the period between animal slaughter and meat consumption. Warner-Bratzler shear force was similar between the four diet groups and it was below 6 kg cm^{-2} , which accounted for acceptable tender samples (Devine, et al., 1993). Similarly, Carson et al. (2001) and Lanza, et al. (2001) studied feed efficiency of different rations on meat quality of sheep and concluded that sarcomere length or WB-shear force values were not affected. Overall, the values for sarcomere length and for WB-shear force values were higher than those reported by Carson et al. (2001) for British sheep breeds.

Conclusions

This study indicated that replacing the commercial concentrate and Rhodes grass hay with a more fibrous feed made from local by-products did not produce significant effects on meat quality characteristics.

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Table 1. Chemical composition of the feeds used in the study (g/100g DM).

Chemical component	Rhodes grass	Date Palm frond silage	Commercial concentrate	By-product concentrate
Crude protein	7	3	19	17
ADF	45	58	6	24
NDF	69	76	18	38
Ash	8	12	7	12
Energy (kg/g)	20.96	19.23	18.32	17.91

Table 2. Effects of date palm frond and prosopis pods on meat quality characteristics of *longissimus dorsi* muscle in Omani sheep.

	Diet				SEM	p-value
	A	B	C	D		
Ultimate pH	5.77	5.83	5.86	5.90	0.081	NS
WB-shear force	4.46	4.85	4.46	4.95	0.509	NS
Sarcomere length	2.08	2.08	2.00	2.02	0.066	NS
Expressed juice	28.83	30.24	29.12	29.05	0.986	NS
Color <i>L</i> *	33.73	33.30	35.03	33.79	0.672	NS
Color <i>b</i> *	11.04	11.10	11.53	11.24	0.425	NS
Color <i>a</i> *	23.69	24.22	24.43	23.01	0.486	NS

THE SIGNIFICANCE OF A MUSCLE GLYCOGEN REDUCING DIET ON PORCINE MEAT AND FAT COLOR

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Key Words: pork; fat color; meat color; diet; aging

Introduction

Meat and fat color are important factors for the appearance of meat. Meat color depends beside the concentration of the muscle pigment on the structure of the meat and the redox-state of the main muscle pigment, myoglobin. Both the structure and the present myoglobin species in fresh meat are dependent on the history of *post mortem* pH and temperature, which directly affect the degree of protein denaturation including metmyoglobin reductase activity (metMbRA) and the oxygen consumption capacity of the meat, and hereby the degree of oxygenation of deoxymyoglobin (Mb) to the cherry-red oxymyoglobin (MbO₂) and the depth of the MbO₂ layer in meat and i.e. degree of blooming. Moreover, the history of *post mortem* pH and temperature during the conversion of muscle to meat continuously affects the activity of metMbRA and oxygen consumption capacity of the meat during aging why the blooming potential of meat during aging has been reported to change, being optimal 3-4 days post slaughter (Ledward, 1992).

Recently, it was reported that a muscle glycogen reducing diet could change the history of *post mortem* pH and temperature in pork resulting in a superior technological quality of the meat, i.e. improved water-holding capacity (Rosenvold et al., 2001). The diet found to have the most optimal effect on the history of *post mortem* pH and temperature was reported to contain a high amount of rapeseed and grass meal. However, other studies have reported that a high content of grass meal in the diet can have inferior influence on fat color, especially, in ruminants (Daly et al., 1999; Priolo et al., 2002). Consequently, the influence of a muscle glycogen reducing diet with a high content of rapeseed and grass meal on fat and meat color has to be elucidated before such a strategy can be recommended as a quality control tool in the production of pork of high quality.

Objectives

The objective of the present study was to elucidate the significance of a muscle glycogen reducing diet containing a high ratio of rapeseed and grass meal on fat color, and the blooming potential of the pork during 1, 2, 4, 8 and 15 days of aging compared to a standard diet.

Methodology

40 pigs (Danish Landrace x Danish Yorkshire sows and Duroc Boars) originating from 20 litters with one female and one castrate in each litter were included in the study. 20 pigs were given a standard grower-finishing diet (control diet) which consisted mainly of barley (55%), soybean meal (20%), wheat (20%), and sugar beet molasses (1%), and 20 pigs were given a diet with a low content of digestible starch (experimental diet) which consisted of high levels of grass meal (24%), rape seed cake (36%), dried sugar beet pulp (25%), soybean meal (7%), animal and vegetable fat (6%) resulting in a 2 (diet) x 2 (sex) experimental design. The experimental diet was offered to the experimental group at a live weight of approximately 65 kg with an initially 1-week habituation period gradually changing from the standard grower-finishing diet into the experimental diet, as described by Rosenvold et al. (2001). All pigs were slaughtered at approximately 90 kg live weight with an initial fasting period of 48 hours for experimental pigs where the animals had free access to water.

On the day of slaughter, the pigs were transported from rearing house to slaughterhouse (200m). The pigs were stunned by 85% CO₂ for three min, exsanguinated, scalded at 62 °C for three min, cleaned and eviscerated within 30 min. pH (pH_{45 min}) and temperature (T_{45 min}) were measured 45 min *post-mortem* in *M. longissimus dorsi* (LD) at the last rib and *M. semimembranosus* (SM). After measurement the carcasses were placed in a chill room at 4 °C.

The day after slaughter the pH (pH_{24 h}) and temperature (T_{24 h}) were measured together with the color of back fat and stripped bacon fat. Moreover, LD (12 cm of the loin 19-31 cm from the last rib) and SM were removed from the carcass. 2 cm thick samples from the loins and SM's were cut and bloomed for 1 h at 4 °C prior to color measurement (1 day *post mortem*). The remaining loins and SM's were vacuum-packed and stored at 4 °C for 2, 4, 8 and 15 days *post mortem* where an identical procedure was applied prior to color measurements. Five color measurements across the individual sample surfaces were carried out and mean values were used for statistical analysis. Color was measured using a Minolta Chroma Meter CR-300 (Osaka, Japan) calibrated against a white tile (L*=93.30, a*=0.32 and b*=0.33).

The Mixed procedure in SAS was applied when calculating least squares means (LSM) and standard errors (SE). The statistical model for fat color parameters included diet, sex and fat as fixed effects, litter, slaughter date and animal nested within sex, diet and slaughter date as random effects. The model analyzing LD and SM color included fixed effects of diet, sex and aging, random effects of litter and slaughter date. Two-way interactions were included when significant. The model for temperature and pH included fixed effects of diet and sex and as random effects litter and slaughter date.

Results & Discussion

Figure 1 shows temperature and pH measured 45 min and 24 h *post mortem* in LD and SM and confirms the previous result of using a muscle glycogen reducing diet on the history of *post mortem* pH and temperature (Rosenvold et al., 2001). Moreover, the muscle glycogen reducing diet resulted in a slightly higher ultimate pH.

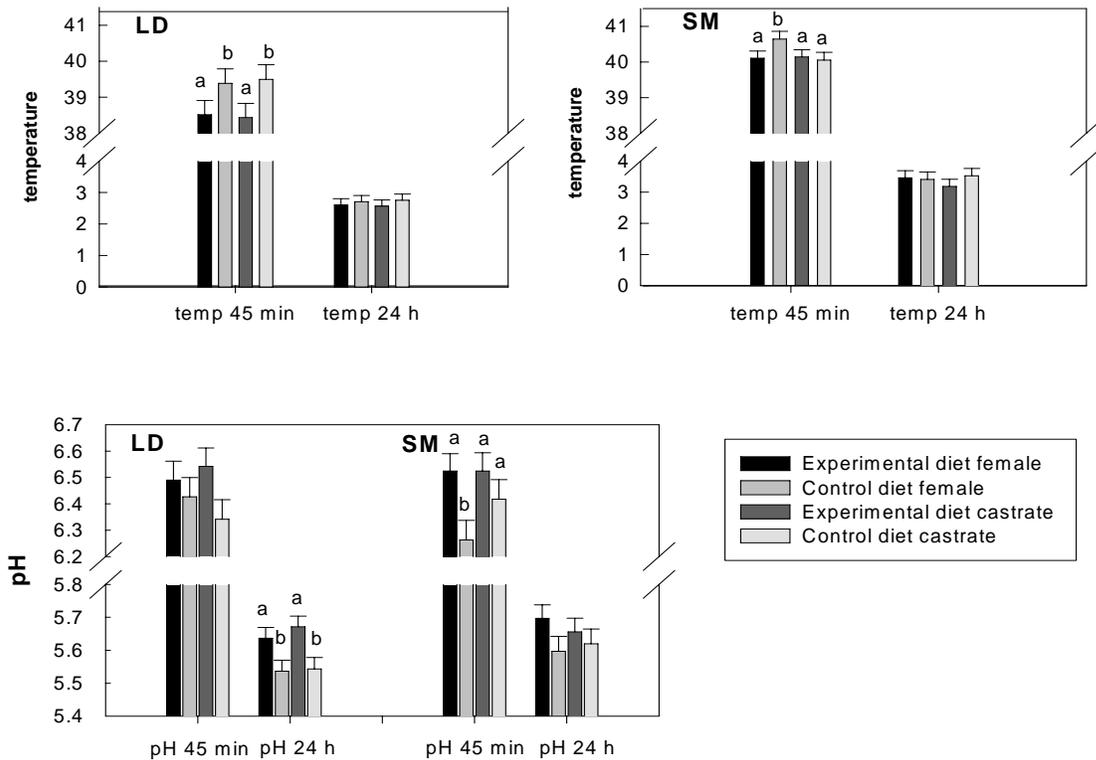


Figure 1. Temperature and pH measured 45 min and 24 h post mortem in LD and SM from pigs fed with strategic finishing feeding (experimental diet) and pigs fed with control feeding, least squares means (LSM) and standard errors (SE) are shown. LSM with different letters (a-b) indicate significant differences between groups, P<0.05.

Figure 2 shows that the experimental diet only had minor effect on fat color, and if any, the experimental diet tended to a higher L*-value while the gender effect was more pronounced, however, without practical importance.

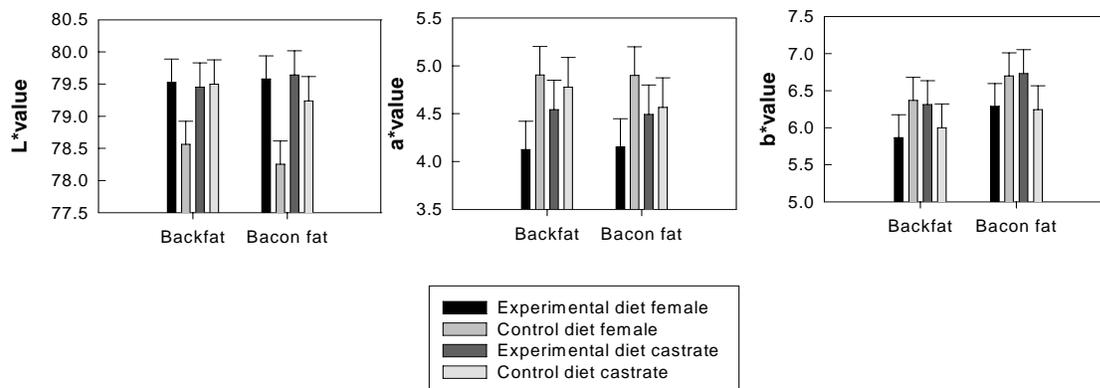


Figure 2. L*, a*- and b*-values measured in back fat and bacon fat from pigs fed with strategic finishing feeding (experimental diet) and pigs fed with control feeding, least squares means (LSM) and standard errors (SE) are shown.

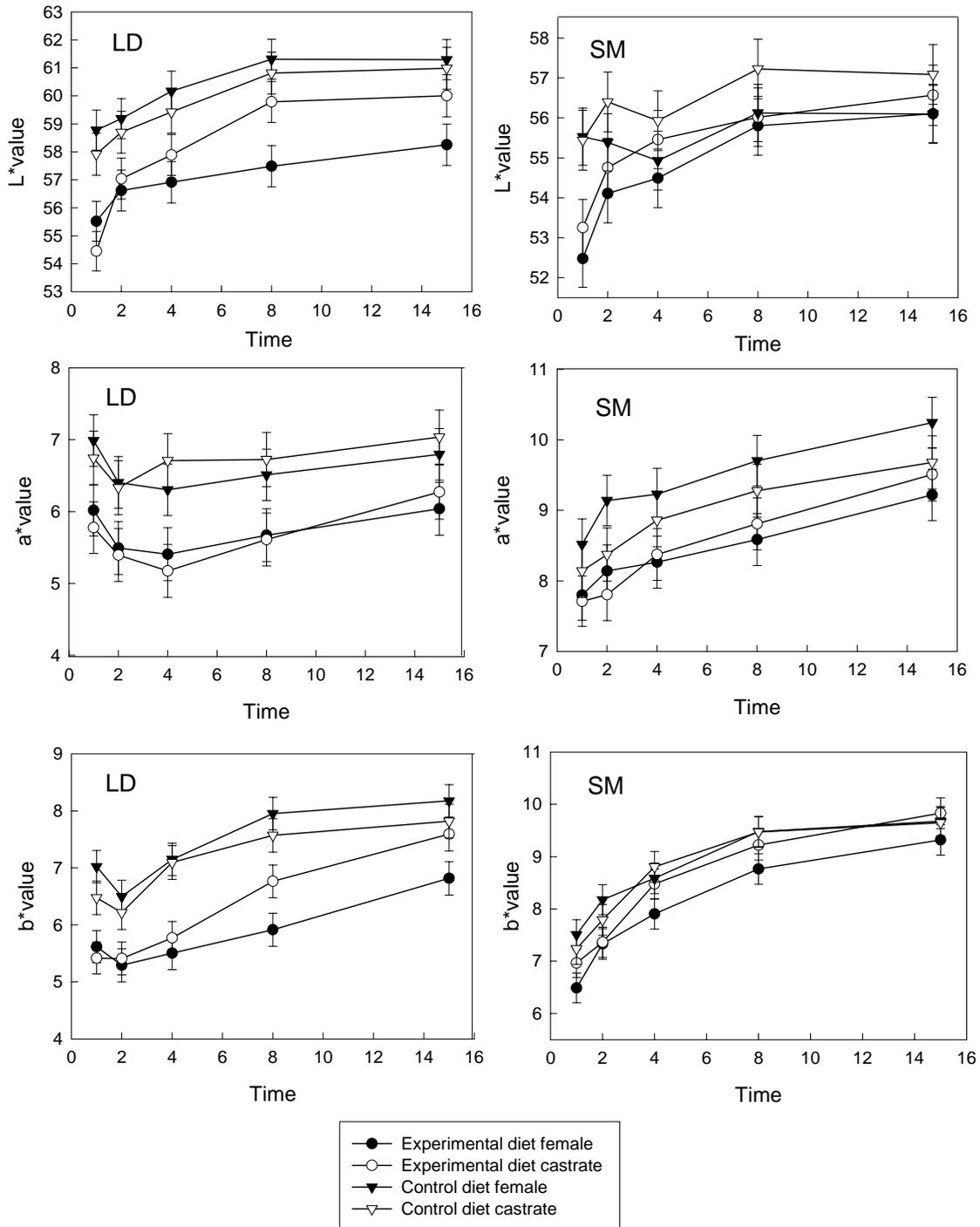


Figure 3. L*, a*- and b*-values measured in *M. longissimus dorsi* (LD) and *M. semimembranosus* from pigs fed with strategic finishing feeding (experimental diet) and pigs fed with control feeding after vacuum-packed storage for 1, 2, 4, 8 and 15 days *post mortem*, least squares means (LSM) and standard errors (SE) are shown.

Figure 3 shows the color (L*, a*- and b*-value) of bloomed cuts of LD and SM as a function of aging time. Due to difference in muscle fiber distribution between the two muscles, with SM being more oxidative than LD, an expected significant difference

($p_{\text{muscle}} < 0.0001$) in L*-, a*- and b*-value between the two muscles was found independent of aging time. Independent of muscle, the color of bloomed pork pigs fed with control diet had higher L*-, a*- and b*-value compared to pork from the experimental diet with the effect being most pronounced in LD (see also Table 1). This diet effect was independent of aging in vacuum of the pork for up to 8 days, however, after 15 days of aging in vacuum the differences in L*-, a*- and b*-value in bloomed pork was found to begin to get closer to each other independent of the diet given to the living pigs. Considering that the experimental diet resulted in higher pH_{45 min} and pH_{24 h} in the muscles, lower T_{45 min} in LD, and that the effect was found to be most pronounced in LD (Table 1) compared to the standard diet, the effect of the experimental diet on pork color can be explained by a higher enzyme activity including metMbRA and higher oxygen consuming activity within the meat together with reduced protein denaturation which together are known to result in the found color characteristics (Lindhahl et al., 2005; van der Wal et al., 1988).

Table 1. The significance (P-value) of diet (D), aging time (A) and sex (S) on the color parameters of bloomed cuts from LD and SM

Muscle	Color	D	A	S	D x A	D x S	A x S
LD	L*-value	0.0022	<.0001	0.5566	0.4619	0.0079	0.2102
	a*-value	0.0369	0.0013	0.3066	0.8322	0.5702	0.5515
	b*-value	0.0016	<.0001	0.3679	0.1595	<.0001	0.1952
SM	L*-value	0.085	<.0001	0.0034	0.0072	0.5773	0.9148
	a*-value	0.0976	<.0001	0.2040	0.9228	0.0005	0.5515
	b*-value	0.0828	<.0001	0.0356	0.1665	0.0005	0.2512

Moreover, Table 1 and Figure 3 show the influence of aging time and gender on the color characteristics of bloomed cuts from LD and SM. In SM the degree of blooming, which is proportional to a simultaneous increase in L*-, a*- and b*-value, was found to be most pronounced preceding 8 days of aging in vacuum, while additional aging in vacuum for seven days did not seem to have any drastic effect on the degree of blooming, even though the degree of redness (a*-value) still was found to increase. In contrast, the degree of blooming in LD after a preceding period of aging in vacuum was found to be more complex, as an initial decrease in a*- and b*-value was found to take place within the first two to four days of aging, after which the degree of blooming again became more pronounced being optimal after eight days of aging in vacuum. An additional seven days of aging in vacuum did not seem to improve the degree of blooming in cuts from LD. This difference between the two muscles with respect to blooming potential as a function of a preceding aging period in vacuum may be explained by differences in heat stability of the enzyme systems known to influence the color of meat (Ledward, 1992). LD is a more superficial muscle compared to SM, and consequently the degree of enzyme activation in SM must be expected to be more pronounced, as the temperature in this muscle will be higher during its conversion of muscle to meat compared to LD, as also seen in Figure 1. The present findings could be explained by the fact that metMb reducing enzymes are more sensitive to the combination of high temperature and the progressing pH decrease *post mortem* in muscles than the inherent oxygen consuming enzymes. A complete inactivation of the metMb reducing enzyme system would render

the picture found in SM where a gradual inactivation of oxygen consuming enzymes during aging in vacuum would result in an increasing depth of the surface MbO₂ layer upon exposure to oxygen and hereby in the progressing blooming potential. In contrast, in LD where both enzyme systems are active on the proposed hypothesis, the activity of these systems will interact. This somehow influences the depth of the surface MbO₂ layer upon exposure to oxygen and might cause the development in blooming potential seen in LD. However, further studies have to be carried out to understand this initial decrease in blooming potential of pork chops from LD exposed to aging in vacuum.

Only minor gender effects on the color characteristics were observed, with the L*- and b*-value being lower in bloomed SM from female pigs (Table 1). However, an interaction between diet and gender was found on the color characteristics indicating that female pigs responded more pronounced to the experimental diet which however, cannot directly be confirmed by observed influence of diet on the history of *post mortem* pH and temperature and the expected effect of these on pork color.

Conclusions

The results of the present study showed that a muscle glycogen reducing diet containing a high ratio of rapeseed and grass meal only had negligible influence on the color of bacon fat and back fat compared to a standard diet. In contrast, the used muscle glycogen reducing diet containing a high ratio of rapeseed and grass meal significantly reduced the blooming potential of the meat and hereby the color of fresh cut pork from LD and SM. Moreover, this difference in fresh meat color was independent of aging in vacuum for up to 15 days. Consequently, further studies need to be carried out, especially the influence on color stability of a muscle glycogen reducing diet, before final recommendations for use of such a potential quality control tool should be given.

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**EFFECT OF PROTECTED LIPID SUPPLEMENTS CONTAINING EITHER
FISH OR LINSEED OIL ON THE PROFILE OF TRANS-OCTADECENOIC AND
CONJUGATED LINOLEIC ACID ISOMERS IN BEEF MUSCLE**

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Background

Conjugated linoleic acid (CLA, 9-cis,11-tr C18:2) and vaccenic acid (TVA, 11-tr C18:1) are both intermediates of ruminal biohydrogenation. Vaccenic acid is derived from dietary linoleic acid, C18:2, in the rumen and is the precursor for 9-cis, 11tr-CLA synthesis in tissues. 10-tr,12-cis CLA is an isomer that is produced only in the rumen, and has been found in milk from lactating cows. Ruminant meat and milk are especially high in CLA and this may have beneficial implications for human health, primarily due to the anti-carcinogenic effect of 9-cis,11-tr CLA as shown in rats (Corl *et al*, 2003), and the partitioning effects of 10-tr,12-cis CLA (Park *et al*, 1999). Studies involving feeding grass silage supplemented with either linseed (rich in C18:3), fish oil (rich in C20:5 and C22:6) or a mixture of the two, resulted in increases in trans- C18:1 and 9-cis, 11-tr CLA in beef muscle when compared with animals fed Megalac, a more saturated supplement containing C16:0 from palm oil (Enser *et al*, 1999, Scollan *et al*, 2001). The fish oil supplement was found to result in higher values for trans-C18:1 than linseed alone, and although the levels of long chain PUFA increased in muscle, the degree of deposition was low (Scollan *et al*, 2001), reflecting a significant degree of biohydrogenation still occurring. In a similar study involving lamb, fish oil was found to increase total trans-C18:1 but not CLA in muscle and adipose tissue (Enser *et al*, 2002). Although it has been shown that there is a strong relationship between total trans C18:1 and 9-cis CLA, it is of interest to evaluate the distribution of the other isomers of trans C18:1 that are not involved with CLA synthesis.

Objectives

In this study we have investigated the effect of dietary lipid on the distribution of the different conjugated C18:2 isomers and trans-C18:1 isomers in beef muscle by using protected lipid supplements to reduce the effects of biohydrogenation, and including some free fish oil to encourage CLA production in the rumen.

Materials and Methods

The profile of trans-C18:1 and conjugated C18:2 fatty acid isomers was studied in muscle from beef cattle fed grass silage *ad libitum* in conjunction with a concentrate (ratio 60:40), where the concentrate contained increasing levels of formaldehyde protected lipid supplement, containing 40% oil, either fish or linseed oil. In order to balance dietary fat intake, Megalac concentrate (high in saturated C16:0 from palm oil) was varied.

In Trial 1, protected linseed supplement (PLS) comprised soya bean, linseed and sunflower oil, with C18:2/C18:3 in a ratio of 1:1, and was fed at 0, 400, 800 and 1000g/d (LControl, PLS1, PLS2 and PLS3). In Trial 2, protected fish oil (soya bean and tuna oil, PFO) was fed at 0, 50, 100 or 200g/d and included 100g/d free fish oil (FFO) in all 4 diets (FControl, PFO1, PFO2 and PFO3).

Both trials comprised 32 Charolais crossbred steers having an initial liveweight of 507 ± 10.3 kg (PLS, Trial 1) and 619.7 ± 7.9 kg (PFO, Trial 2). They were fed the experimental diets for 100d.

Following slaughter, lipids were extracted from m. *longissimus thoracis et lumborum* using chloroform/methanol, followed by alkaline hydrolysis before preparation of methyl esters using diazomethane. FAME, including conjugated C18:2 isomers, were analysed on a CP Sil88 100m x 0.25mm i.d. capillary column using hydrogen as the carrier gas, and a flame ionisation detector. Fatty acids were quantified by the use of an internal standard, C23:0, and identified using standards purchased from Matreya, Inc. USA (Universal Biologicals, Cambridge Ltd) and Sigma, UK. Linearity of the detector was checked by using a C16-C24-monoenoic fatty acid mixture (FAME#5, Restek, UK).

Fatty acid methyl esters taken from animals fed PFO3 and PLS3 as well as their control –fed groups were analysed for trans-C18:1 isomers, using argentation thin-layer chromatography to isolate the trans-monoenoic fatty acid fraction (IUPAC-AOAC method 985.20, 1990), followed by GC analysis.

Statistical analysis was carried out by ANOVA using Genstat 5, Release 4.1(1995),

Results and Discussion

Three major isomers of CLA were quantified in lipid extracts (Table 1), 9-cis, 11-tr CLA, 11-tr, 13-cis CLA and 11-cis, 13-tr CLA, comprising 83%, 10.3% and 6.13% of total CLA respectively. This value for 9-cis, 11-tr CLA compares well with that of 80% found by Chin *et al* (1992) in beef muscle. Comparison with HPLC analysis suggests that this peak includes a small amount of 7-tr, 9-cis and 8-cis,10-trans CLA (Fritsche *et al*, 2000).

9-cis, 11-tr CLA was significantly higher in steers fed PLS3 ($P < 0.01$, Table 1). In contrast, there was no significant effect of PFO supplementation on levels of this isomer although PFO1 appeared higher than the other groups. Looor *et al* (2002), in a study with lactating cows, suggested that linseed oil may increase endogenous synthesis of this isomer in tissues by enhancing post-absorptive availability of 11-tr C18:1. The lack of an effect in the PFO trial may be due to insufficient levels of free fish oil in the rumen, and a lack of inhibition of rumen reductase activity.

11-tr, 13-cis CLA showed a similar pattern to that of 9-cis, 11-tr CLA in the PLS trial except that PLS2 as well as PLS3 were also significantly higher than LControl fed steers ($P < 0.001$). Animals fed PFO showed no change in the level of this isomer.

While 11-tr, 13-tr CLA appeared to decrease, but not significantly, in animals fed all levels of PLS compared with their LControls, inclusion of PFO had no effect on the level of this isomer in steers in Trial 2.

Levels of 10-tr, 12-cis CLA were found to be below the analytical threshold in all groups of animals, regardless of supplementation. This is not unexpected since these animals have been fed a high forage diet, which has been shown to result in lower levels of the isomer (Dannenberger *et al*, 2004)

Analysis of trans-C18:1 isomers showed that, when comparing level 3 of either protected lipid supplement with its control group, PLS3 resulted in a significant decrease in 6-8-trans, 9-trans, and 12-trans C18:1 when expressed as % of total C18:1 isomers, whereas feeding PFO had no effect (Fig.1). In contrast, 11-trans C18:1 (TVA) was significantly raised in PLS3 fed animals ($P < 0.05$). There was a small increase in PFO3-fed animals compared with their FControls but this was not significant. Overall there was no difference in the amount of total trans-C18:1 present, with levels ranging from 164-204mg/100g tissue.

There was a strong relationship between 11-tr C18:1 and 9-cis, 11-tr CLA ($y = 0.2366x + 0.0614$, $R^2 = 0.622$, Figure 2)

Conclusions

Feeding increasing levels of protected linseed supplement resulted in changes in the pattern of isomer distribution within the total trans-C18:1 fraction and the three major isomers of CLA in beef muscle.

The lack of effect of PFO supplementation on levels of all CLA and trans-C18:1 isomers may have been due to insufficient quantities of oil when compared with that given in the PLS trial.

The distribution of various isomers of trans-C18:1 was affected by dietary PLS although overall amounts of total trans-C18:1 was not different between the groups of steers. Regardless of the effects of diet on the other trans C18:1 isomers, 11-trans C18:1 (TVA) was strongly related to 9-cis, 11-trans CLA.

Acknowledgements

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Table 1. CLA isomers in beef muscle (% of total fatty acids).

Numbers within rows with different superscripts differ significantly, (P<0.01, ** P<0.001,***) § includes 7tr,9c and 8c,10tr.

	L- Control	PLS1	PLS2	PLS3	F- Control	PFO1	PFO2	PFO3	sed	P
9c,11t [§]	0.421 ^a	0.497 ^{ab}	0.495 ^{ab}	0.639 ^c	0.441 ^{ab}	0.545 ^{bc}	0.518 ^{ab}	0.491 ^{ab}	0.057	0.009
11t,13c	0.050 ^a	0.057 ^{ab}	0.072 ^{bc}	0.076 ^c	0.046 ^a	0.041 ^a	0.044 ^a	0.049 ^a	0.009	<0.001
11t,13t	0.039	0.019	0.021	0.019	0.018	0.018	0.018	0.019	0.009	0.365

Figure 1. Effect of protected oils on trans C18:1 isomers in beef muscle (% total C18:1)

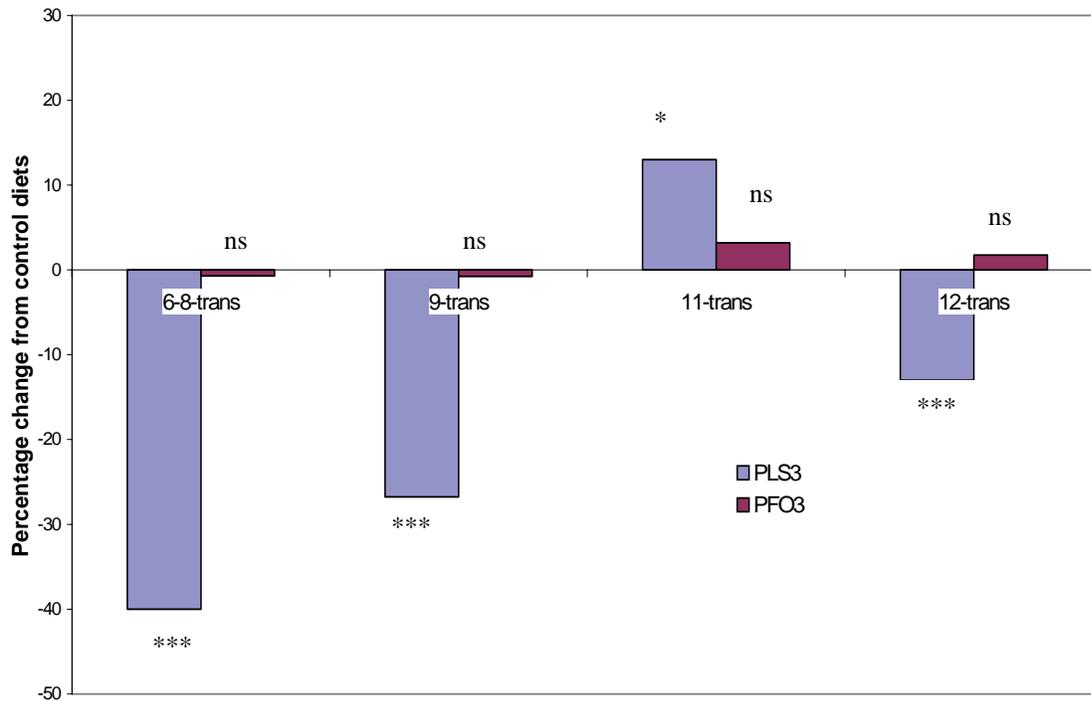
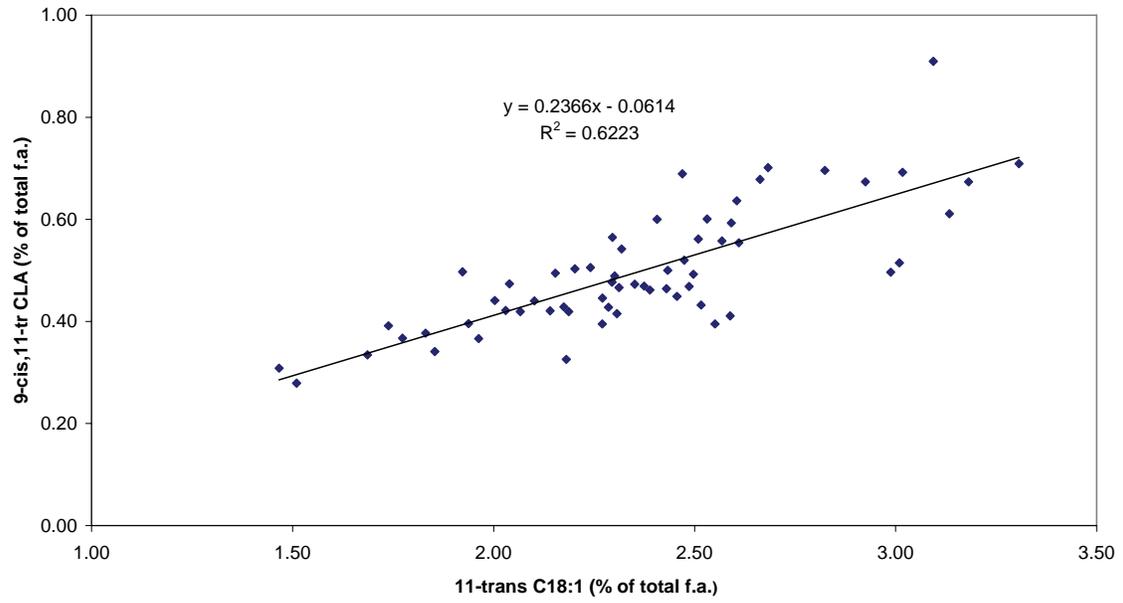


Figure 2. Relationship between 11-trans C18:1 and 9-cis,11-tr CLA in beef muscle



EFFECTS OF FEEDING PERIODS OF DIETARY *RHUS VERNICIFLUA* STOKES ON MEAT QUALITY CHARACTERISTICS OF HANWOO (KOREAN CATTLE) BEEF DURING REFRIGERATED STORAGE

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Key Words: *Rhus verniciflua* Stokes, feeding periods, color, WHC, MUFA, Hanwoo beef.

Introduction

Rhus verniciflua Stokes (RVS) belongs to Anacardiaceae and has been used traditionally for medicinal purposes and for the protection of antiquities in Korea for a long time (Kim 1996). Recently, it was shown that RVS has an antioxidant function. Antioxidant activity of RVS has been reported to correspond to well known enzymatic and non-enzymatic antioxidants in model linoleic emulsion experiments (Lim and Shim 1997). The stem bark of *Rhus verniciflua* contains a high level of urushiols, which are polymerized formation of a lacquer film by the radical-chain reaction (Hirota et al. 1998). The exudate was previously found to have anti-AIDS, a strong antioxidant and immune-enhancing activities (Miller et al. 1996). However, urushiol was the main irritating component of exudate constituents of *Rhus verniciflua*. The heartwood of *Rhus verniciflua* does not cause this type of allergenic action, which implies that it does not contain urushiols (Park et al. 2004). However, information on the application of RVS is still limited.

Objectives

The objective of this study was to determine the effects of feeding periods (0, 3, 4, 5, and 6 months) of dietary *Rhus verniciflua* Stokes with 4%/feed on meat color, water-holding capacity, lipid oxidation and fatty acid composition in *M. Semimembranosus* from Hanwoo (Korean cattle) beef during refrigerated storage.

Methodology

Animals, diets and treatments

Rhus verniciflua Stokes of 8 years was obtained from Wonju, Kangwon province, Korea. The stem bark and heartwood of RVS were naturally dried and reduced to sawdust by an electrical mill. Hanwoo (Korean cattle) steers were divided into five

groups. Control group (n = 3) was fed a common basal diet for 28 months. The other groups (n = 3/group) were fed a supplemented concentrate diet with a RVS supplement of 4%/feed for 3-6 months before slaughter. The *Semimembranosus* muscles were sliced (1.2 cm thickness), then overwrapped in polyethylene wrap film (oxygen transmission rate 35,273 cc/m²/24hr/tm, thickness 0.01 mm). Samples were then held 7 days at 3 °C.

Analytical procedures

CIE L*, a*, and b* values for Illuminant C were measured by a color difference meter (CR-310, Minolta Co., Tokyo, Japan). Also, chroma (C*) and hue-angle (h°) values were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$, and $h^{\circ} = \tan^{-1}(b^*/a^*)$, respectively. The relative content of metmyoglobin at the meat surface was calculated by the method of Kryzwicki (1979) using reflectance at 525, 572, and 730 nm. Reflectance readings were converted to absorbance [$2 - \log(\% \text{ reflectance})$] and used in the equation (Demos et al., 1996). The pH value was determined by homogenizing 10 g sample with 100 ml distilled water for 1 min. A press technique reported by Grau and Hamm (1953) was used to determine water-holding capacity (WHC). Thiobarbituric acid reactive substances (TBARS) was measured according to the modified method of Sinnhuber and Yu (1977). Total lipids for fatty acid analysis were extracted from muscle using the method of Folch et al (1957). Fatty acid methyl esters were prepared according to the procedure of Sukhija and Palmquist (1988). Data were analyzed as a 5 (feeding period) by 4 (storage day) factorial design using the General Linear Model procedure of SAS.

Results & Discussion

As shown in Table 1, the pH value was significantly ($P < 0.05$) lower in RVS-supplemented groups for 3-5 months than in control group. Metmyoglobin (%) was significantly ($P < 0.05$) increased during storage time in all of the groups, but RVS-supplemented groups for 3-5 months had a lower rate of metmyoglobin accumulation during storage. As a whole, TBARS value was significantly ($P < 0.05$) lower in RVS-supplemented group for 6 months than in the other groups, and the TBARS value of day 7 was significantly ($P < 0.05$) higher in control group than in other groups. WHC was significantly increased during refrigerated storage except RVS-supplemented group for 5 months. WHC was significantly ($P < 0.05$) higher in RVS-supplemented groups for 4-5 months than in the other groups. The CIE L*, a*, b* and C* values of RVS-supplemented group for 4 months were significantly ($P < 0.05$) higher than those of the other groups over time (Table 2). The CIE a* and C* values were significantly ($P < 0.05$) decreased during refrigerated storage in all of the groups. In particular, control and RVS-supplemented group for 6 months were more accelerated compared to the other groups. The a* value of day 7 was significantly ($P < 0.05$) higher in RVS-supplemented group for 4 months than in the other groups. Hue angle increased ($P < 0.05$) as storage time increased. And control group at day 7 had higher ($P < 0.05$) hue angle than the other groups. As shown in Table 3, the proportion of C18:1, was significantly ($P < 0.05$) higher in RVS-supplemented group for 5 months. MUFA was significantly ($P < 0.05$) higher in RVS-supplemented groups for 4-5 months than in the other groups.

Conclusions

The meat from 4% *Rhus verniciflua* Stokes-supplemented Hanwoo (Korean cattle) for 4 months was effective in increasing color stability, WHC and monounsaturated fatty acids (MUFA) than was the other meat.

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Tables and Figures

Table 1: Effects of feeding periods of dietary *Rhus verniciflua* Stokes on pH, myoglobin, TBARS, and WHC in Hanwoo (Korean cattle) beef during refrigerated storage

Items	Storage days	Feeding periods (Months)				
		Control	3	4	5	6
pH	0	5.56 ^{abB}	5.49 ^{aC}	5.45 ^{CD}	5.41 ^{aD}	5.62 ^{aA}
	2	5.63 ^{aA}	5.41 ^{bC}	5.40 ^C	5.41 ^{aC}	5.51 ^{bB}
	5	5.56 ^{abA}	5.40 ^{bB}	5.40 ^B	5.39 ^{abB}	5.55 ^{bA}
	7	5.50 ^{bA}	5.36 ^{bB}	5.38 ^B	5.37 ^{bB}	5.50 ^{bA}
Metmyoglobin	0	16.74 ^{dB}	17.42 ^{dAB}	12.84 ^{dC}	14.23 ^{dC}	18.69 ^{dA}
	2	24.32 ^{cA}	19.01 ^{cB}	17.84 ^{cB}	18.81 ^{cB}	23.78 ^{cA}
	5	31.17 ^{bA}	20.54 ^{bC}	21.60 ^{bC}	21.80 ^{bC}	27.39 ^{bB}
	7	35.29 ^{aA}	23.38 ^{aC}	24.23 ^{aC}	24.53 ^{aC}	30.77 ^{aB}
TBARS	0	0.17 ^d	0.16 ^d	0.16 ^d	0.16 ^d	0.16 ^d
	2	0.27 ^{cA}	0.26 ^{cA}	0.27 ^{cA}	0.24 ^{cAB}	0.23 ^{cB}
	5	0.38 ^{bA}	0.32 ^{bAB}	0.34 ^{bA}	0.33 ^{bAB}	0.28 ^{bB}
	7	0.57 ^{aA}	0.40 ^{aB}	0.40 ^{aB}	0.38 ^{aB}	0.38 ^{aB}
WHC	0	37.70 ^{dC}	34.47 ^{dD}	40.83 ^{cB}	46.67 ^A	30.59 ^{cE}
	2	39.93 ^{cB}	37.19 ^{bC}	42.38 ^{bcA}	42.85 ^A	32.75 ^{bD}
	5	40.70 ^{bA}	41.53 ^{aA}	44.95 ^{abA}	45.17 ^A	34.03 ^{abB}
	7	42.51 ^{aB}	40.85 ^{aB}	46.65 ^{aA}	42.85 ^B	34.74 ^{aC}

^{abcd} Means in the same column with different superscripts are significantly different ($P < 0.05$).

^{ABCDE} Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 2: Effect of feeding periods of dietary *Rhus verniciflua* Stokes on meat color in Hanwoo (Korean cattle) beef during refrigerated storage

Items	Storage days	Feeding periods (Months)				
		Control	3	4	5	6
<i>L</i> *	0	42.65 ^A	40.51 ^{bB}	42.74 ^{bA}	41.94 ^{bAB}	40.47 ^{bB}
	2	41.51 ^B	43.98 ^{aA}	43.61 ^{bA}	42.14 ^{bB}	41.58 ^{aB}
	5	43.29 ^A	43.73 ^{aA}	43.93 ^{bA}	42.60 ^{bAB}	41.56 ^{aB}
	7	43.88 ^B	44.86 ^{aAB}	45.93 ^{aA}	44.41 ^{aAB}	41.69 ^{aC}
<i>a</i> *	0	22.52 ^{aA}	19.39 ^{abB}	22.63 ^{aA}	23.30 ^{aA}	19.60 ^{aB}
	2	20.92 ^{bB}	19.47 ^{abC}	22.05 ^{aA}	22.20 ^{abA}	18.01 ^{bD}
	5	17.92 ^{cB}	20.08 ^{aA}	21.32 ^{abA}	20.94 ^{bA}	16.56 ^{cC}
	7	17.14 ^{cC}	18.97 ^{bC}	20.00 ^{bA}	18.34 ^{cB}	16.13 ^{cC}
<i>b</i> *	0	12.27 ^{aA}	10.47 ^{bC}	12.18 ^A	11.43 ^B	10.62 ^{aC}
	2	11.86 ^{abAB}	11.29 ^{aB}	12.11 ^A	11.68 ^{AB}	10.49 ^{aC}
	5	11.19 ^{abB}	11.58 ^{aAB}	12.11 ^A	11.45 ^{AB}	10.19 ^{abC}
	7	11.53 ^{bA}	11.66 ^{aA}	11.83 ^A	11.31 ^A	9.82 ^{bB}
<i>C</i> *	0	25.64 ^{aA}	22.04 ^{bB}	25.70 ^{aA}	25.95 ^{aA}	22.25 ^{aB}
	2	24.05 ^{bA}	22.51 ^{abB}	25.15 ^{aA}	25.09 ^{abA}	20.73 ^{bC}
	5	21.14 ^{cB}	23.18 ^{aA}	24.52 ^{abA}	23.87 ^{bA}	19.43 ^{cC}
	7	20.67 ^{cB}	22.25 ^{abA}	23.24 ^{bA}	21.81 ^{cAB}	18.74 ^{cC}
<i>h</i> ^o	0	28.60 ^{bA}	28.37 ^{aA}	28.22 ^{cA}	26.04 ^{cB}	28.43 ^{cA}
	2	29.51 ^{bAB}	30.12 ^{bA}	28.67 ^{bcBC}	27.73 ^{bC}	30.38 ^{bA}
	5	32.03 ^{aA}	29.96 ^{bB}	29.52 ^{abB}	28.64 ^{bB}	31.57 ^{aA}
	7	33.80 ^{aA}	31.56 ^{aB}	30.53 ^{aB}	31.69 ^{aB}	31.56 ^{aB}

^{abc}Means in the same column with different superscripts are significantly different ($P < 0.05$).

^{ABCD}Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 3: Effect of feeding periods of dietary *Rhus verniciflua* Stokes on fatty acid composition in Hanwoo (Korean cattle) beef

Fatty acids	Feeding periods (Months)				
	Control	3	4	5	6
C14:0	2.72	3.05	2.58	2.78	2.39
C16:0	24.97	25.27	25.30	25.45	24.97
C16:1	5.26 ^{AB}	6.82 ^A	6.48 ^{AB}	6.53 ^{AB}	5.04 ^B
C18:0	13.19	12.11	10.15	9.71	12.16
C18:1	45.19 ^B	46.92 ^{AB}	48.69 ^{AB}	49.16 ^A	45.25 ^B
C18:2	5.41	4.29	4.64	4.38	6.66
C18:3	0.42 ^A	0.18 ^C	0.26 ^{BC}	0.22 ^C	0.32 ^B
C20:1	0.42 ^A	0.09 ^B	0.14 ^B	0.15 ^B	0.39 ^A
C20:4	1.94 ^{AB}	1.24 ^B	1.69 ^{AB}	1.56 ^{AB}	2.35 ^A
C22:4	0.48 ^A	0.02 ^B	0.08 ^B	0.04 ^B	0.45 ^A
SFA ¹	40.87	40.43	38.02	37.94	39.52
MUFA ²	50.87 ^B	53.83 ^A	55.32 ^A	55.85 ^A	50.68 ^B
PUFA ³	8.25 ^{AB}	5.74 ^B	6.66 ^A	6.21 ^B	9.79 ^A
UFA ⁴	59.13	59.57	61.98	62.06	60.48
MUFA/SFA	1.26	1.33	1.46	1.47	1.28

^{ABC}Means in the same row with different superscripts are significantly different ($P < 0.05$).

¹Saturated fatty acids, ²Monounsaturated fatty acids, ³Polyunsaturated fatty acids, ⁴Unsaturated fatty acids.

EFFECT OF DIETARY ENERGY LEVELS ON THE MEAT QUALITY OF KOREAN NATIVE BLACK PIGS

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Key Words: Korean native black pig, intramuscular fat, drip loss, color, TBARS, sensory evaluation

Introduction

Recently in Korea, importance is given to native breeds of livestock under the preservation of its gene resources and the consumer's demand for good taste and quality of meat products. Hence the number of native livestock-breeding farmers that bred native livestock is gradually increasing. Korean native black pig (KNP) has been originated from small, middle, and large species of black pig that had originally inhabited in Mauturia almost 2,000 years ago and has been settled in the Korean Peninsula. KNP has ability to adapt easily to the climatic variations, and have better resistance to diseases (RDA 2001). Muscle color of KNP is redder than that of Landrace and back fat color is also lighter than Landrace (Jin et al. 2001a). Even sensory quality of raw and cooked meat is higher in KNP than to Landrace (Jin et al. 2001b). Glutamate and essential amino acid content in pork from KNP is greater as compared with Landrace (Park et al. 2005). But breeding system and nutrient requirements suited to inherent characteristics of KNP is not still clearly established and KNP is supplied with diet fed to modern-genotype pig, thereby KNP does not bring an economical value for productivity and meat quality.

Objectives

The objective of this experiment was to determine the effect of dietary energy level on the meat quality of Korean native black pigs.

Methodology

Animals, diets and sample

Fifty-four KNPs with a live weight of 25 kg were allotted to three dietary treatments (9 gilts and 9 barrows per treatment). The treatments included three levels (high, medium, and low) of metabolizable energy (ME) at the growing stage (25-45 kg) comprising high (3265 kcal/kg), medium (3165 kcal/kg) and low (3065 kcal/kg) energy and during finishing stage (45-65 kg) the energy levels were high = 3065 kcal/kg, medium = 2965

kcal/kg, and low = 2865 kcal/kg. Three gilts and three barrows in each treatment were slaughtered at a live weight of 65 kg. The Longissimus muscles were utilized for the meat quality measurement. Following cut into 3 cm, samples were individually packaged in low-density polyethylene zipper bags (LDPE, Clean zipper bag, Cleanwrap Co., LTD, South Korea) and placed in dark room at 4 °C for 9 days.

Analytical methods

The IMF (intramuscular fat) content was analyzed according to AOAC (1995). The drip loss was determined according to Honikel (1998) and expressed as percentage of the initial weight. Color of the sample surface was measured by a Minolta Chroma meter (CR-301, Minolta Co., Japan), which recorded the lightness (L^*), redness (a^*), and yellowness (b^*). The TBARS (2-thiobarbituric acid reactive substances) value was determined as described by Sinnhuber and Yu (1977) and reported as mg malonaldehyde (MA)/kg meat. Sensory analyses of raw and cooked meat were performed by trained ten-member laboratory panel. They rated muscle color, back fat color, marbling score, and overall liking of raw meat, taste, flavor, texture, juiciness, and overall liking of cooked meat using 9-point hedonic scales (1 = extremely unlike; 5 = normally like; 9 = extremely like). Data was analyzed using the General Linear Model procedure of SAS (1999) program. Differences among means at the 5% level were determined by the Duncan's multiple range tests.

Results & Discussion

The IMF (Fig. 1) was higher ($P < 0.05$) in barrows fed high-energy diets than that fed low energy diets and also in gilts fed medium energy levels. But IMF content remained comparable to barrows fed medium energy diets. Increasing the dietary energy levels decreased the drip loss (Fig. 2) but it was not affected by sex. It was significantly higher ($P < 0.05$) in high energy fed animals as compared with other treatments and increased linearly with increasing the refrigerated storage in all treatments. The lightness (L^*) of the muscle surface (Fig. 3) decreased by increasing the dietary energy level and was lowest ($P < 0.05$) in high energy group than to other treatments at all measurements after storage. The redness (a^*) (Fig. 4) increased as dietary energy level increased and was also higher in the barrows fed medium energy diets as compared with other treatments but it get decreased ($P < 0.05$) during refrigerated storage in all treatments. In addition, the lightness (L^*) and redness (a^*) in medium energy group were affected by sex, since the lightness (L^*) was lower and the redness was higher in barrow than in gilt ($P < 0.05$). The yellowness (b^*) (Fig. 5) was lower ($P < 0.05$) in the gilt fed low energy diets than to other treatments. The TBARS value (Fig. 6) was more in high and medium energy fed animals as compared with low energy from 6 d onwards ($P < 0.05$) and increased in all treatments during refrigerated storage ($P < 0.05$). Muscle color, back fat color, marbling score, and overall liking of raw meat and taste, flavor, texture, juiciness, and overall liking of cooked meat (Table 1) were higher ($P < 0.05$) in high and medium energy treatments than to low energy.

Conclusions

As dietary energy level of Korean native black pig increased, the intramuscular fat content, redness (a^*), lipid oxidation, and sensory evaluation of the meat increased but the drip loss and lightness (L^*) decreased.

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Tables and Figures

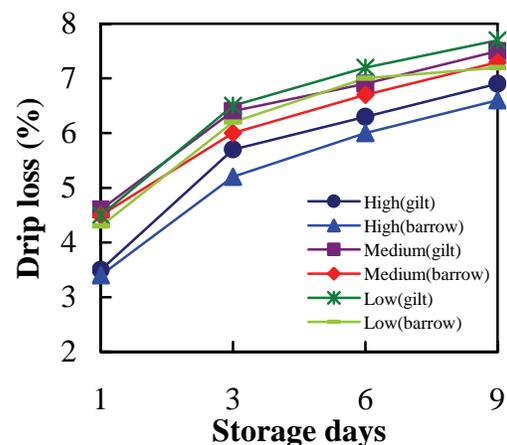
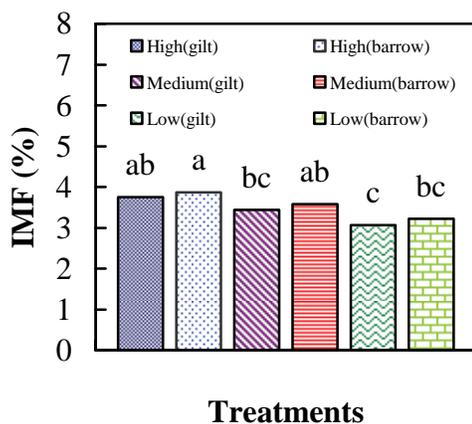


Fig. 1: Effect of dietary energy level on the IMF of Korean native black pig.

Fig. 2: Effect of dietary energy level on the drip loss in pork of Korean native black pig during refrigerated storage.

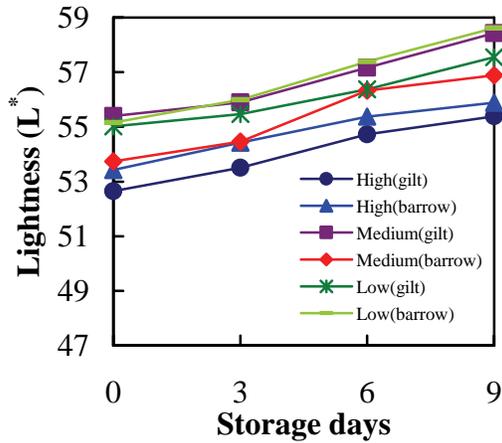


Fig. 3: Effect of dietary energy level on the muscle lightness of Korean native black pig during refrigerated storage.

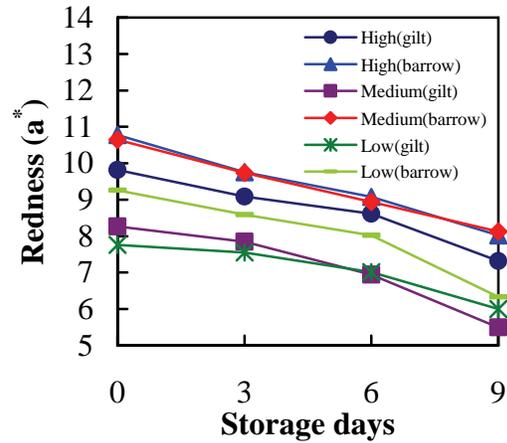


Fig. 4: Effect of dietary energy level on the muscle redness of Korean native black pig during refrigerated storage.

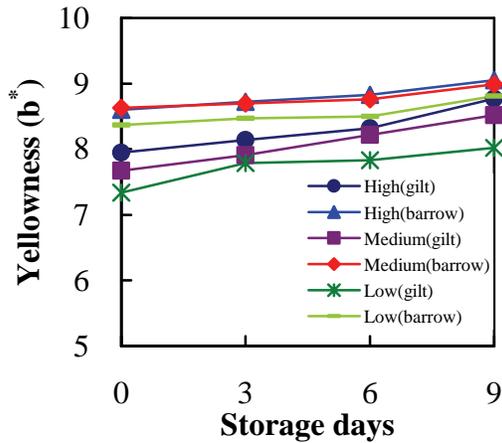


Fig. 5: Effect of dietary energy level on the muscle yellowness of Korean native black pig during refrigerated storage.

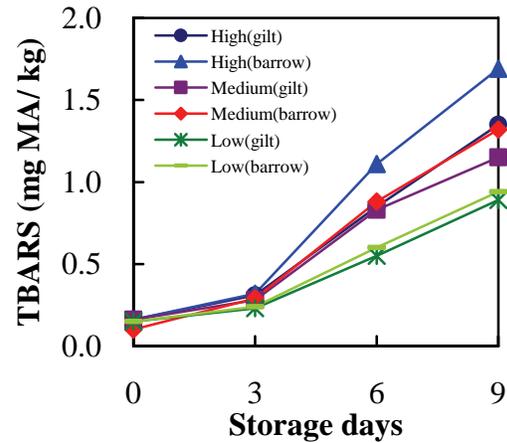


Fig. 6: Effect of dietary energy level on the TBARS value of Korean native black pig during refrigerated storage.

Table 1: Effect of dietary energy level on the sensory evaluation of raw and cooked pork from Korean native black pig.

Sensory evaluation *	Dietary energy level					
	High		Medium		Low	
	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow
Raw meat						
Muscle color	7.22 ^{AB}	7.44 ^A	6.56 ^{AB}	6.78 ^B	5.56 ^C	5.75 ^C
Back fat color	7.11 ^A	7.22 ^A	6.44 ^A	6.44 ^A	5.22 ^C	5.25 ^C
Marbling score	7.22 ^A	7.33 ^A	6.67 ^A	6.78 ^A	5.33 ^B	5.50 ^B
Overall liking	7.11 ^{AB}	7.44 ^A	6.33 ^{AB}	6.56 ^B	5.56 ^C	5.63 ^C
Cooked meat						
Taste	7.00 ^A	7.13 ^A	6.63 ^A	6.75 ^A	5.38 ^B	5.63 ^B
Flavor	6.88 ^A	7.00 ^A	6.50 ^{AB}	6.50 ^{AB}	5.63 ^B	5.50 ^B
Texture	7.13 ^A	7.25 ^A	6.50 ^A	6.88 ^A	5.50 ^B	5.38 ^B
Juiciness	6.88 ^{AB}	7.13 ^A	6.63 ^{AB}	6.75 ^{AB}	5.63 ^B	5.88 ^{AB}
Overall liking	7.00 ^A	7.25 ^A	6.75 ^A	6.88 ^A	5.38 ^B	5.50 ^B

^{ABC} Means in the same rows with different superscripts are significantly ($P < 0.05$).

* 9-point hedonic scale (1=Extremely unlike, 5=normally like, 9=extremely like).

SENSORIALY EVALUATION OF LAMB MEAT PRODUCED UNDER DIFFERENT PRODUCTION SYSTEMS OF URUGUAY

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Key Words: lamb meat quality, sensory analysis, production systems.

Introduction

Marketing and promotion strategies for meat products in most of the current export markets, mainly in those with high competitiveness, have demonstrated the advantage of being based on solid scientific and technique knowledge in order to certify and assure the nourishing innocuousness, animal welfare, human health and product quality. In spite of this world tendency, in Uruguay, there is a recent concern about this matter, so there is a need of positioning Uruguayan meat products in an international context based on this modern approach.

There are many different sheep production systems in the world, with very specialized lamb meat products oriented to certain niche markets. This reality is very clear in Europe, where in the Mediterranean area, young animals are produced almost at the foot of the ewe (“lechales”) with a short period of fattening under very intensive production conditions, whereas in the North Europe, lambs are produced on intensive grazing systems with strategic use of concentrates, but remarkably heavier than those coming from the South. In this sense, the average carcass weight in 2001 was 11.1, 20.1 and 21.7 kg for Spain, UK and Germany, respectively.

These differences in lamb types and production systems are also associated with a high variation in both cooking and consumption habits, determining a wide range of preferences for certain type of meats and, therefore, generating some constraints in the commercial exchange (Hernando *et al.*, 1996 and Sañudo *et al.*, 1998).

These difficulties are greater in sheep than in bovine species, given that lamb meat is more aromatic, so, it has more probability of being rejected by the most sensible consumers.

There are some reasons to believe that significant differences may exist in lamb palatability attributes of different geographical sources due to variations in genetics, nutrition, slaughter weight and age, chilling rates, processing, and extent of postmortem ageing (Jeremiah, 1988).

As a result, the better knowledge gathering in relation to those factors influencing the preferences of the end consumers around the world will generate more information for the future design of marketing and promoting campaigns of the Uruguayan lamb.

Objective

To characterize and to analyze comparatively the sensorial quality of two Uruguayan lamb products in relation to European products coming from three different countries.

Material and methods

Animals of Uruguay

In Uruguay, 60 animals coming from two types of products were analyzed: light lambs (11.1±1.4 kg of cold carcass weight and 3-4 months of age) and heavy lambs (19.4±2.2 kg of cold carcass weight and 12-13 months of age). All the animals were castrated males of the Corriedale breed (the majority breed of Uruguay) raised under grazing conditions on rangelands and finished between two to three months on improved pastures at the Research Unit "Glencoe" of INIA Tacuarembó. The lambs were slaughtered at commercial conditions.

Animals of Europe

In Europe, in order to compare with the two Uruguayan products, one representative type of commercial lamb was used in each country (Germany, Spain and the United Kingdom) with two ageing times (7 and 20 days), using 20 animals by country. The Table 1 shows the comparisons in carcass and meat quality of the Uruguayan and European lambs.

In Germany, the animals were males of Suffolk breed or Schwarzköpfe Fleischschaf x Merino Landschaf crossbreed with a CCW of 23.2±3.65 kg. The animals were finished in pastures dominated by clover, with a strategic use of concentrates, mainly a mixture of wheat, pea and beans. The age at slaughter was on average 4 to 6 months. Spanish animals were entire males of the Rasa Aragonesa breed, weaned around 55 days of age and reared under confinement from birth under intensive feeding system, using concentrate and cereal straw *ad libitum*. The age at slaughter was under 3 months and CCW average was 10.2±0.6 kg.

In the United Kingdom, the lambs were castrated males from commercial crossbreeding type with a 22.8±1.7 kg CCW, bred mainly on grass, with a strategic use of concentrates.

General sampling

The muscle *Longissimus dorsi* (LD) was taken of all the animals for each country and lamb type at 48 hours after slaughtering. It was divided into pieces that were vacuum packaged and aged for 20 days for the Uruguayan lamb types, which is the regular aging period of the lamb meat exported to Europe.

In Europe, the meat was aged 7 days (most common in Europe) or 20 days (similar of the Uruguayan ageing time).

Sensorial evaluation

The sensorial analysis was performed by a trained panel of 8 people, in individual cabins with red light. Each one of the panelists valued, in a non-structured 1-100 line scale, the following variables: lamb odor intensity, strange odors intensity, tenderness, juiciness, lamb flavor intensity, fatty flavors, strange flavors, quality of the flavor and global appreciation.

The meat was previously defrosted in water until reaching an internal temperature of 16-18 °C. This meat was later cut into slices of 2 cm thickness, which were then cooked in a double plate grill, preheated to 200 °C, until reaching an internal temperature of 70 °C. Finally, each piece was cut into prisms of 2 cm side, and maintained warm until the tasting time.

The analysis was made with plates serving four samples at each time, where were compared the 8 types of lamb meats, resulting of the following possible combinations: 2 Uruguayan products and the lamb meat of Germany, Spain and United Kingdom, aged for 7 or 20 days.

Statistical analysis

For the statistical analysis it was used the GLM procedure (General Linear Model) of the SAS.

The applied model used for evaluations of the results of the sensorial analysis determined differences between type of samples, considering the plate within each session, and the panelist as fixed effects. Also, the interaction panelist by animal type was introduced into the model. The statistical differences ($P < 0,05$) between types of samples of the least square means of each attribute were obtained by the Tukey test.

Results and Discussion

Characterization of Uruguayan products

In Table 2, the results obtained in carcass and meat quality traits (tenderness, WB Warner Bratzler, pH and meat color) for both Uruguayan products are summarized in table 2, in relation to the average of the European carcass and meat samples.

It was observed that the Uruguayan carcasses have a similar morphology and composition to those coming from the European lambs. The linear measures and the percentage of different tissues from the Uruguayan light lamb carcass were within the expected ranks observed for the European light lamb carcass. The composition of the Uruguayan heavy lamb carcasses was also located within the expectable ranks for this type of product under European conditions, but much closer to the lowest values reported.

The pH of the Uruguayan lamb meat was slightly high, and its texture and meat color (as much for the light lambs as for the heavy ones) fell down within the expected values. The explanation of higher red intensity of Uruguayan meat could be associated with the grass fed production system.

Sensorial evaluation

The results of the sensorial panel evaluation are presented in Tables 3 and 4 and in Figure 1. In Table 3, can be seen the importance of the different main effects as well as those related to the interaction occurred between lamb type and panelist. In Table 4, are shown the corrected averages of the different sensorial attributes for the eight lamb types analyzed. The results of the analysis of main components are represented in the Figure 1.

The results presented in Table 3 highlighted that the effect of the lamb type was significant, or at least showed a tendency, for all the attributes of the sensorial profile, with the exception for the intensity of lamb flavor.

From the information gathered by the panel (Table 4), can be appreciated that, the greater lamb odor intensity occurred in the Spanish lamb aged by 7 days, because this product is familiar for the Spanish panel, which is associated to the "natural" lamb aroma. Nevertheless, this intensity of lamb odor was significantly higher ($P < 0,05$) than the Uruguayan light lamb. Within the European lambs, the greater notes for the strange odors were associated with the longer ageing period. This could be related to the normal effect caused by ageing, which results in the development of some aromas derived from rancidity and microbial contaminations, modifying the normal odor of meat. This increase in strange odors is especially remarkable in the meat of younger animals of Spain and Germany which, by their peculiar structure and muscular physiology, are more sensitive to longer ageing periods. This effect did not occur for the lamb meat of the Uruguayan animals.

The highest values of tenderness were obtained in the meat of the youngest animals aged by 20 days, followed by the rest of the lamb meats aged by 20 days and finally the meat provided by the olderer lambs with 7 days of ageing. These results give an idea about the importance of the ageing, over other productive, industrial or processing aspects, in the attainment of tender meats. Possibly, because of its greater intra muscular fat content and, to a certain extent, for its high pH values, the meat of the Uruguayan heavy lambs was juicier than the meat of the German animals aged by 7 days.

Lamb flavor was not significantly different ($P > 0.05$) between the different types of meats evaluated. On the other hand, the greater greasy flavor was obtained in the meat of Spanish and the heavy Uruguayan lambs, although the differences were significant only between the Spanish and German lamb meat aged by 7 days. The fat of the Spanish animals was presumably more unsaturated, with lower temperature of fusion, and the greater fat cover of the Uruguayan heavy lamb, can help to explain these results. Greater strange flavors were detected, within each country, particularly in the meat with greater ageing times. In general, the German meat, mainly aged for 7 days, was the one that presented the lowest notes of strange flavors.

For the group of panelists, the Spanish meat, especially the most aged, and the one of heavy Uruguayan lambs, were those that obtained the lowest acceptability notes. This result can be related to the greater presence of abnormal and greasy flavors, not well accepted by panelists, and to the excessive ageing period of the local meat.

In Figure 1, it can be shown the way that the different types of lambs are grouped according to the principal component analysis performed. The panel separates by the vertical axis the youngest, Spanish and German animals, from the older animals of less intensive production system. This axis is mainly associated to lamb odor and flavor. The horizontal axis, which is associated to descriptors of acceptability and tenderness as well

as greasy and strange flavors and odors, separate basically the lambs of Germany and Spain, leaving grass animals in an intermediate situation.

Conclusions

The carcass of the Uruguayan light lambs presented, despite of the differences in size, very similar characteristics to those of the heavy lambs. In general, the Uruguayan products could be penalized for an excessive degree of fatness, but just in the case of the countries of the Mediterranean area.

The Uruguayan meat gives values of pH slightly elevated, which could be indicated that some pre-slaughter management techniques might be improved.

The Uruguayan lamb meat has aromatic and textural characteristics not very different from those of the European lamb types compared, especially to the German and British types aged by 20 days.

Meat of the youngest animals aged for 20 days was the tenderest, highlighting the importance of the ageing period to obtain good tender values for the lamb meat.

Long ageing periods (20 days) of the lamb meat, on the other hand, tend to develop especial aromas (abnormal and greasy flavors) that modify the normal odor of meat, which were not well accepted by the tasting panelists.

It does not appear a clear relationship between morphology, carcass weight and acceptability of the Uruguayan lamb types, there was a slight tendency from the experts to reject the meat coming from a carcass with more fat content.

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Tables and Figures

Table 1. Comparisons of carcass and meat quality of the Uruguayan and European lamb types analyzed.

	CCW (kg)	Conformation (1-5)	Fat cover (1-5)	pH
Uruguayan Light	11.1	2.5 (O+)	2.3	5.78
Uruguayan Heavy	19.4	2.8 (R)	2.9	5.80
German	23.2	2.6 (R-)	2.8	-
Spanish	10.2	1.0 (P)	1.8	-
British	22.8	3.6 (O-)	3.0	-

Note: CCW = cold carcass weight.

The conformation and fat cover scores were determined according to the European Union standards (SEUROP) (CEE (1991)).

Table 2. Carcass and meat quality traits compared between Uruguayan and European products. European information was obtained from Sañudo (personal communication).

Products	Uruguayan Light lamb	Uruguayan Heavy lamb	Variability in Europe	
			Light intensive lamb (local breed)	Grass lamb (meat breed)
Carcass quality				
Carcass length (cm)	55.8	63.1	50-55	60-65
Leg length (cm)	34.3	26.0	35-37	33-36
Fat (%)	10.3	11.6	9-11	10-14
Muscle (%)	60.9	61.5	59-61	60-63
Bone (%)	25.0	22.4	24-27	22-25
Other (%)	3.8	4.5	2-4	2-4
Meat quality				
pH	5.77	5.85	5.5-5.6	5.6-5.7
Tenderness (7 days)	3.41	2.85	3-4	2-3
Tenderness (20 days)		2.47	1.5-2.0	
L *	41.5	40.2	40-45	35-40
a *	18.5	20.0	10-15	15-20
b *	9.3	10.0	5-7	6.0-8.0

Table 3. Statistical significance of the different main effects and their interactions on diverse sensorial attributes of lamb meat.

	Lamb odor intensity	Strange odor intensity	Tenderness	Juiciness	Lamb flavor intensity	Fat flavor intensity	Strange flavor Intensity	Flavor quality	Global acceptability
Lamb type (T)	+	***	***	+	NS	**	***	***	***
Panelist (P)	***	***	***	***	***	***	***	***	***
Plate (Session)	***	*	***	***	***	***	***	***	***
T*P	+	*	NS	NS	***	***	**	***	***
Root MSE	1.24	1.63	1.24	1.53	1.15	1.35	1.87	1.35	1.41

Note: NS= not significant differences; + = P<0.10; * = P< 0.05; ** = P<0.01; *** = P< 0.001.

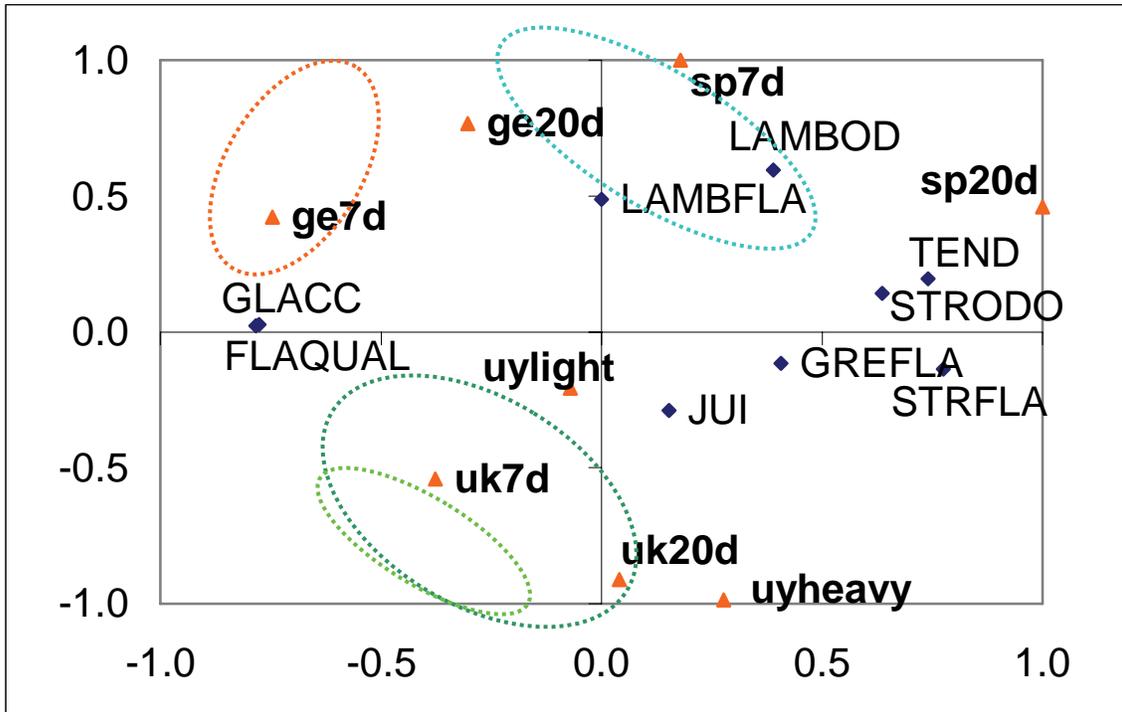
RMSE: Residual Mean Square Error

Table 4. Comparison of different sensorial quality traits between Uruguayan and European lamb types.

	Lamb odor intensity	Strange odor intensity	Tenderness	Juiciness	Lamb flavor intensity	Fat flavor intensity	Strange flavor Intensity	Flavor quality	Global acceptability
Uruguayan Light	4.51 b	2,80 c	6,96 bc	4,94 ab	5,91	4,55 ab	4.29 cd	4,08 ab	3,94 ab
Uruguayan heavy	4,71 ab	3,02 c	7.23 b	5,19 a	5,80	4,74 ab	4.78 b	3.96 b	3.87 b
Spain 7 days	5,03 a	3,12 c	7,38 a	5,07 ab	6,11	4,83 a	4.76 b	3.87 b	3.78 b
Spain 20 days	4,87 ab	3,35 a	7,73 a	4,89 ab	5,95	4,57 ab	5,58 a	3,25 c	3,14 c
Germany 7 days	4,82 ab	2,59 c	6,53 c	4.64 b	5,83	4.28 b	3.69 d	4,62 a	4,43 a
Germany 20 days	4,84 ab	3,35 a	7,31 a	4,82 ab	6,01	4,47 ab	3.90 cd	4,41 ab	4,29 ab
UK 7 days	4,77 ab	2,74 c	6,65 c	4,81 ab	5,76	4,29 ab	4.39 cd	4,25 ab	4,06 ab
UK 20 days	4,80 ab	3.13 b	7.15 b	4,96 ab	5,75	4,43 ab	4,55 bc	4,09 ab	3,97 ab

Note: a, b, c, d: different letters between the lamb type indicate significant differences (P<0.05).

Figure 1. Analysis of main components between variables and lamb types (ge: German; sp: Spanish; uk: British; 7d and 20d: meat aged 7 and 20 days; uylight: Uruguayan light; uyheavy: Uruguayan heavy).



Note: LAMBOD: lamb odor intensity; LAMBFLA: lamb flavor intensity; STRODO: strange odor intensity; TEND: tenderness; JUI: juiciness; GLAPP: global acceptability; FLAQUAL: flavor quality; GREFLA: greasy flavor intensity and STRFLA: strange flavor intensity.

SENSORY EVALUATION OF BEEF MEAT PRODUCED UNDER DIFFERENT PRODUCTION SYSTEMS OF URUGUAY

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Key Words: beef meat quality, sensorial evaluation, production systems.

Introduction

The world-wide marketing for beef meat is very complex, mainly due to the presence of certain animal diseases, production costs and sale prices.

Within the global consumption of meat, the competition between animal species, different cooking and eating habits in each country impose limits for the growth of a determined sector. In Europe, there is a possibility for increasing beef meat market, since European countries stay with annual beef meat consumptions quite low (18.9 kg *per capita*) (Simpson, 2004).

The beef meat sector in Europe, mainly intensive orientated systems, has faced, in the last years, so many problems originated by the increasing constrain linked with the concept that red meat is a food product of low quality, no tender and potentially dangerous for human health. The excellent image given by consumers to those products which are coming from extensive production systems could benefit countries like Uruguay.

The scientific and technical information are basic and necessary platforms to promote and to valorize red meat products at world-wide level. Uruguayan exports of beef meat products towards to the international market can be limited if this country does not face key strategies for generating information in this especial area.

Objectives

To characterize and to analyze comparatively the sensorial quality of two Uruguayan beef products in relation to alternative products coming from three different European countries.

Material and methods

Animals of Uruguay

In Uruguay, 40 animals coming from two types of products were analyzed: steers of 2 or 3 years of age at slaughter. All the animals were castrated male of the Hereford breed (the main beef breed of Uruguay), raised under exclusive grazing conditions on native rangelands and fattened on improved pastures at the Central Progeny Test of the Uruguayan Hereford Breed Association.. The steers were slaughtered under commercial conditions, with 428.5 and 519.0 kg of average live weight for the 2 and 3 years old, respectively. Both types of steers generate the main beef products that Uruguay export to the international market.

Animals of Europe

In Europe, one commercial local product in three European countries (Germany, Spain and the United Kingdom) with two ageing times (7 and 20 days) were compared with the Uruguayan beef samples, using 20 animals by country. In Table 1, the main characteristics of the European products are compared with Uruguayan types.

In Germany, the animals were bulls, from Fleckvieh breed, although some Limousine crosses were included. Animals were finished in confinement with corn silage *ad libitum*, complemented with restricted amounts of concentrates, made of soybean meal and cereal grains. The slaughter age and carcass weight ranged from 19 to 24 months and from 283.2 to 447 kg, respectively.

Spanish animals were Holstein breed bulls, weaned and fed in an intensive concentrate system, were animals received diets with high energy concentration and cereal straw *ad libitum*. The slaughter age and carcass weight ranged from 19 to 24 months and from 209 to 254 kg, respectively.

In the United Kingdom, the animals were castrated males with very different genetic basis (crossbreeds between Devon, Hereford, Charolaise and Limousine and pure breeds like Friesian and Fleckvieh), finished basically on a grass diet, complemented with concentrates. The slaughter age and carcass weight ranged from 18 to 22 months and from 280 to 352 kg, respectively.

General Sampling

The muscle *Longissimus dorsi* (LD) was taken of all the animals for each country after 48 hours of slaughtering. In Uruguay, it was divided into pieces, which were vacuum packaged and aged for 20 days, which represents the regular aging period of the meat exported to Europe.

In Europe, the meat samples were aged during 7 days (most common situation in Europe) and for 20 days (similar to the Uruguayan ageing time).

Sensorial evaluation

The sensorial analysis was made with a trained panel of 10 people, in individual cabins with red light. Each panelists valued in a non-structured 1-100 mm line scales

(Sañudo *et al*, 2003) the following variables: beef odor intensity, strange odors intensity, tenderness, juiciness, beef flavor intensity, greasy flavors, strange flavors, quality of the flavor and global appreciation.

The meat was previously defrosted in water until reaching an internal temperature of 16-18 °C. The meat pieces were later cut into slices of 2 cm thickness, which were cooked in a double plate grill, preheated to 200 °C, until reaching an internal temperature of 70 °C. Each piece was cut in prisms of 2 cm side, and maintained warm until the tasting time began.

The analysis, performed with plates with four samples offered to panelists, included 8 types of beef, where all possible combinations were tested: 2 Uruguayan products and 6 meat samples coming from Germany, Spain and United Kingdom, aged for 7 or 20 days.

Statistical analysis

For the statistical analysis, it was used the GLM procedure (General Linear Model) of the SAS.

The applied model used for evaluations of the results of the sensorial analysis determined differences between type of samples, considering the plate within each session, and the panelist as fixed effects. Also, the interaction panelist by animal type was introduced into the model. The statistical differences ($P < 0,05$) between types of samples of the least square averages of each attribute were obtained by the Tukey test.

Results and Discussion

Characterization of Uruguayan products

In Table 2, the results obtained in carcass and meat quality traits (tenderness, WB Warner Bratzler, pH and meat color) for both Uruguayan products are summarized in table 2, in relation to the average of the European carcass and meat samples.

It is observed that the Uruguayan carcass have similar tissue composition compared to those of the young bulls of local breeds, they are longer and they have legs of similar conformation to the European ones. With the exception of the pH value (a little high), the rest of the characteristics defined by instrumental quality analysis, were located within the normal limits of acceptance for the beef meat in Europe.

Sensorial evaluation

The results of the sensorial panel evaluation are shown in Tables 3 and 4 and in Figure 1. In Table 3, it is presented the importance of the different main effects and the interaction between animal type and panelist. The corrected averages of the different sensorial attributes for the different beef types analyzed are presented in Table 4. The results of the analysis of main components are shown in Figure 1.

It was observed that the effect of the beef type was very significant for all the attributes of the sensorial profile (Table 3).

The Table 4 shows that, in the opinion of the tasting panel, the smaller intensity of beef odor occurred in the youngest animals, particularly for bull calves of Spanish origin and in the British meat aged for 20 days. The higher strange odors were associated to the Spanish and UK meats aged by 20 days, which is an expected result because the ageing tends to develop aromas from rancidity and microbial contamination that modify the normal odor of the meat. The lower note was for the British meat aged 7 days, possibly due to the fact that this test was developed by an English panel that would be familiarized with this type of meat.

The greater values of tenderness were observed in the meat of the British animals, followed by the meat of the youngest animals aged for 7 or 20 days and the Uruguayan meat, and finally in the meat of the German animals aged during 7 days. These results indicate the importance of the ageing time over other productive, industrial or processing aspects in order to obtain tender meats. Possibly, given by its greater fat content and its higher pH, the meat of the Uruguayan animals was the juiciest, followed by the 7 days aged Spanish meat, associated to younger animals, and the British meat of 7 days of ageing. The British and Spanish meat aged during 20 days, together to the German of 7 days, had the lower notes of juiciness.

The higher values of beef flavor were obtained for the British meat aged for 7 days, followed by the Uruguayan meat, possibly because it came from older animals. The lower notes of beef flavor were for the British meat aged for 20 days. The greater greasy flavor was observed in the Uruguayan and German meats and the lower in the Spanish and British meats, not presenting a clear relationship between these notes and the amount of intramuscular fat observed in the different meat types under studying, with the exception of the Spanish meat. The higher strange flavors were detected clearly in the British meat with 20 days of ageing, with very important differences compared to the rest of the meat types tested. These results can be explained by the occurrence of a particular problem, probably happened during ageing, since the meat coming from this country, aged only for 7 days, was the one that presented the lowest value on this parameter.

For the group of panelists, the British meat with 7 days of ageing was the most accepted, followed by the Uruguayan and German meats aged by 20 days. The Spanish meat, a product not well known because it came from a very intensive production system, and the German and British meats with 20 days of ageing, obtained the lower notes of acceptability (Figure 1). This result can be related to a greater presence of strange, little tempting flavors, more than associations with problems of texture, since, in fact, the tenderness would be more associated, as it can be appreciated in the Figure 1, to the located meats in a quadrant opposed to the one of the acceptability.

Conclusions

The Uruguayan meat presents values of pH a little elevated, which could be indicating that some pre-slaughter management techniques might be improved.

In general, the greater values of tenderness were observed in the meat of the youngest animals aged for 7 or 20 days and in the British and Uruguayan meats.

Long ageing periods (20 days) for beef meat, tend to develop especial aromas (abnormal and greasy flavors) that modify the normal odor and flavor of the meat, which are not well accepted by the tasting panelists.

Under the sensorial point of view, the most accepted meat was the British aged for 7 days, while both Uruguayan types occupied an intermediate position between the different European meats analyzed, demonstrating their good adaptability to the long ageing periods. There were not differences in meat characteristics between the Uruguayan steers with different ages.

It can not be assessed that the carcass weight, fat content or index of compactness of the carcass affect the acceptability of the Uruguayan meat, determined by a panel of experts.

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Tables and Figures

Table 1. Summary of some carcass and meat quality traits of the Uruguayan and European beef types analyzed.

	CCW (kg)	Conformation (1-5)	Fat cover (1-5)	pH 24 hours
Uruguayan 2 years	224.8	2.8 (R)	3.0	5.60
Uruguayan 3 years	282.0	3.2 (R)	3.5	5.71
German	382.4	3.4 (R+)	2.6	5.69
Spanish	229.0	2.0 (O)	2.0	5.42
British	313.3	2.5 (O+)	3.2	5.57

Note: CCW = cold carcass weight. Conformation and fat cover according to European Union System (SEUROP), CEE (1991).

Table 2. Carcass and meat quality traits compared between Uruguayan and European products. European information was obtained from Sañudo (personal communication).

	Two years steer	Three years steers	Intensive beef (local breed)	Intensive beef (meat breed)
Carcass quality				
Carcass length (cm)	132,6	138,2	130	120
Leg perimeter (cm)	103,6	110,1	105	110
Leg length (cm)	76,8	80,3	80	75
Fat (%)	11,9	13,6	12-16	5-10
Muscle (%)	63,2	62,7	58-65	70-75
Bone (%)	22,1	21,0	18-21	15-17
Others (%)	2,8	2,7	2-4	2-4
Meat quality				
pH	5,60	5,71	5,5-5,6	5,6-5,7
Tenderness (7 days)	4,9	4,7	4,5-5	4,5-5
Tenderness (20 days)	4,4	4,8	4-4,5	4-4,5
L *	37,0	35,6	40-41	37-38
a *	20,0	15,9	15-16	17-18
b *	10,4	6,9	9-10	9-10

Table 3. Statistical significance of the different effects on a broad sensorial attributes of beef meat.

	Beef odor intensity	Strange odor intensity	Tenderne ss	Juicines s	Beef flavor intensit y	Greasy flavor intensity	Strange flavor intensity	Flavor quality	Global acceptabili ty
Beef type (T)	***	***	***	***	***	***	***	***	***
Panelist (P)	***	***	***	***	***	***	***	***	***
Plate (session)	***	***	***	***	***	**	***	***	***
T*P	***	***	NS	NS	***	***	***	***	***
RMSE	1,21	1,59	1,78	1,64	1,44	1,03	1,70	1,68	1,60

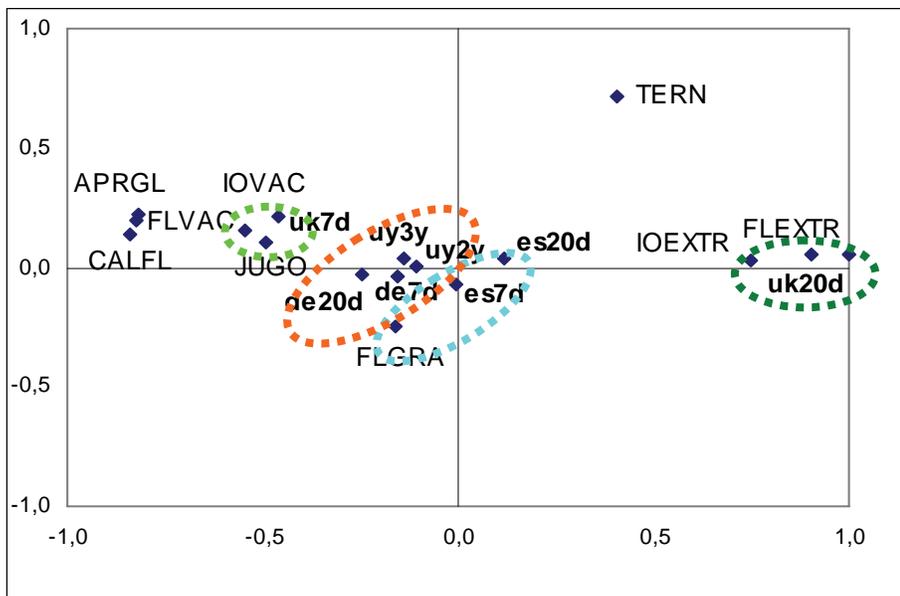
Note: NS: not significant differences; *** = P < 0.0001.
RMSE: Residual Mean Square Error

Table 4. Average global score of the beef meat given by the tasters.

Type of animal	Beef odor intensity	Strange odor intensity	Tenderness	Juiciness	Beef flavor intensity	Greasy flavor intensity	Strange flavor intensity	Flavor quality	Global acceptability
UY	2,82 ^{ab}	2,24 ^b	3,79 ^b	3,62 ^{ab}	2,77 ^{bc}	1,60 ^a	2,61 ^{bcd}	2,92 ^{bc}	2,65 ^{bc}
2 years									
UY	2,69 ^{bc}	2,13 ^b	3,83 ^b	3,93 ^a	2,93 ^b	1,56 ^{ab}	2,49 ^{cd}	2,93 ^{bc}	2,67 ^{bc}
3 years									
DE 7d	2,81 ^{ab}	1,82 ^{bc}	2,62 ^c	3,24 ^b	2,66 ^{bcd}	1,52 ^{ab}	2,43 ^{cd}	2,88 ^{bc}	2,22 ^c
DE 20d	2,90 ^{ab}	1,77 ^{bc}	3,73 ^b	3,58 ^{ab}	3,11 ^b	1,52 ^{ab}	2,24 ^d	3,28 ^b	2,87 ^b
ES 7d	2,52 ^{bc}	2,22 ^b	3,96 ^{ab}	3,62 ^{ab}	2,43 ^{cd}	1,30 ^{abc}	2,94 ^{bc}	2,71 ^{bc}	2,41 ^{bc}
ES 20d	2,51 ^{bc}	2,79 ^a	4,11 ^{ab}	3,21 ^b	2,27 ^d	1,13 ^c	3,19 ^b	2,56 ^c	2,24 ^c
UK 7d	3,19 ^a	1,44 ^c	4,25 ^{ab}	3,71 ^{ab}	3,82 ^a	1,35 ^{abc}	1,56 ^e	4,16 ^a	3,49 ^a
UK 20d	2,38 ^c	3,25 ^a	4,54 ^a	3,20 ^b	1,22 ^e	1,22 ^{bc}	6,78 ^a	0,74 ^d	0,77 ^d

Note: different letters between a type indicate significant differences (P<0.05).
 UY = Uruguay, DE = Germany; ES = Spain; UK = United Kingdom.

Figure 1. Analysis of main components between variables and beef type (de: German; es: Spanish; uk: British: 7 and 20 days: aged meat for 7 or 20 days; uy2y: Uruguayan 2 years; uy3y: Uruguayan 3 years).



Note: BEEFOD: beef odor intensity; GLAPP: global appreciation; FLAQUAL: flavor quality; STRFLA: strange flavor intensity; GREFLA: greasy flavor intensity; BEEFLA: beef flavor intensity; JUIC: juiciness; STRODO: strange odor intensity; TEND: tenderness.

EFFECT OF GRAIN-BASED VERSUS FORAGE-BASED FEEDING PRACTICES ON THE CONJUGATED LINOLEIC ACID CONTENT OF LAMB

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Key Words: Conjugated Linoleic Acid, Lamb, Fatty Acids, Forage

Introduction

Lamb per capita consumption has been relatively constant over the past several years, representing less than one half of a percent of the total per capita consumption (AMI, 2003). With poultry, seafood, and red meat per capita consumption at 221 lbs., there appears to be opportunity for the lamb industry to capture a bigger portion of the consumer's plate.

Lamb is positioned to take advantage of the current trends surrounding consumer preference toward food products raised under natural conditions with beneficial nutritional attributes. For example, there appears to be considerable interest in the potential health benefits of ruminant, grass-fed animal products versus grain fed products. Chin et al. (1994) reported that ruminant derived animal products contained higher levels of conjugated linoleic acid (CLA), a fatty acid isomer that has been reported to have potential human health benefits. Such benefits include anticarcinogenic (Ip et al., 1994; Parodi, 1994; Visonneau et al., 1997) as well as antidiabetic (Houseknecht et al., 1998) effects.

In particular, grass-fed, beef and dairy products appear to be higher in conjugated linoleic acid relative to food products raised under other feeding practices (Stanton et al., 1997; Kelly et al., 1998; French et al., 2000; Poulson et al., 2004; Noci et al., 2005). While there appears to be an increasing pool of knowledge surrounding beef and dairy products, much less is known regarding the health benefits of lamb, specifically the concentration of conjugated linoleic acid.

Objectives

The objective of this study was to determine the effect of grain-based versus forage-based feeding practices on the conjugated linoleic acid content of lamb.

Methodology

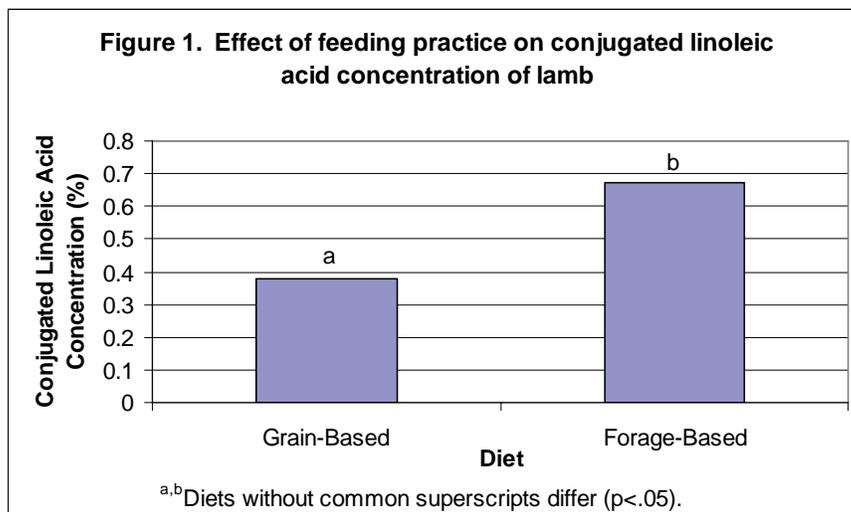
Twenty-three crossbred, wether lambs from the California State University, Chico Agricultural Teaching and Research Center were randomly assigned to one of two finishing diets (grain-based or forage-based) across three sire breed types (Silverdale, White Dorper, and Blackface) post-weaning. The grain-based diet consisted primarily of corn supplemented with alfalfa hay at a rate of 39% while the forage-based diet consisted

primarily of alfalfa hay supplemented with almonds hulls at a rate of 18%. Lambs were maintained on a raised floor facility in diet X breed treatment groups during the finishing phase of the trial. Animals in all treatment groups had ad libitum access to feed and water. Lambs were harvested at a constant backfat of .16 in. as determined by real-time ultrasound (Aloka 500) between the 12th and 13th ribs. All lambs were processed through a federally inspected plant. At fabrication of each lamb carcass, *longissimus* muscle was obtained from matching locations (post-12th rib) from each lamb, aged 14 d at 34° C, and then frozen until the lipid analysis.

Prior to lipid extraction, *longissimus* samples were thawed and ground. Lipids were extracted from lamb samples in triplicate using a modified version of the Stanton et al. (1997) procedure. Lipid extraction included three phases: sample preparation and lipid extraction, lipid separation, and methylation of total fatty acids with sodium methoxide. Conjugated linoleic acid (cis-9, trans-11-18:2; CLA) concentration of each *longissimus* sample was then determined using gas chromatography using procedures described by French et al. (2000). Data were analyzed using ANOVA as a 2X3 factorial, fitting diet and sire breed as main effects (Statistix 8, 2003).

Results & Discussion

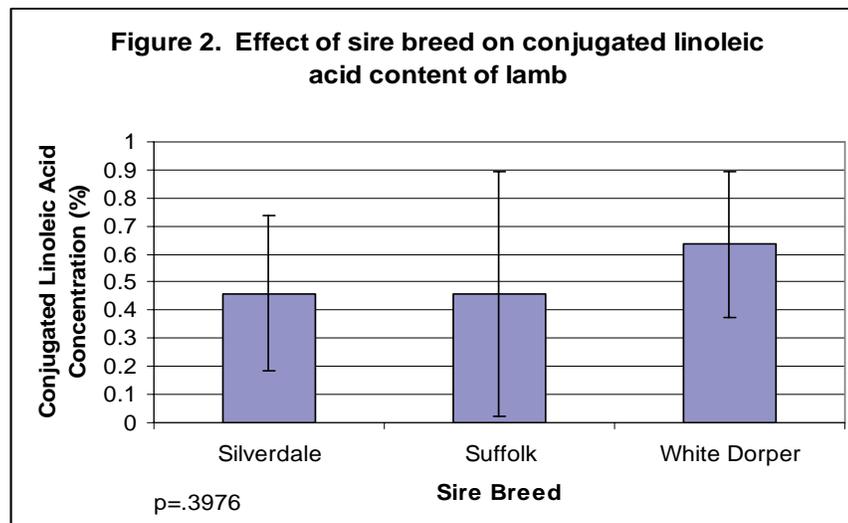
The CLA content of lamb finished on a forage-based diet was significantly higher ($p=.0256$) compared to lamb finished on a grain-based diet (Figure 1). Forage-finished lamb appeared to be .34% higher in CLA content compared to grain-finished lamb. Nuernberg et al. (2001) found similar findings, reporting enhanced CLA concentrations in pasture-fed lamb. Similarly, Aurousseau et al. (2004) reported that grass fed lamb was preferred compared to stall fed lamb from a human health perspective due to its higher concentration of CLA. Researchers also found grass fed lamb exhibited a more favorable omega-6 to omega-3 ratio.



Similar trends have been found in beef and dairy products. French et al. (2000) reported favorable fatty acid profiles for beef fed forage-based rations relative to those with higher levels of concentrates. Researchers reported higher concentrations of

polyunsaturated fatty acids, lower concentrations of saturated fatty acids, more favorable omega-6:omega-3 ratio, and increased CLA as the forage content of rations increased. Similarly, Poulson et al. (2004) reported that beef raised on forage and pasture exhibited enhanced CLA concentrations. Stanton et al. (1997) and Kelly et al. (1998) both reported that the inclusion of forage in the diet of dairy cows increased the CLA content of milk. Research suggests that the inclusion of forage appears to improve the CLA concentration in ruminant animal products.

Sire breed (Figure 2) did not appear to have a significant effect on CLA content of lamb finished on forage versus grain, nor was there a significant diet X breed interaction ($p > .05$). Demirel et al. (2004) also investigated breed effects along with the addition of polyunsaturated fatty acids to diets as potential factors influencing CLA concentration in lamb; as with the present study, breed did not appear to be a significant source of variation. However, researchers did find that adding polyunsaturated fats to diets enhanced CLA concentrations in lamb meat.



Conclusions

Results suggest that the conjugated linoleic acid profile of lamb may be improved from a human health perspective when forage is included in finishing diets of lamb. However, further research is needed to investigate potential sire breed effects on the CLA content in lamb *longissimus* muscle.

Acknowledgements

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EFFECT OF CARCASS LEANNESS IN PIGS ON PROTEIN CONTENT AND CHEMICAL COMPOSITION OF PRIME CUTS

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Key words: pork, leanness, protein content, chemical composition

Introduction

In recent years, there have been significant changes in the way of meat raw materials are produced in Poland. These changes mainly concern the choice of breeds and pig fattening methods. Previously dominant pure native breeds of pigs, raised for meat and lard (mainly Polish Large White and Puławska) have been replaced by crossbreds with an increasing percentage of genes from the high-lean breeds Pietrain, Duroc and Hampshire. One positive result has been a relatively rapid increase in pig leanness, estimated in the 1990s to be approximately 1% per year for the whole population. Recently, this growing trend has slowed, and is likely to disappear once uniform and viable fattening methods are introduced. The negative effect of genetic change is a considerable increase in the output of poorer quality meat (mainly PSE and acidic meat). Whether and how the increase in leanness affects the level of protein and intramuscular fat has yet to be established.

Objectives

The objective of this study was to assess the current protein content and chemical composition of lean pork in Poland due to changes in pig leanness.

Methodology

Study material: Taking advantage of an opportunity provided by experiments aimed at testing carcass leanness equipment in slaughter lines, we made detailed analyses of the chemical composition of meat from the prime cuts (ham, shoulder and loin) of 59 carcass sides of pigs (33 gilts and 26 barrows) differing in slaughter weight and leanness. The experimental animals were purchased in two different regions of Poland.

Methods: The carcass sides of the analysed animals were dissected according to Walstra and Markus (1) and the quantity of lean meat from particular elements of the dissected carcass was determined. Protein content was determined according to the Kjeldahl method, fat content using the method of Soxhlet, collagen content according to the Stegemann-Stalder method, and water content using the drying method. All the analytical methods were compliant with ISO standards.

The results were analysed statistically by estimating the distribution of the analysed parameters and their interrelationships in the correlation and regression calculus using Statgraphics Plus v. 3.0 software.

Results and Discussion

The data and range of leanness and weight postmortem are given in Table 1.

The data, frequency, mean \bar{X} , standard deviation S_x and coefficient of variance V_x obtained for intramuscular fat content (IFC), crude protein content (PC) and connective tissue protein (collagen) (CC) are given in Table 2. Table 3 presents the relative parameters characterizing the protein content of meat, Feder's number (FN) which is a ratio between the water content and crude protein content of meat, protein fat free (PFF), and the protein content ratio (PCR) percentage of CC in PC.

Comparison of the results obtained and statistical analysis showed that sex difference had no significant effect on the parameters studied, while differences between the mean parameters of particular carcass elements generally proved significant.

The intramuscular fat content of the meat of the analysed carcass elements is not considerably different from that of the meat of Polish Large White pigs tested in the 1970s (2). The protein content of loin and ham is approximately 1.2-2.2% lower and the protein content of shoulder is comparable. We have no comparative data for the content of connective tissue proteins, but it is worth noting the low level of these proteins, especially in ham. It is known that connective tissue proteins bind much less water than do muscle proteins and their reduced level may be reflected in greater quantities of meat and thus a lower meat protein content.

The results of the correlation analyses are illustrated in Table 4. The correlation analysis revealed that increased carcass leanness is accompanied by significant increases in the protein content of meat (PC) and in PFF, and by a decreased, or of Feder's number (FN). This finding contradicts the popular belief among the practitioners that increased carcass leanness reduces the protein content and thus negatively affects the suitability of meat for processing. There was no significant effect of carcass weight on the chemical composition of the meat.

The correlation analysis confirmed the well-known fact that the chemical composition of meat is specific to individual animals, and a high level of a component (e.g. protein) in one carcass element is indicative of a high level in the other elements. This relationship is particularly close for loin compared to ham, and for loin compared to shoulder.

Conclusions

- Pig carcass leanness affects the intramuscular fat content (IFC) of carcass elements to a small degree, but significantly affects the level of crude protein (PC), Feder's number (FN) and the protein fat free (PFF).
- Postmortem carcass weight had little effect on the chemical composition of lean meat in the analysed elements.
- Animal sex was found to have no significant effect on the chemical composition of the lean meat of the elements tested.

- The protein content of the meat of loins and hams in the analysed carcasses was considerably lower compared to carcasses studied at our institute in the 1970s.
- The chemical composition of the lean meat of pigs is specific to individual animals, and the composition of loin is most representative of the chemical composition of the other carcass elements.

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Table 1. Number of animals and variation in carcass leanness and weight postmortem.

Animals	Frequency/n/	Leanness [%]			Carcass weight postmortem [kg]		
		maximum	mean	minimum	maximum	mean	minimum
gilts	33	59.8	52.3	43.5	92.0	77.1	61.4
barrows	26	59.5	51.5	42.0	99.3	79.2	62.2

Table 2. Data obtained for intramuscular fat (IFC), crude protein (PC) and connective tissue protein – collagen (CC)

parameter	n	Loin			Ham			Shoulder		
		\bar{X}	Sx	Vx	\bar{X}	Sx	Vx	\bar{X}	Sx	Vx
Fat content (IFC) %										
gilts	33	5.0	1.4	28.0	3.9	1.0	26.0	4.5	2.5	35.0
barrows	26	5.1	2.1	41.4	4.0	1.3	31.1	4.2	1.1	26.6
total	59	5.0	1.7	34.5	4.0	1.1	28.2	4.3	1.4	31.9
Weighted mean	59	4.34				1.05				24.21
Crude protein content (PC) %										
gilts	33	21.6	1.3	5.9	20.5	1.2	5.6	19.7	0.9	4.5
barrows	26	21.4	1.3	6.2	20.1	1.3	6.5	19.5	0.9	4.7
total	59	21.5	1.3	6.0	20.3	1.2	6.0	19.6	0.9	4.6
Weighted mean	59	20.5				1.1				5.2
Content of connective tissue protein (CC)%										
gilts	33	0.99	0.23	23.2	0.76	0.16	21.4	1.07	0.16	14.7
barrows	26	0.95	0.14	14.7	0.75	0.11	14.0	1.09	0.24	21.6
total	59	0.97	0.19	20.0	0.75	0.11	15.9	1.08	0.20	18.0

Table 3. Relative parameters of meat protein content

elements		loin			ham			shoulder		
Parameter	animals	\bar{X}	Sx	Vx	\bar{X}	Sx	Vx	\bar{X}	Sx	Vx
Feder's number (FN) [-]	gilts	3.35	0.25	7.5	3.63	0.23	6.4	3.78	0.17	4.6
	barrows	3.38	0.26	7.6	3.71	0.29	7.8	3.83	0.20	5.3
	total	3.36	0.25	7.5	3.66	0.26	7.1	3.80	0.19	4.9
Protein fat free (PFF) [%]	gilts	22.8	1.4	6.1	21.4	1.1	5.4	20.7	0.8	4.0
	barrows	22.5	1.4	6.1	20.9	1.4	6.7	20.3	1.0	4.7
	total	22.6	1.4	6.1	21.2	1.3	6.0	20.5	0.9	4.4
Protein content ratio (PCR) [%]	gilts	4.58	1.14	24.9	3.69	0.90	24.4	5.28	1.22	23.2
	barrows	4.45	0.70	15.8	3.75	0.62	16.5	5.62	1.22	21.6
	total	4.52	0.96	21.3	3.62	0.64	17.6	5.52	1.04	18.9

Table 4. Correlation coefficients between carcass leanness and weight and parameters of chemical composition of meat for carcasses, gilts and barrows in total (n=59)

	Parameters elements	fat content (IFC)	protein content (PC)	Feder's number (FN)	Protein fat free (PFF)	Protein content ratio (PCR)
Leanness	loin	- 0.238	0.570 ***	- 0.460 ***	0.487 ***	- 0.342 **
	ham	- 0.275 *	0.459 ***	- 0.408 **	0.393 **	- 0.187
	shoulder	- 0.206	0.438 ***	- 0.376 **	0.393 **	- 0.194
Carcass weight	loin	0.202	0.144	- 0.255 *	0.208	- 0.316 *
	ham	- 0.266 *	0.266 *	- 0.336 *	0.316 *	- 0.114
	shoulder	0.009	0.086	- 0.148	0.096	0.025

*** significant at probability level (spl.)0.001, **spl. 0,01, * spl. 0,05

Table 5. Correlation coefficients between parameters of chemical composition of meat of particular elements for gilt and barrow carcasses in total (n=59)

Parameters elements	Fat content (RFC)	Protein content (PC)	Feder's number (FN)	Protein fat free (PFF)	Protein content ratio (PCR)
Loin/ham	0.555 ***	0.782 ***	0.840 ***	0.835 ***	0.333 **
Loin/shoulder	0.336 **	0.652 ***	0.632 ***	0.624 ***	0.295 **
Ham/shoulder	0.251	0.589 ***	0.609 ***	0.586 ***	0.174

**EFFECT OF ELECTRICAL STIMULATION ON THE SENSORY
CHARACTERISTICS OF SPRINGBOK (ANTIDORCAS MARSUPIALIS)**

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Abstract

The effect of electrical stimulation of Springbok carcasses on the sensory qualities of *M.longissimus*, was evaluated in a completely randomised experimental design. Analysis of variance was performed on the data and Tukey's LSD were calculated at a 5% significance level to compare treatment means (SAS, 1990). Descriptive sensory analyses were performed on the meat samples. A trained six-member panel evaluated the meat for the following sensory attributes: Springbok aroma intensity, initial impression of juiciness, sustained juiciness, tenderness, residue and overall Springbok flavour by means of a eight-point structured line scale. The panellists were seated in individual booths in a temperature and light controlled room, receiving a set of two samples served in a complete randomised order. Applying electrical stimulation to Springbok carcasses were found to have had no significant effect ($P>0.05$) on the sensory characteristics of aroma intensity, initial juiciness, sustained juiciness, tenderness, residue and flavour.

Preparation of samples

Preparations of carcasses: Electrical Stimulation (ES) was applied on Springbok carcasses after death and the sensory qualities compared to samples where no electrical stimulation was applied (NES). *M.longissimus* samples from the two treatments (ES and NES) were defrosted at a temperature of 2-4°C for a period of 24 h. Meat samples was cut to uniform size and placed on foil-covered metal racks. Each metal rack was placed in a coded oven bag and a probe inserted into the centre of the meat. Samples were cooked at 160°C in two Defy 835 ovens connected to a computerised electronic temperature control system (Viljoen, Muller, De Swardt, Sadie & Vosloo, 2001) to an internal temperature of 68°C. The meat was allowed to rest for 5 minutes, in which time an endpoint temperature of 72°C were reached. Cubed samples (1.5 x 1.5 cm) were taken from the middle of each sample and individually wrapped in aluminium foil. The samples were placed in preheated, coded glass ramekins in a preheated oven of 100°C and evaluated within 10 minutes.

Sensory analysis

Descriptive sensory analyses were performed on the meat samples. A trained six-member panel evaluated the meat for the following sensory attributes: Springbok aroma intensity, initial impression of juiciness, sustained juiciness, tenderness, residue and overall Springbok flavour by means of a eight-point structured line scale. Table 1 depicts the definitions of the attributes used in the sensory analyses. The panellists were seated in individual booths in a temperature and light controlled room, receiving a set of two samples served in a complete randomised order. Crackers, apple slices and distilled water were used to cleanse the palate between samples.

Table 1: Verbal definitions of sensory attributes evaluated

ATTRIBUTE	VERBAL DEFINITION
Springbok aroma intensity	Take a few short sniffs as soon as you remove the foil.
Initial impression of juiciness	The amount of fluid exuded on the cut surface when pressed between the thumb and forefinger
Sustained juiciness	The impression that you form after the first two to three chews between the molar teeth
First bite	The impression of tenderness after the first two the three chews between the molar teeth
Residue	The amount of residue left in the mouth after the first twenty to thirty chews
Overall Springbok flavour	This is a combination of taste and swallowing

Statistical analysis of data

The experimental design consisted of a randomized complete block design with two treatments (ES and NES) replicated in 10 blocks. Analysis of variance was performed on

the data and Tukey's LSD were calculated at a 5% significance level to compare treatment means (SAS, 1990). See addendum B for statistical analysis of the data.

Results and discussion

The panel could not detect a significant difference ($P>0.05$) between the two treatments (electrical stimulated and not electrical stimulated) in any of the sensory attributes evaluated. Mean values for the attributes evaluated are given in Table 2. Although not significant ($P>0.05$), mean values for the electrical stimulated sample (ES) was higher than that of the NES sample for initial juiciness, sustained juiciness, tenderness and flavour. Mean values for the NES sample was higher for aroma intensity and residue, although this was not significant ($P>0.05$).

Table 2: Mean values for the sensory attributes evaluated

Attribute	Treatments		Tukey's LSD (5%)
	NES	ES	
Springbok aroma intensity	6.05 ^a	6.00 ^a	0.360
Initial juiciness	6.60 ^a	6.75 ^a	0.286
Sustained juiciness	6.43 ^a	6.52 ^a	0.256
Tenderness	6.43 ^a	6.55 ^a	0.390
Residue	6.68 ^a	6.67 ^a	0.245
Flavour	6.15 ^a	6.32 ^a	0.418

^aValues in the same row with different superscripts differ significantly ($P\leq 0.05$)

Conclusions

The panel could not detect a significant difference ($P>0.05$) between the two treatments (electrical stimulated and not electrical stimulated) in any of the sensory attributes evaluated (aroma intensity, initial juiciness, sustained juiciness, tenderness, residue and flavour).

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THE EFFECT OF DAY AND NIGHT CROPPING ON THE MEAT QUALITY OF IMPALA (*AEPYCEROS MELAMPUS*)

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Key Words: pH; game meat; harvesting; water holding capacity; colour; drip loss; cooking loss; shear force

Introduction

The impala's wide distribution in southern Africa and its relative abundance make it well suited to continuous cropping for game meat production (Bourgarel *et al*, 2002). In South Africa, the impala is the single most important species in the Lowveld and Bushveld areas in terms of its population numbers (Hoffman, 2000). A recent study by Eloff (2002) showed that impala are the most popular game species at game auctions, making up almost a third of the total animals sold. This is the case because of their relative abundance when compared to other species and the fact that new game farmers who wish to establish game populations on their farms are able to buy them without stretching their financial resources. They tend to be seen as the "bread and butter" of a game farming operation because of their rapid population growth rates (Fairall, 1985) and so they are easily traded. The smaller and newer game farms do not usually have resident predator populations. Without this natural form of population control it soon becomes necessary to crop animals in order to prevent overgrazing and destructive interspecies competition. In the light of this growing industry the development of efficient cropping methods for game has become an aspect that requires urgent attention.

Objectives

This study was conducted to determine the effects of day- and nighttime cropping on the meat quality parameters of impala.

Methodology

The cropping of impala herds took place at the Mara Research Station (23° 05' S and 29° 25' E; 961 m.a.s.l.) in the Limpopo Province, South Africa. During the nighttime operation 16 animals of random age and sex were harvested. Targeted animals were shot high in the neck or the head. Ambient temperatures varied between 2-11 °C. During the daytime, 24 animals were harvested. The animals were hunted on foot and high neck shots were used. Ambient temperatures varied between 19-27 °C.

Following the shooting, the dead animals were immediately exsanguinated. pH (pH₄₅) and temperature (temp₄₅) readings were taken in the *M. longissimus lumborum* using a calibrated Crison 506 portable pH meter. The animals were then transported to the slaughtering facility where they were skinned, eviscerated and the carcasses cleaned and cooled (4°C). pH profiles (measuring the pH and temperature every two hours for the first 12h, and then every four hours for the following 12h post mortem) were taken from five animals shot in the day and ten animals from those cropped at night. pH_u readings were taken from all of the carcasses 24 hours post mortem. Loin (*M. longissimus lumborum* between the 1st and 4th) samples for physical analysis were taken from the carcasses 36 hours after cropping. Steaks (15mm in thickness) cut perpendicular to the longitudinal axis of the muscle on the caudal side of the sample were used to determine the drip loss and cooking loss according to the methods set out by Honikel (1998). For the Warner Bratzler shear force test, five 12.7mm diameter samples were cut randomly from the cooked block of meat perpendicular to the longitudinal axis of the muscle fibre, at a crosshead speed of 200mm/min. Freshly cut steaks were allowed to bloom for 20 minutes where-after the colour (L*, a*, b* h_{ab} and chroma) was measured using a Color-guide 45°/0° colorimeter (BYK-Gardener, USA).

Analyses of variance were performed on all the variables measured within treatments (SAS, 1989). No significant age or sex differences were found and so the data were pooled for further analysis. Standard t-tests were then conducted with the time of cropping as the main effect. The non-linear regression procedure (Proc NLIN) of SAS (1989) was used to fit exponential decay models ($y = a + b e^{(-ct)}$) to the rate of pH decline (t=time, h) and the rate of temperature decline for both the day and night cropped groups. The a, b, and c values from the above mentioned regression model were then analysed using the t-test procedure of SAS (1989) to test for differences between the time of cropping. As there were differences found in the rate of muscle temperature decrease between the day and night treatments, the pH readings were standardized at 4 °C using the formula of Bruce *et al* (2001). The pH_{adjusted} was then re-analysed.

Results & Discussion

The temperature drops for both treatments could be represented by an exponential decay model (Day: a = 8.03, b = 31.66, c = -0.15; Night: a = 4.49, b = 45.33, c = -0.31). The temperature drop of the night cropped group fell twice as fast as that of the day cropped group, thus indicating rapid cooling in the night cropped muscles.

The pH decline under the prevailing ambient temperatures in the day and night differed significantly (Table 1), with the day cropped group having a more rapid rate of pH decline. Although the analysis was repeated after the temperature was adjusted to a standard 4°C for both treatments, this difference still persisted. The pH₄₅ of the animals cropped at night were significantly higher than those shot in the day (Table 2). However, the pH_u of the day cropped animals were higher than those of the group harvested at night. The night-cropped animals had a lower average drip-loss compared to the day-cropped animals whilst the cooking loss did not differ (Table 2). The Warner-Bratzler shear force tests showed that the meat from the night cropped group was significantly more tender than that of the day cropped group. No significant differences between the two treatments were noted in so far as the colour of the meat was concerned.

The difference in pH profiles is likely as a result of the difference in ante mortem conditions experienced by the animals prior to death between the day and night treatments. It is likely that the difference arose as a result of the heightened stress level of the day cropped animals because of their awareness of the hunters. Another influence could be the heightened level of physical activity during the day, particularly during the rut, which would cause the glycolytic enzyme activity to remain high for a longer period inducing a more rapid pH decline. The relative unawareness of the animals of the croppers (and thus unstressed state) during the night cropping, would result in a lowered glycolytic enzyme activity and a slower rate of pH decline.

All the other physical quality attributes can be correlated with the difference in pH decline profiles between the day and night cropping.

Conclusions

From the results of this study it is evident that night cropping of impala has a beneficial effect on the meat quality as opposed to day cropping. The results of the pH data, drip loss and shear force analyses clearly show that the method of night cropping yields a better meat quality than the day cropping method. Night cropping does not seem to have any detrimental effects on meat quality and it can be deduced that this is as a result of lower ante mortem stress to the animals.

Owing to the very low nighttime ambient temperatures, it is possible that animals cropped at night could develop cold shortening, however this specific aspect requires further research.

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Tables and Figures

Table 1: The calculated constants (LS Mean \pm SE) for the exponential equations fitted to the pH decline under normal temperature conditions and under adjusted standard temperature (4 °C) for day (n = 5) and night (n = 10) cropped impala

$y = a + be^{-ct}$ constants	T	Normal pH decline	P < t	pH decline adjusted to std temp	P < t
a	D	5.38 \pm 0.006	< 0.01	5.46 \pm 0.006	< 0.01
	N	5.41 \pm 0.004		5.42 \pm 0.004	
b	D	2.93 \pm 0.124	< 0.01	2.90 \pm 0.086	< 0.01
	N	2.14 \pm 0.083		2.47 \pm 0.057	
c	D	-0.72 \pm 0.040	< 0.01	-0.58 \pm 0.027	< 0.01
	N	-0.53 \pm 0.026		-0.45 \pm 0.018	

T (Time) = D (Day) or N (Night)

Table 2: Mean pH values and physical meat quality parameters (LS Mean \pm SE) for the day (n = 24) and night (n = 16) cropped impala at Mara

	Day cropped	Night cropped	P < t
Mean pH ₄₅	6.55 \pm 0.235	6.67 \pm 0.111	0.05
Mean pH _u	5.45 \pm 0.108	5.39 \pm 0.081	0.05
Drip loss (%)	4.15 \pm 2.339	2.93 \pm 1.597	0.05
Cooking loss (%)	32.87 \pm 4.101	32.99 \pm 5.109	0.90
Warner Bratzler shear force (g/mm ²)	23.42 \pm 8.128	19.11 \pm 5.675	0.05

SHEAR FORCE AND SARCOMERE LENGTH IN FIVE PELVIC SUSPENDED MUSCLES FROM DIFFERENT BOVINE GENDERS

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Key Words: Beef, Sarcomere length, Shear force, Pelvic suspension, Tenderness

Introduction

Differences in tenderness and other quality aspects exist among muscles with different function and location in the live animal. These differences generally can be explained by a variation in collagen content, proteolytic activity and in contraction or stretching during rigor mortis (King *et al.*, 2003). Pelvic suspension is known to stretch muscles in the hind leg and thereby improve tenderness (Hostetler *et al.*, 1970). In previously reported data (Lundesjö Ahnström *et al.*, 2003) we found that pelvic suspension significantly reduced Warner-Bratzler shear force in *M. longissimus dorsi*, *M. gluteus medius* and *M. adductor* from young bulls but no equivalent reduction in shear force was found for the same muscles from heifers. Since pelvic suspension has been shown to stretch sarcomeres this study was made to see how sarcomeres and shear force values responded to pelvic suspension.

Objectives

The objective of this study was to investigate the combined effects of gender and pelvic suspension on sarcomere length and shear force of beef from five different muscles.

Methodology

Carcasses from Swedish Red and White cattle were selected on the slaughter line and met the following criteria; 7 heifers (age 24-28 months, weight 249-301 kg), 7 young bulls (*young bulls 24mo*, age 22-28 months, weight 235-310 kg), 7 young bulls (*young bulls 34mo*, age 32-35 months, weight 300-349 kg) and 6 cows (age 47-88 months, weight 261-317 kg). All carcasses were electrically stimulated (low voltage, 30 sec) within 30 minutes after bleeding. The left side from each carcass was hung by the pelvic bone approximately 60 minutes after exsanguination while the right side was hung by the Achilles tendon. The carcasses were chilled (50 min) in a chilling tunnel, starting at -2°C , passing a zone at -4°C and then back again to -2°C . The carcasses were stored at $2-4^{\circ}\text{C}$ for 2 days and then fabricated. To avoid DFD-carcasses, the pH was measured in the

centre of the *M. longissimus dorsi* at the 11th rib using a probe electrode. The whole muscles, *M. semimembranosus* (SM), *M. longissimus dorsi* (LD), *M. psoas major* (PM), *M. gluteus medius* (GM) and *M. adductor* (AD), were vacuum-packed and aged for 7 days at 4°C. Then samples for Warner-Bratzler measurements were removed and frozen at -20°C. GM was cut into two pieces along the thick connective tissue band in the central part of the muscle and only the smaller piece was used for analyses.

Samples for sarcomere length measurements were removed from the middle part of the muscles after 7 days of ageing, put in glutaraldehyde fixative and prepared according to Cross *et al.* (1980). The lengths of 10 sarcomeres were measured in 10 myofibrils from each sample.

Warner-Bratzler (WB) shear forces (Honikel, 1998) were used for measurement of tenderness. WB samples were thawed over night at 4°C and then placed in a water bath (20°C) for 1 hour. The samples were vacuum-packed and heated in a water bath for 2 h at 70°C. The weight of the meat samples were recorded before freezing, after thawing and after cooking. The cooked meat samples were stored at 4°C until the measurement of shear force was performed the next day. From each sample, strips (40 x 10 x 10 mm) were cut following the muscle fibre structure. A minimum of 8 strips from each sample were analysed using a Stable Micro System Texture Analyser HD 100 (Godalning, UK) equipped with a Warner-Bratzler shear force blade with a rectangular hole (Honikel, 1998). Statistical evaluation was performed using the Procedure Mixed in SAS (Version 8e, SAS Institute Inc., Cary, NC, USA). The statistical analyses were made separately for each muscle. The model contained the fixed effects of sex and suspension method and the interaction between these, and the random effect of animal.

Results

The investigated muscles were affected differently by pelvic suspension depending on gender. The genders also responded differently to pelvic suspension. The cows and heifers were not affected as much as the bulls. Of the investigated muscles, SM and AD were stretched the most after pelvic suspension with an increase in muscle length of 36% and 46% respectively. Our earlier results have shown a 30% increase in length of SM in young bulls (Lundesjö, 2001).

M. semimembranosus

In the SM a 1µm increase in sarcomere length was found in pelvic suspended sides compared with achilles suspended sides for young bulls 24mo, young bulls 34mo and cows ($p < 0.001$) whereas the heifers had a 0.3µm increase ($p = 0.007$) in sarcomere length. This increase in sarcomere length was not clearly related to the values for Warner-Bratzler shear force where we found a strong relationship in the bull groups and a slightly less pronounced effect in the heifer/cow groups (Figure 1a). Pelvic suspension compared with achilles suspension gave a 24% decrease ($p < 0.001$) in maximum shear force for the young bulls 24mo and 29% ($p < 0.001$) decrease for young bulls 34mo whereas the heifers had a decrease of 17% ($p = 0.006$) and the cows had no significant decrease in shear force for pelvic suspended sides. There were no significant differences in shear force between genders after pelvic suspension. The variation between samples, across genders, within treatment decreased from 21% in achilles suspended SM to 12% in pelvic suspended SM.

Of the genders young bulls 24mo had the greatest reduction in variation with a 66% decrease in shear force variation for pelvic suspended sides followed by a 52% reduction found in cows (Figure 2a). The within sample variation did not decrease in any gender or treatment due to pelvic suspension.

M. longissimus dorsi

Maximum shear force in LD was reduced in all four genders due to pelvic suspension. No significant differences in shear force occurred between genders after pelvic suspension. The effect was largest in young bulls 24mo where shear force was reduced 35% ($p<0.001$) followed by a 22% difference ($p=0.006$) for young bulls 34mo (Figure 1 b). Heifers and cows had no significant reductions in maximum shear force, which does not agree with previous findings (Lundesjö Ahnström *et al.*, 2004) where a significant reduction in LD shear force due to pelvic suspension was found for both Charolais and Aberdeen Angus heifers. The variation in shear force between samples, across genders and within treatment decreased from 32% for achilles suspended sides to 16% for pelvic suspended sides. Large reductions in variation due to pelvic suspension could be seen in young bulls 24mo, young bulls 34mo and cows, which had smaller variations in shear force by 70, 60 and 50%, respectively (Figure 2b). The response in sarcomere length did not follow the same pattern as the shear force since the cows had a 20% increase in sarcomere length of the LD from pelvic suspended carcasses ($p<0.001$). However, in heifers, sarcomere length change was not significant. The young bulls 24mo and 34mo had significant increases in sarcomere length of 12% ($p=0.002$) and 19% ($p<0.001$), respectively.

M. adductor

For the AD, pelvic suspension (compared to achilles suspension) reduced shear force by 9% ($p=0.1$) for young bulls 24mo. The same group also had a reduction in between sample variation in shear force of 15% in achilles suspended sides compared to 7% for pelvic suspended sides. The cows had an opposite effect where the achilles suspended sides had an 8% ($p=0.007$) lower shear force value but no difference in variation for the different treatments. The heifers and young bulls 34mo showed no significant differences in shear force due to suspension method. The sarcomere lengths for young bulls 24mo and heifers (data not collected for the other groups) had a significant increase of 70% ($p<0.001$) in sarcomere length for pelvic suspended muscles from young bulls whereas the heifers did not have any significant changes in sarcomere length.

M. psoas major

Heifers and cows had slightly higher shear force values for pelvic suspended sides than achilles suspended sides. This can be expected since PM could contract due to pelvic suspension. Although no significant differences in shear force could be attributed to suspension method in any of the genders, it is interesting that the young bull groups had lower shear force value for pelvic suspended sides. This is contradictory to the female groups. However, in young bulls, this reduction in shear force was not confirmed by the sarcomere length measurements that showed a 15% decrease in sarcomere length for

pelvic suspended sides. No significant differences were found in sarcomere length for heifers although the length increased by 8% for pelvic suspended sides.

M. gluteus medius

GM had an inconsistent response to pelvic suspension in different genders. For young bulls 24mo and 34mo, the shear force decreased 23% ($p=0.004$) and 35% ($p<0.001$), respectively. The sarcomere length for young bulls 24mo was $0.9\mu\text{m}$ (52%) longer for pelvic suspended sides ($p<0.001$). For heifers and cows, pelvic suspension did not affect shear force or sarcomere length.

Discussion

The aim of our study was to diminish variation in tenderness in different cuts from different genders. Earlier work (Lundesjö et al., 2001) has shown that the variation in shear force between animals decreased from 26% for achilles suspended sides to 12% for young bulls suspended by their pelvis. Pelvic- compared to achilles suspension diminished variation in shear force for all muscles in the present study. The greatest reductions in variation were found in the male groups. The males also had the greatest response to pelvic suspension for shear force values where muscles from pelvic suspended sides were more tender compared to achilles suspended sides. This was not the case for the female groups where some of the muscles were less tender after pelvic suspension (heifers, negative response in PM and GM and cows in AD and PM). The differences between treatments were also smaller in the female group. This could be related to the relatively low shear values and good tenderness in these genders. The sarcomere length generally was longer in pelvic suspended sides. The bull groups had the greatest lengthening of sarcomeres, but contradictory to the shear force results the cows showed a large response to pelvic suspension leading to longer sarcomeres for those sides. We propose that greater stretching occurred in the sarcomeres in young bulls, but the same increase in sarcomere length in cows did not result in additional improvement of tenderness. One explanation of these differences might be due to differences within the pelvic region between genders and between females before and after calving. This could result in different angles of the hind leg during pelvic suspension and therefore different impact on the muscles. Another explanation could be differences in collagen stability between sexes which could interact with sarcomere stretching during rigor development.

Muscles were affected differently by pelvic suspension, which is in agreement with Barnier and Smulders (1994) who investigated effects of pelvic suspension on shear force values of five beef muscles. Although treatment increased sarcomere length of all muscles, the effect on the shear force ranged from positive, to negligible, to negative between muscles. Hostetler *et al.* (1970) also showed inconsistency in response to the treatment by different muscles where similar changes in sarcomere length did not produce proportionate changes in tenderness. Our results across genders showed an increase in sarcomere length ranging from 48% (SM, $p<0.0001$) > 30% (GM, $p<0.0001$) > 24% (AD, $p=0.001$) > 13% (LD, $p<0.0001$) to a negative response of -4% (PM, $p=0.34$). This can be compared to the results for shear force which ranged from 22% (LD, $p<0.0001$) > 20% (SM, $p<0.0001$) > 17% (GM, $p=0.0001$) > 0.07% (PM, $p=0.97$) > -0.1% (AD, $p=0.98$). However, it should be emphasized that the interaction, gender x

suspension method was significant for both sarcomere length and shear force in all muscles (with an exception for shear force in LD).

Conclusions

Pelvic suspension is a useful tool when the goal is to reduce tenderness variation in beef muscles. It has a more pronounced effect for muscles from carcasses from bulls compared to cows and heifers. Shear force and sarcomere length does not respond equally to the treatment.

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Figures

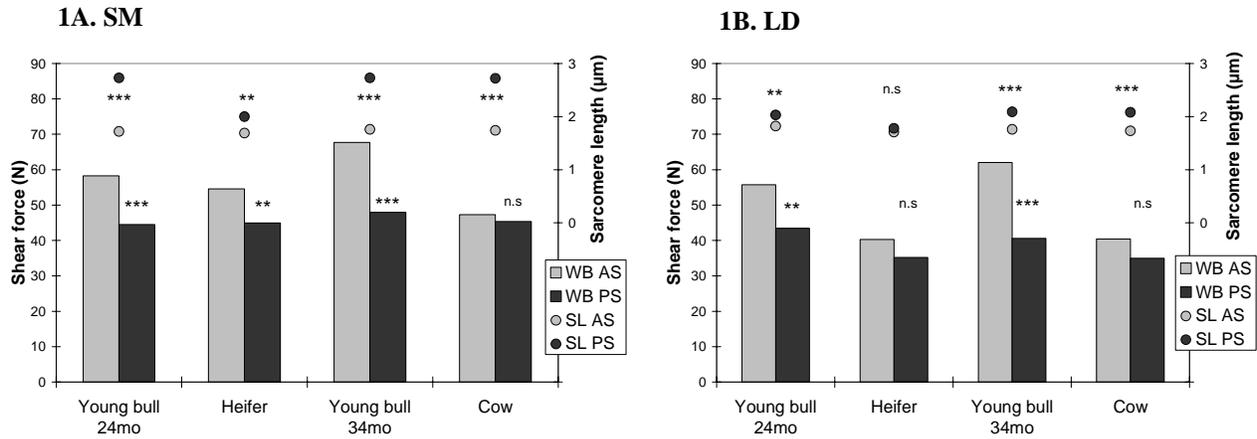


Figure 1 A & B.

The effect of pelvic (PS) and achilles (AS) suspension on WB Shear force (WB) and sarcomere length (SL) in four genders and two muscles A) SM and B) LD

(Levels of significance: ns = $p > 0.10$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.)

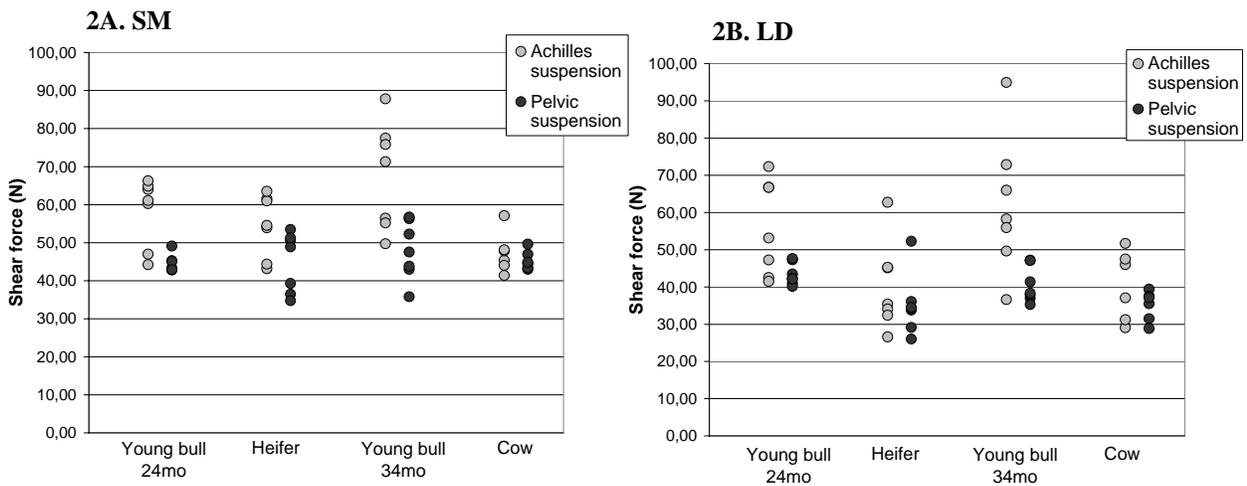


Figure 2 A & B

The variation in shear force for two achilles and pelvic suspended muscles from four genders, A) SM, B) LD.

VERY FAST CHILLING AND QUALITY PARAMETERS OF MUSCLE L. DORSI FROM *BOS INDICUS* NELORE STEER

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Key Words: Tenderness, Meat quality, Very fast chilling, Hot boning, Beef, Nelore, Aging

Introduction

Tenderness is one of the most important properties of meat to the consumer. The bos indicus breed, largely produced in Brazil, gives a tougher meat when compared to European breeds. The hot boning is a well established process to accelerate meat processing and several studies have been done showing its economical advantages and enhancement of the functional properties of the meat (KASTNER, 1977, CUTHBERTSON, 1984). Until now no meat company adopted this kind of boning in Brazil. In Europe recently, an alternative chilling practice known as ultra rapid chilling has been investigated for beef (JOSEPH, 1996; O'MAHONY, 1997) and lamb (REDMOND, 2000) carcass processing. Results from some studies indicate that there are minimal differences in tenderness between ultra fast and conventionally chilled products but other studies demonstrated that its commercial application would seem somewhat limited.

Objectives

The purpose of this study was to investigate the effects of very fast chilling and aging of electrically stimulated hot boned cuts on quality parameters and sarcomere length of Longissimus dorsi muscle from bos indicus Nelore steers.

Methodology

Twenty four forage fed Nelore steers with 30-36 months of age were slaughtered at 4 different times over a period of 3 months. The animals were randomly assigned to 3 treatments and 2 replications for session. In all treatments low voltage electrical stimulation (JARVIS BV 80) was applied immediately after bleeding. The L. dorsi (LD) muscles were excised at approximately 45 min post mortem (PM). The muscles of the right side of carcasses were chilled very fast in a tunnel freezer with forced air circulating at 2m/s and -20°C (HBVFC). The cooling time lasted, in average, 2h and 30 min until the muscle surface reached -1 or -2°C. Temperatures near the surface and at the center of muscle were measured with T type thermocouples and recorded in a Grant Datalogger. After chilling muscles were divided into 3 pieces and vacuum repacked. The muscles of the left side of carcasses were also divided into 3 pieces, vacuum packed in barrier bags and stored at 0°C up to 14 days (HB0). In the control treatment the boned muscles from

carcasses hanged by the Achilles tendon were conventionally chilled 24h at 0°C (AT). Muscles were also divided into 3 pieces, vacuum packed and conditioned at 0°C for 14 days. The meat pieces were randomly taken for analysis at 2, 7 and 14 days PM. The temperatures of the cooling rooms, carcasses and cuts were recorded in a datalogger Field Chart Novus.

The pH and R values were sampled at 1, 2, 4, 6, 8, 24 hours PM and at 7 and 14 days PM. pH was determined as described by BENDALL, 1973 and R-Values (A 250/A 260), according to HONIKEL & FISCHER (1977) with a spectrophotometer VARIAN Cary 1E.

The meat pieces were cut into three 2.54cm slices for shear force determinations and sensory analysis at 2nd, 7th and 14th days PM. The steaks were cooked according to AMSA (1995) guidelines in an electrical grill (150°C) till its internal temperature reached 74°C. Total cooking losses were calculated weighting steaks before and after cooking. Temperatures in the center of steaks were monitored with digital thermometer NOVUS 51. Shear forces were determined with a TA.XT2i Texture Analyzer with a Warner-Bratzler probe (TA-7 USDA).

Tenderness, juiciness and flavor were evaluated by 15 trained panelists utilizing structured scales with 10 cm, where 0 meant slightly tender or slightly juicy or slightly aged flavor, 5 meant tender or juicy or aged flavor and 10 meant very much tender or very much juicy or intense aged flavor. It was statistically analyzed as an incomplete balanced blocks (Compusense Inc 4.2).

Sarcomere length was measured following a general protocol of histological processing for morphometric analysis of length. The images were captured with a microscope (Eclipse 800 Nikon Japan), a digital camera (CoolSnap-Pro Digital Media Cybernetics USA) and processed with the ImagePro-Plus Software Media Cybernetics USA.

Analysis of variance was used to test for treatment effects significance and the Duncan Test means was used to detect means differences ($p < 0.05$).

Results & Discussion

According to HONIKEL (2003), very fast chilling of muscle is conveniently defined as such that a temperature of 0°C is reached within 5 hours PM. Figure 1 shows that HBVFC regime caused a rapid temperature fall. After two hours the surface temperature was near to -1°C and the inner temperature was close to 0°C.

HBO muscles (LD) took 10h PM to reach 5°C at the center of the muscle and the AT muscles, needed 24h to reach 5°C (data not shown).

The pH values in Table 1 show that at 24 h PM there was a significant difference between HBO and TA treatments ($p < 0.05$). Muscles cooled in the carcass (TA) had lower pH values than hot boned muscles cooled at 0°C (HBO).

No significant difference between treatments was observed until 8 hours PM ($p > 0.05$) and in the 7th and 14th day PM.

The rate of pH fall was not affected by the HBVFC treatment.

In the first 6 hours, the HBO muscles had high R values at 1h and 6h PM ($p < 0.05$) (Table 1). As suggested by HONIKEL (1981) R values > 1.1 and the pH values < 5.9 ,

means that the muscle is in rigor mortis. According to this statement, the results from Table 1 indicate that at six hour PM the HBO and HBVFC muscles were in rigor mortis.

There was a great variability in R values for the HBVFC treatment; it increased very fast from the 4th to 6th hour PM and decreased from 6th to 8th hour PM.

At 24h PM HBVFC muscles had mean R value lower than HBO ones ($p < 0,05$) and this indicates that from 6 to 24 h PM the rate of conversion of ATP decreased for the HBVFC muscles.

AT treated muscles presented the lowest mean R value at 6 hour PM indicating that their rigor mortis was delayed.

Sarcomere length was measured at boning and after 14 days PM. As can be seen in Table 2 at boning there was no statistical difference in the sarcomere length for all treatments. At the 14th day PM, HBVFC samples had their mean sarcomere drastically reduced to 1,23 μ m and significantly lower than those of other treatments ($p < 0,05$). This length of sarcomere clearly indicates that cold shortening occurred.

The HBO muscles had their sarcomeres slightly reduced after 14 days of aging, showing an average length of 1,90 μ m and there was no significant difference in the sarcomere length of HBO and AT muscles.

Even though the time-temperature and pH values of HBO muscles after 10h PM could induce cold shortening according to THOMPSON (2002), the average sarcomere length at the 14th day can be seen as an average sarcomere length for a muscle not shortened after rigor resolution has occurred.

No difference was found in shear force values amongst any of the treatments at 2nd day PM (Table 3). However, at 7th and 14th day PM the HBVFC muscles were significantly tougher than the conventionally chilled (AT) ($p < 0,05$) but there was no difference to HBO muscles ($p > 0,05$).

The reduction of shear force with aging was observed in treatments HBO and AT. In the HBO and AT samples the shear force values decreased significantly after 7 days, but not from the 7th to 14th day PM. In the case of HBVFC samples, there were no significant differences in shear forces during all the aging period ($p < 0,05$).

After 14 days HBVFC samples, which had the shortest sarcomere length, had also the highest shear force values. Tenderness scores (Table 3) clearly indicated higher meat toughness for HBVFC muscles after 14 days PM ($p < 0,05$).

Tenderness scores for HBO samples do not show statistical difference in relation to AT samples after 14 days of aging ($p > 0,05$).

Tenderness scores shows that from the 2nd to 7th day PM HBVFC samples tenderized with aging. In the case of HBO and AT samples, tenderization occurred from the 2nd to 14th day ($p < 0,05$).

The panelists did not perceive differences in juiciness neither between treatments nor for each treatment during aging (Table3).

“Aged flavor” scores in Table 3 show no significant differences between treatments in 2, 7 and 14 days PM ($p > 0,05$) but analyzing each treatment separately, “aged flavor” scores were significant different from the 2nd to 7th and from 7th to 14th day PM.

Drip losses of HBVFC muscles were significantly higher ($p < 0,05$) than other treatments at 2, 7 and 14 days PM (Table 4). At 2nd day PM, HBVFC muscles showed 1,6 times more mean dripping losses than the HBO muscles and 3 times more than AT muscles. At 14th day PM HBVFC muscles had 2,95 times more dripping losses than

HBO muscles and 2,25 times more than AT muscles. This result agrees with VAN MOESEKE (2001) and HONIKEL (2003).

There was an increase in drip losses during the aging period for all treatments ($p < 0,05$). HBO and HBVFC muscles showed significant differences in the average drip losses from the 2nd to the 14th day PM. AT muscles were significantly different from the 2nd to 7th day but not from 7th to 14th day PM.

As can be seen in Table 4, there were no differences in cooking losses between treatments at 2, 7 and 14 days PM and for each treatment with aging time ($p < 0,05$).

Conclusions

The results suggest that application of very fast chilling to Nelore steer meat is not practical commercially. HBVFC toughens vacuum packed hot boned beef, increases drip losses and aging is not effective.

The HBO treatment showed no difference to HBVFC considering muscle shear force while sensory analysis showed no significant differences in tenderness scores between HBO and AT cuts, being both more tender than HBVFC muscles.

Considering the effect of aging, the treatments AT and HBO showed the smaller shear force values after 7 days PM, but this improvement in tenderness was noticed by the sensory panel only after 14 days PM, what means that full tenderization will happen only after two weeks.

In spite of differences in drip losses, differences in juiciness were not perceived by trained panelists.

Aging improved “aged meat flavor” for all treatments.

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Tables and Figures

Table 1. Mean pH and R values and standard errors (SE) for muscle *L. dorsi* from different treatments at 1, 2, 4, 6, 8, 24h and at 7 and 14 days PM

Measurement	Treatment					
	HBO		HBVF		AT	
	Mean	SE	Mean	SE	Mean	SE
pH						
1h PM	6,27	±0,10	6,15	±0,10	6,37	±0,07
2h PM	6,16	±0,13	6,16	±0,10	6,21	±0,07
4h PM	5,99	±0,10	6,10	±0,07	6,17	±0,09
6h PM	5,96	±0,08	5,90	±0,05	5,94	±0,10
8h PM	5,92	±0,09	5,72	±0,06	5,82	±0,12
24h PM	5,71 ^{a,x}	±0,04	5,60 ^{ab}	±0,05	5,53 ^b	±0,07
7 days PM	5,52 ^y	±0,02	5,46	±0,04	5,48	±0,03
14days PM	5,52 ^y	±0,03	5,45	±0,08	5,45	±0,05
R value						
1h PM	1,01 ^a	±0,02	0,88 ^b	±0,01	0,92 ^b	±0,03
2h PM	1,05	±0,05	0,93	±0,02	0,93	±0,04
4h PM	1,10	±0,05	0,94	±0,11	0,97	±0,02
6h PM	1,25 ^a	±0,06	1,26 ^a	±0,03	1,07 ^b	±0,04
8h PM	1,30	±0,05	1,15	±0,12	1,11	±0,06
24h PM	1,41 ^a	±0,01	1,22 ^b	±0,06	1,32 ^{ab}	±0,05
7 days PM	1,45	±0,01	1,41	±0,15	1,37	±0,06
14days PM	1,41	±0,02	1,37	±0,11	1,32	±0,08

Table 2. Mean sarcomere length (SL) and standard error (SE) for muscle *L. dorsi* from different treatments at 0, 2 and 14 days PM

Measurement	Treatment					
	HBO		HBVFC		AT	
	Mean	SE	Mean	SE	Mean	SE
SL	μm		μm		μm	
0 days PM	2,04 ^x	$\pm 0,05$	1,71	$\pm 0,20$	-	-
2 days PM	-	-	-	-	2,09	$\pm 0,09$
14days PM	1,90 ^{a,y}	$\pm 0,02$	1,23 ^b	$\pm 0,04$	1,98 ^a	$\pm 0,06$

a, b, Treatment effect. Means in the same line with unlike superscripts are different $p < 0,05$

x, y, z Aging effect. Means in the same column with unlike superscripts are different $p < 0,05$

Table 3. Mean shear force and sensory analysis scores for tenderness, juiciness and flavor and standard error (SE) for muscle *L. dorsi* from different treatments at 2, 7 and 14 days PM.

Measurement	Treatment					
	HBO		HBVFC		AT	
	Mean	SE	Mean	SE	Mean	SE
Shear Force	kgf		kgf		kgf	
2 days PM	7,39 ^x	$\pm 0,50$	7,10	$\pm 0,42$	6,93 ^x	$\pm 0,47$
7 days PM	5,90 ^{ab,y}	$\pm 0,34$	6,77 ^a	$\pm 0,47$	5,36 ^{b,y}	$\pm 0,36$
14days PM	5,14 ^{ab,y}	$\pm 0,32$	6,13 ^a	$\pm 0,32$	4,86 ^{b,y}	$\pm 0,52$
Tenderness						
2 days PM	5,10 ^y	$\pm 0,49$	4,66 ^y	$\pm 0,15$	5,44 ^y	$\pm 0,27$
7 days PM	5,66 ^y	$\pm 0,40$	5,56 ^x	$\pm 0,32$	6,07 ^{xy}	$\pm 0,32$
14days PM	6,96 ^{a,x}	$\pm 0,32$	5,56 ^{b,x}	$\pm 0,25$	6,73 ^{a,x}	$\pm 0,39$
Juiciness						
2 days PM	6,55	$\pm 0,09$	6,37	$\pm 0,18$	6,16	$\pm 0,25$
7 days PM	6,41	$\pm 0,17$	6,40	$\pm 0,14$	6,30	$\pm 0,19$
14days PM	6,44	$\pm 0,13$	6,26	$\pm 0,16$	6,54	$\pm 0,21$
Flavor						
2 days PM	2,64 ^z	$\pm 0,23$	2,41 ^z	$\pm 0,13$	2,91 ^z	$\pm 0,22$
7 days PM	3,77 ^y	$\pm 0,17$	3,75 ^y	$\pm 0,15$	4,01 ^y	$\pm 0,14$
14days PM	5,05 ^x	$\pm 0,18$	4,76 ^x	$\pm 0,17$	4,96 ^x	$\pm 0,28$

Table 4. Mean drip loss and cooking loss and standard error (SE) for muscle *L. dorsi* from different treatments at 2, 7 and 14 days PM

Measurement	Treatment					
	HBO		HBVFC		AT	
	Mean	SE	Mean	SE	Mean	SE
Drip loss	%		%		%	
2 days PM	0,67 ^{b,y}	±0,11	1,59 ^{a,y}	±0,29	0,53 ^{b,y}	±0,15
7 days PM	0,97 ^{b,xy}	±0,12	3,45 ^{a,xy}	±0,87	1,46 ^{b,x}	±0,28
14days PM	1,35 ^{b,x}	±0,25	3,99 ^{a,x}	±0,84	1,77 ^{b,x}	±0,36
Cooking loss						
2 days PM	26,19	±0,52	28,83	±1,34	30,54	±2,39
7 days PM	26,03	±1,77	29,95	±1,44	28,58	±0,94
14days PM	29,70	±1,53	28,51	±1,29	27,64	±1,12

a, b, Treatment effect. Means in the same line with unlike superscripts are different $p < 0,05$

x, y, z Aging effect. Means in the same column with unlike superscripts are different $p < 0,05$

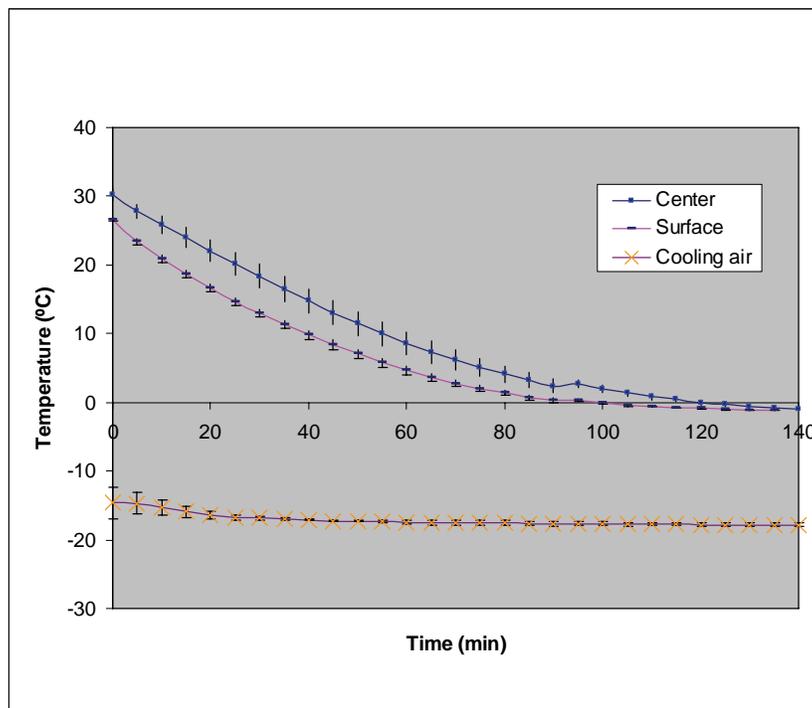


Figure 1. Chilling times in the surface and center of hot boned Longissimus dorsi vacuum packed cut chilled in a tunnel freezer at -20°C .

TIMING OF NITRIC OXIDE INHIBITION PRE-SLAUGHTER INFLUENCES LAMB MEAT TENDERNESS AND PROTEOLYSIS

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Key Words: lamb, tenderness, nitric oxide

Abstract

Nitric oxide (NO) is a free radical that is constantly produced or released throughout the body by diverse tissues and is known to influence proteolytic activity in human and rodent skeletal muscle as well as being involved in regulation of calcium homeostasis in the muscle cell. The influence of nitric oxide (NO) on development of meat tenderness has been demonstrated through postmortem manipulation and this experiment was designed to manipulate NO synthase activity *in vivo*. The aim was to investigate the effect of timing of NOS inhibition, on meat quality attributes of two different lamb muscles. Sixty-four second cross lambs were used. Endogenous NOS activity was manipulated using infusion of L-NAME (0, vs 30 mg/kg) at either 24 hrs or 3 hrs post-slaughter. Lambs were slaughtered and meat quality attributes measured in the *semimembranosus* (SM) and *longissimus thoracis* (LT). Muscle pH at 1 hr and 24 hrs postmortem was not influenced by L-NAME infusion or timing of infusion in either muscle ($P>0.05$). Inhibition of endogenous NOS using L-NAME resulted in lower WBSF values in the LT ($P<0.05$) and tended to result in lower WBSF values for the SM ($P<0.10$) which was more pronounced in the LT in lambs infused at 24 hrs pre-slaughter ($P<0.05$). Similarly, MFI values in the LT and in the SM were higher in L-NAME infused lambs compared to those infused with saline ($P0.05$ for both). Sarcomere length tended to be reduced in the LT of L-NAME infused lambs ($P<0.10$), which was more pronounced in the lambs infused at 24 hrs pre-slaughter. Sarcomere length in the SM was not influenced by treatments ($P>0.05$). In conclusion, inhibition of endogenous NOS activity caused an increase in tenderness, as measured by shear force and an increase in proteolysis, as measured by Myofibrillar Fragmentation Index values, in both the LT and SM muscles. The effect was more pronounced in lambs that were infused at 24 hrs pre-slaughter compared to 3 hrs pre-slaughter. The role of skeletal muscle NOS activity pre-slaughter in determining meat tenderness deserves further investigation.

Introduction

Nitric oxide (NO) is a free radical that is constantly produced or released throughout the body by diverse tissues and is known to influence proteolytic activity in human and rodent skeletal muscle (Michetti et al., 1995) as well as being involved in regulation of

calcium homeostasis in the muscle cell (Hare, 2003). Injection of nitric oxide (NO) donors and inhibitors into hot-boned beef *longissimus thoracis* (LT) at 2 hrs post-slaughter has been found to reduce and increase shear force respectively (Cook et al., 1998). Nitric oxide synthase (NOS) activity post-slaughter has been found at 0 hrs post-slaughter in chicken, turkey, pork diaphragm and trout muscle but only the pork diaphragm retained NOS activity until 24 hrs post-slaughter and even then, it was in an *in vitro* system where ample substrate and co-factors were provided (Brannan and Decker, 2002). Cottrell et al. (2002) were the first to show an effect of manipulation of NO levels *in vivo* pre-slaughter on meat tenderness postmortem. They showed that infusion of a NOS inhibitor, L-NAME, into lambs at 3 hrs pre-slaughter resulted in more tender meat in the LT muscle at 3 days post-slaughter but tougher meat in the *semimembranosus* (SM) at 1 and 3 days post-slaughter. The effect of changing the time of infusion of the NOS inhibitor on meat tenderness is unknown and thus was proposed for investigation.

Objectives

To investigate the effect of timing of NOS inhibition on meat quality attributes of two different lamb muscles.

Methodology

Sixty-four second cross (Border Leicester /Merino dam x Poll Dorset sire) lambs approximately six months old, ranging between 33.5 and 51kg live weight, were selected from a flock, blocked on liveweight and were allocated to one of four slaughters (n=16 per slaughter). On the day prior to slaughter, lambs were brought into a shed and an indwelling jugular catheter was inserted intravenously into the jugular vein. Animals were deprived of food approximately 12 hrs pre-slaughter, but retained *ad libitum* access to water. Lambs were allocated to an L-NAME infusion treatment (30 mg/kg of L-NAME in 0.9% saline vs saline; infused via the indwelling catheter) and two infusion times (3 hrs vs 24 hrs pre-slaughter) in a 2 x 2 factorial with a balanced randomised block design. Lambs were quietly moved from pens in the shed to the abattoir and then stunned using 200 volts and 1 amp for 4 seconds applied to the head. Animals were exsanguinated and carcasses eviscerated before entering the chiller (2 °C ±1°C). The postmortem pH was measured at 1 and 24 hours post-slaughter in the SM and LTL. The LT and SM were removed at 24 hrs postmortem. Tenderness was measured by Warner-Bratzler shear force (WBSF) after 1, 3 or 9 days of ageing by methods described in Channon et al. (2000). Samples were removed from the LT at 24 hrs postmortem for snap freezing in liquid nitrogen and freezer storage prior to measurement of sarcomere length and from the LT and SM for measurement of myofibrillar fragmentation index (MFI). Muscle surface colour (L*a*b*) was measured after 30 min. bloom at 1, 3 and 9 days postmortem. All data were tested for significance with an analysis of variance (ANOVA) blocked for the slaughter day. For MFI and WBSF, days of ageing was analysed in the AOV. As there were no interactions between time of infusion pre-slaughter, L-NAME treatment and days of ageing, means are presented as least squares means across all ageing periods.

Results & Discussion

The effects of L-NAME infusion and timing of infusion on major meat quality traits are shown in Table 1. Muscle pH at 1 hr and 24 hrs postmortem was not influenced by L-NAME infusion or timing of infusion in either muscle ($P>0.05$). Inhibition of endogenous NOS using L-NAME resulted in lower WBSF values in the LT ($P<0.05$) and tended to result in lower WBSF values for the SM ($P<0.10$) which was more pronounced in the LT in lambs infused at 24 hrs pre-slaughter ($P<0.05$). Similarly, MFI values in the LT and in the SM were higher in L-NAME infused lambs compared to those infused with saline ($P=0.05$ for both). Sarcomere length tended to be reduced in the LT of L-NAME infused lambs ($P<0.10$), which was more pronounced in the lambs infused at 24 hrs pre-slaughter. Sarcomere length in the SM was not influenced by treatments ($P>0.05$). There were no major effects of treatments on any surface colour measurements ($P>0.05$; data not presented).

Conclusions

Inhibition of endogenous NOS activity caused an increase in tenderness, as measured by shear force and an increase in proteolysis, as indicated by Myofibrillar Fragmentation Index values, in both the LT and SM muscles. The effect was more pronounced in lambs that were infused at 24 hrs pre-slaughter compared to 3 hrs pre-slaughter. The role of skeletal muscle NOS activity pre-slaughter in determining meat tenderness deserves further investigation.

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Tables and Figures

Table 1: Effect of injection time (IT, 3 hrs vs 24 hrs pre-slaughter) and L-NAME infusion (L, 0 vs 30 mg/kg) on Warner-Bratzler shear force (WBSF), Myofibrillar Fragmentation Index (MFI), sarcomere length (SL) and the pH and 1 hr or 24 hrs (ultimate) post-slaughter for the muscles *longissimus thoracis* and *semimembranosus*.

Injection time	3 hrs		24 hrs		SED	F-value		
	L-NAME 0	L-NAME 30	L-NAME 0	L-NAME 30		L	IT	L x IT
<i>Longissimus thoracis</i>								
WBSF (kg)	3.88	3.81	4.39	3.61	0.195	0.002	0.262	0.010
MFI	74.2	80.8	70.5	77.2	3.31	0.005	0.121	0.981
SL (um)	2.08	2.09	2.22	2.01	0.077	0.072	0.688	0.048
pH 1 hr	6.58	6.67	6.58	6.57	0.084	0.495	0.374	0.403
pH ultimate	5.53	5.52	5.54	5.52	0.013	0.221	0.565	0.755
<i>Semimembranosus</i>								
WBSF (kg)	3.78	3.67	3.99	3.84	0.147	0.088	0.057	0.897
MFI	70.2	73.7	66.8	73.2	3.57	0.049	0.440	0.570
SL (um)	2.10	2.07	2.01	2.06	0.064	0.876	0.316	0.384
pH 1 hr	6.35	6.43	6.43	6.47	0.055	0.144	0.137	0.580
pH ultimate	5.51	5.50	5.50	5.50	0.015	0.349	0.513	0.711

EFFECT OF TRANSPORT TIME AND SEASON ON SENSORIAL ASPECTS OF RABBIT MEAT QUALITY¹

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Key Words: rabbit, transport, sensory quality, sensory panel.

Introduction

The production chain involves all processes from the farm to the fork. Within the transport chain, from farm to abattoir, there are many factors that may induce stress in rabbits (Jolley, 1990) and affect meat quality (Masoero et al., 1992). These factors can decrease instrumental meat quality, but little is known about changes in sensory quality. End consumer values quality products, as the set of characteristics that determines its ability to satisfy their needs. In this regard, assessing rabbit meat quality with a sensory panel is a good way to analyse consumer requirements. Sensory analyses provide quantitative data on the effects of different treatments (transport time or season) in terms of consumers. In Spain, transport of commercial rabbits to the abattoir is relatively short, typically less than three hours (Buil et al., 2004). Recently, a report by the Scientific Committee on Animal Health and Animal Welfare proposes important limitations on transport time. The European Parliament has suggested decreasing all journeys for all species to less than nine hours. Bad handling during transport could put Good Manufacturing Practice (GMP) at risk. Therefore, it is necessary to maintain GMP during the whole process without compromising animal welfare to offer a high quality product.

Objectives

The aim of this study was to analyze whether transport times of up to 7 h can have a significant effect on sensory meat quality, in terms of odour, texture, flavour intensity and acceptability. We also considered how meat quality could be affected by the position of the rabbits in the truck. Repeats journeys were performed in two seasons (winter and summer) in continental Spain, which has very hot summers and cold winters.

Methodology

The study was performed using 156 commercial rabbits slaughtered at two months of age (live weight 2300g.). The experiment was performed in June and December (2003), the hot and cold season, respectively. We studied two transport times, short (1h) and long (7h) with three replicates in both seasons.

We selected animals randomly from upper, middle and bottom positions within the truck to study the effect of the position on meat quality. The stocking density during

transport was 360 cm² per animal (cage size 57 x 57 x 25 cm). The average temperature was recorded every 5 minutes with a Testo thermometer at the level of the study animals. Carcasses were chilled under commercial conditions at 0°C for 24 h. The meat pH was measured at 24 h post-mortem on the lumbar region with a Crison 52-00 electrode. The *longissimus dorsi* muscles were removed from both sides, and left sides were vacuum-packaged and chilled at -18°C until sensory analysis. For sensory analyses we followed the procedure described in Campo et al. (1999) using a tasting room with individual booths. Ten taste sessions were carried out by an eight-member sensory panel; each session was a multi-sample test with three main plates containing four samples each. Steaks were thawed in tap water for 2h until they reached an internal temperature of 17-19° C. Meat was cooked in a double plate grill (SAMMIC P8D-2) at 200°C wrapped with aluminium foil until an internal temperature of 70°C. In every session, samples were served to the panellists under red lighting to mask differences in meat colour. Samples were presented in a different order to each panellist within the same plate; each treatment was analyzed and compared 20 times for each member of the panel with a total of 160 judges. Samples were evaluated by panellists using a 10cm linear scale where different descriptors were assessed: rabbit odour intensity, strange odour intensity, tenderness, juiciness, fibrousness, and greasiness, rabbit flavour intensity, strange flavour intensity and overall liking. A score of 0 represents low odour and flavour intensity, bad texture and low overall liking. A score of 10 represents samples with high odour and flavour intensity, good texture and high acceptability. Data were analyzed using least squared method of the GLM procedure of the SAS (SAS, 1985), with a fixed effect of holding time and position within the truck.

Results & Discussion

The average temperatures during transport were 11°C ± 3 and 28°C ± 2 in winter and summer respectively. Average relative humidity was 63.5 % ± 7.6 in winter and 47.5 % ± 6 in summer.

There were significant ($p \leq 0.05$) differences in odd odour between seasons (Table 1). The highest value of this trait was in the hot season (Table 2). The scores given for rabbit odour and rabbit flavour were intermediate (between 4 and 5), which shows that flavour is normally related to odour. There was no significant effect of position (up, middle, and bottom) or transport time on meat sensory quality (Table 1). Meat odour, rabbit or odd odour, did not vary significantly between journey times. Although transport time did not have a significant effect on rabbit odour intensity, there was a significant interaction between transport time and season ($p \leq 0.05$) for this sensory trait (Fig. 1). Tenderness varied with respect to the transport time and season interaction. Meat from the short journeys in summer was the most tender, and least tender after long journeys in winter (Fig.1). In studies on sensory quality, tenderness seems to improve after short journeys (2 h-Autom times) (Xicatto et al. 1995). Other stress factors, such as the lack of environmental control and the extreme temperatures within the truck during transportation, could also contribute.

Although there were not statistically significant effects on texture sensory traits, there were seen some tendencies. In general, tenderness was in the middle point of the scale. Meat from rabbits that underwent the short journey had the lowest tenderness and the

highest fibrousness. The judgments regarding fibrousness were highly associated with the scores for tenderness. Fibrousness was slightly higher when tenderness was lower. There were no significant effects of transport time, season or position in the truck on juiciness, but the highest juiciness value was found in animals that underwent a short journey in the hot season.

The interaction between transport time and season was also observed for Warner Bratzler texture traits (María et al., 2004). Other studies (Dalle Zotte et al., 1995) indicate that there is a high correlation between instrumental tenderness and sensory tenderness. Therefore, more instrumental and sensory analyses within a same experiment need to be assessed in this research field.

Conclusions

In conclusion, transport time did not have a significant effect on sensory meat quality, but the interaction between transport time and season affected significantly some sensorial descriptors. Therefore, even under optimum commercial conditions, rabbit meat quality could be affected. This may not imply a lack of animal welfare, but increased awareness of animal welfare during the commercial process. Hence, a new quality concept within the meat industry is developing which involves ethical aspects during the process. It is possible that in future, consumers will be ready to pay more for an animal product produced under systems that are highly concerned about animal welfare (María et al. 2004).

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Tables and Figures

Table 1: Summary table of the significance of the main effects and their interaction for instrumental meat quality parameters in rabbits in Spain.

	Transport time	Position the truck	on Season	Transport Position	x Transport Season	x Position Season	x Transport Position
ODOUR							
Rabbit odour intensity	NS	NS	NS	NS	*	NS	NS
Odd dours	NS	NS	*	NS	NS	NS	NS
TEXTURE:							
Tenderness	NS	NS	NS	NS	*	NS	NS
Juiciness	NS	NS	NS	NS	NS	NS	NS
Fibrousness	NS	NS	NS	NS	NS	NS	NS
Greasiness	NS	NS	NS	NS	NS	NS	NS
FLAVOUR							
INTENSITY:							
Rabbit flavour	NS	NS	NS	NS	NS	NS	NS
Odd flavours	NS	NS	NS	NS	NS	NS	NS

Levels of significance: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Season refers to summer or winter.

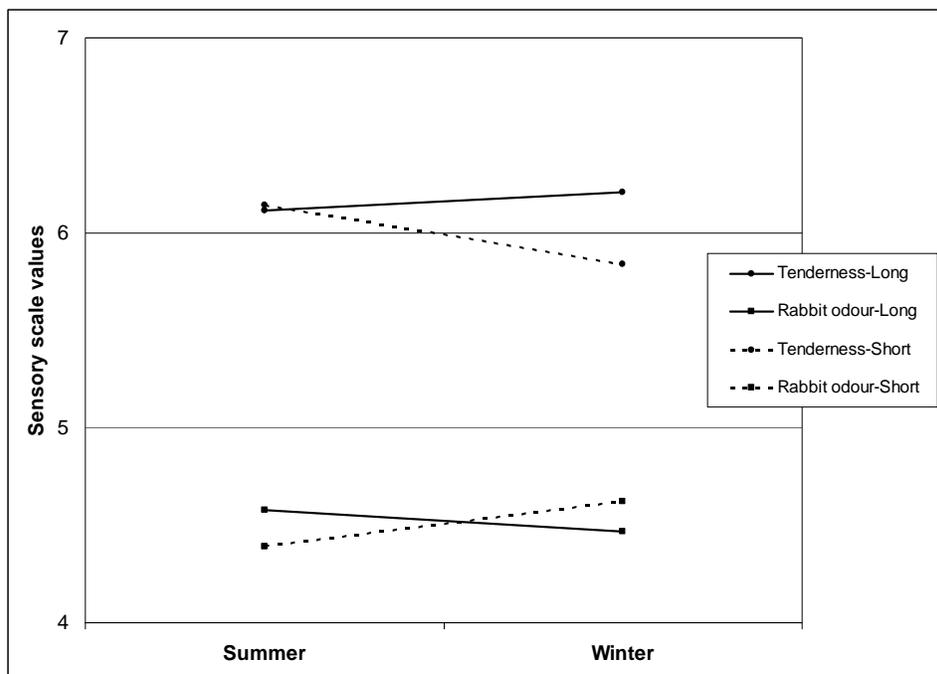
Time: journey time (1 hours or 7 hours). Position: position in the multi floor cage rolling stand (top, middle or bottom cages) during transport.

Table 2: Least squared means (\pm s.d.) of sensory traits analyzed according to transport time, season and position within the truck in commercial rabbits (2kg.) in Spain.

	Transport time		Season		Position on the truck		
	Long (7h)	Short (1h)	Hot	Cold	upper	middle	bottom
ODOUR							
INTENSITY							
Rabbit odour	4.52 \pm 0.06	4.51 \pm 0.06	4.48 \pm 0.06	4.54 \pm 0.06	4.52 \pm 0.07	4.49 \pm 0.07	4.53 \pm 0.07
Strange odours	1.62 \pm 0.07	1.74 \pm 0.07	1.78 \pm 0.07 ^a	1.58 \pm 0.07 ^b	1.7 \pm 0.08	1.74 \pm 0.08	1.6 \pm 0.08
TEXTURE							
Tenderness	6.16 \pm 0.09	5.99 \pm 0.09	6.13 \pm 0.09	6.02 \pm 0.09	6.04 \pm 0.11	6.11 \pm 0.11	6.08 \pm 0.11
Juiciness	3.78 \pm 0.07	3.86 \pm 0.07	3.84 \pm 0.07	3.8 \pm 0.07	3.78 \pm 0.08	3.87 \pm 0.08	3.82 \pm 0.08
Fibrousness	3.14 \pm 0.09	3.33 \pm 0.09	3.17 \pm 0.09	3.3 \pm 0.09	3.25 \pm 0.11	3.11 \pm 0.11	3.34 \pm 0.11
Greasiness	3.42 \pm 0.06	3.35 \pm 0.06	3.36 \pm 0.06	3.41 \pm 0.06	3.36 \pm 0.08	3.35 \pm 0.08	3.45 \pm 0.08
FLAVOUR							
INTENSITY							
Rabbit flavour	5.15 \pm 0.05	5.23 \pm 0.05	5.22 \pm 0.05	5.15 \pm 0.05	5.09 \pm 0.07	5.19 \pm 0.07	5.28 \pm 0.07
Strange flavours	1.98 \pm 0.07	2.14 \pm 0.07	2.12 \pm 0.07	2.01 \pm 0.07	2.09 \pm 0.08	2.06 \pm 0.08	2.04 \pm 0.08

Different letters in the same row indicate significant differences, $p < 0.05$.

Figure 1. Interaction effect between transport time (short or long) and season (winter or summer) for two sensory traits, tenderness and rabbit odour intensity.



CONJUGATED ISOMERS OF LINOLEIC ACID IN MERTOLENGA-PDO BEEF AS AFFECTED BY SLAUGHTER SEASON AND MUSCLE TYPE

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Key Words: beef, conjugated linoleic acid, muscle type, slaughter season.

Introduction

Meat from autochthonous bovine breeds based on traditional production systems has been progressively reintroduced in Portuguese market as a result of its putative highly intrinsic quality (Costa *et al.*, 2003). One of such example is Mertolenga beef with Protected Designation of Origin (PDO) (Commission Regulation n°1107/96 of 12/06, EC), obtained from Mertolenga breed bullocks raised on a semi-extensive production system, in the south of Portugal (Alentejo), according to the product specifications.

Foods from ruminants, such as milk and meat, are the most important dietary sources of conjugated isomers of linoleic acid (CLA), a minor group of fatty acids formed in the rumen as intermediates during the biohydrogenation of long-chain unsaturated fatty acids or through endogenous Δ^9 desaturation of *trans*-11 octadecenoic acid (Poulson *et al.*, 2004). In recent years, much research has been focused on CLA, particularly in *9c11t*, the main CLA occurring naturally in foods, together with *10t12c*, due to its health-promoting biological activities in animals, including anticarcinogenic, antithrombotic, antiatherogenic, antidiabetogenic and immunomodulatory properties (Belury, 2002).

Meat fatty acid composition is influenced by dietary factors, and in lower extent, by genetic factors (De Smet *et al.*, 2004). The dietary factors are often linked with a particular feeding strategy and production system (Raes *et al.*, 2004). It is well documented that, including grass in the diet of steers, caused a desirable increase in the CLA concentration (French *et al.*, 2000). Thus, meat from grazing ruminants is expected to reflect the variability of pasture biomass and the nutritive value of pasture is highly dependent on cultural practices, season and geographical factors (Moloney *et al.*, 2001).

Objectives

The aim of this research was to evaluate seasonal changes (based on early autumn and late spring sampling) and the influence of muscle type (*longissimus thoracis*, *longissimus*

lumborum and *semitendinosus* muscles) on the profile of conjugated isomers of linoleic acid in Mertolenga-PDO beef.

Methodology

Mertolenga breed bullocks were raised on pasture and finished on concentrate in the last 3 to 6 months. Bullocks were slaughtered in early autumn (n=15; mean \pm SE of age and carcass weight were 17 \pm 0.7 months and 239 \pm 7 kg) and in late spring (n=15; 24 \pm 0.7 months and 231 \pm 8 kg). Meat samples were taken from *longissimus thoracis* (LT), *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of bullocks.

Intramuscular fat was extracted with methylene chloride-methanol (4:1 v/v) and *n*-hexane, as described by Fritsche *et al.* (2000). CLA isomers were converted to methyl esters by base-catalysed transesterification with sodium methoxide 0.5 M solution in anhydrous methanol, in order to avoid isomerisation of isomers. The methyl esters of CLA isomers were individually separated and quantified by triple column silver-ion (ChromSpher 5 Lipids, 4.6 mm ID \times 250 mm, 5 μ m particle size), using an HPLC system (HP 1100 Series) equipped with autosampler and diode array detector (DAD) adjusted at 233 nm, with a solvent (0.1 % acetonitrile in *n*-hexane) flow rate of 1 ml/min and injection volumes of 20 μ l. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial standards and with values published in the literature (Fritsche *et al.*, 2000). Total and individual CLA isomers contents in meat were determined based on the external standard technique (using 9*c*,11*t*, 10*t*,12*c*, 9*c*,11*c* and 9*t*,11*t* as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000).

The data were analysed using the GLM procedure of SAS (1989). Total and specific CLA contents and the proportion of each CLA isomer were studied by analysis of variance, including the effects of animal inside slaughter season (A(S)), slaughter season (S), muscle type (M) and interaction between S and M. The effect of slaughter season was tested using A(S) as error term. When the *F*-test was significant, the least-squares means were compared.

Results & Discussion

Total CLA (mg/g muscle) and specific CLA (mg/g fat) contents were not affected ($p > 0.05$) by slaughter season. Total and specific CLA contents were higher ($p < 0.001$) in LT and LL muscles than in ST muscle. The values of specific CLA contents ranged between 2.6 and 4.5 mg/g fat. These values are in accordance with those reported by Realini *et al.* (2004), which found average values of 2.5 mg/g fat for concentrate-fed beef and 5.3 mg/g fat for grass-fed beef.

The slaughter season affected several some individual and the sums of CLA isomers in Mertolenga-PDO beef fat. The isomers 12*t*,14*t* ($p < 0.01$), 11*t*,13*t* ($p < 0.001$), 11*t*,13*c* ($p < 0.01$), 9*c*,11*c* ($p < 0.05$) and the sum of total *t,t* ($p < 0.01$) showed higher percentages in spring slaughtered animals than in autumn slaughtered animals. In contrast, the isomers 10*t*,12*c* ($p < 0.05$), 7*t*,9*c* and total *c/t* CLA ($p < 0.001$) showed higher percentage in autumn slaughtered animals than in spring slaughtered animals. Although little information is currently available to assess seasonal changes in beef CLA, Lock and Garnsworthy

(2003) observed that CLA percentages on milk fat varied throughout the year, with the highest values registered in the summer months (May to July), when cows received fresh grass. The CLA isomeric distribution showed a clear predominance of the bioactive 9*c*,11*t* isomer (rumenic acid) in both seasons. However, the relative proportion of 9*c*,11*t* to the total CLA in Mertolenga-PDO beef (68.5-74.5%), which did not show neither seasonal nor muscle location effects, was lower than the values presented by Fritsche *et al.* (2000). The major difference between muscles was observed for 11*t*,13*t* CLA isomer ($p < 0.001$). The interaction between slaughter season and muscle type was only significant for the percentages of the bioactive isomers (10*t*,12*c* and 9*c*,11*t*) and for 12,14*c/t* and 11*t*,13*t* CLA isomers.

Conclusions

The results of the present study indicate that total and specific CLA contents of Mertolenga-PDO beef are not significantly affected by slaughter season, although important seasonal variations were observed in CLA isomer distribution. Additionally, the CLA profile is also affected by muscle type, resulting lower amounts of CLA in ST muscle. The rumenic acid (9*c*11*t*) was not affected by slaughter season and muscle type although their significant interaction. The characterization of the Mertolenga-PDO beef based on conjugated isomers of linoleic acid content throughout the year provide added value to the consumers (selection based on the quality/prize ratio) and to the producers (health claims, with higher prices in the market).

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Tables and Figures

Table 1. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers of intramuscular fat in *longissimus thoracis* (LT), *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of beef from Mertolenga bullocks reared according Mertolenga-PDO specifications and slaughtered in early autumn and late spring.

	Mertolenga-PDO beef						SEM	Effects ¹		
	Autumn			Spring				S	M	S×M
	LT	LL	ST	LT	LL	ST				
total CLA	0.062	0.072	0.039	0.066	0.061	0.031	0.007	ns	***	ns
specific CLA	3.65	4.53	3.39	3.51	4.12	2.63	0.314	ns	***	ns
CLA isomers (% total CLA)										
12 <i>t</i> ,14 <i>t</i>	0.97	0.96	0.81	2.05	1.75	1.22	0.151	**	**	ns
11 <i>t</i> ,13 <i>t</i>	1.47 ^a	1.37 ^{a,b}	0.92 ^b	4.43 ^c	3.68 ^d	2.51 ^e	0.187	***	***	**
10 <i>t</i> ,12 <i>t</i>	0.98	0.90	0.66	0.92	0.91	1.12	0.191	ns	ns	ns
9 <i>t</i> ,11 <i>t</i>	2.80	2.88	2.99	3.36	3.70	4.03	0.260	ns	ns	ns
8 <i>t</i> ,10 <i>t</i>	0.70	0.65	0.71	0.67	0.77	0.40	0.092	ns	ns	ns
7 <i>t</i> ,9 <i>t</i>	0.99	0.83	1.32	1.02	1.01	0.88	0.176	ns	ns	ns
6 <i>t</i> ,8 <i>t</i>	0.28	0.31	0.06	0.32	0.44	0.20	0.074	ns	**	ns
12 <i>t</i> ,14 <i>c/t</i>	1.05 ^a	1.25 ^a	2.66 ^b	2.29 ^{b,c}	2.32 ^{b,c}	1.50 ^{a,c}	0.341	ns	ns	**
11 <i>t</i> ,13 <i>c</i>	1.16	1.40	2.62	3.55	3.41	3.56	0.312	**	*	ns
11 <i>c</i> ,13 <i>t</i>	0.51	0.68	1.15	0.33	0.42	0.34	0.175	ns	ns	ns
10 <i>t</i> ,12 <i>c</i>	2.44 ^{a,b}	2.13 ^{a,d}	2.99 ^b	1.33 ^c	1.69 ^{c,d}	1.48 ^c	0.209	*	ns	*
9 <i>c</i> ,11 <i>t</i>	74.50 ^a	71.70 ^{b,d}	68.68 ^c	69.51 ^{b,c}	68.52 ^c	72.56 ^{a,d}	0.906	ns	ns	***
7 <i>t</i> ,9 <i>c</i>	2.44	2.13	2.99	8.90	9.96	8.47	0.479	***	ns	ns
9<i>c</i>,11<i>c</i>	1.05	1.05	0.98	1.32	1.42	1.73	0.159	*	ns	ns
<i>total t,t</i>	8.20	7.89	7.46	12.76	12.26	10.36	0.506	**	**	ns
<i>total c,t</i>	90.75	91.06	91.56	85.91	86.32	87.91	0.544	***	*	ns

¹Levels of significance: ns, $p>0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; means in the same row with different superscripts are significantly different ($p<0.05$); SEM, standard error of mean. The symbols used mean as follow: S, season; M, muscle type.

BACKFAT QUALITY OF SOUTH AFRICAN BACONER PIGS WITH DIFFERENT CARCASS CLASSIFICATION CHARACTERISTICS

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Key Words: Backfat, South Africa, classification, pigs, fat quality, survey

Introduction

Wood (1984) defined good quality fat in pigs as firm and white. He defined poor quality fat as soft, oily, wet, grey and floppy. Processed meat products like bacon and fermented sausages are especially affected by soft, unsaturated fat of poor quality (Fischer, 1989). Feed ingredients commonly used in pig diets in South Africa are rich in unsaturated fatty acids and have the potential to produce soft backfat with poor technological properties (van der Merwe, 1985; Madsen, Jakobsen & Mortensen, 1992). Other factors that may contribute to inferior fat quality are the very low slaughter weights and constant decrease in backfat thickness of South African pigs over the last decade (SAMIC, 2005).

Iodine value is the parameter mostly used to evaluate subcutaneous pork fat quality (Häuser & Rhyner, 1991). Extractable fat content, refraction index, content of individual fatty acids and ratios thereof have also been used to predict fat quality (Hart, 1956; Enser, 1983; Houben & Krol, 1983; Honkavaara, 1989; Häuser & Prabucki, 1990; Prabucki, 1991; Warnants, Van Oeckel & Boucqué, 1998). A need exists for a rapid method for identifying poor fat quality. A system worth evaluating for application in South Africa is the French system of predicting fat quality. This system entails the selection of adipose tissue using an indirect method based on carcass grading information (Davenel et al., 1999). In the current South African pork classification system, lean meat content is calculated by means of a single backfat and muscle thickness measurement (between the 2nd and 3rd last rib, 45 mm from the carcass midline) in the case of the Hennesey Grading Probe or only backfat thickness in the case of the Intrascop (Bruwer, 1991).

Objectives

The objectives of this study were to obtain an overview of the situation regarding fat quality of pig carcasses in South Africa, to determine whether fat quality of pigs in different classification groups differed and whether backfat thickness and lean meat content groups in the South African pig classification system (PORCUS) could be used to predict fat quality of pig carcasses.

Methodology

Over a one year period, a total of 2107 pig carcasses from all classification groups were sampled at a major South African pig abattoir. A backfat sample was removed at the midline position of each carcass perpendicular to the hole made by the Hennessey Grading probe. Total lipid was extracted from the backfat, according to the method of Folch, Lees & Sloane-Stanley (1957). Fatty acid methyl esters (FAME) was prepared using methanol-BF₃ (Slover & Lanza, 1979). Fatty acid methyl esters were quantified using a Varian GX 3400 flame ionization gas chromatograph, with a capillary column (Chrompack CPSIL 88, 100 m length, 0.25 µm ID, 0.2 µm film thickness). Identification of FAME was made by comparing retention times with those of standards obtained from SIGMA (189-19). The following fatty acid ratios were calculated: saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), stearic to linoleic acid ratio (C18:0/C18:2) and double bond index (DBI). Iodine value was determined by means of the Hanus method (AOAC, 2000). Refraction index was determined with an Abbe refractometer (AOAC, 2000). Analysis of variance (ANOVA) and the Tukey-Kramer multiple comparison test was used to determine whether fat quality parameters of pig carcasses in different classification groups differ significantly (NCSS, 2004).

Results & Discussion

Significant differences were observed between classification groups for carcass mass, backfat thickness, muscle thickness and lean meat content (Table 1). Backfat thickness values of the O, R, C, U and S classification groups, conformed to the minimum backfat thickness value of 15 mm proposed by Davenel et al. (1999). The P classification group had an average lean meat content of 70.10 % and the S classification group an average lean meat content of 60.44 %. Lean meat content from all classification groups were higher than the maximum value of 57 % proposed by Davenel et al. (1999) for good fat quality.

In Table 1, extractable fat content showed a significant ($P < 0.001$) increase with decreased lean meat content and increased backfat thickness (i.e. from the P to the S classification group). None of the groups reached the minimum value of 84 % proposed by Prabucki (1991) for good fat quality. Iodine values showed a significant decrease with increased backfat thickness and decreased lean meat content (Table 1). Only the C, U and S classification groups had average iodine values lower than 70 proposed by Barton-Gade (1987) as the maximum for good fat quality. No classification group could reach a refraction index value of below 1.4598 proposed by Hart (1956) as the maximum for good fat quality.

Both palmitic (C16:0) and C18:0 levels increased significantly ($P < 0.001$) from the P to S classification groups. Good quality fat should contain more than 12 % C18:0 (Houben & Krol, 1983), a criterion fulfilled only by the R, C, U and S classification groups. A significant ($P < 0.001$) increase in oleic acid (C18:1) was observed with increased backfat thickness and decreased lean meat content. Linoleic acid content decreased significantly

Table 1. Average carcass classification and fat quality measurements of pig carcasses within the different classification groups of the SA pig classification system.

Classification group	P	O	R	C	U	S	All pigs
% Lean meat content range	≥ 70	68–69	66–67	64–65	62–63	≤ 61	-
Backfat thickness range (mm)	≤ 12	13–17	18–22	23–27	28–32	> 32	-
Number of pigs	539	539	544	347	102	36	2107
Classification parameters:							
Carcass mass (kg)	69.83^a (5.73)	73.25^b (5.70)	74.72^c (5.78)	76.44^d (5.52)	78.10^d (5.59)	77.36^{cd} (6.10)	73.58 (6.24)
Backfat thickness (45mm)	11.61^a (1.15)	15.05^b (1.26)	18.77^c (1.32)	22.97^d (1.35)	26.95^e (1.39)	31.41^f (2.55)	17.29 (5.00)
Muscle thickness (mm)	53.86^c (5.32)	52.55^d (5.13)	51.20^c (5.23)	50.19^c (5.74)	48.22^b (5.72)	44.54^a (7.70)	51.80 (5.68)
Lean meat content (%)	70.10^f (0.50)	68.43^e (0.56)	66.64^d (0.56)	64.65^c (0.57)	62.70^b (0.57)	60.44^a (1.15)	67.36 (2.40)
Chemical parameters:							
Extractable fat (%)	70.22^a (5.07)	74.43^b (3.89)	77.00^c (3.01)	78.69^d (2.80)	79.94^e (2.16)	79.95^{de} (3.28)	75.08 (5.03)
Iodine value	76.95^d (5.15)	73.01^c (4.61)	70.65^b (4.20)	68.97^a (4.00)	68.21^a (3.86)	67.46^a (3.67)	72.42 (5.45)
Refraction index	1.46152^d (0.00061)	1.46102^c (0.00054)	1.46073^b (0.00049)	1.46056^a (0.00042)	1.46047^a (0.00044)	1.46036^a (0.00047)	1.46096 (0.00064)
Individual fatty acids:							
C16:0	20.73^a (1.37)	21.73^b (1.29)	22.43^c (1.22)	22.92^d (1.14)	23.19^d (1.05)	23.53^d (1.16)	21.95 (1.52)
C18:0	11.06^a (1.33)	11.68^b (1.27)	12.15^c (1.31)	12.51^d (1.26)	12.66^d (1.24)	13.00^d (1.25)	11.85 (1.41)
C18:1	39.13^a (2.26)	40.52^b (2.00)	41.15^c (1.88)	41.41^c (1.96)	41.45^c (1.60)	41.25^{bc} (2.02)	40.53 (2.20)
C18:2	20.13^b (3.44)	17.27^b (3.03)	15.76^b (2.63)	14.89^a (2.67)	14.55^a (2.42)	14.27^a (2.59)	17.04 (3.58)
Fatty acid ratios:							
SFA (%)	33.61^a (2.54)	35.20^b (2.35)	36.36^c (2.33)	37.17^d (2.16)	37.60^d (2.13)	38.27^d (2.17)	35.59 (2.73)
MUFA (%)	43.42^a (2.45)	45.08^b (2.19)	45.64^c (1.89)	45.85^c (2.11)	45.79^c (1.81)	45.47^{bc} (1.98)	44.97 (2.35)
PUFA (%)	22.33^d (3.74)	19.20^c (3.23)	17.53^b (2.81)	16.59^a (2.88)	16.23^a (2.66)	15.88^a (2.73)	18.94 (3.87)
C18:0/C18:2	0.57^a (0.15)	0.70^b (0.17)	0.80^c (0.20)	0.88^d (0.23)	0.90^d (0.22)	0.95^d (0.22)	0.74 (0.22)
DBI	90.49^d (6.12)	85.61^c (5.27)	82.56^b (4.86)	80.73^a (4.65)	79.84^a (4.59)	78.79^a (4.51)	84.87 (6.48)

The value in brackets refer to standard deviation. Means with different superscripts within the same row differ significantly ($P < 0.001$)

($P < 0.001$) from the P to S classification groups. Only the C, U and S classification groups complied with the 15 % maximum C18:2 content proposed by Enser (1983) for good fat quality.

A significant ($P < 0.001$) increase in total SFA content was observed from the P to S classification groups. No group as a whole could comply with the 41 % minimum SFA content proposed by Häuser & Prabucki (1990) for good fat quality. A significant ($P < 0.001$) increase in the total MUFA content was also observed from the P to C classification groups, but a decrease in the MUFA content in the U and S groups were found. All classification groups complied with the 57 % maximum MUFA content proposed by Häuser & Prabucki (1990) for good fat quality. A significant ($P < 0.001$) decrease was observed in total PUFA content from the P to the S classification groups. No classification group could comply with the 15 % maximum PUFA content proposed by Warnants et al. (1998). The C18:0/C18:2 ratio increased significantly with increased backfat thickness and decreased lean meat content. No group could comply with the minimum C18:0/C18:2 ratio of 1.2 proposed by Honkavaara (1989) for good fat quality. A significant decrease was observed in DBI from the P to the S groups. Only the U and S groups could comply with the maximum DBI value of 80 proposed by Prabucki (1991) for good fat quality, while individual pigs from all the groups had DBI values of less than 80.

In spite of the fact that the Hennessey measured backfat thickness from most classification groups conformed to Davenel et al.'s (1999) requirement of more than 15 mm, no classification group could conform to their requirement of < 57 % lean meat for good fat quality. Fat quality from the R, C, U and S classification groups conformed to some of the international fat quality requirements (Table 1). This clearly illustrated that the maximum value of 57 % lean meat content and minimum value of 15 mm backfat thickness used by the French to predict good fat quality are not applicable to South African conditions and that these values will have to be recalculated for the South African pig classification system.

Conclusions

This research indicated that significant differences exist in backfat quality of pigs in different classification groups. Increased backfat thickness and decreased lean meat content were associated with good fat quality. From the data discussed above it is clear that the fat quality of pigs in the P and O classification groups sampled in this survey was generally inferior to those from the R, C, U and S classification groups. Due to the demand for the carcasses of leaner pigs in South Africa, higher prices per kg are generally paid for carcasses from the P and O classes than from the R, C, U and S classes (i.e. those with thicker backfat of better quality).

In pigs and other monogastric animals, the fatty acid composition of the fat tissue triglycerides (particularly in subcutaneous fat) can be changed by altering the fatty acid composition of dietary fat, which is absorbed intact from the small intestine and incorporated directly into fat tissue (Rhee, Davidson, Cross, & Ziprin, 1990). This means that it is possible to modify the fatty acid composition of pigs by the strategic use of specific dietary fat sources (Morgan, Noble, Cocchi & McCartney, 1992). This implies that by including feedstuffs with a more saturated fatty acid profile, dietary manipulation may be used to solve the problem of soft and low quality backfat of pigs in especially the P and O classification groups.

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THERMAL STABILITY OF CONNECTIVE TISSUE AND MEAT QUALITY OF LOOSE STRUCTURED PORK *SEMIMEMBRANOSUS* MUSCLES

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Key Words: Connective tissue, pork, *semimembranosus*, meat quality, differential scanning calorimetry

Introduction

The main roles of connective tissue in muscle are to ensure the passive elastic response of muscle transform the force from muscle fibers into mechanical movement and to mechanically support the vessels and nerves. Collagen is the main protein of all connective tissues and type I collagen is the most abundant one in intramuscular connective tissue (Bailey & Light 1989, Kjær 2004). Researchers have found that collagen solubility decreases with animal age and varies between muscles and animal species (Hill, 1966; Kovanen et al. 1980; Nakano & Thompson 1980). This is due to the maturation of collagen cross-links in muscles. Also different proportions of collagen types have an effect on thermal stability of collagen (Bailey & Light 1989). Thermal stability of collagen has originally been measured to obtain information on the relationship between collagen solubility and meat tenderness (Goll et al. 1963; Carmichael & Lawrie 1967).

Zones of PSE (pale, soft, exudative) like meat, which are accompanied by loose fibre structure have been observed on the lateral surface of pork *semimembranosus* muscle (Minvielle et al. 2001; Franck et al. 2002; Franck et al. 2003; Laville et al. 2005). Risk factors for these zones, seem to include high carcass weight and low ultimate pH in *semimembranosus* muscle (Minvielle et al. 2001). There are also studies showing that PSE zones appear in higher percentage of hams from pigs carrying *n* allele at HAL locus (Aubry et al. 2000) and RN⁻ allele at the RN locus (Le Roy et al. 2001). Higher collagen solubility, glycolytic potential, free glucose, glucose-6-phosphate and lactate have been observed in defective muscles compared to normal muscles (Minvielle et al. 2001).

This loose structured meat is also characterised by the ease at which bundles can be pulled away by hand (personal observations on Finnish and Irish samples). It is possible that these abnormalities can be attributed in part to connective tissue properties. Therefore it would be of interest to study these properties and any subsequent influence on quality attributes.

Objectives

The objective of this study was to compare thermal stability of connective tissue from normal and loose structured *semimembranosus* muscle and to measure general meat quality parameters of the two groups of meat.

Methodology

Semimembranosus muscles were visually assessed at 24hr postmortem in the boning hall of commercial abattoirs in Ireland following standard slaughter, chilling and boning procedures. Approximately 100g of sample was taken from muscles showing 'loose structure' (n=7) and 'normal structure' (n=7) (photo 1).

On return to The National Food Centre, reflectance [Opto-star (SFK technology, Denmark)], conductivity [Pork Quality Meter (PQM) (Inteck GmbH, Industriestrasse 9, D-86551 Aichach, Germany)] and colour [Miniscan XE Plus (Hunter Associates Laboratory Inc., Virginia, USA)] measurements were recorded on all samples. A rough estimate of drip loss was obtained by holding the samples (approx. 100g) at +1°C for three days (modification of method of Honikel 1998). pH was recorded after drip loss assessment [meter model no: 420A (Orion Research Inc., Boston, MA 02129, USA); Amagruss pH electrode (pH/mV Sensors Ltd., Murrisk-Westport, Co. Mayo, Ireland)]. Meat was blended with Robot coupe blender (R301 Ultra, Robot coupe SA, France) in order to blend the drip back into meat before pH measurements. Results for individual meat samples for reflectance, conductivity and color were an average of four readings across each sample surface.

Connective tissue was extracted from myofibril proteins according to King (1987) and Aktas & Kaya (2001) with few modifications. Thermal properties of connective tissue were determined in DSC2010 with refrigerating cooling system (RCS) (TA Instruments, AGB Scientific, Dublin, Ireland). The samples were heated +10 – +95°C with the rate 5°C/min in the DSC which was calibrated with mercury (mp -38.8°C, ΔH_m 11.4Jg⁻¹) distilled water (mp -0.0°C, ΔH_m 334.5Jg⁻¹), gallium (mp 29.8°C, ΔH_m 80Jg⁻¹) and indium (mp 156.6°C, ΔH_m 28.45Jg⁻¹). Both the onset temperature and the peak of thermal transition temperature of connective tissue were recorded from the thermogram. Statistical analysis was carried out using SPSS12 (SPSS for Windows).

Results & Discussion

Following analysis of the data it was noted that a number of the 'normal structure' muscles were exhibiting DFD like characteristics. Therefore statistical analyses were conducted including and excluding these data. Table 1 shows the results obtained with the DFD like muscles removed. No significant differences were noted in ultimate pH (pH_u), conductivity, Hunter a* value and drip loss values. Color in loose structured meat was lighter (L*), more yellow (b*) and had higher Reflectance% than in 'normal structured' meat. This was anticipated as 'loose structured' muscle was visibly paler.

The onset and peak of thermal transition temperature of connective tissue (in Table 1.) measured with DSC were lower (p<0.001) in loose structured meat than in normal structured meat. Although the number of samples was very small the results were

statistically significant. It was interesting to note that when the full set of data was analysed (n=7 for both groups) similar results were obtained (results not presented). As the selection of samples was based on visual structure this is not so surprising. Franck et al. (2002) concluded that the defective *semimembranosus* muscles show PSE characteristics, low pH at 30-45 post mortem with high temperature and lower pH_u than normal looking meat. It was not feasible in this experiment to obtain early *post mortem* pH data therefore we do not have direct evidence of fast pH fall which could have related the loose structure to PSE defect. Although, Horgan et al. (1991) already found that decreasing the pH of connective tissue decreases the thermal transition temperature. They also noted that in addition to pH thermal transition temperature of connective tissue is dependent on the composition of the cross-links of collagen. Even though the conditions of Horgan et al. (1991) study were not corresponding to raw meat, this could indicate that lower pH_u in loose structured muscles had some effect on connective tissue by lowering the thermal transition temperature. Also findings of Aktas & Kaya (2001) support the theory that low pH_u lowers thermal transition temperature. After marinating connective tissue in lactic acid they recorded thermal transition temperatures around 39°C which was 23-25°C lower than that of control group but again, the conditions were not corresponding to raw meat.

Table 1. Mean values and standard deviations of meat quality and DSC traits of loose and normal structured pork *semimembranosus* muscles.

	n=7)	(n=3)	P
7 3)	0.128	0.038	NS
3)	3.311	2.794	**
)	1.948	.529	NS
)	1.074	0.528	***
ctivity (day 1), mS/cm	0.588	4.003	NS
ance (day 1), %	8.013	6.526	**
estimate for drip loss, %	03	06	NS
set, °C	0.784	0.766	***
ak, °C	0.396	0.397	***

*** p<0.001, ** p<0.01, * p<0.05, NS not significant

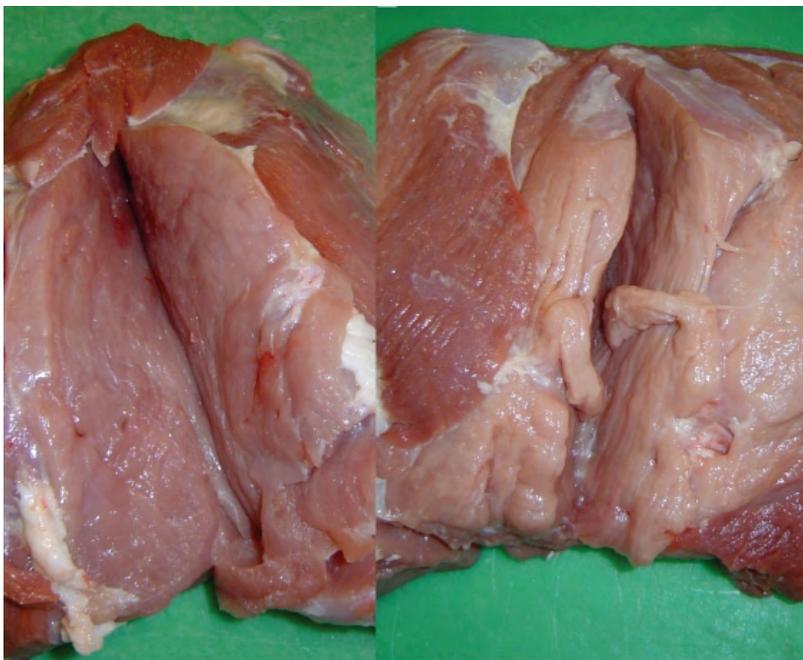


Photo 1. Examples of ‘normal structure’ (RHS) and ‘loose structure’ (LHS) *semimembranosus* muscle.

Conclusions

Thermal properties of connective tissue were compared in ‘loose structure’ and ‘normal structure’ pork *semimembranosus* muscles. A significantly lower thermal transition temperature of intramuscular connective tissue in was observed in the ‘loose structure’ muscle. It would be interesting to further clarify the role of connective tissue in weakening of the structure in *semimembranosus* muscles and the interaction with meat quality characteristics.

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TOTAL AND HEAT SOLUBLE COLLAGEN CONTENTS IN DIFFERENT EUROPEAN CATTLE BREEDS – PRELIMINARY RESULTS

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Key Words: collagen, soluble collagen, beef, breed, compression

Introduction

Selective breeding in cattle has been very successful in increasing production factors such as daily gain and feed efficiency for beef breeds and milk yield for dairy breeds. Until now there is limited information and subsequent little knowledge to allow selection programs to be designed to improve product quality. Identification of the genes involved in aspects of carcass and meat quality will provide the basic information to devise breeding programs that enhance quality. The present study is part of an ongoing EU-project which examines young bulls from 15 different breeds (both dairy and beef) representing the genetic diversity of European cattle. In vivo measurements covering production, slaughter data, carcass quality, meat quality parameters (both physical and chemical) and qualitative traits and their relationship with meat quality characteristics are investigated.

Tenderness is one of the most important quality attributes in beef (reviewed by Geay et al., 2001). The two main components of meat toughness are the myofibrillar component and the connective tissue (reviewed by Maltin et al., 2003). The connective tissue is believed to contribute to the background toughness. The total amount and chemical composition of the collagen within the connective tissue is thus of large importance for the background toughness.

Objectives

The objectives of this study were to investigate the effect of breed on total and heat soluble collagen content in *M. longissimus thoracis* and to study the correlation between total collagen and compression data.

Methodology

The material for connective tissue analysis consisted of 437 young bulls from 15 different European cattle breeds (30 animals per breed on average); Jersey, South Devon, Aberdeen Angus and Highland from United Kingdom; Holstein, Danish Red Cattle and Simmental from Denmark; Asturiana de los Valles, Casina, Avileña and Pirenaica from Spain; Piemontese and Marchigiana from Italy and Limousin and Charolais from France. Diet composition and slaughtering method are described in Sañudo *et al.* (2004).

At 24 hours post-mortem (p.m.) the *M. Longissimus thoracis* (between 8th and 13th rib) was excised from the left side of the carcass. One slice was cut at 24 hours p.m., vacuum packed and frozen at -20°C , the remaining was stored at $3^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until 48 hours. A section was subdivided in slices, vacuum packaged and frozen; another section was stored at $3^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until 10 days p.m., then cut, vacuum packaged and frozen. Samples were stored at -20°C until use.

Total and heat soluble collagen was determined using a slight modification of the method described by Kristensen *et al.*, 2002. Briefly; 6 grams of meat were finely chopped, mixed with 20 mL of 0.9% NaCl and heat-treated for 2 h at 90°C . After homogenisation and centrifugation (15 min at $4,000 \times g$), 30 mL of 6.0 M HCl was added to the supernatant and 50 mL of 6.0 M HCl was added to the pellet. Both were then hydrolyzed overnight in a sand bath at 110°C . The concentration of hydroxyproline was determined and a factor of 7.14 was used to convert hydroxyproline to collagen. The amount of soluble collagen was calculated from the hydroxyproline concentration in the supernatant, and the total collagen was calculated from the sum of the hydroxyproline concentration in the pellet and in the supernatant.

The procedure for instrumental analysis of raw meat samples using a modified compression device is described in Olleta *et al.* (2005).

Data were analysed by the method of least squares using the GLM procedure (SAS, 1999). The statistical model included the fixed effect of breed. In order to calculate partial correlations (i.e. corrected pearson correlations for the breed differences) between total collagen content and compression at 80%, the Manova statement was included in the GLM analysis.

Results & Discussion

As shown in Table 1 breed differences were observed in the total and heat soluble collagen contents. Overall the breeds ranged from 2.70 to 4.08 mg/g meat in total collagen and from 21.63 to 27.34% in heat soluble collagen. The smallest significant difference between breeds which have been detected in collagen content was low (5 to 10% on average), which fits well with the calculation made by Listrat and Hocquette

(2004) for the same muscle. This good sensitivity is likely to be explained by the high number of animals (on average 30) analyzed per breed.

The dairy breeds in general contained higher amounts of collagen than the meat specialized breeds. Thus, Jersey contained significantly higher amounts of collagen compared to most other breeds; however, the Jersey also had the highest proportion of heat soluble collagen (Table 1). Piemontese, and in general meat specialized animals, contained significantly less collagen than the other breeds and less heat soluble collagen than most of them (Table 1). Less collagen and a high proportion of heat soluble collagen might contribute positively to the mechanical and sensory assessments of meat toughness. Piemontese contained significantly less heat soluble collagen than most of the breeds, however, the lowest proportion of heat soluble collagen was found in Holstein and Danish Red Cattle.

The influence of breed on compression test values at 20% (C20) and at 80% (C80) is presented by Olleta et al. (2005). Lepetit and Culioli (1994) reported that C80 were mainly related to the connective tissue component of meat toughness. It was therefore interesting to investigate the correlation between C80 and the total collagen content found in the different breeds. The partial correlation, corrected for the breed differences, showed a positive correlation between total collagen and C80 at 48 hours p.m. and at 10 days p.m. (Table 2). From table 2 it also appears that for only few of the 15 breeds was there a correlation between C80 determined both 48 hours p.m. and 10 days p.m. and total collagen content. This may be explained by the relative low amount of total collagen in the *M. longissimus thoracis* by comparison with other bovine muscles. The results strongly suggest that the relationship between total collagen and C80 is breed dependent. It was initially expected that collagen would play a significant role in toughness for the breeds where its total content was high. It was not observed in practice suggesting that, not only total collagen content, but also solubility and any other connective tissue characteristics (perimysium thickness, collagen isoforms, spatial distribution and organization of connective tissue, etc) are likely to play also an important role in the determination of basal toughness (reviewed from Purslow, 2005).

Conclusions

In conclusion, the total and heat soluble collagen varies between breeds. The correlation between total collagen and compression at 80% is breed-dependent.

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Table 1. Total and heat soluble collagen in young bulls from 15 European cattle breeds slaughtered at similar age. Values are expressed as $\bar{x} \pm \text{SE}$ ($n \approx 30$).

Breed	Total collagen (mg/g muscle)	Soluble collagen (%)
Jersey	4.08 ± 0.10 ^a	27.34 ± 0.59 ^a
Holstein	3.88 ± 0.11 ^{ab}	21.63 ± 0.61 ^g
Red Danish Cattle	3.85 ± 0.11 ^b	21.05 ± 0.61 ^g
Aberdeen Angus	3.71 ± 0.11 ^{bc}	26.98 ± 0.61 ^{ab}
Charolais	3.68 ± 0.10 ^{bc}	24.12 ± 0.59 ^{ef}
Simmental	3.60 ± 0.13 ^c	25.07 ± 0.73 ^{cde}
Casina	3.60 ± 0.10 ^c	25.53 ± 0.59 ^{cd}
Marchigiana	3.58 ± 0.11 ^c	23.71 ± 0.63 ^{ef}
Highland	3.55 ± 0.11 ^c	24.14 ± 0.62 ^{ef}
Avileña	3.51 ± 0.11 ^c	24.23 ± 0.60 ^{ef}
Pirenaica	3.18 ± 0.10 ^d	23.37 ± 0.59 ^f
South Devon	3.12 ± 0.11 ^{de}	25.99 ± 0.63 ^{bc}
Asturiana de los Valles	2.96 ± 0.11 ^{ef}	24.46 ± 0.60 ^{def}
Limousin	2.85 ± 0.10 ^{fg}	24.74 ± 0.59 ^{cde}
Piemontese	2.70 ± 0.11 ^g	23.37 ± 0.60 ^f

a-g Within column, values with the same letter are not significantly different ($P > 0.05$)

Table 2. Correlation between total collagen content and compression data from 15 European cattle breeds slaughtered at similar age.

Breed	Compression 80% 48 hours p.m.	Compression 80% 10 days p.m.
Partial correlation^a	0.17^{***}	0.20^{***}
Asturiana de los Valles	0.56 ^{***}	0.71 ^{***}
Pirenaica	0.48 ^{**}	0.61 ^{***}
Holstein	0.15 ^{ns}	0.41 [*]
South Devon	0.48 [*]	0.33 ^{ns}
Simmental	0.16 ^{ns}	0.28 ^{ns}
Marchigiana	0.28 ^{ns}	0.23 ^{ns}
Limousin	0.12 ^{ns}	0.20 ^{ns}
Charolais	0.23 ^{ns}	0.14 ^{ns}
Aberdeen Angus	0.11 ^{ns}	0.04 ^{ns}
Avileña	0.22 ^{ns}	0.01 ^{ns}
Jersey	0.02 ^{ns}	-0.02 ^{ns}
Highland	0.24 ^{ns}	-0.06 ^{ns}
Red Danish Cattle	-0.24 ^{ns}	-0.07 ^{ns}
Casina	-0.13 ^{ns}	-0.10 ^{ns}
Piemontese	-0.04 ^{ns}	-0.10 ^{ns}

Superscript designates the level of significance: ns: non significant, * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), ^aPartial correlation is corrected for the breed effects

ASSESSMENT OF LAMB CARCASS COMPOSITION BY X-RAY COMPUTER TOMOGRAPHY (CT) AND IMAGE ANALYSIS

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Key Words: lamb, carcass composition, computer tomography, image analysis

Introduction

Lean, fat and bone content are not uniformly distributed in lamb carcasses. Carcass composition is used as a reference for grading systems world-wide. The purpose of grading of carcasses is usually split in two parts:

- Sorting of carcasses into groups of similar values
- Communicating needs and demands among all segments of the meat industry, from farm to fork

To assess carcass composition, one needs to know the total distribution of lean, fat and bone throughout the whole carcass. Traditionally, this has been done by full dissection, which is a costly, invasive and a time-consuming method. Therefore, less expensive, more cost-effective and non-invasive methods are required for assessment of carcass composition. X-ray computer tomography (CT) was developed by Cormack and Hounsfield, for which they received the Nobel Price Award in Medicine (1979). Since then, several applications have been tested within various fields of science, such as animal and food science. CT scanning has shown promising results as an alternative method for dissection of pork carcasses [2].

Objectives

The main objectives of this study were to:

1. study lean, fat and bone content distribution in lamb carcasses using X-ray computer CT
2. test the predictive ability of X-ray CT on lamb carcass composition using multivariate statistical methods

Methodology

Sampling

A total of 140 lambs were sampled randomly from one Norwegian abattoir during autumn 2004. The abattoir is one of the largest abattoirs in Norway and is located in the central part of southern Norway. Lambs are supplied from mountain pastures (inland). Crossbred was the dominant breed (56%) and ram was the most numerous sex in our samples (63%).

Scanning

The carcasses were scanned using a Siemens Somatom Emotion CT scanner [3]. Images were obtained by operating with a “Spine” algorithm [3], using the following set-up: voltage 130 kV, mAs 120, 512x512 pixels per scan, FOW 400 mm, pixel size 0.78 mm, slice thickness 3 mm and scan time 1s. The images were produced in DICOM format, 12-bit grayscale (2^{12}) with a range from 0 to 4,096, where 0 is total black and 4,096 is total white. This corresponds with the Hounsfield (HU) scale by an offset of -1,024 to 3071 [3], where 0 is the value of water. The total number of images per lamb varied from 25 to 27, according to carcass length, resulting in a [4 cm x n] grid, where n is number of images according to carcass length.

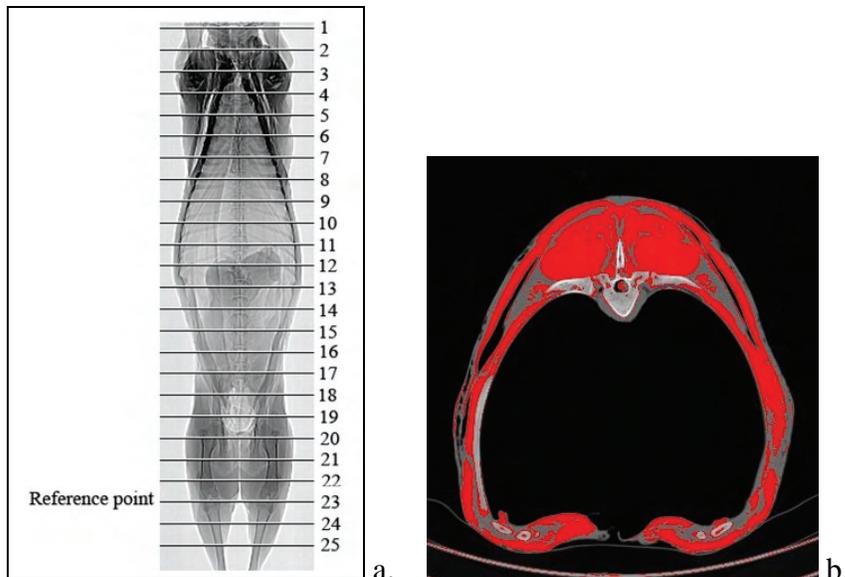


Fig. 1. CT scanning of lamb carcass.

1. Fixed scanning sites, starting point 1. Reference point (23) at distal femur.
2. Scan at 12th vertebra. Lean area is highlighted in red color.

Starting point was cranial end of carcass (neck). Distal point of leg bone (femur) was used as a reference point for each carcass (fig.1). The reference point was used to aim the [4 cm x n] grid.

Image analysis

The images from the CT scanning were pre-processed and analyzed using Image J 1.31v [4]. The various tissues in each scan slice were segmented into fat, lean and bone according to their gray value range (fig.2).

Gray value ranges were [830, 1001] for fat, [1002, 1170] for meat (lean) and [1171, 4096] for bone, according to their corresponding HU ranges: [-194, -23], [-22, 146] and [147, 3072], respectively [5].

The area of each tissue segment was obtained from the images, using the integral of pixels to calculate fat, lean and bone content from each image:

$$\text{Fat area} = \int_{830}^{1001} f(x) dx$$

$$\text{Lean area} = \int_{1002}^{1170} f(x) dx$$

$$\text{Bone area} = \int_{1171}^{2747} f(x) dx$$

$$\text{Total area} = \int_{830}^{2747} f(x) dx$$

where $f(x)$ is a function of the number of pixels per gray value range.

For the entire carcass, the sums of the images 1 through 27 were calculated:

$$\text{Total lean content, entire carcass} = \sum_{27}^1 \int_{1170}^{1002} f(x) dx$$

$$\text{Total fat content, entire carcass} = \sum_{27}^1 \int_{1001}^{830} f(x) dx$$

$$\text{Total bone content, entire carcass} = \sum_{27}^1 \int_{2747}^{1171} f(x) dx$$

The total tissue contents for each lamb were estimated and given as sum, mean, standard deviation, minimum and maximum variables.

Cutting

The carcasses were cut at the pilot plant of the Norwegian Meat Research Centre. Lean, fat and bone were separated visually and weighed according to EU regulations for pig dissection [1]. Fat content in trimmings and cuts were measured using a Scanalyzer, Scanio, Denmark. Measuring error (MSE) for Scanalyzer was 1.0% for fat [6]. Saleable meat content was calculated using weight of saleable meat, including saleable subcutaneous, inter- and intramuscular fat [7]. In this trial, cutting error was not estimated. This source of error will inevitably add to the error of prediction.

Statistical Data Analysis

Statistical data analysis was performed in The Unscrambler®v9.1 [8]. Prediction models were calculated using the partial least square (PLS) regression routine with full leave-one-out cross validation [8]. Fat, lean and bone contents from cutting were used as dependent variables, while total tissue content estimations from image analysis were used as independent variables. Two samples had to be left out due to obvious cutting and measuring errors. For PLS-modelling, 138 samples were used. Validation, uncertainty testing and selection of most important variables were estimated using full cross validation with jack-knifing and stability plots [8].

Results & Discussion

Table 1. Descriptive statistics, carcass characteristics (dependent variables)

Dependent variable	n	Mean	Median	St.dev	Min	Max
Carcass weight (kg)	140	19.77	19.40	3.49	9.20	29.60
Lean content(per cent)	140	61.19	61.35	2.72	50.73	67.51
Fat content(per cent)	140	13.04	12.39	3.24	7.36	26.09
Bone content(per cent)	140	22.09	22.21	2.16	16.54	30.04
Saleable meat (kg)	140	12.98	12.63	2.55	4.80	20.48
Saleable meat content (per cent)	140	65.46	65.38	2.69	52.19	75.44

Table 2. Correlation coefficients (Pearson) and significance test for correlation, carcass characteristics (dependent variables)

	CCW ^a	Lean ^b	Fat ^c	Bone ^d	SM ^e	SMY ^f
Carcass weight ^a	1					
Lean content ^b	-0.17*	1				
Fat content ^c	0.51**	-0.72**	1			
Bone content ^d	-0.59**	0.05 ^{ns}	-0.69**	1		
Saleable meat ^e	0.99**	-0.05 ^{ns}	0.45**	-0.64**	1	
Saleable meat content ^f	0.48**	0.62**	-0.05 ^{ns}	-0.54**	0.60**	1

- ns no significant correlation $p > 0.05$
 * $0.01 < p < 0.05$
 ** $p < 0.01$

The fixed position images [4 cm x n] were not sampled from the same anatomical sites due to variation in carcass length. Only the reference site (distal end, femur) was anatomically accurate each time. By plotting tissue contents from image analysis, a systematic skewness depending on carcass length was detected, causing small dissimilar changes over short distances. Therefore, sums of images for the entire carcass were used for modeling, which is independent of carcass length. Anatomical site selection or denser fixed grids may lead to more accurate prediction in future trials.

The distribution of lean, fat and bone varied throughout lamb carcasses:

- For lean, the largest content (per cent) occurred in the shoulder and leg region
- For fat, the largest content (per cent) occurred in the shoulder and belly region
- For bone, the largest content (per cent) occurred in the belly region

Table 3. Results form regression analysis. Cutting variables as dependent, CT variables as independent

Dependent	R ²	RMSEP*	Important CT image variable	Number of PC's (PLS-modelling)
Lean content	0.85	1.42	Mean lean area	4
Fat content	0.91	1.36	Median fat area	4
Bone content	0.84	1.16	Median lean area	4
Carcass weight	0.98	0.66	Sum lean area	2
Saleable meat	0.98	0.48	Sum lean area	2
Saleable meat content	0.81	1.50	Mean lean area	5

* Prediction error, RMSEP (Root Mean Square Error of Prediction)

Explained carcass composition variation (R²) spanned from 81.2 to 90.8 (saleable meat content and fat content, respectively) (tab. 3). The fat predictions were more accurate than the other carcass components. Prediction errors for lean, fat, bone and saleable meat content were 1.42, 1.37, 1.16 and 1.50 percent. For carcass weight and saleable meat weight, the prediction errors were 0.63 kg and 0.48 kg. The results showed that CT scanning could be used as a predictor of carcass composition, with further improvement of accuracy by adjusting imaging techniques.

The number of principal components (PC) in table 3 shows the complexity of source of variation in the model. Originally, 18 independent image variables were used to model 5 dependent cutting variables. Due to collinearity and the need to reveal data structure, latent variables or PC's were estimated by the PLS-procedure. Weight of saleable meat and carcass has low complexity, and may be modeled by 2 PC's. Saleable meat content has higher complexity, and may be modeled by using 5 PC's. 4 PC's could model the tissue contents (lean, fat and bone).

Lean image variables were the most important variables for modeling 5 of 6 cutting (dependent) variables. Only for modeling fat, fat image variables were most important.

Conclusions

Computer Tomography (CT) is a versatile method for visualizing and assessing lamb carcass composition, especially for carcass + tissue weights and fat content. For lean, bone and saleable meat content, CT showed promising results, but needs to be improved. A further study of optimal scanning sites is necessary to achieve more accurate prediction models.

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NON-DESTRUCTIVE DETERMINATION OF INTRAMUSCULAR FAT (IMF) IN PIGS BY MEANS OF ACOUSTIC PARAMETERS DERIVED FROM A CLINICAL B-MODE ULTRASOUND DEVICE

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Key Words: ultrasound spectral analysis, intramuscular fat, pig, m. longissimus

Introduction

The intramuscular fat content (IMF) of the m. longissimus (MLD) of pigs is widely regarded as an important parameter influencing sensory characteristics of porcine meat (Fernandez et al. 1999a,b; Kipfmüller et al. 2000; Eggert et al. 2002). Several authors consider an IMF content of 2 – 2.5 % (at 13th/14th lumbal vertebra / with acid pre-treatment extraction) as the optimal level in terms of sensory properties of porcine meat. (Fernandez et al. 1999) reported positive relationships up to 3,5 % IMF. Both the implementation of IMF in breeding programs in order to raise the intramuscular fat content and payment systems that also focus on quality still require an objective and reliable real-time procedure to non-destructively determine IMF in living pigs and carcasses, respectively.

Ultrasound has increasingly been investigated as a non destructive method to analyse tissue for medical applications. Compared to image analysis, spectral analysis of ultrasound echo signals provides more extensive information. (Lizzi et al. 2003) Thus, it has become one of the prominent tools for quantitative tissue characterization and differentiation of soft tissue in medical research, e.g. to classify cancer or atherosclerotic plaque (Watson et al. 2000; Raju and Srinivasan 2001; Scheipers et al. 2003).

Objectives

The aim of this study was to investigate the use of acoustic parameters obtained by spectral analysis of ultrasound echo signals to non-destructively predict intramuscular fat content of porcine longissimus muscle.

Methodology

The ultrasound data acquisition system consists of a clinical B-mode-device (KONTRON SIGMA 44 HVCD, C.K. Medical GmbH, D-84030 Ergolding) that is equipped with a mechanical sector scanner (KONTRON type Wobbler AA5A). The centre frequency of the probe is 3.5 MHz. The corresponding -6dB bandwidth ranges from 2.8 to 3.8 MHz. The B-mode device is equipped with an interface to obtain the

backscatter data (voltages) prior to digitalisation by means of a SPECTRUM PCI.212 A/D converter-card (Spectrum Systementwicklung, D-22962 Siek) with a sampling frequency of 20 MHz.

Ultrasound data acquisition was performed about 45 min post mortem after carcass classification at hot carcasses and about 20 hours p.m. at chilled loins (with and without back fat, respectively). Table 1 illustrates the measurement conditions.

Table 1: Survey of the measurement conditions of ultrasound data acquisition.

time	object	temperature [°C]
45 min post mortem	carcass warm	37.2 ± 2.5
20 h post mortem	loin w/backfat	4.5 ± 1.4
20 h post mortem	loin w/o backfat	4.5 ± 1.4

Scanning localization at m. longissimus was chosen with respect to the official site of carcass classification at slaughter (13th/14th rib). The ultrasound transducer was placed about 6 cm from the middle line of the pig back towards ventral. As for scanning direction, 3 measurements each were conducted both perpendicular and parallel to the muscle fibre direction. This is with respect to reported anisotropy of acoustic parameters. Sonogel was used as coupling medium. All measurements were performed by the same person experienced in diagnostic ultrasound.

Parameter calculation was done off-line from the raw data that have been stored. Therefore, regions of interest (ROI) were selected covering approximately 8,4 cm² of longissimus muscle. The corresponding ultrasound echo signals were then transformed into spectra by means of Fast Fourier Transform (FFT). From the spectra, 60 parameters were derived that mainly describe attenuation and backscattering of the ultrasound. (Mörlein, Rosner et al. 2005) Further laboratory work aimed at both the compensation of system specific effects and user settings, e.g. time gain compensation (TGC). That included measurements on tissue mimicking phantoms and sound pressure distribution measurements by means of the thin-wire method as reported in detail in. (Brand 2004)

In total, 115 carcasses of the same genetic origin were investigated. At the site of ultrasound data acquisition one chop was removed from the longissimus muscle for chemical determination of intramuscular fat content in duplicate by means of petrol ether using Soxtherm-apparatus with acid pre-treatment. The IMF content is related to fresh matter.

Statistical analysis was done using SAS (The SAS institute, version 8e), STATISTICA (STATSOFT, version 6.1) and The Unscrambler (Camo, version 9.0) software packages. Regression and discriminant analyses were performed with stepwise procedures. The obtained acoustic parameters were averaged, i.e. 3 measurements per scanning direction each, and a total average over both scanning directions.

Results & Discussion

Table 2 displays descriptive statistics of the carcasses including mean and range. The chemically determined intramuscular fat content of the carcasses used here ranged from 0.71 to 3.18 %.

Table 2: Descriptive statistics of carcass and meat quality characteristics (number of observations = 115 ; s.d. = standard deviation of the mean).

parameter	mean	s.d.	minimum	maximum
carcass weight [kg]	94.8	7.6	76.8	113.4
lean meat [%]	55.1	3.1	45.3	61.4
backfat width [mm]	17.0	3.4	10.2	28.5
muscle width [mm]	57.3	5.2	43.7	69.1
intramuscular fat [%]	1.51	0.55	0.71	3.18
protein [%]	23.80	0.57	21.73	24.97
dry matter [%]	25.72	0.66	23.80	27.32
ash [%]	1.22	0.04	1.08	1.37

Correlations between acoustic parameters and intramuscular fat range up to $r = 0.40$ dependent on measurement conditions. Among the acoustic parameters there is extensive collinearity that is also confirmed by principal component analysis (not shown in detail here).

In table 3 results of multiple linear regression analyses are shown. Best results are obtained using mean acoustic parameters that are averaged from 6 measurements in both perpendicular and parallel orientation referring to the muscle fiber orientation. Thus, 60 percent of the variation of intramuscular fat content is explained by the ultrasound information, and root mean square error of prediction (SEP) amounts 0.36 % IMF. In this case, 80 % of the residuals between ultrasound predicted IMF and chemical IMF is below 0.40 IMF %. Using averages of 3 corresponding scans per scanning direction, the predictions of IMF are worse as indicated by the coefficient of determination R^2 (table 3). Perpendicular scans seem to outperform parallel scans in terms of predicting the fat content.

Table 3: Coefficients of determination (R^2) and corresponding root mean square errors of prediction (SEP) in % IMF obtained with multiple regression analysis with relation to measurement conditions.

		perpendicular	parallel	both directions
hot carcasses	R^2	0.46	0.24	0.60
	SEP [% IMF]	0.42	0.49	0.36
chilled loins with backfat	R^2	0.26	0.26	0.25
	SEP [% IMF]	0.48	0.48	0.49
chilled loins w/o backfat	R^2	0.35	0.03	0.10
	SEP [% IMF]	0.46	0.55	0.53

Much closer relations between the intramuscular fat content and the calculated ultrasound parameters, and higher precision of prediction arise when exclusively the carcasses with an IMF content greater than 2% IMF are included in the regression analyses. This indicates a detection threshold for the estimation of the IMF by means of ultrasound scans on pigs. Whether or not this is confirmed would however have to be investigated in additional tests with a much greater number of carcasses.

Discriminant analyses were performed to discriminate carcasses according to their intramuscular fat content. Threshold for fat classes HIGH/LOW was set at 2 % IMF. As seen with regression results, discrimination was most successful for data acquisitions at hot carcasses. Results are given in table 4 as relative proportion of correctly classified carcasses. Best results are obtained by use of averaged parameters derived from repeated scans in perpendicular direction at hot carcasses. i.e. 77 % of the carcasses are correctly classified.

Table 4: Relative proportion of correctly classified carcasses into HIGH/LOW fat class (threshold level: 2.0 % IMF) by means of acoustic parameters dependent on measurement conditions.

objects	perpendicular	parallel	both directions
hot carcasses	77	73	70
chilled loins w/ back fat	65	72	76
chilled loins w/o back fat	58	-	63

Both regression and discriminant analyses were performed by means of stepwise procedures. Regarding to the measurement conditions, the resulting functions widely vary in terms of the significant regression variables included in the models. That is probably due to the collinearity amongst the acoustic parameters. Besides further data acquisition to verify the above findings, future work is also aimed at clarifying these relationships within the spectral parameters.

Conclusions

For the first time, comprehensive investigations on the use of acoustic parameters derived from spectral analysis of backscattered ultrasound to estimate the intramuscular fat content in porcine longissimus muscle have been reported. The data acquisition system was developed to transfer echo signals from a clinical B-mode system to a personal computer for further signal processing. Over and above the results presented here appear promising to estimate intramuscular fat in pig carcasses with ultrasound spectral parameters. The analyses carried out, and simulations in the ultrasound laboratory provide further valuable information with respect to the ideal ultrasound system if the potential of the ultrasound echo signals for characterising tissues and predicting the fat content is to be used in full. Furthermore, our study also showed that only a small share of the marketed pigs have the - from a sensorial point of view, desired - fat content greater than 2% in the cutlet (here: 17% of the carcasses).

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PREDICTING BEEF TENDERNESS USING OPTICAL SCATTERING

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Key Words: Fiber optics, Scattering, Tenderness, Shear force

Introduction

Beef tenderness is an important beef quality attribute. In beef industry, an accurate, non-invasive, fast and economic method is favorable for beef tenderness prediction. NIR/VIS spectra analysis is one of the most promising techniques that could be possibly used to evaluate beef tenderness non-destructively.

Optical spectrum reflects both the chemical composition and the microstructure characteristic of meat. When light transports in meat, different chemical compositions absorb the light, and meanwhile, light is scattered by sample micro structural components. In general, meat microstructures such as myofibril and collagen are considered the main factors determining meat tenderness. Thus the meat scattering properties would be a better physical parameter that could reflect the tenderness of meat.

The measured NIR reflection or transmission spectra are the combined results of light absorption and scattering in meat. In order to improve the measurement sensitivity and accuracy, it is necessary to separate scattering from absorption. Birth et al. (1978) and Macdougall (1970) measured scattering coefficients of pork samples using Kubeka-Munk model. However, in their methods, the sample need to be cut into slices for layered transmission measurement, which is impractical for the on-line application. A practical and accurate method for measuring meat scattering coefficients is still desirable.

Objectives

In this study, a fiber optic scanner was built to measure the spatially resolved diffuse reflectance distribution above meat sample surface. Scattering coefficients were calculated by fitting the measured data with light diffuse equation. Our objective is to investigate the potential of using optical scattering properties for beef tenderness prediction.

Methodology

The experimental apparatus was shown in Fig.1. A 20W broadband halogen light (Ocean Optics Inc., HL-2000-FHSA-HP) was used as the light source. A fixed optical fiber with an oblique angle 56° relative to the vertical direction was used to deliver incident light. A scanning optical fiber was used to collect the spatial-resolved diffuse reflectance light at different positions. The scanning optical fiber was connected to a spectrometer (Ocean Optics Inc., USB2000) to record the diffuse reflectance spectra. The

acquired spectra were then processed by a personal computer. The scanning fiber was controlled by a translation stage and scanned above the sample. The sample scattering coefficients were derived using methods proposed by Wang et al., 1995.

Beef samples were acquired from the meat laboratory at the University of Missouri-Columbia. Each sample was from a different animal and had been aged for at least 14 days. Samples were stored at 2°C –3°C before each measurement. Warner-Bratzler Shear Force Tests were performed on the same sample after the optical measurement. Samples were then cooked on a Farberware electric broiler (Farberware Inc., Bronx, NY). During cooking, sample was turned over as the internal temperature reached 41°C; then was continued to cook to the final temperature 71°C. Internal temperature was monitored using a thermocouple thermometer (MODEL 8112-20, Cole-Parmer Instrument Company, Vernon Hills, Illinois). Two cores (diameter =1.27cm) were removed from each sample for shear force test. Each core was sheared for three times perpendicular to the longitudinal orientation of the muscle fiber using TA.XT2 Texture Analyzers (Texture Technologies, Corp., Scarsdale, NY) with WB shear force attachment. The crosshead speed was set at 3.3 mm/sec. The average of six peak shear values represented the WB shear force value for each sample.

Results & Discussion

In order to have the largest variance in tenderness, extreme cuts were chosen as our test samples according to the US National Beef Tenderness Survey 1991 (Morgan et al., 1991). Scattering coefficients at 721nm of six top round steaks and five tenderloin steaks were measured and shown in Fig. 2. Tenderloin steaks had smaller scattering coefficients than top round steaks except two samples. Tenderloin steaks are from middle parts and top round steaks are from end parts in an animal body. In general, top round steak is tough and tenderloin steak is tender. According to previous research, muscles at end parts, which mainly support animal movements, have lower oxidative capacity and contain thicker muscle fibers; while muscles at middle parts, which mainly involved in posture, have higher oxidative capacity and contain thinner muscle fibers (Klont, 1998; Totland and Kryvi, 1991). Thicker muscle fibers increase light scattering cross section and have higher scattering coefficients, while thinner fibers reduce light scattering cross section and have smaller scattering coefficients. Meanwhile, thicker muscle fibers need more force to chew, bite and mince them, and are tougher, while thinner muscle fibers are easier to be chewed, minced and taste tender.

However, variations existed among different animals, and the final tenderness is also affected by many factors such as slaughter handling and post-mortem processing. For the two exceptions in tenderloin, we measured their raw beef Warner-Bratzler shear force. The results showed that the two tenderloin samples with higher scattering coefficients also had higher WB shear force than the rest tenderloin samples. The WB shear forces were 26.85N and 27.15N respectively for the two higher scattering tenderloin samples, while all other tenderloin steaks had shear force smaller than 20.58N.

Scattering coefficients at 721nm wavelength of USDA Utility and USDA Select rib eye steaks were also compared in this study (Fig. 3). Scattering coefficients of three Utility steaks were larger than all Select steaks and one Utility steak had lower scattering coefficient than the Select steaks. According to USDA meat quality grade standards,

Utility beef are usually from older animals, and generally are tougher than USDA Select beef. As animal gets older, the amount of connective tissues (collagen) and the degree of interconnectivity of the collagen in muscles increase and meat becomes tougher (Sinex, 1968; Fang et al., 1999). The increased amount of connective tissues and the interconnectivity of collagen also increase the light scattering cross section and result in higher scattering in meat. However, exceptions also existed among different grades because of the natural variations among different animals and post-mortem development.

The exceptional Utility sample with lower scattering coefficient also had lower WB shear force (17.44N) than the Select sample (23.52N).

A more comprehensive investigation on the correlation between scattering coefficients and beef tenderness was also conducted in this study. A total 32 samples including bottom round, top round, tenderloin, and ribeye steaks were used. The scattering coefficients at 721nm wavelength were measured. A linear correlation plot was plotted in Fig. 4. The linear regression results indicated that the shear force value of cooked beef was significantly ($P < 0.05$) correlated to the reduced scattering coefficient with a coefficient of determination of 0.59. ($R^2 = 0.59$). These results indicated that the optical scattering coefficients do reflect meat mechanical properties.

Conclusions

Our preliminary results indicated that the scattering coefficients of beef are significantly ($p < 0.05$) correlated with the Warner-Bratzler shear force. The tougher beef has higher scattering coefficients and the tender beef has smaller scattering coefficients.

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Tables and Figures

Figure 1.

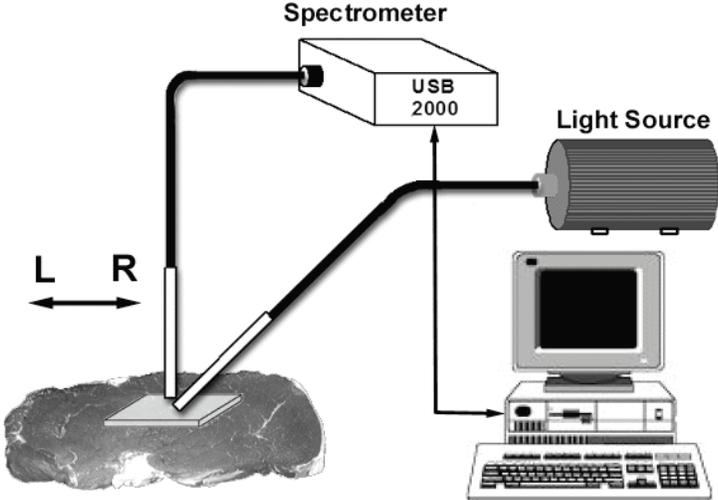


Figure 1. The schematic diagram of the experimental setup.

Figure. 2.

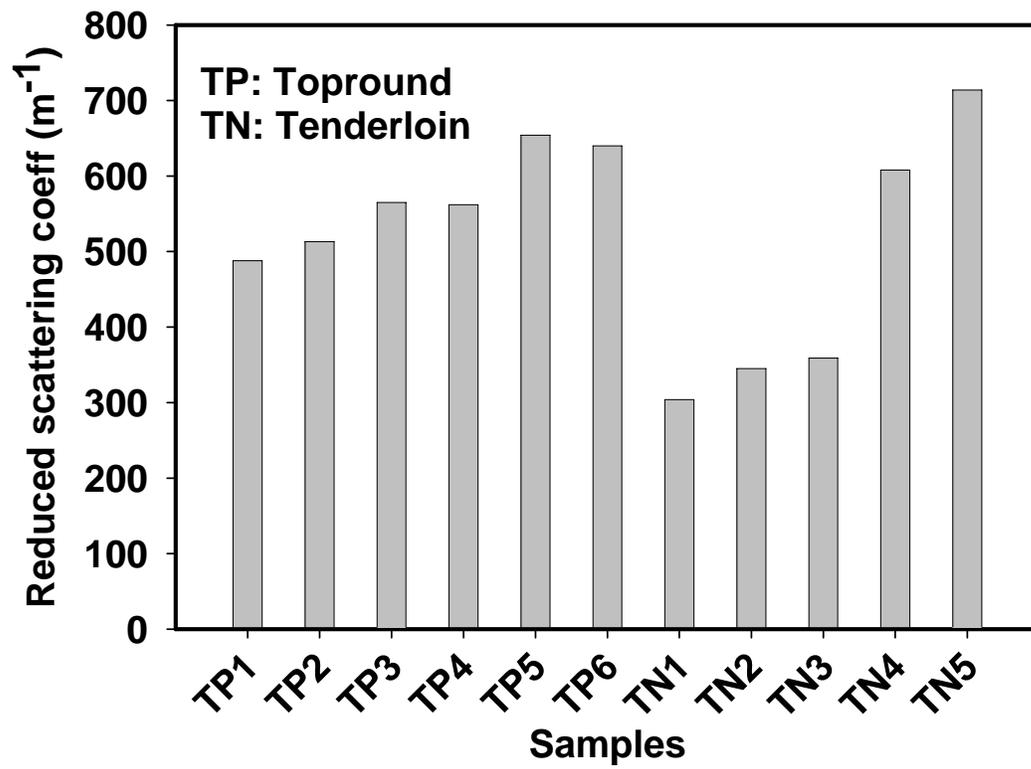


Figure 2. The reduced scattering coefficients at 721nm wavelength of tenderloin and top round steaks.

Figure. 3.

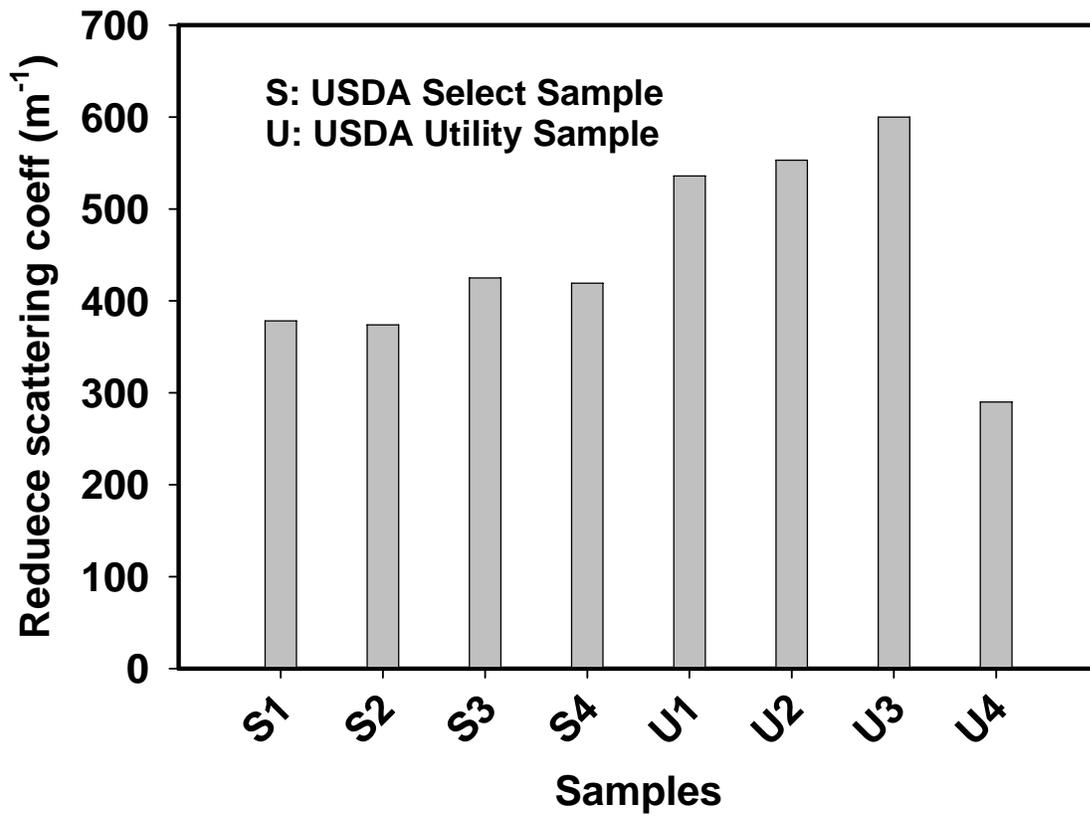


Figure 3. The reduced scattering coefficients at 721nm wavelength of USDA Utility and USDA Select steaks.

Figure 4.

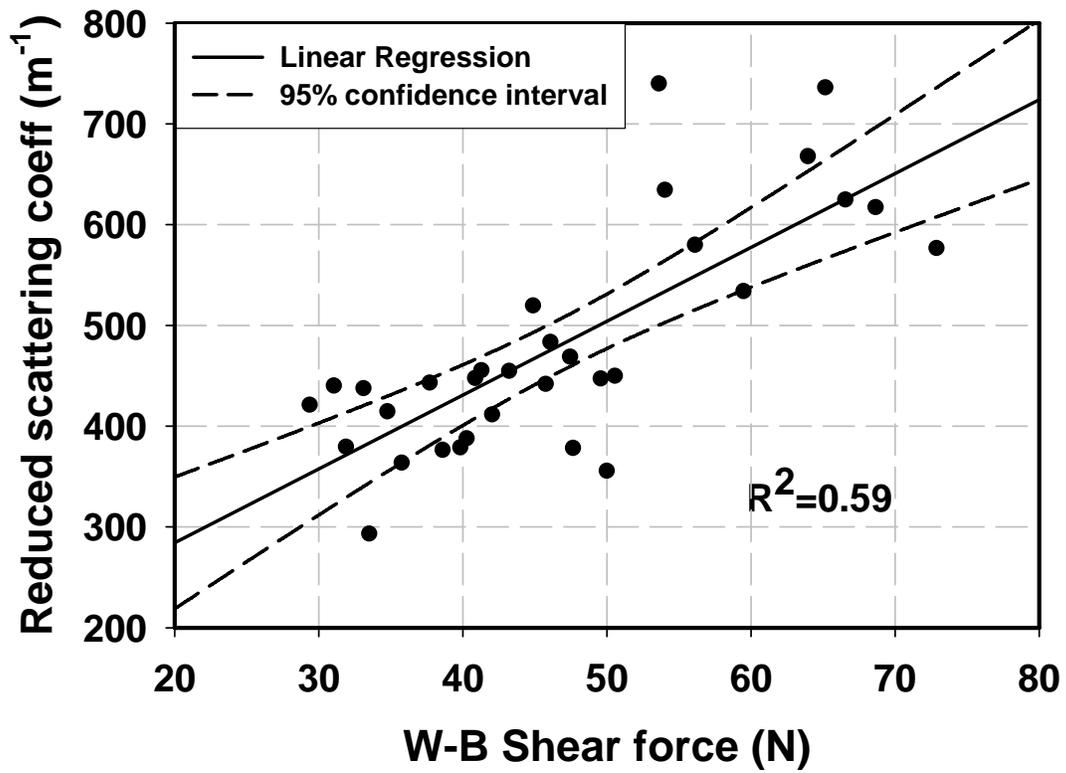


Figure 4. The correlation between WB shear forces of cooked beef and reduced scattering coefficients at 721nm.

EVALUATION OF CURRENT UNITED STATES PORK INDUSTRY CARCASS GRADING EQUIPMENT^a

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Marketing Service.

Key Words: Pork, Carcass, Yield, Instrument

Introduction

Mandatory Price Reporting (MPR) was developed as an information system to enable the United States Department of Agriculture (USDA) to implement the intent of the 1999 Livestock Mandatory Reporting Act. One component of MPR requires reporting of pork pricing data based on pork carcass compositional yield. In accordance with MPR, market data must be reported in an accurate and timely fashion to the public. Existing pork carcass grading equipment could be used to report an industry standard that could be commonly evaluated and reported on a daily basis. Reporting a common endpoint of pork carcass yield and (or) primal and subprimal cuts manufactured and sold in the U.S., would be well received and readily understood by packers, further processors, and producers.

Objectives

The objective of this project was to provide the USDA Agricultural Marketing Service (AMS) a means of standardizing data across different pork carcass composition assessment equipment to a common end point. This would create a base of consistency for reporting national pork pricing information.

Methodology

The numbers of barrow and gilt carcasses used in the trial to meet the average backfat and carcass weight for each of the nine selection cells were selected over an eight week span (Table 1). Carcasses were pre-selected based on visual composition appraisal for inclusion within the selection grid below. Each cell included carcasses selected over the eight week period, to represent multiple days of plant operation and no one cell was filled on a single day's operation. Final evaluation of the carcass condition was conducted prior to the grading station. Carcasses with skin removed, possessing lean tissue or bone removal, and (or) a poor carcass split were not considered for inclusion.

Table 1. Final carcass selection grid

Fat Thickness (mm)		Carcass Weight Range (kg)					
		79.4 – 87.1		87.5 – 93.9		94.3 – 104.3	
		Barrows	Gilts	Barrows	Gilts	Barrows	Gilts
< 20.3	No.	17	18	17	17	17	19
	Hot Carc. wt (kg)	83	83	90.2	90.6	97.0	96.9
	Last rib FT (mm)	17.0	17.0	18.0	18.0	17.8	18.3
20.3-27.9	No.	17	20	17	18	17	15
	Hot Carc. wt (kg)	82.4	82.4	91.7	90.9	98.5	98.3
	Last rib FT (mm)	23.9	23.9	23.9	23.4	23.4	23.6
> 28.0	No.	17	14	17	17	17	20
	Hot Carc. wt (kg)	82.5	82.4	90.8	90.3	100.3	99.8
	Last rib FT (mm)	30.5	29.0	30.0	30.0	32.5	30.5

Carcass weight and last-rib backfat thickness were obtained on the warm, pre-rigor carcasses railed off-line at the carcass grading station. Carcasses were included in the study based on the gender, hot carcass weight, and last rib backfat thickness. Carcasses not fitting the grid were returned to normal plant processing. Carcasses fitting the grid were measured with the following equipment; Animal Ultrasound Services (AUS; Ithaca, NY) Carcass Value Technology instrument 1 (CVT1; 17cm long 5049 ultrasonic transducer head) and CVT2 (12.5cm long 5011 ultrasonic transducer head), Hennessy Optical Grading Probe (Auckland, NZ), and SFK (Peosta, IA) Fat-O-Meater (FOM) optical grading probe, Ultrasonic FOM, and the Automatic FOM (AutoFOM). Data collection personnel operated the same pieces of equipment throughout the trial and were properly trained for operation. Right carcass sides were removed from the processing line after an overnight chill, wrapped, and transported via refrigerated truck to the University of Missouri Meat Science Laboratory. The weekly schedule is outlined in Figure 1.

Figure 1. Weekly schedule of carcass selection, cutout, and data collection

Monday	Tuesday	Wednesday	Thursday	Friday
AM: Carcass selection and equipment data collection PM: Carcass identification labeling in the cooler.	AM: Pork sides removed from processing line & wrapped PM: Sides transported to UMC via refrigerated truck.	Carcass cutout & data collection.	Continued cutout (if necessary).	Data entry.

Carcass cutout data sheets were designed to obtain the weight of all primal and subprimal cuts reported in the USDA National Carlot Pork Report. Cutout sheets and cut specifications were supervised then approved by USDA Market News personnel. After collection of cutout data, all boneless soft tissue was collected for each side, mixed, and ground, then re-mixed and subsampled for analysis of total ether extractable lipid and moisture. Fat-free carcass lean (FFL) was determined according to Schinckel et al. (2001) whereby; $FFL = \text{dissected soft tissue (ST) wt} - [\text{ST lipid weight} / \% \text{lipid of pure fat sample}]$ and lipid-free lean (LFL) calculated as $LFL = \text{dissected soft tissue (ST) wt} - (\text{ST weight} * \% \text{lipid of ST})$.

Statistics. Linear regression analysis was conducted using the representative fat and lean tissue depths (or loin muscle area as output by the AutoFOM) obtained from each electronic grading instrument. Tissue measurements and hot carcass weight were used as independent variables to predict pork carcass yield to various trim levels, bone-

in/boneless, and fat-free status (Table 2). Final prediction equations were determined using the STEPWISE procedure of SAS (Raleigh, NC). Independent variables were selected for inclusion in the final regression equation at a level of $P < 0.10$.

Results & Discussion

The precision and accuracy of each instrument are similar, but for the Ultrasonic Fat-O-Meater Table 2. The CVT probes (also ultrasonic equipment) recorded the highest R^2 and lowest RMSE statistics. Therefore, it appears that ultrasonic attenuation due to carcass external fat cooling was not a problem. When the new equations (developed from these data) were applied to predict compositional yield, no differences were observed across instrument for predicted carcass yield. This suggests that the equations generated from this project can be used as an industry standard.

Table 2. Accuracy (coefficient of determination; R^2) and precision (residual standard deviation; RSD) of pork carcass grading instruments to predict proportional carcass yield.

% carcass yield ^b	Ruler	Pork carcass grading equipment ^a						
		AFOM	CVT1	CVT2	FOM	HGP	UFOM	
BI-6FLC	R^2 (RSD)	0.568 (1.67)	0.682 (1.44)	0.704 (1.38)	0.708 (1.37)	0.649 (1.51)	0.664 (1.47)	0.355 (2.04)
BI-3FLC		0.592 (1.73)	0.708 (1.47)	0.734 (1.39)	0.728 (1.42)	0.678 (1.55)	0.692 (1.50)	0.363 (2.16)
BI-0FLC		0.664 (2.07)	0.754 (1.78)	0.791 (1.62)	0.784 (1.66)	0.726 (1.88)	0.729 (1.86)	0.392 (2.78)
BL-0FLC		0.472 (2.29)	0.639 (1.91)	0.688 (1.76)	0.672 (1.81)	0.593 (2.03)	0.599 (2.00)	0.313 (2.62)
FFL		0.282 (4.55)	0.387 (4.24)	0.454 (4.00)	0.416 (4.12)	0.366 (4.30)	0.368 (4.27)	0.225 (4.72)
LFL		0.479 (2.99)	0.600 (2.64)	0.678 (2.35)	0.662 (2.42)	0.554 (2.78)	0.561 (2.75)	0.321 (3.41)

^a**Ruler:** lastrib fat thickness measured with a stainless steel ruler. **AFOM:** Automatic Fat-O-Meater, SFK, Industries, Inc. Peosta, IA. **CVT1:** Carcass Value Technology system 1, Animal Ultrasound Services (AUS), Ithaca, NY (17 cm, 5049 transducer). **CVT2:** Carcass Value Technology system 2, AUS 12.5 cm 5011 transducer and *tailhook*). **FOM,** Fat-O-Meater, SFK. **HGP:** Hennessy Grading Probe, Hennessy Grading Systems, Inc., Auckland, NZ. **UFOM:** Ultra-Fat-O-Meater, SFK.

^b**BI-6FLC:** Bone-in, four lean cuts (Ham, loin, picnic shoulder, Boston butt) trimmed to 6.35 mm external fat depth. **BI-3FLC:** Bone-in, four lean cuts trimmed to 3.18 mm external fat depth. **BI-0FLC:** Bone-in, four lean cuts trimmed to 0.0 mm external fat depth. **BL-0FLC:** Boneless four lean cuts (five piece ham, full-length loin, strap on, picnic shoulder, and Boston butt) trimmed to 0 mm external fat. **FFL:** Fat-free lean = carcass soft tissue wt – (soft tissue lipid / %lipid of pure fat tissue). **LFL:** Lipid-free lean = carcass soft tissue wt – (carcass soft tissue wt * %lipid soft tissue)

Hot carcass weight (HCWT) was not significantly correlated (Pearson Coefficient of Correlation) with any proportional carcass yield variable. The fat depth as recorded by the CVT1 possessed the highest correlation with all carcass yield variables, followed closely by CVT2 and FOM derived fat depth. The highest correlations between fat depth

and proportional product yield were seen for three yield estimates of bone-in closely trimmed four lean cuts. Of the two definitions of fat-free mass, LFL possessed higher correlation with subcutaneous fat measurements. All subcutaneous fat measurements were significantly ($P < 0.0001$) correlated to one another. The highest correlations were seen between the FOM and HGP ($r = 0.94$), CVT1 and CVT2 ($r = 0.93$), and last rib fat thickness and AFOM last rib fat thickness ($r = 0.915$). Correlations between muscle depth measurements from the various pieces of equipment were significant, yet not as highly correlated to one another as were the fat depth measures.

Conclusions

The different yield endpoints predicted by carcass grading equipment will possess different meanings and levels of importance to the individual utilizing them. Packers and wholesalers may find the estimate of trimmed or boneless yield to be the most beneficial while swine producers using the National Research Council's nutrition management software will use and appreciate the fat-free lean estimate and researchers may obtain the most information from lipid-free lean. The data and results generated from the completion of this project will benefit all segments of the pork production chain.

CHALLENGES IN THE APPROVAL OF CT AS FUTURE REFERENCE FOR GRADING OF FARMED ANIMALS

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Key Words: CT, grading, protocol, reconstruction, pre-processing, Hounsfield spectrum, reproducibility

Introduction

In recent years, there has been an increased interest in adaptation of computerized tomography (CT) for applications within the animal science community. The pioneering work by Vangen et al. (1981) has been followed by several European institutions, Glasbey et al. (2002) and Doeschl-Wilson et al. (2005), some participating in the European EUPIGCLASS project (Growth G6RD-CT-1999-00127). This collaborative project suggests the application of tomographic scanning as the approved reference for porcine grading in the European Union.

The potential benefit from the suggestion is obvious, as the reference can be made with a minimum of actors' influence on the result. The topic of actors' influence on dissection results was a major focus in the EUPIGCLASS project. The influence of the butchers on the dissection result between different European countries was quantified for the first time. This socio-cultural impact on the dissection result is still to be analyzed in detail.

Very recently some quite encouraging results have been presented - Dobrowolski, et al. (2005), Romvari et al. (2005) - to demonstrate the potential of the tomographic technology in grading, growth and breeding of farmed animals. Until now, however, very limited information on the reproducibility (instrument to instrument variation and differences in reconstruction algorithms for creating the images) of this new scanning technique has been published, Christensen et al. (2005). Some aspects of this important topic will be addressed here.

In this study, we will emphasize on the importance of using only the relevant parts of CT-images of porcine carcasses when estimating e.g. the lean meat content. Instead of using the raw CT-images as basis for our calculations we suggest using a pre-processing step prior to applying multivariate modelling methods for predicting the lean meat content. Also the importance of ones choice of the reconstruction algorithm will be discussed.

Being developed as a supportive tool in human medical diagnostics, the CT technology is closely tied up to a subjective evaluation of the output from the CT scanner, a series of consecutive slices displaying internal features of a human body under investigation. The evaluation is thus based on the imaging qualities of the CT technology. The features of highlighting certain tissue abnormalities and changes (over time) are developed almost to perfection to support and ease the diagnosis.

A special application of tomographic technology is quantification of bone density for osteoporosis diagnostics. This application applies a three-point calibration phantom to account for subtle non-linearity in the standard CT system. The software tools developed for Quantitative CT (QCT) can be adjusted for meat science applications to improve the reproducibility of the technique.

After the initial Norwegian work, progress of computer power and image software has been tremendous, and the development of new applications in the field of farmed animals can benefit from these advances.

The signal analysis of CT images is related to the absorption of x-rays, described on the Hounsfield-scale. Apart from this basic fact, the additional analyses can be divided into two very distinct methodologies: a) Spectral approach where the pixel (or voxel) distribution is quantified by spectral analysis tools (e.g. multivariate calibration) and b) imaging approach where the images (tomograms) are evaluated by image analysis tools (e.g. segmentation). After the premature work in the early eighties, a considerable progress has been made within both fields, driven by other scientific and industrial communities than meat science.

We have investigated the influence of different recording (scanning) protocols on the tomographic data generated at our Siemens AR.T. CT scanner. The investigation implies significance of pre-processing the generated tomograms prior to the spectral calibration of the total voxel spectra. The implication is related to the final robustness of the spectral CT approach and thus extremely pertinent for the approval of CT as a future European reference for pig or sheep grading. Even though the discussion here will originate from a sequential (single slice) system, the discussion is highly relevant for helical and multi-slice systems as well.

This preliminary study of the robustness is made to focus the attention of the grading community to agree on necessary and sufficient requirements to establish CT as a transparent and objective reference for pig grading.

Objectives

The objective here is to discuss different reconstruction protocols to illustrate their impact on spectral analyses of the generated tomograms. The work also reveals the necessity of pre-processing data to cope with the influence of the scanning environment, i.e. the object couch material and other fixation means. The influence will be studied with respect to calibration of the lean meat/fat ratio: The lean meat percentage (LMP).

One other objective is to discuss the effect of data reduction in a full dataset consisting of 130 tomograms of a pig carcass. Due to time constraints, the smaller number of images the more efficient the grading procedure. Our aim is to demonstrate the performance at the lower limit of such a spatial sampling, one single slice representing the entire carcass, recorded at a fixed anatomical landmark.

Spectral analysis can be considered as a very coarse analysis of the CT images as the spatial information of the voxels is neglected. We suggest including a pre-processing of the image data prior to the spectral analysis, in which the environment of the carcass is removed by segmentation, clipping or other image processing means. Even with a static environment, i.e. same couch/cradle for all carcasses, the relative influence on the LMP

can be significant. The suggested pre-processing is believed to improve the robustness of a CT grading system.

Methodology

A dataset of 40 left half carcasses (pure breed DUROC with an average (dissected) LMP = 59.1% and SD = 2.5% (Min - Max = 53.3 - 63.7)) was calibrated to the dissected LMP using the approved EU reference method as described in Walstra et al. (1996). The datasets each consist of 130 tomograms (10 mm thickness and 10 mm feed) for each of the 40 animals. All carcasses were placed on the couch with the top of the pubis bone as anatomic landmark positioned on the same slice number (slice no. 30).

The scanning was made on a Siemens Somatom Plus (single slice mode) with the AB9 High Resolution kernel number 9 (AB_07041, Highest spatial resolution; for finest bone details) reconstruction algorithm. The animals were scanned the day after slaughter thus fully equalized to approx. 5 °C.

The day after scanning (two days post mortem), the carcasses were dissected according to the approved EU reference method.

Two different analysis schemes were used to predict the LMP. The first scheme included no pre-processing of the tomograms, but simply used the spectral distribution of the total content of the tomograms, i.e. the carcass and the couch. In the other approach, the couch was extracted from the tomograms prior to the spectral calculation.

Another dataset, describing various reconstruction algorithms provided in the CT scanner, was used as a preliminary investigation of the spectral impact from these, conventionally very obscured, elements of the tomogram generation. Five belly products were scanned with 10 mm thickness/10 mm feed using four different reconstruction algorithms (AB1, AB3, AB6 and AB9) with increasing sharpness. The products were each scanned once, and then the raw data projections were used to reconstruct four different tomogram sets of each product. For all tomogram data sets, a pre-processing of removing all material but the tissue was made prior to the Hounsfield spectral calculation. The Hounsfield spectra were used to predict the total product weight as reference parameter for the preliminary study.

Results & Discussion

The DUROC dataset was pre-processed by manual segmentation and extraction of the couch material from the tomograms, see Fig. 2e. Initially the pre-processed image of slice 30 was used to calculate the Hounsfield spectra in the range of -150HU to 250HU, for all individuals of the dataset, see Fig. 2a and b. The 40 spectra were then used as input to make a multivariate prediction of the dissected LMP of the entire carcass. This gave a correlation of $R^2=0.76$ and an error of prediction (RMSEP) of 1.22%. To improve the performance, a further pre-processing of scaling to unit variance is performed thus improving the result to $R^2=0.83$ with a RMSEP=1.01% made from the information contained in one single tomographic slice. Of course it has to be noted that the biological input is a pure breed DUROC sample, thus the limited anatomical diversity might overestimate the performance of the one single slice analysis.

The dataset is based on a Siemens Ultra High AB9 reconstruction kernel to improve high-contrast details (bones) on the cost of artefacts, see Fig. 2c and d. Therefore images may exhibit a quite high noise level in the low contrast regions of the tomogram, i.e. the fat/meat tissue. To reduce this noise, an average of five pre-processed Hounsfield spectra (slices 30-34) are used as input to a prediction model. The noise reduction improves the prediction performance to $R^2=0.93$ with a RMSEP = 0.64%, still with a 2 PC model, see Fig. 1.

The five slices are assumed to contain very highly redundant anatomical information, as the longitudinal anatomical variation within five cm cranial to the pubis bone can be taken as quite small. Thus the improvement in prediction performance can be caused by the noise reduction. Again the limited amount of samples of pure breed animals can overestimate the performance in general.

A 2 PC model based on 60 pre-processed slices (the entire middle part of the carcass) of the carcass improved the prediction performance additionally to $R^2=0.96$ with a RMSEP = 0.55.

Taking all 130 slices from each pig was expected to produce even further improvement of the prediction performance. The inclusion of all slices surprisingly deteriorated the performance to $R^2=0.93$ with a RMSEP = 0.65, still for a 2 PC model.

For the reason of contrast only, the Hounsfield spectrum of the entire content of all 130 slices were averaged, leading to a 6 PC model with $R^2=0.56$ and an error of prediction RMSEP=1.7%. Compared even to the single-slice input model above the result is not encouraging. The couch will show up as a constant volume, but the relative contribution to animals of dissimilar weight (volume) is different. In a LMP estimation the presence of the couch seems to affect the performance of the prediction model, see Fig. 2e.

The influence of the couch origins from a mixed pixel problem. The mixed pixels can end up in the spectral range of interest even with a couch material with a Hounsfield spectrum outside the -150 HU to +250HU. The mixed pixel problem is described in detail by Glasbey et al.

Compared to the five-slice model, the improvements by applying the prediction models based on 60 and 130 tomograms are surprisingly modest. The reason for the absent improvement will be addressed in the preliminary study below in which scanning of five belly products are reconstructed using four different algorithms. The five boneless products represent the weight range of 800 to 1200 grams and for the cause of demonstration only; the aim here is to predict the product weight from the tomographic dataset. The products are scanned with a 10 mm slice/10 mm feed protocol as used in the DUROC study above.

An example of the spectral variation due to reconstruction features is shown in Fig. 3. The impact of the reconstruction is very clearly seen, especially the difference between AB9 h39 and AB1 b21. The difference is tested using a conventional GLM procedure (SAS Institute Inc.). The test reveals that algorithm AB9 h39 is significantly ($p<0.0001$) different from the other three. Correspondingly AB1 b21 is different ($p=0.007$) from AB6 b26, whereas AB3 b23 and AB6 b26 are considered to result in similar spectral appearances. Even the meat peak height at approx. 50 HU is calculated with quite diverging results. The peak position of respectively fat and meat is, however, constant and thus appears to be independent of the reconstruction algorithms applied here. In other

words, the variation in peak position observed between the five products is not caused by reconstruction artefacts.

The full width half maximum (FWHM) of the two peaks, however, differ considerably between the four applied reconstruction algorithms. In this preliminary study we are not able to judge which of the algorithms is giving the most precise distribution, a tissue phantom will be suitable for such an investigation.

As the reconstruction is a core feature of medical CT scanners, the detailed information on the reconstruction functionality is in general a restricted area, not open to users of the CT systems. An area, however, that will be of utmost importance in the future work in approval of CT as a new reference for European pig carcass grading.

In this preliminary work, we predict the weight of five belly products from the individual Hounsfield spectra, using the same reconstruction algorithm. Using partial least squares regression (Unscrambler v. 9.2, from Camo Norway) the modelling is repeated with all four reconstruction algorithms. The prediction performance of the different models appears to be quite similar, see table I.

The main topic here is, however, not the prediction performance but the effect of the reconstruction on the spectral appearance. We investigate this by applying say the AB3 b23-spectrum in the AB9 h39 prediction models.

We use the AB9 h39 based prediction model and try to predict the weight of each of the five products by using the remaining spectral appearances of the same products as input for the AB9 h39 prediction model.

From table II it can be deduced that the main difference between spectral predictions of product weight based on different reconstruction algorithms is a bias term.

Choosing the reconstruction algorithm AB9 h39 as base for this example is only to illustrate the topic. The preliminary data does not support any recommendation of either of the algorithms used here, even though it appears from the comparison in Table I that a weight prediction model based on the high-resolution reconstruction algorithm AB9 h39 is slightly superior.

Conclusions

In a study based on carcasses from 40 pure breed DUROC pigs from which CT-images were acquired, the importance of pre-processing the images prior to using the CT-data for predicting e.g. the lean meat percentage (LMP) is demonstrated. Apart from improving the prediction performance, the robustness of CT determination of LMP is enhanced by the pre-processing.

In this study, five slices from a specific region of the carcass middle of the CT-images were converted to Hounsfield scale histograms that in turn were used as input for multivariate, partial least squares (PLS), models designed to predict the lean meat content in each carcass. The conversion to histograms was made in two distinct ways: by using the entire CT-image, meaning images containing both carcass and background (e.g. couch on which the carcass is laid) and by using pre-processed images where only the parts of the CT-images containing carcass information is utilized. It is demonstrated that PLS models based on the pre-processed images are much more robust than similar models based on raw CT-images. With pre-processing, cross validation revealed that the optimal PLS model should use only 2 factors for predicting LMP whereas the PLS model

based on the raw CT-images used 6 factors. Also the PLS model based on pre-processed data gave a root mean square error of cross validation (RMSECV) of 0.64% lean meat compared to 1.70% for the PLS model based on the raw CT-data.

When 60 slices representing the entire middle part of the carcass were used as a basis for creating the input histograms for the PLS model, the prediction performance was improved to an impressive RMSECV = 0.55%.

Furthermore, a preliminary study of the impact on the spectral appearance of the reconstruction algorithm shows that this topic must be of crucial importance to the future work of approving CT as reference for porcine grading within Europe.

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Tables and Figures

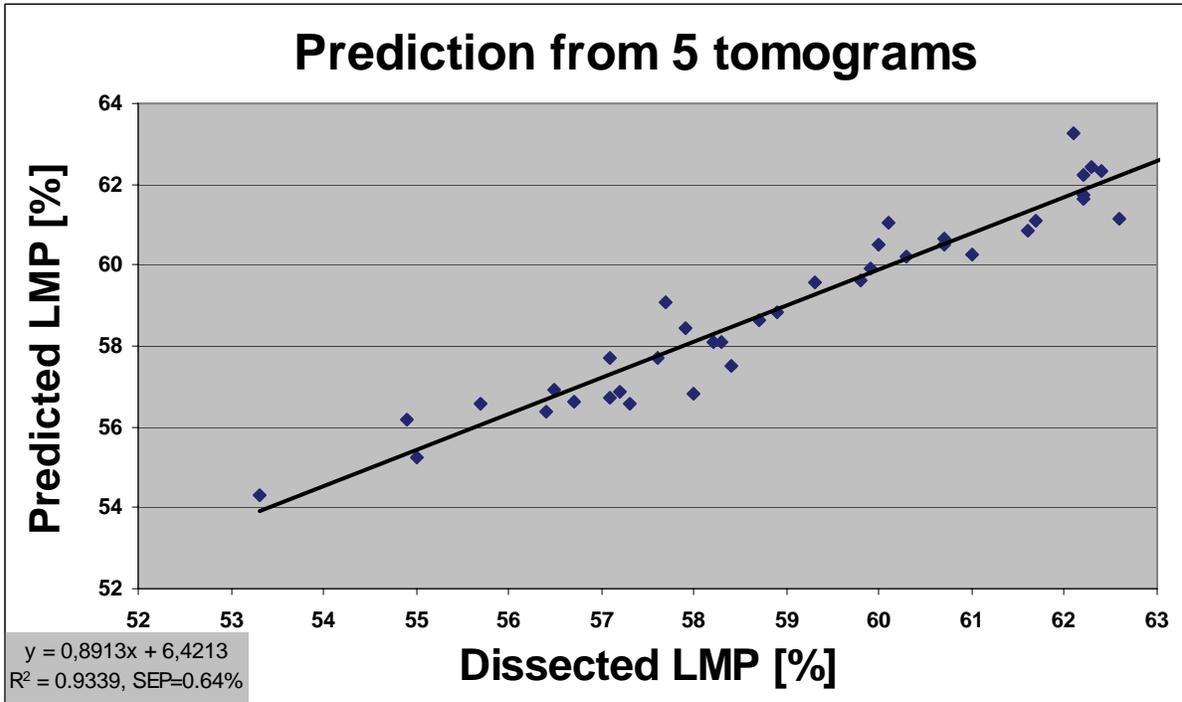


Figure 1. Slice 30 to 34 for all 40 (39) individuals used to predict (Predicted Y) the dissected LMP (Measured Y) with a 2 PC partial least squares model.

Slice 30org



2a

Slice 30segm



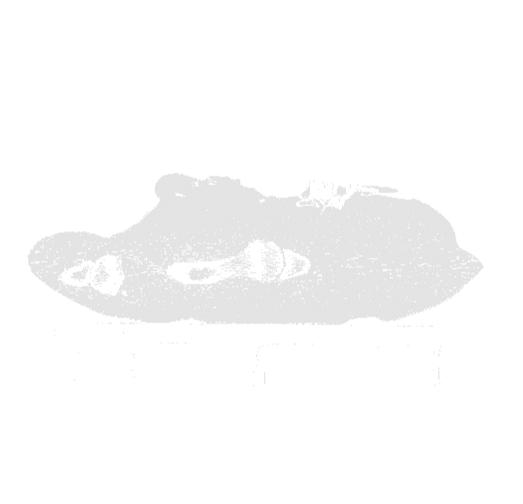
2b

Slice 112org



2c

Slice 112segm



2d



Figure 2. The potential of pre-processing the tomograms is illustrated here. Fig 2a shows the reconstructed tomogram of slice 30 and Fig 2b shows the pixels between -150HU and +250HU of slice 30. It can be seen that parts of the couch appears in this spectral range, thus contributing to the prediction model. Fig. 2c and 2d illustrate the same duality. Here the effect of reconstruction artefacts in the shoulder can be seen to remove some tissue pixels from the spectral range of interest. The four illustrations are shown with a reversed look-up-table (gray scale). Fig 2e illustrate the pre-processing with a manual segmentation of the couch material, which then is excluded from the spectral calculation. The segmented couch is lined with a solid red line

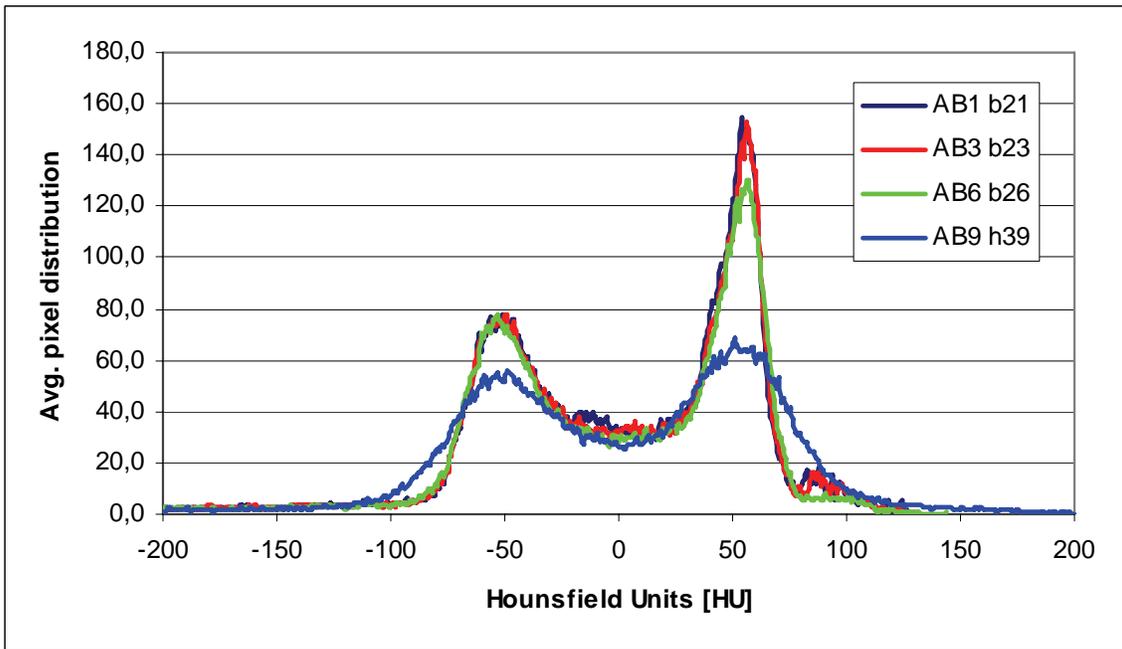


Figure 3. An example of reconstructed tomogram spectra of the same belly product (Weight: 1023 gram), using four different commercial reconstruction algorithms.

Table I. Prediction performance of the four different partial least squares models

	AB1 b21	AB3 b23	AB6 b26	AB9 h39
Correlation R^2	0.9892	0.9839	0.9848	0.9998
RMSECV [g]	21	25	25	3

Table II. Impact from the reconstruction algorithm on the weight prediction

AB9 h39	Product 1	Product 2	Product 3	Product 4	Product 5	R^2	Bias	SEP
Reference	1242	1350	1262	1023	801	-	-	-
AB1 pred.	1411	1487	1371	1163	890	0.9840	129	31
AB3 pred.	1397	1431	1359	1135	890	0.9837	107	29
AB6 pred.	1367	1419	1320	1082	885	0.9849	79.4	28
AB9 pred.	1237	1352	1263	1025	801	0.9998	0	3

**ON-LINE ANALYSIS OF THE PROXIMAL COMPOSITION OF WHOLE
ENTRECÔTES (CHUCK ENDS) BY A NIR TRANSFLECTANCE INSTRUMENT
- A PRELIMINARY REPORT**

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Key Words: NIR, transreflectance, analysis, entrecotes

Introduction

Un-processed meat raw materials are heterogeneous with regard to distribution of the major constituents, i.e. moisture, fat and protein. In reflectance spectroscopy, where the surface of the sample is monitored, good estimates of the chemical composition of such commodities are not easy to obtain. The surface to measure is limited, and frequently this surface does not represent the average contents of the constituents of interest.

NIR has in the last two decades found many applications in food analysis. However, the NIR sampling step has often proved critical¹. There are three basically different ways of obtaining NIR spectra from a sample: I) By reflectance measurements, where the energy reflected mainly from the surface of the sample is detected. II) By transmittance measurements, where the energy that has been transmitted through the sample is detected. III) By transflectance measurements, which is a combination the two above. The illumination and detection take place on the same side of the sample, like in reflectance measurements, but the energy has been transported some distance into the sample and back to the surface prior to the detection.

NIR reflectance measures mainly the surface and is normally not well suited for measuring heterogeneous samples such as high fat beef cuts. Transmittance measurements, where the total samples can be monitored by NIR, are a better solution for analysis of such small heterogeneous samples. However, for on-line purposes, transmittance measurements pose problems as sample thickness varies and gives rise to undesirable offset variations in the spectra. Many products are also too thick to transilluminate. Another possibility is NIR transflectance analysis, where the light probes deeper into the material as compared to reflectance, and a more representative sampling is obtained.

Normally, transflectance measurements require contact between the sample and the instrument probe. This can introduce difficulties, both with regard to mechanics and hygiene in its practical use. However, a new instrument has recently been patented, that measures in remote transflectance with no sample contact.

Objectives

The objective of this study was to explore the potential of a newly developed transfectance NIR instrument in on-line estimation of the proximal composition of whole meat cuts. Entrecôte (corresponds to chuck end) was chosen as a suitable model. Entrecôtes are highly heterogenous beef cuts, with a variation in fat content that makes it interesting for the abattoirs to be able to sort them.

Methodology

40 entrecôtes (chuck end) were sampled from a commercial slaughter house. Each muscle was scanned on a conveyer belt at 4°C for about 6-8sec on both the fat and the lean side. The whole entrecôtes were ground and mixed and 300g from each sample was homogenized in a mini-chopper with horizontal mounted knives for up to 1min. The following analyses were done in duplicates;

- Fat (Soxtec extraction) - AOAC 991.36
- Water (drying overnight at 102-105°C for 16-18hrs) - NMKL 23 (1991)
- Protein (Kjeldahl) – AOAC 981.10 (1983)

The transfectance NIR scanner is an on-line measurement system, modified from a commercial system used for automatic plastic waste sorting (Titech Visionsort, Norway). It is very fast and can measure and analyze objects on a conveyor belt at a high speed (3 m/sec). It is also a spectral imaging system, which produces images with a NIR spectrum in each pixel of the image.

A powerful illumination line was projected down onto samples on the conveyor belt. A vertical black shield protected the detector from the main part of the direct reflected light. Adjusted optics enabled measurement of light emerging from the samples approx. 2cm from the illumination line. This means that both the surface and the interior of the sample were being measured simultaneously. The average NIR spectrum from each image was used for calibration. Measurements on the fat and lean sides of the cut were analyzed both separately and in combination.

The prediction models for each constituent were calculated by partial least squares methods (PLS) and validated by full cross validation. The prediction results were presented as root mean square error of cross validation (RMSECV):

$$RMSECV = \sqrt{I^{-1} * \sum_{i=1}^I (y - \hat{y})^2}$$

where the sample number is represented by i [1,2,3,...,I], while y and \hat{y} represents the reference method value and the NIR predicted value, respectively. The PLS regression results for the on-line data were calculated using the software Unscrambler[®], version 9.1, (Camo AS, Oslo, Norway).

Results & Discussion

To obtain wide ranges of fat (3.5-26.6%), protein (16.0-21.1%) and water (54.0-74.3%) contents, a broad range of weights of the muscles was obtained in the sampling (1.2-6.1kg). Plotting the content of fat against the contents of water and protein in the cuts showed high correlations, as could be expected (Figure 1). The sums of the components for each sample were checked to trace any outliers.

In Figure 2, an image with an averaged NIR spectrum in each pixel is shown. Fat and lean areas of the surface can easily be identified. Earlier studies on cheese and fish have shown that the penetration depth is as great as 15-40mm, all depending of product. Figure 3 indicates that the penetration of visible light into the cut, from the fat side, is at least 2-3cm. The penetration depth in the wavelength region 800-1000nm is known to be even higher than that of the visible range.

Table 1 gives an overview of the prediction results for fat, water and protein on a set of 39 samples, both vacuum packed cuts and cuts and without package. When scanning on both sides, the average scans were used in the models. Due to the limited number of samples, no more than five factors were allowed in the prediction models. One sample was deleted from the original sample set of 40.

Generally the prediction results for water was better than for fat content. The models for protein were not satisfactory. As expected, un-packed meat yielded improved predictions over packed, but the difference was not large. Scanning both sides of the cut yielded better predictions than scanning only one side, and scanning the fat side gave better prediction results than scanning the lean side.

In Figure 4 the PLS prediction results are presented for fat analysis (39 samples) of un-packed cuts, when both sides are scanned. The correlation coefficient is 0.89 and the RMSECV=2.56%. For water estimation the correlation coefficient and RMSECV improved to 0.91 and 2.01%, respectively (Figure 5).

In the NIR analysis, fat, water and protein were measured simultaneously. The information in the strong relationships between the components is obviously used in the calibrations, which is shown in the similarity of the regression coefficient plots for the components (Figure6). The plot for water and protein are almost identical, while the fat plot is the inverse image of those. This means that the estimations of the individual components are not independent. I.e. a calibration that is developed for muscle meat with a fixed relationship between fat and water can therefore not be used for meat where the ratio between water, fat and other ingredients has been altered 2.

Conclusions

Promising prediction models were observed. Cross-validated partial least square regressions showed that the prediction results for water content was better than for fat content. The models for protein were not satisfactory. As expected, measurements on un-packed meat yielded improved predictions over packed, but the difference was not large. Scanning both sides of the cut yielded better predictions than scanning only one side, and scanning only the fat side gave better predictions than scanning only the lean side. The best models yielded explained variances of approx. 90%, while the cross-validated prediction errors were 2.0-2.5%.

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Tables and Figures

Figure 1. Relationships between fat, water and protein (+SUM) in the entrecôtes.

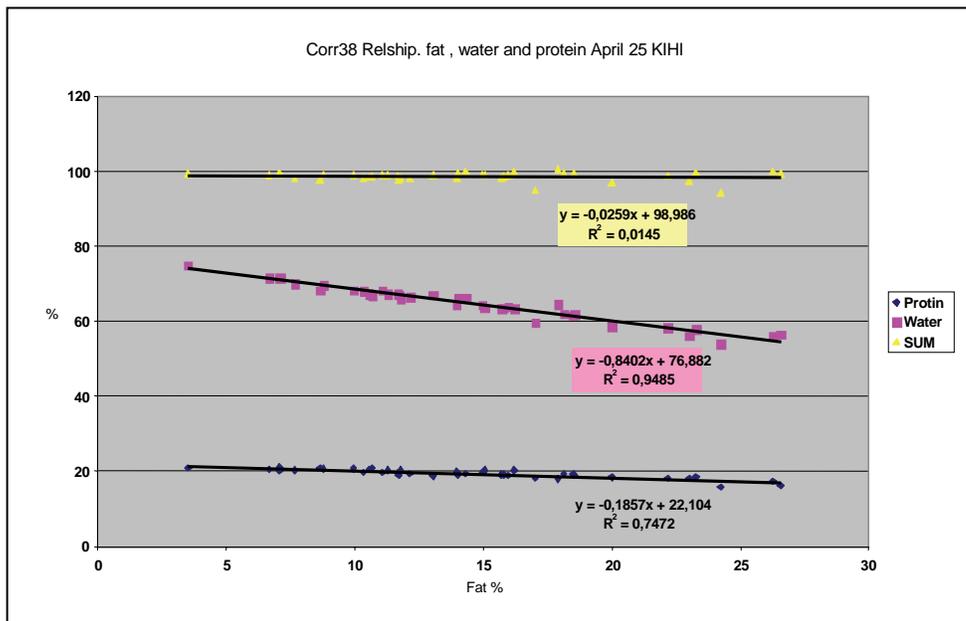


Figure 2. Spectral images of entrecôte (Titech)

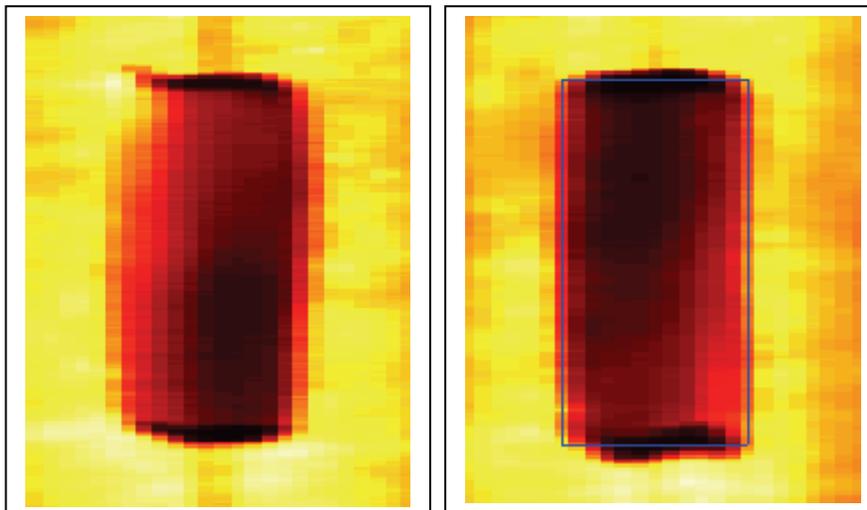


Figure 3. Entrecôte measurement from fat side (Titech Visionsort)



Table 1. Prediction results on- line NIR analysis of proximal composition of whole beef

Measuring mode	Packaging	Meat constituent	Correlation coefficients	RMSECV %	No of PC's in PLS models
Both sides	Packed	Fat	0.83	3.12	4
		Water	0.85	2.52	5
		Protein	0.61	0.96	3
	Un-packed	Fat	0.89	2.56	5
		Water	0.91	2.01	5
		Protein	0.69	0.88	5
Fat side	Packed	Fat	0.78	3.50	2
		Water	0.79	2.96	5
		Protein	0.62	0.95	2
	Un-packed	Fat	0.86	3.38	4
		Water	0.87	2.77	5
		Protein	0.66	0.92	5
Lean side	Packed	Fat	0.72	3.92	4
		Water	0.70	3.45	4
		Protein	0.57	0.99	2
	Un-packed	Fat	0.73	3.84	4
		Water			
		Protein	0.63	0.93	2

Figure 4. Prediction of fat content (samples scanned both sides, unpacked cuts) (Titech Visionsort)

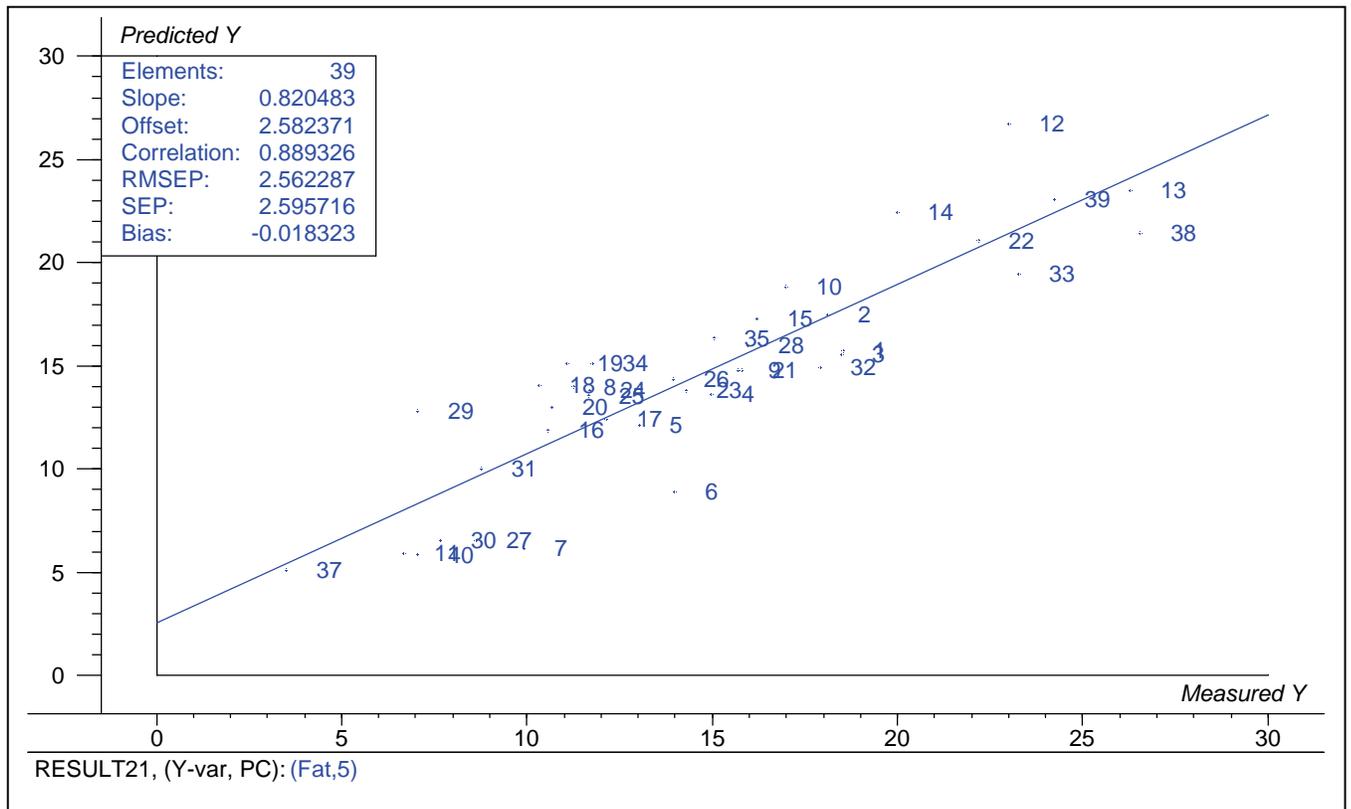


Figure 5. Prediction of water content (samples scanned both sides, un-packed cuts) (Titech Visionsort)

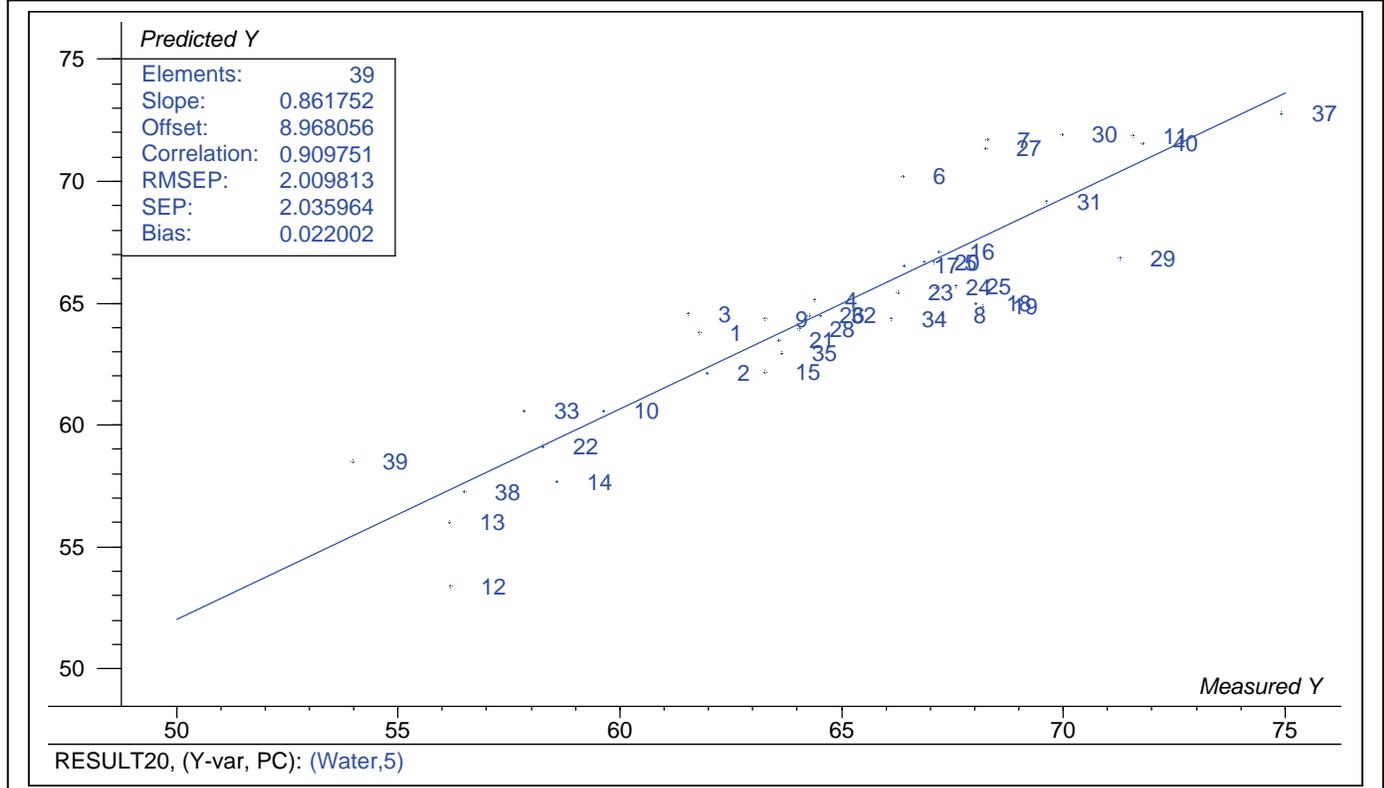
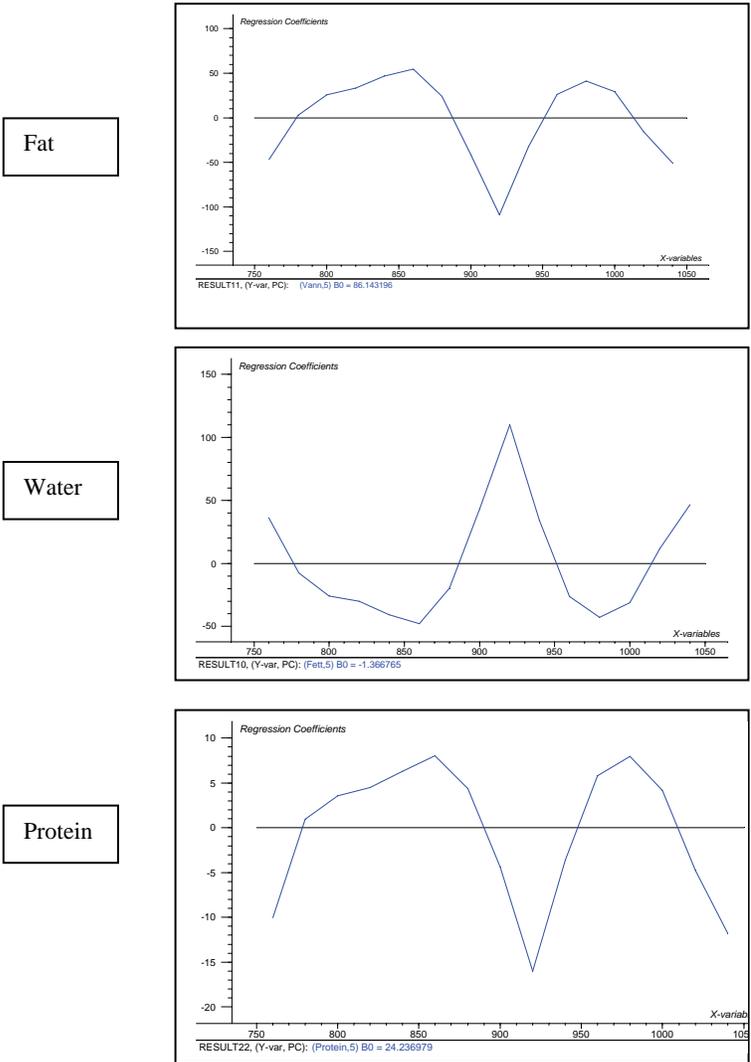


Figure 6. Regression coefficients in PLS- models (samples scanned both sides,



ON-LINE ASSESSMENT OF THE PROXIMAL COMPOSITION OF GROUND MEAT BY NIR AND OTHER INSTRUMENTAL TECHNIQUES

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Introduction

The variation in composition of meat raw materials intended for industrial processing is a considerable problem for the meat industry. This is mainly due to variability in individual carcass composition and causes reduced stability in the following manufacturing processes. In turn the customer is exposed to unwanted variation in product quality in the stores. Standardisation of raw material composition is usually based on off-line analyses on small samples from large batches of ground meat, which is a procedure prone to high sampling errors. A range of instrumentation has in recent years been developed for on-line meat process analysis. These make it possible to continuously measure the flow of ground meat, which gives a better representation of the proximal composition of the whole batch and reduces the problems regarding sampling.

Objectives

An on-line instrumentation should be tested under the same conditions it is to be used later. The aim of this project was to test different commercially available instrumentations for on-line analyses of fat, water and protein contents of ground beef under industry conditions.

Methodology

The instrument sensors were either mounted at the outlet of a meat grinder, on a conveyer belt or in a pipeline with moving ground meat. The 60 batches ranged between 150-500kg, and the grinding sizes were 18 or 40mm. The following instruments were selected for testing;

- Reflectance diode array NIR, Corona 45 (Zeiss)
- Reflectance NIR, MM710 (NDC-IE)
- X-ray AVS Raytech (Safeline)

In addition, a microwave instrument assembled and operated by the Danish Meat Research Institute was included in the testing. Much emphasis was placed on obtaining representative samples for the following reference analysis;

- Total fat (extraction and acid hydrolysis) - ASN 3121
- Water (drying overnight at 102-105°C for 16-18hrs) - NMKL 23 (1991)
- Protein (Kjeldahl) – AOAC 981.10 (1983)

Results & Discussion

There is known to be a close, inverse relationship between fat and water in meat. Plotting the content of fat against the contents of water and protein in the cuts showed high correlations. The sums of the components for each sample were checked to trace any outliers. The information in the strong relationships between the components is obviously used in the calibrations.

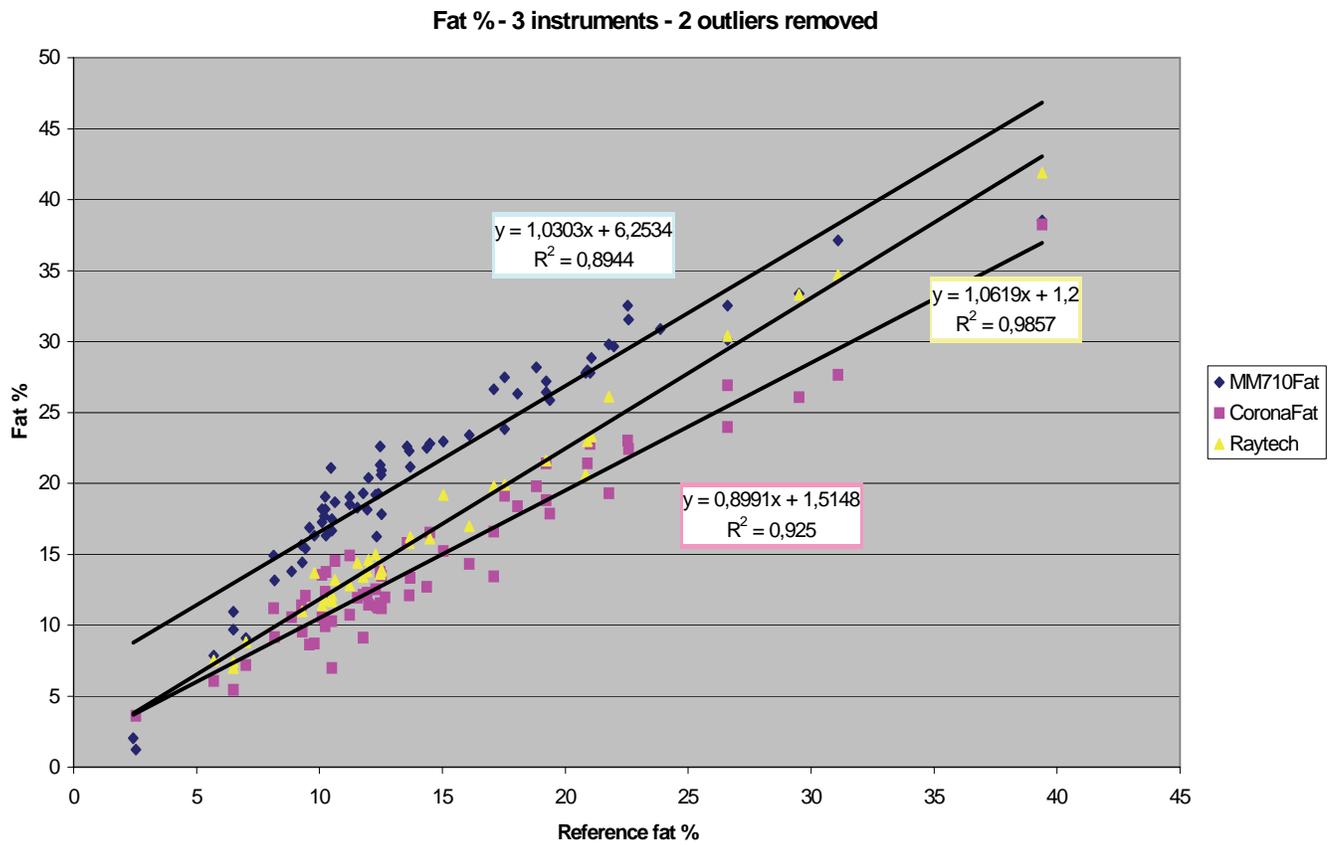
As both the 2 NIR instruments and the X-ray instrument performed well, they can be recommended for implementation in meat processes for the analysis of fat and water (Figure 1). The X-ray instrument yielded the best prediction results, with a standard error down to 0.5 % for the best model. The coarseness of the ground meat did not seem to have a large effect on the performance of the analysis. The largest contributor to the overall error in the analyses was evidently the sampling error. An on-line instrument for a specific meat application should be selected on the basis of the particular process and monitoring site in that process.

Conclusions

1. Prediction results for fat or water in ground meat for all 3 instruments were in general promising with explained variances in the range 89-99%. Prediction of protein by the NIR instruments was not satisfactory with explained variances as low as 71-75%.
2. Coarsely ground meat (40mm) was as well predicted as finer ground meat (18mm).
3. The non-contact microwave instrument did not perform satisfactory in this test due to various interferences and needs to be developed further.

Tables and Figures

Figure 1. On line analysis of fat by 3 different commercially available instrumentations.



DETERMINATION OF PORK QUALITY CHARACTERISTICS USING VIS/NIR SPECTROSCOPY

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Key Words: Near-infrared spectroscopy, meat quality, pork

Introduction

Rapid screening techniques to determine quality characteristics of meat are of great interest to the industry. In this respect, near-infrared spectroscopy (NIRS) is one of the most promising techniques (Monin, 1998). In recent years, the usefulness of NIRS has been investigated for a number of quality aspects of pork. Amongst these are the fatty acid composition of pork fat (García-Olmo et al., 2001; González-Martín et al., 2002), on-line determination of fat, water and protein content of ground pork (Togersen et al., 1999), measurement of pH (Andersen et al., 1999), measurement of intramuscular fat (Brøndum et al., 2000; Schwörer et al., 2000) prediction of drip loss (Brøndum et al., 2000; Forrest et al., 2000; Geesink et al., 2003), prediction of tenderness (Geesink et al., 2003), and determination of the RN- phenotype (Josell et al., 2001; Josell et al., 2000). Most of these applications were moderately successful to successful, with the exception of prediction of tenderness. Prediction models for drip loss were moderately successful with correlation coefficients and prediction errors of 0.64 and 2.43% (Brøndum et al., 2000) and 0.74 and 1.1% (Geesink et al., 2003) when spectra were recorded post rigor, and a correlation coefficient and prediction error of 0.84 and 1.8% when spectra were recorded pre rigor (Forrest et al., 2000). In all of these studies the models were validated by full cross validation (leave one out). Thus, these models have not been tested on an independent data set.

Objectives

The purpose of the present study was to further investigate the usefulness of NIRS to predict water-holding capacity. In addition, the predictive power of NIRS for intramuscular fat, color characteristics and pH was tested further.

Methodology

Animals and slaughter

Visible/near-infrared spectra and meat quality characteristics of the longissimus muscle were collected from three batches of pigs, slaughtered at different days. Pigs in the first batch (n = 38) were boars and gilts from crossbreed Dutch landrace x Finnish

landrace (sow) and Yorkshire (boar). Pigs in the second (n = 87) and third batch (n = 82) were barrows and gilts from crossbreed Yorkshire (sow) and Pietrain (boar). Pigs were slaughtered at a live weight of about 105 kg at a commercial slaughter plant. Pigs were electrically stunned and killed by exsanguination. Further processing of the carcasses was according to routine procedures of the slaughter plant.

Meat quality traits

One day after slaughter ca. 25 cm of the longissimus muscle of the right carcass side, starting at the 4th lumbar vertebra, was collected and transported to CCL Research and stored at 4°C. The following day the muscles were divided in 1.8 cm slices starting at the rostral end of the muscles. Slices 2, 4, 6, and 8 were used for determination of drip loss. The 3rd slice was used for visible/near infrared spectroscopy, pH determination, and determination of intramuscular fat. The 5th slice was used for color measurements. For determination of drip loss, two circular samples with a diameter of 4 cm were removed from the slices using a cork borer. The samples, 8 per muscle, were weighed, placed on display trays, covered with foil and stored for 5 days at 4°C. After storage, the samples were patted dry with paper towel, and drip loss (%) was determined by reweighing the samples. Color was determined, after blooming for 30 minutes, by measurement of L*-, a*-, and b* values using a Minolta Chromameter CR-210 (Minolta Co., Osaka, Japan). A Radiometer PHM85 Precision pH meter equipped with a Radiometer PHC 2431 glass electrode (Radiometer, Brønshøj, Denmark) was used to determine the pH of the muscles. Intramuscular fat content was determined by a commercial laboratory (Nutricontrol, Veghel, The Netherlands) according to ISO/IEC 17025:2000.

Visible/Near Infrared Spectroscopy and chemometric analyses

Reflectance spectra of meat were recorded between 400 and 2500 nm using a NIRSystem 6500 scanning spectrophotometer (Foss NIRSystems, Silversprings, MD, USA) with a transport module. Samples were placed in a sample holder (5 x 6 cm) with a quartz window. Twenty five spectra per sample with a resolution of 2 nm were recorded. Spectrophotometer control and preliminary spectral file management were performed using WINISI software (version 1.50; Infracsoft International, Port Matilda, MD, USA). Exploratory data analysis, calibration, and validation were performed using WINISI.

Calibrations were developed for each meat quality trait testing a number of wavelength ranges (400-800 nm, 400-1100 nm, 800-1100 nm, 800-2500, 1100-2500, and 400-2500), derivative orders (0-2), and by using or not using scatter correction (SNV and Detrend). The remaining settings were the defaults of the software package. Regression equations, using modified partial least squares (MPLS), were first developed for the complete data set. Based on the standard error of cross validation (SECV) and coefficient of determination, the wavelength range and mathematical treatments of the most promising models were used to develop calibration models based on the data of two batches of pigs. The resulting models were validated using the data from the remaining batch. All batch combinations were tested this way to test the stability of the NIR calibrations.

Results & Discussion

Summary statistics of the meat quality attributes are given in Table 1. In all measured parameters, the standard deviation (SD) was 18 - 20% of the difference between the maximum and minimum values of that parameter. This indicates that the data set contained a sufficiently large variation to allow for a meaningful calibration.

Characteristics of the predictive models are given in Table 2. The ratio between the standard error of calibration (SEC) and the standard error of cross-validation within the calibrated data set (SECV) varied between 1.02 and 1.53, indicating a sufficiently robust calibration. The ratio between the standard error of prediction (SEP) and the SEC ranged from 0.84 to 2.15 (1.27 on average). Assuming the SEC is approximately equal to the standard error of the laboratory (SEL), this ratio is very acceptable with regard to the accuracy of the calibration.

Among the 207 samples in the validation data set, 54.1, and 86.5% of the samples were predicted within 1 and 2% of the observed drip loss. On an arbitrary basis samples with a drip loss < 6% can be classified as superior water-holding capacity and > 8% as inferior water-holding capacity (Figure 1). Of all samples 34 were predicted to be superior and 33 inferior. Of the samples classified as superior, 50% exhibited a drip loss < 6%, whereas 6% exhibited a drip loss > 8%. Of the samples classified as inferior, 64% exhibited a drip loss > 8% and none had a drip loss < 6%. This example shows that the model is not robust enough to correctly classify each individual sample, but it may be used to select batches of meat with an on average superior or inferior water-holding capacity.

For the color parameters 80.2% of the samples were predicted within 2 units of the actual L*-value, and 85% and 95.7% within 1 unit of the actual a*- and b*-value, respectively. Given that both the Minolta Chromameter and the NIRSystem measure light reflectance in the visual spectrum a stronger correlation between both measurements could have been expected (Table 2). However, considerable variation in color exists within the porcine longissimus (Van Oeckel and Warnants, 2003). Since both measurements were not performed on the same muscle slice, color variation within the muscle may explain the relatively moderate correlation between both measurements.

In accordance with the results of Andersen et al. (1999), pH could be predicted relatively well despite the narrow pH range of the samples (Tables 1 & 2). Of all samples, 84% were predicted within 0.1 pH unit of the measured value. Given that measurements with a pH probe are relatively slow and, in our opinion, do not offer good precision during routine use under processing conditions, NIRS may be a suitable alternative to measurement with pH probes.

Intramuscular fat was predicted with good accuracy (Table 2). Of all samples, 83.9% and 97.6% were predicted within 0.5% and 1%, respectively, of the measured amount.

This experiment assessed the potential use of NIRS for measuring meat quality traits by doing a proper validation of successful calibrations described in other studies. When calibrating, the robustness and accuracy are usually judged by using cross-validation methods. However, this judgement is of limited value unless it holds true for independent samples as well. This study showed that calibrations for pH, intramuscular fat, drip loss, and L*, a*, and b* color values in the porcine longissimus muscle can be used on samples from an independent batch of pigs with about 1.27 times the accuracy of the

calibration itself. The batches of pigs used consisted of different crossbreeds. Combinations of different batches of pigs were used for calibration, but the similarity of the results implies that breed, at least for modern commercial type pigs, does not affect the accuracy of the calibration.

The potential for use of NIRS depends on the parameter to be predicted. Intramuscular fat can be determined with good accuracy. Muscle pH and color values are reasonably well predicted with NIRS. It can be expected that predictions for color can be improved if both Minolta and NIRS measurements are done on the same slice of meat. These measurements are sufficiently accurate to use either quantitatively or for classification of quality categories of pieces of meat. Drip loss can not be determined with sufficient accuracy using NIRS, but classification of quality groups is possible. The average drip loss between groups will be different if selected on the basis of NIRS. For use in practice, a validation should be made for an industrial type NIRS apparatus.

Conclusions

After validation with sets of independent samples, NIRS has the potential to become a valuable rapid screening method for the meat industry. Intramuscular fat, pH and color values may be predicted quantitatively or be classified. Drip loss may be used for selecting quality subgroups. NIRS calibrations appear breed independent and thus allow use of this technique in most slaughter houses.

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Tables and Figures

Table 1. Meat quality traits (mean, range, and standard deviation) of three batches of pigs, slaughtered on different days.

Trait	Batch 1 (n = 38)			Batch 2 (n = 87)			Batch 3 (n = 82)		
	Mean	Range	S.D.	Mean	Range	S.D.	Mean	Range	S.D.
Drip loss (%)	8.0	5.6-10.9	1.2	6.7	2.9-11.3	1.8	6.9	2.5-11.6	1.7
L*-value	53.5	48.4-59.5	2.7	51.0	45.2-58.4	2.6	50.1	43.7-58.0	2.5
a*-value	14.7	13.0-17.6	1.0	15.1	13.5-17.5	0.9	15.4	13.1-17.0	0.8
b*-value	6.5	4.8-8.0	0.7	6.0	4.3-8.1	0.7	5.9	4.6-8.0	0.7
Muscle pH	5.39	5.25-5.53	0.06	5.46	5.31-5.66	0.08	5.46	5.27-5.71	0.09
I.M. Fat (%)	1.2	0.4-2.3	0.4	1.1	0.1-3.6	0.6	1.1	0.2-4.3	0.5

Table 2. Correlation coefficients (r), standard error of calibration (SEC), and standard error of prediction in the PLSR models for meat quality attributes.

Item	Wavelength	Treatment	Model	Calibration			Validation		
				N	SEC	SECV	r	SEP	r
Drip (%)	400-800	None	Exp. 1 + 2	123	1.11	1.24	0.74	1.41	0.58
			Exp. 1 + 3	117	1.27	1.35	0.56	1.42	0.59
			Exp. 2 + 3	165	1.19	1.35	0.70	1.14	0.56
L*-value	400-800	None	Exp. 1 + 2	124	1.28	1.57	0.89	1.42	0.83
			Exp. 1 + 3	119	1.57	1.65	0.85	1.64	0.79
			Exp. 2 + 3	165	1.27	1.39	0.87	1.25	0.89
a*-value	400-1100	1st deriv.	Exp. 1 + 2	122	0.46	0.57	0.88	0.67	0.66
		SNV +	Exp. 1 + 3	116	0.51	0.59	0.83	0.67	0.72
		Detrend	Exp. 2 + 3	165	0.57	0.58	0.76	0.74	0.68
b*-value	400-800	None	Exp. 1 + 2	123	0.44	0.46	0.81	0.52	0.67
			Exp. 1 + 3	117	0.44	0.49	0.81	0.51	0.71
			Exp. 2 + 3	168	0.50	0.52	0.68	0.42	0.84
I.m. fat (%)	800-2500	2nd deriv.	Exp. 1 + 2	122	0.33	0.36	0.70	0.39	0.69
			Exp. 1 + 3	115	0.20	0.26	0.86	0.37	0.76
			Exp. 2 + 3	164	0.28	0.33	0.83	0.40	0.63
pH	400-1100	2nd deriv.	Exp. 1 + 2	123	0.033	0.045	0.91	0.071	0.66
			Exp. 1 + 3	117	0.047	0.060	0.85	0.070	0.63
			Exp. 2 + 3	166	0.049	0.063	0.83	0.047	0.84

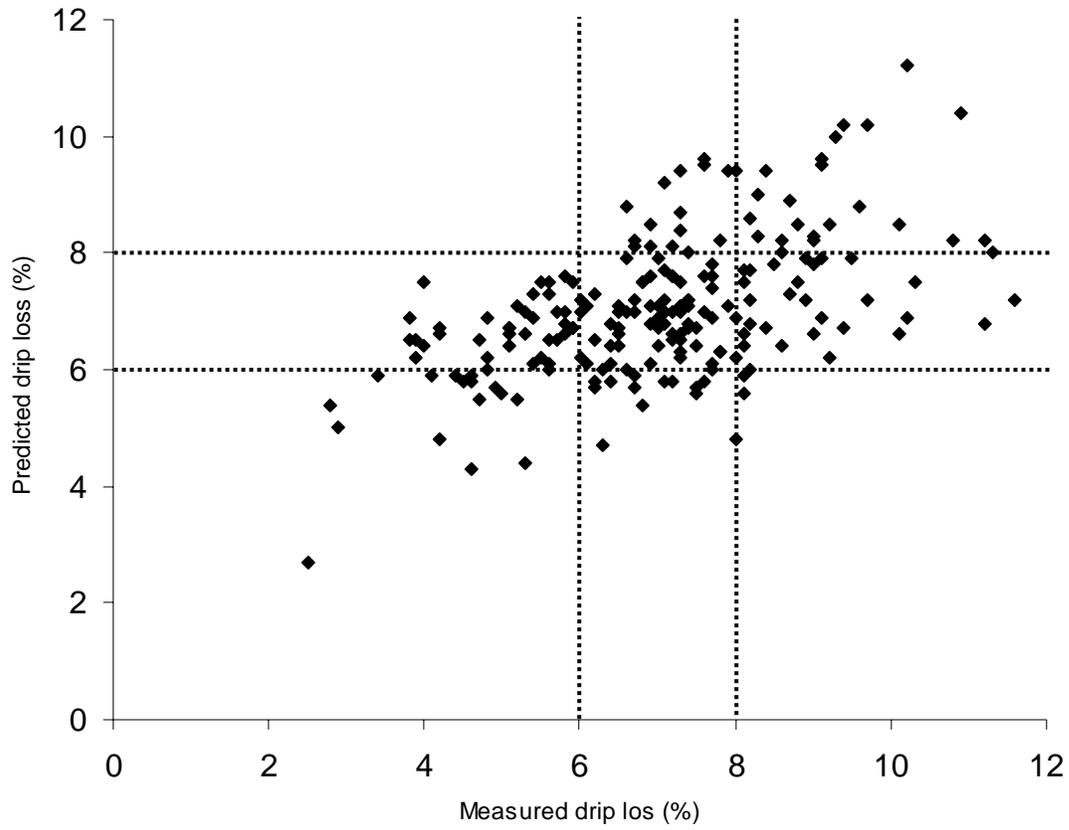


Figure 1. Predicted versus measured drip loss in 207 loin samples as assessed by NIRS

NONDESTRUCTIVE NIR SPECTROSCOPY FOR DETECTING FRESH AND FROZEN-THAWED FISH MEATS

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Key Words: NIR spectroscopy, fresh and frozen fish, PCA, SIMCA, LDA

Introduction

Given the perishable nature of fish or fillets, extension of its shelf-life is a requirement of normal trading. According to the Food and Agricultural Organization (FAO) and the Japan Agriculture Standard (JAS) regulations, labeling should state that the fish has been frozen and must not be refrozen (FAO 1982; JAS 2000). Fresh fish is, indeed, understood as being fish freshly caught or which has been chilled and stored for a short period at normal refrigeration temperature prior to purchase or use. For storage over longer periods freezing is normally utilized. The consumer perception of frozen fish is inferior to that of the fresh material and this is reflected in the price which it realises. In practice, a considerable number of frozen fish are thawed in fish shops, stored on ice and sold as unfrozen fish without being labeled as such. Control of labeling is only possible, if there any rapid and reliable methods exist, which allow food control authorities to distinguish between fresh and frozen-thawed fish or fillets.

To detect fresh and frozen-thawed fish, measurement of the electric properties of fish tissues, visual inspection of the eye lens, judgments of the integrity of red blood cells by microscopy, or estimation of the hematocrit value were proposed (Yoshioka and Kitamikado 1988; Rehbin 1992). In practice, proposed methods cannot be applied to those fish or fillets possessing no blood, eye lens or skin. Determination of enzyme activity (Rehbein and Cakli 2000) is also time-consuming or destructive. Near-infrared (NIR) spectroscopy is a physical and faster technique, requiring minimal or no sample preparation/reagent and its precision can be high. The method offers the possibility of measuring physical and chemical properties of fish or fillets. It has been widely used in the food industry, is based on the electromagnetic absorption of organic compounds (Uddin and others 2002; Blazquez and others 2004). No extractions are needed and no wastes are produced in visible/NIR spectroscopy using fibre optic probe, which would be an eco-friendly instrumental technique.

Objectives

To develop a nondestructive fast technique for identification of fresh and frozen-thawed fish meats.

Methodology

Live 108 each of red sea bream *Pagrus major* and horse mackerel *Trachurus japonicus*, were purchased from Kanazawa Prefecture, Japan. For fresh or unfrozen, 54 samples were used soon after killed while another 54 fish was kept at -40°C . After 30 days of frozen stored samples were removed and thawed overnight at 5°C then evaluated as frozen-thawed samples.

Samples were scanned using a NIRSystems 6500 spectrophotometer (Silver Spring, MD, USA) equipped with a surface interactance fibre optic accessory. Before spectra were measured on the fish, a reference spectrum was obtained by measuring the reflected radiation from a white ceramic plate. Spectra were recorded the wavelength range 400-1098 nm at 2 nm intervals. The spectra were stored in optical density units $\log(1/T)$, where T represents the percent of energy transmitted. Spectral data were analyzed with “The Unscrambler” software (Version 8.05, Camo, USA). The 108 samples in total, 54 of them fresh and 54 of them frozen-then-thawed, were divided into a modeling set and a prediction set. The modeling set contained 35 samples for the fresh and 35 for the frozen fish. Twenty-seven of those samples were picked as every odd numbered sample in the order of recording, and the remaining 8 samples were selected randomly. Thus, 19 samples for both fresh and frozen fish were allocated to the prediction set. Sample spectra for both sets were treated in exactly the same way with second derivative or multiplicative scatter correction (MSC), or no treatment was applied at all.

We used the classification method called Soft Independent Modeling of Class Analogy (SIMCA) and Linear Discriminant Analysis (LDA) using PCA (principal components analysis) scores. The former method is based on disjoint PCA models where for each group an independent PCA model is constructed which are then used to classify new, unknown samples. Later one uses the so-called scores values of PCA results as input variables to the LDA. By performing PCA first, we reduce the number of variables and make them independent and by doing so, only a small fraction of information is lost.

Results & Discussion

For a classification to be successful two things are needed. Firstly, samples belonging to the same group should be as similar as possible and secondly, the groups should be as far away from each other as possible. In our case, defining groups was easy, since the absorbance spectra of the fresh and frozen-thawed fish samples are very much different as seen in Figure 1. The second derivative spectra of fresh and frozen samples shown with the water band featuring strongly around 966 nm as a negative peak and similar observations were also noted in horse mackerel (data not shown). The major effect of freeze-thawing treatment involves a gross change in total absorbance after freezing and thawing; this arises from changes in light scatter presumably arising from alterations in the physical structure of at least the surface layer of fish. The existence of these differences suggests that it may, indeed, be possible to detect freeze-thaw treatment by means of this spectroscopic procedure.

Water absorbs strongly in specific wavelengths which is expected and usually exhibits a broad band because of H-bonding interactions with itself and with other components in the meat (Figure 1). In visible-NIR spectroscopy, the regions from 740–

760 nm and 960–980 nm are related to O–H bond of the water in the sample (Buning-pfaue 2003). In this figure, only the 900-1098 nm is displayed as we cut off the visible region and excluded it from any calculations. We have tested other spectral intervals also, but the distance of the two groups was the biggest and the spread of data points within each group the smallest when the 900-1098 nm interval with original absorbance spectra was used. The PCA score plot clearly shows us that the fresh and the frozen-thawed samples are well separated (Figure 2). Similar separation was also observed in DESIR analysis of fresh and frozen-thawed fish was performed on the meat juices (Uddin and Okazaki 2004). Using the results of this exploratory stage for all spectral treatments applied, two independent PCA models were generated with the modeling sets and then they were used to build SIMCA models. SIMCA models were applied to the prediction set and results of the prediction can be best visualized by plotting the sample-to-model distances for all samples as shown in Figure 3 where the two groups are well defined and separated. All prediction samples are much closer to the group that they should belong to. However, not every sample is within membership limits for both the modeling and the prediction samples. As can be seen some samples are located in the upper right quadrant, indicating that they belong to none of the defined models. No sample is in the lower left quadrant, meaning that no sample was classified to both groups simultaneously. The upper left and lower right quadrants define samples which belong to one group. However when the sample spectra were subjected to MSC transformation, modeling and classification seem much more uncertain (data not shown). The two groups are very close; in fact, they almost overlap even at the modeling stage. This means that the MSC transformation removed information, i.e. scattering, on which the previous model is based, therefore models are not that far apart.

As regards LDA, the results are much more clear-cut as seen in Table 1. To perform modeling and classification the same wavelength range, spectral transformation and prediction samples were used for LDA analysis as well. It is clear from the table that the model using original absorbance spectra achieved much better (100%) classification accuracy for the prediction samples. The same figures for MSC treated spectra are considerably worse, indicating again that scattering is the information that makes classification work. We think that for fresh fish, the cellular structure is intact. When light enters the fresh fish, cells not only absorb the light but also change its direction until the light reaches the next cell. This multiple changes in the direction of light is called scattering, which increases the distance the light travels from the entry point to the exit point of the sample. This increase results in increased absorbance as seen in Figure 1a. On the other hand, when freezing and thawing is done, the cell membranes get damaged leaking the intracellular contents into the extracellular space. Thus, there is much smaller number of cells that can scatter light as it travels through the sample, reducing the distance the light has to cover. As a result, light interacts with a smaller number of molecules, which in turn results in a decrease of absorbance (Figure 1). Nevertheless, results are promising that a fast measurement method can be developed to detect fraud such as when frozen-thawed fish are sold as fresh.

Conclusions

The applicability of visible/NIR technique has been successfully demonstrated to differentiate between fresh and frozen-thawed fish. The technique uses the fact that fish muscle absorbs and reflects light in different ways during storage and thawing. Spectroscopically measuring raw materials using known characteristics, models can be developed that can again be used to estimate the characteristics for unknown specimen. Once have the models, differentiation between fresh and frozen thawed fish could be a matter of seconds.

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Tables and Figures

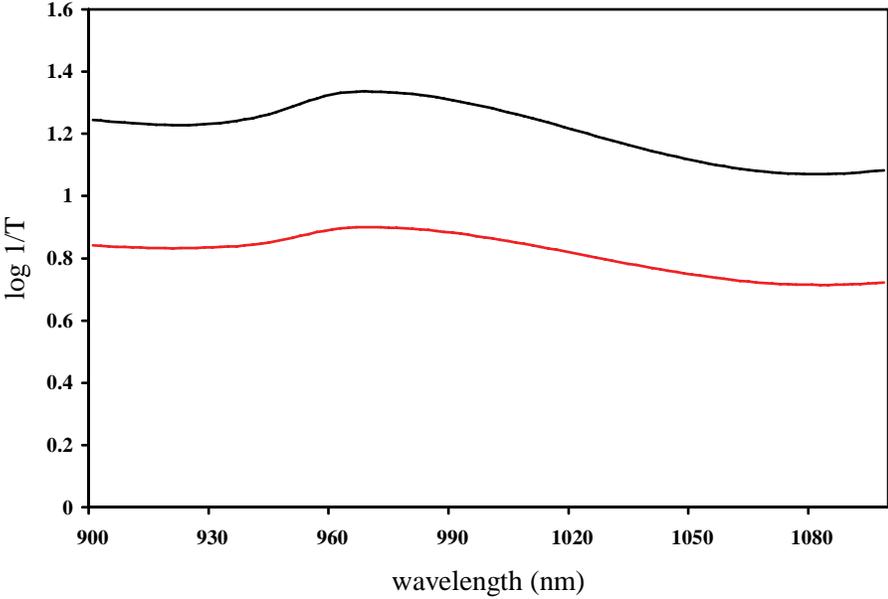


Figure 1. Average original absorbance spectra of fresh (upper line) and frozen (lower line) red sea bream sample spectra in the 900-1098 nm wavelength range

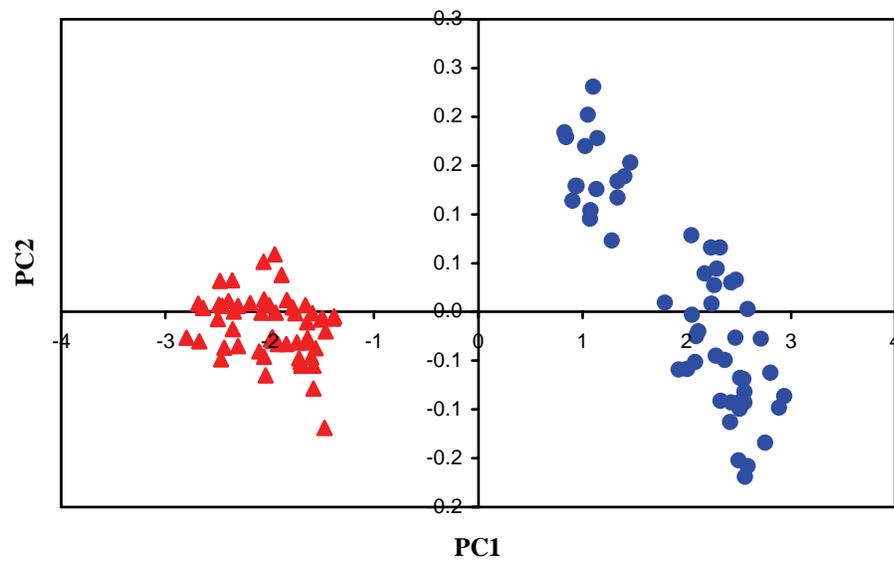


Figure 2-Two dimensional PCA score plot of all 108 red sea bream samples. Samples on the left side of the ordinate axis are frozen samples (triangles), while those on the right are fresh samples (circles).

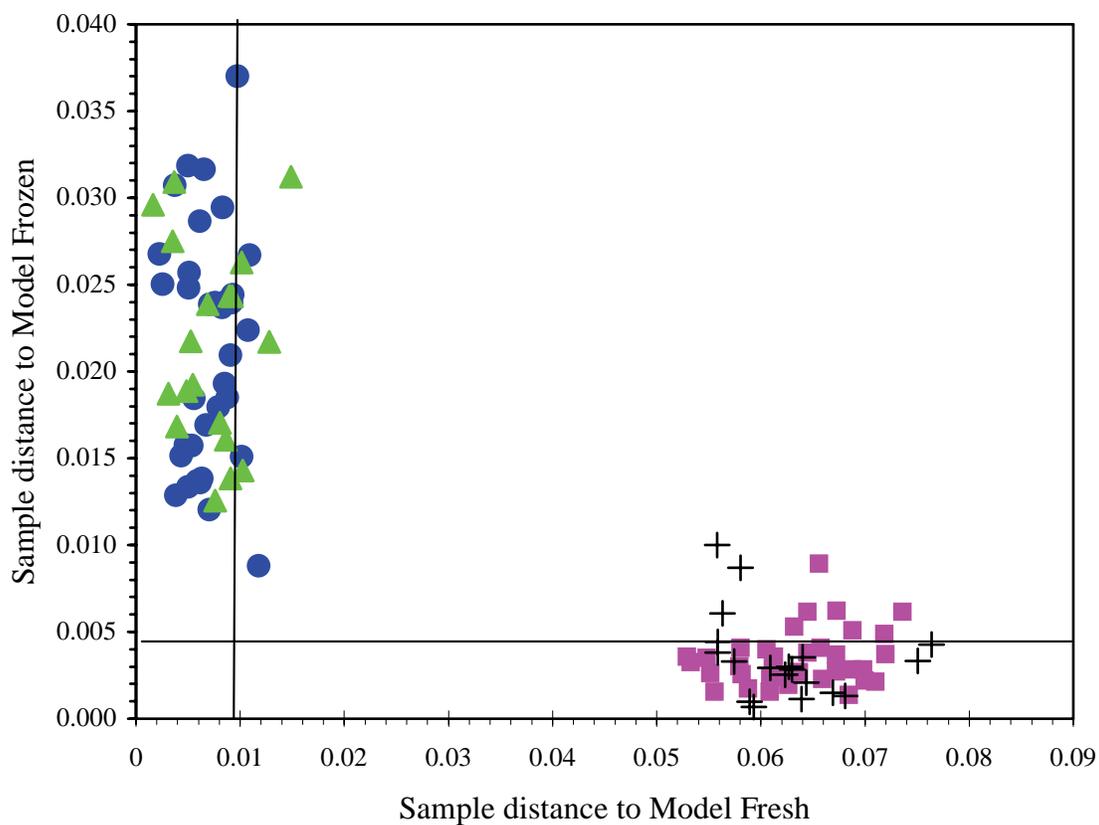


Figure 3-Cooman's plot for discrimination between fresh and frozen-thawed red sea bream. Spectra were submitted to SIMCA without any treatment. Circle Rs are fresh modeling samples, square Fs are frozen-thawed modeling samples, and triangle Rs and plus Fs are fresh and frozen-thawed prediction samples, respectively. The horizontal and vertical gray lines are class memberships limits calculated at a 5% confidence limit.

Table-1. Discrimination results between fresh and frozen-thawed prediction red sea bream using LDA with PCA scores as input variables

Spectral transformation	N correct in Groups ^a		Group proportion correct		Overall proportion correct
	Type of fish		Type of fish		
	Fresh	Frozen	Fresh	Frozen	
None	19	19	100%	100%	100%
MSC	15	16	79%	84%	81%

^aThe number of correctly classified samples out of 19 prediction samples for the fresh and frozen-thawed red sea bream groups, respectively

STUDY ON THE USE OF NEW OPTICAL-NEEDLE DEVICE CGM FOR ESTIMATING THE LEAN MEAT PERCENTAGE IN PIG CARCASSES

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Key-words: meat percentage, pig carcasses, estimating

Introduction

Among many methods of estimating meat percentage in pig carcasses the most important one is optical-needle and ultrasound technique. Meat percentage estimation performed by the means of this method is based on regression equations provided for domestic pig population with reference to analysis of correlations between fat thickness and *longissimus dorsi* (LD) muscle thickness on the one side, and meat percentage obtained from dissection (Borzuta 1998, Dobrowolski *et al.* 1993, Dumas and Dhone 1998).

In Poland, as new classification devices were introduced to domestic slaughterhouses, many regression equations were provided for the purpose of estimating meat percentage. In 2002, researches were begun on re-testing various devices within the framework of twinning project PHARE no. PL.01.04.06, co-financed by European Union. One of the devices tested in this project is French probe CGM, in which modern optical-digital converter have been used, the resolution of which is twenty times higher than in optic-needle devices of older generation (Komender 2003).

Objectives

The objective of the research was to provide the regression equation for the purpose of meat percentage estimation in pig carcasses with new optical-needle CGM device.

Methodology

On slaughter lines of three slaughterhouses 286 carcasses were chosen for measurements and dissection, arranged in three groups of similar size, differing as for back fat thickness, i.e. group 1 from 4 to 16 mm, group 2 from 17 to 28 mm, group 3 from 29 to 44 mm (Borzuta *et al.* 2003). The carcasses were chosen from the weight range 60 to 120 kg, and they originated half from gilts and half from castrated males.

On the chosen, left half-carcasses the measurements of fat and LD muscle thickness were performed with CGM probe in two points, i.e. at the level of last rib and between 3rd and 4th thoracic vertebrae, counting vertebrae from the end. The needle was

inserted 6 cm from split line, paralelly to cut surface. Dissection was performed by a team of 10 trained burchers supervised by experts from Poland and Denmark. The team used the referential dissection method obligatory in European Union (Walstra and Markus 1996), which anticipates dissection of 4 main joints, i.e. ham without shank, shoulder without shank, loin, belly and tenderloin. Regression equation for estimating meat percentage was calculated with multiple linear regression MLR.

Results & Discussion

Examined carcasses had average meat percentage amounting to 52,84% and represented population of normal distribution. High, statistically significant correlation coefficients were obtained ($P \leq 0,01$) between performed fat and muscle thickness measurements and meat percentage in carcass (tab. 1). Considerably higher correlations were obtained for fat thickness rather than LD muscle thickness, what is commonly known (Daumas *et al.* 1998, Blicharski *et al.* 2000, Borzuta 1998). Correlation coefficients for manual measurements performed on cross-section areas of half-carcasses turned to be only slightly higher than for measurements performed with the probe, what proves high accuracy of the probe.

In the set of meat percentage measurement results there were not any outliers found and therefore regression equations were also provided for all dissected half-carcasses.

The equations are as follows:

a/ Equation with carcass weight (W) and four measurement points

$$Y = 50,17026 - 0,30012F1 - 0,36252F2 + 0,11475M1 + 0,19995M2 - 0,04152 W$$

Estimating error RMSE = 2,21 $R^2 = 0,82$

b/ Equation with four measurement points, without carcass weight

$$Y = 48,80655 - 0,33616F1 - 0,36445F2 + 0,10173M1 + 0,18911M2$$

Estimating error RMSE = 2,23 $R^2 = 0,82$

c/ Equation with two measurement points

$$Y = 50,11930 - 0,62421F2 + 0,26979M2$$

Estimating error RMSE = 2,38 $R^2 = 0,79$

Calculation results prove that each of three regression equations mentioned above can be used for implementation into industry as they meet the European Union regulations as for estimating error, which cannot exceed 2,5% (EC Regulation 3127/94). Analysis of those equations indicates, however, that including carcass weight into regression analysis decreased the estimating error RMSE only by 0,02, what practically is of no importance whatsoever for classification results. Abandoning the measurement points F1 and M1, however, causes increase of estimating error from 2,23 to 2,38. As in two-point measurement RMSE does not exceed the value of 2,5%, the producer of the probe decided to introduce the device with that simplified measurement procedure into the industry, what significantly increases the CGM probe efficiency. A little bit lower

estimating error was provided only for France, RMSE = 2,17 for castrated males and in Belgium (RSME = 2,08), but in both countries 4 measurement points were included into the equation (Daumas and Dhorne 1998).

The results of regression analysis for one-point measurement equation were given in tab. 2

Conclusions

1. Research performed on numerous population of dissected pig carcass proved high, statistically significant correlation between meat percentage in carcass and fat and *longissimus dorsi* muscle thickness at the level of last rib and in the cross-section area between 3rd and 4th thoracic vertebrae, counting vertebrae from the end.
2. Multiple linear regression equation provided for CGM probe for the purpose of estimating lean meat percentage in pig half-carcasses takes into account two measurement features of fat thickness and the *longissimus dorsi* muscle thickness defined in the thesis results and its estimating error RMSE = 2,38.

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Tables and Figures

Table 1. Coefficient correlations between muscling indexes and meat content of pig carcasses

Correlated trait	Indexes measured handly by ryler	Indexes measured by CGM
Thickness of backfat	- 0,43	-
F ₁	- 0,82	- 0,77
M ₁	0,59	0,45
F ₂	- 0,83	- 0,78
M ₂	0,58	0,48

F₁ – fat thickness under the last rib, 6 cm from midline, measured parallelly to this line

F₂ – as F₁ but measurement was made between 3 and 4 last back vertebra

M₁ – thickness of m. LD measured in the same point as F₁

M₂ – thickness of m. LD measured in the same point as F₂

Table 2. Regression analysis for CGM device

Traits	Regression coefficient	s	t	P
intercept	50,11930	1,08476	46,20	0,0001
F ₂	-0,62421	0,02289	-27,27	0,0001
M ₂	0,26979	0,01767	15,27	0,0001

AN OBJECTIVE METHOD TO MEASURE FIRMNESS IN FRESH PORK LOINS

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Key Words: Firmness, Pork, Quality

Introduction

The pork industry currently uses several different meat characteristics to separate fresh pork into quality groups. These characteristics commonly include pH, color, marbling, and firmness. While most of these quality measurements can be obtained through both subjective and objective methods, there is currently no objective method to quantify firmness of fresh meat either in the laboratory setting or in the plant. The pH of a fresh product is can be quickly measured with a portable pH meter such as the pH Star from SFK (SFK Technologies). There is no standard for subjective determination of pH. Color can be scored subjectively using standards such as the NPPC pork quality standards (NPPC 1991; NPPC 1999) or the Japanese Color Score tiles. Color can also be measured objectively with the use of colorimeters such as those produced by Minolta™, Hunter™, and ColorTec™. Subjective marbling scores may be obtained through visual evaluation, however, objective measurements, such as extraction with solvent or acid hydrolysis, are generally considered to be more precise. Of the previously mentioned quality characteristics, firmness is the only one that cannot currently be objectively quantified. Methods for measuring firmness include visual and hands on evaluation combined with knowledge from previous experiences. Consistency, repeatability, and uniformity throughout the industry are some potential problems with these methods. Standards currently utilized for firmness evaluation of fresh pork are the three-point scale described by the NPPC in 1999 and the five-point scale described by the Wisconsin pork quality standards in 1963 and the NPPC in 1991. These scales range from a score of one, being very soft and very watery, to the top score of either three or five, being very firm and dry.

Objectives

The primary goal of the following research was to identify a method, either destructive or nondestructive, to objectively quantify firmness of fresh pork loins. Focus was placed on the use of either a universal testing machine (i.e. Instron) or a texture analyzer.

Methodology

Part 1.

Forty-two fresh pork loins were selected from the boneless loin line of a commercial slaughter facility at 24 hours post mortem. Product was selected based on subjective firmness of the whole boneless loin. Using the NPPC 5-point scale, twenty-one loins of subjective firmness score less than 3 as well as twenty-one loins with subjective firmness score of greater than 3 were selected in hopes of finding forty-two total loins with a somewhat uniform subjective firmness distribution.

At two days post mortem, the blade end of each boneless loin was removed at the area of the tenth rib and the loin was placed randomly on a table for evaluation by a 5 member panel utilizing a 5-point scale (NPPC 1991). Four boneless loins with subjective firmness scores of 1, 2, 3, and 4 were made available to panelists as standards during evaluation. A loin with a subjective firmness score of 5 was not readily available. Panelist scores were evaluated for uniformity and all scores were averaged to determine the subjective firmness of each loin for use during analysis of data.

Following evaluation by the panelists, loins were prepared for evaluation with the TA.XT2 texture analyzer (Texture Technologies Corporation). It was decided from preliminary data that a compression of less than 40 % was most effective in identifying differences amongst various firmness scores of loins and that utilizing 2.54 cm chops worked better than utilizing the intact loin. With this in mind, four separate compression tests were performed on four separate chops from each loin. Duplicate compressions of a set distance (5 mm and 7.5 mm) and two compressions based on percent of chop height (20% and 30%) were performed on chops from each loin. The rationale for this is that if all of the chops were cut to the exact same width and did not deform or sag when placed flat then the 5 mm and 20 % compressions should yield very similar results (because 20 % of 25.4 mm is 5.1 mm and 30 % of 25.4 mm is 7.62 mm). However, because it was assumed that the less firm chops would sag when laid flat, it was not immediately clear which method would be more beneficial.

Chops were consistently cut to 2.54 cm by use of two knives mounted together. Two 10-inch Forschner cimeter knives (available from Koch Industries, Kansas City, MO, item no. 3367 00581) were bolted together at the handle to yield 2.54 cm between the blades. The double bladed knife was drawn through the loin to yield a chop of 2.54 cm and a chop of approximately 1.0 cm with each slice. The 1.0 cm chop was discarded and the 2.54 cm chop was utilized for analysis. This ensured that the texture test was performed on a fresh cut surface each time and dehydration did not affect the results. Chops were placed on the platform of the TA.XT2 and the test initiated within 30 seconds of cutting. After it was placed on the platform, it was allowed a 5 sec rest period prior to being compressed in the center of the chop. Random sampling of loin temperature determined that they were not different and averaged 3.0° C.

The TA.XT2 was set up in a cold room at the meat science laboratory with an air temperature of 3.1° C overnight and allowed to equilibrate. The texture analyzer was equipped with a 5 kg load cell and 25 mm diameter flat acrylic cylindrical plunger for the tests. It was calibrated using a 2 kg check weight prior to each day's activities. The analyzer was set with a pre test speed of 4.0 mm / sec, a test speed of 2.0 mm / sec, a post test speed of 2.0 mm / sec, and a trigger force of 1.0 g (when the load cell detected 1.0 g

of force being applied to it, it would capture this point as the initial height of the product and start the appropriate compression test from the four described earlier.) Data was collected using Texture Expert, version 1.11. Dependent variables captured and/or calculated by the Texture Expert software included initial height of product (when the probe contacted the meat), peak force of the second curve, area under the first curve, area under the second curve, area over the curve measuring adhesiveness, hardness (peak force of the first curve), adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. Because the data generated a force * time curve, all area calculations will have the units of grams * seconds or g*s. Although it has been documented that gumminess and chewiness should not be calculated on the same product because gumminess is a measure of semisolid foods and chewiness is a measure of solid foods, both were calculated initially (Szczesniak 1995).

Statistical analysis was performed using the REG procedure of SAS on all measurements independently in addition to using the MAXR option to create the best fit equation using all variables available and setting them equal to subjective firmness in the model (SAS Institute, Inc. version 8.2 1999). This was done for all four compression tests previously described in this chapter.

Part 2.

A total of 233 fresh boneless pork loins were transported to the University of Illinois Meat Science Laboratory for further evaluation. Loins were evaluated over a 2-day period with 100 loins evaluated on day 1 and 133 loins evaluated on day 2. Vacuum bags were opened and blade ends were removed at the area of the 10th rib for panelist subjective firmness evaluation. Loins with subjective firmness scores of 1, 2, 3, 4, and 5 were made available for the 7-member panel during evaluation. Panelist subjective firmness scores were averaged to determine the subjective firmness score for each individual loin.

Following panel evaluation, loins were analyzed using the TA.XT2 texture analyzer (Texture Technologies Corporation). Compression values of 7.5 mm from contact with the chop and 30 % of initial chop height were utilized for this population of loins. Chop preparation and machine setup were identical to that in Part 1.

Dependent variables utilized for the analysis included initial chop height, area 1 (area under the first curve), and area 4 (area under the curve from the first compression but stopping at the peak of that curve). Two separate regressions were run to determine the effect of using the equation calculated from area 1 versus using the equation calculated from area 4 to predict subjective firmness. The reg procedure of SAS was utilized to assess the effectiveness of predicting subjective firmness scores (SAS Institute, Inc. version 8.2 1999).

Results & Discussion

Part 1.

Subjective firmness scores as determined by the panel ranged from 1.0 to 4.0 for the population of 42 loins. Ideally, the range would have been from 1.0 to 5.0, however, no loins of firmness score 5 could be identified during loin selection. Figure 1 displays a

histogram for the population of loins as they relate to subjective firmness scores. Even though loins were selected to be above and below a firmness score of 3, it is difficult to not select loins of average firmness.

Results from the analysis of the data from all four compression types indicated that certain calculations correlated consistently better with subjective firmness scores than others. Based on these results, the five following dependent variables were selected to build the prediction equation: initial height, hardness (peak force of the first compression), force 1 (peak force of the second compression), area 1 (area under the curve from the first compression, and area 4 (area under the curve from the first compression but stopping at the peak of that curve). Not much was gained concerning the R^2 when the MAXR option of SAS was utilized. The final prediction equations for each compression method included four models. Subjective firmness was regressed against 1) height and force 1, 2) height and area 1, 3) height and area 4, and 4) height and hardness. The R^2 values for each of these equations can be seen in Table 1. Multiple prediction equations were created to use in conjunction with future data sets. However, based upon the results, the 7.5 mm compression coupled with the area under the first compression curve was the most likely candidate to create the prediction equation for this population of fresh pork loins. Figure 2 displays the plot of subjective firmness by height and the area under the curve from the first compression (area 1) that yielded a regression line with an R^2 of 0.54. Figure 3 displays a similar plot, but from area 4 rather than area 1. The R^2 from the equation with area 4 is 0.55. The range of values for the area under the curve from the first compression (area 1) was from 246.55 to 1295.73 while area 4 was 218.06 to 1122.09.

Part 2.

Subjective firmness scores as determined by panel evaluation ranged from 1.7 to 5.0 with a mean of 3.0 for the population of 233 loins. Predicted firmness values were calculated using two separate equations, one with height plus area 1 and one with height plus area 4, and yielded ranges from 2.4 to 5.8. The prediction equations utilized were previously generated from the population of 42 loins using height, area 1, and area 4 (Part 1).

Figure 4, a histogram displaying the subjective firmness scores of the population of 233 loins, demonstrates that this would be considered a normal distribution of loins centered around an average score of 2.98. The bulk of the values are between a subjective firmness score of 2.5 and 3.5. One must keep in mind that when looking at a population that is a small segment of the overall scale, regression can be very difficult because one can not predict how the population would have looked beyond the present data. Histograms of predicted values from equations utilizing area 1 and area 4 can be found in Figures 5 and 6, respectively.

Keeping this in mind, regressions were performed to compare the predicted firmness values to the subjective panel firmness values. Figure 7 displays the regression of subjective firmness with predicted from height plus area. The heavy concentration of loins with average firmness (scores of 2.5 to 3.5) can be easily detected in this figure. The R^2 value from this regression was 0.30. Had this population contained more loins with low and high firmness scores, the plot may have been extended and a higher R^2 might have been achieved. Figure 8 displays similar results, but utilizes the dependent variables

height and area 7 from the texture analyzer. The same trends visible in Figure 4 are also visible in Figure 8. The R^2 value from the regression involving area 4 was 0.29.

Conclusions

The prediction equation generated with this data did a moderately successful job of correlating objective values obtained by the TA.XT2 texture analyzer to subjective values as assessed by a panel. The authors feel that the approach used herein was successful to a point. It is believed that a better prediction equation could be built with a larger and more uniformly distributed population of loins concerning firmness scores. Upon building a more precise equation, it would then be possible to create a standard protocol to be used in future studies. Future experiments should not only evaluate the accuracy and repeatability of the method, but also search for quick and possibly hand held devices to be utilized in conjunction with or separately from the texture analyzer.

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Tables and Figures

Table 1. R^2 Values for Selected Variables Regressed with Initial Height vs. Compression Setting

Compression Method	Force1(g)	Area4(g*s)	Area1(g*s)	Hardness(g)
5.0mm	0.40	0.32	0.32	0.38
7.5mm	0.49	0.55	0.54	0.49
20%	0.46	0.36	0.38	0.43
30%	0.35	0.39	0.36	0.35

Figure 1. Histogram displaying the subjective firmness distribution of 42 loins.

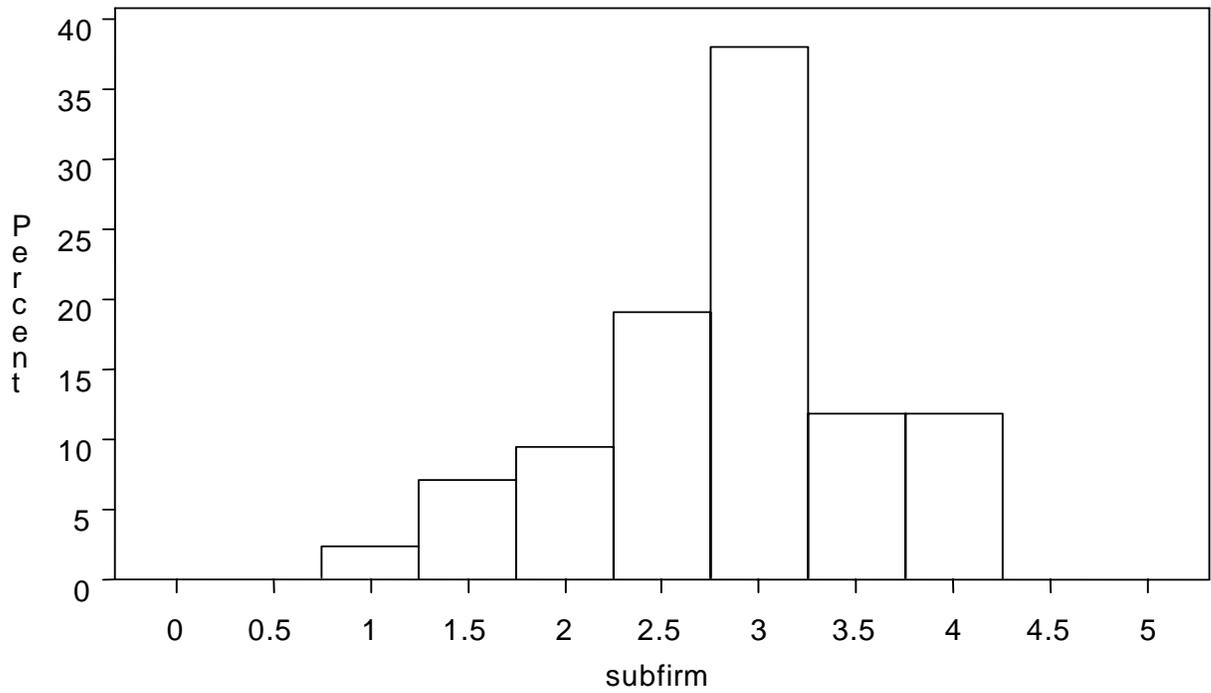
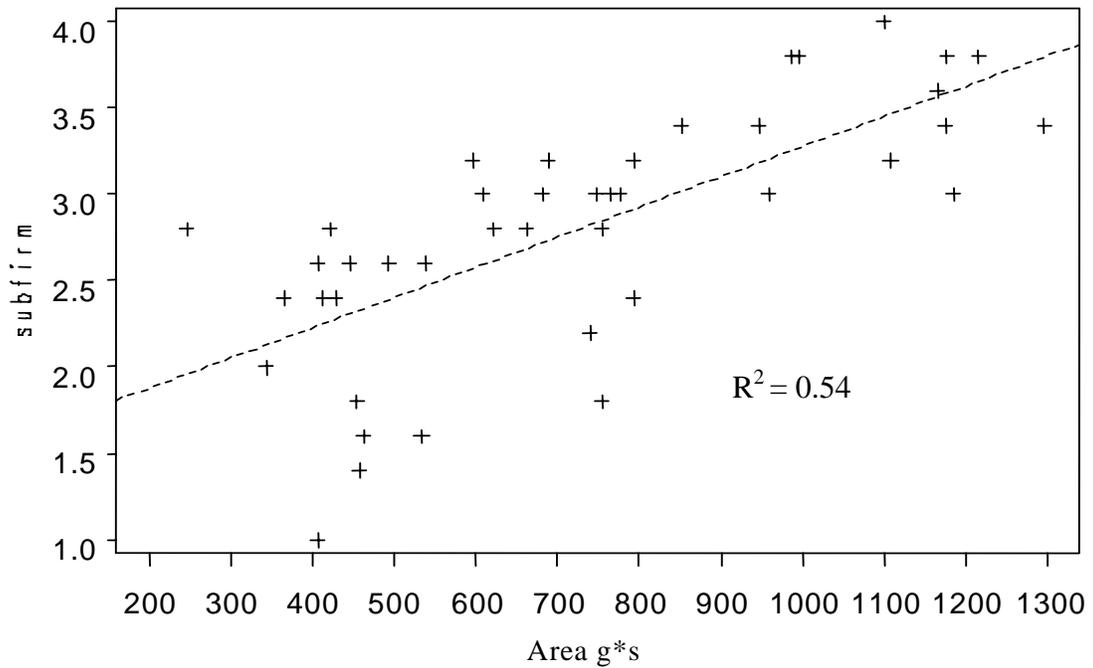
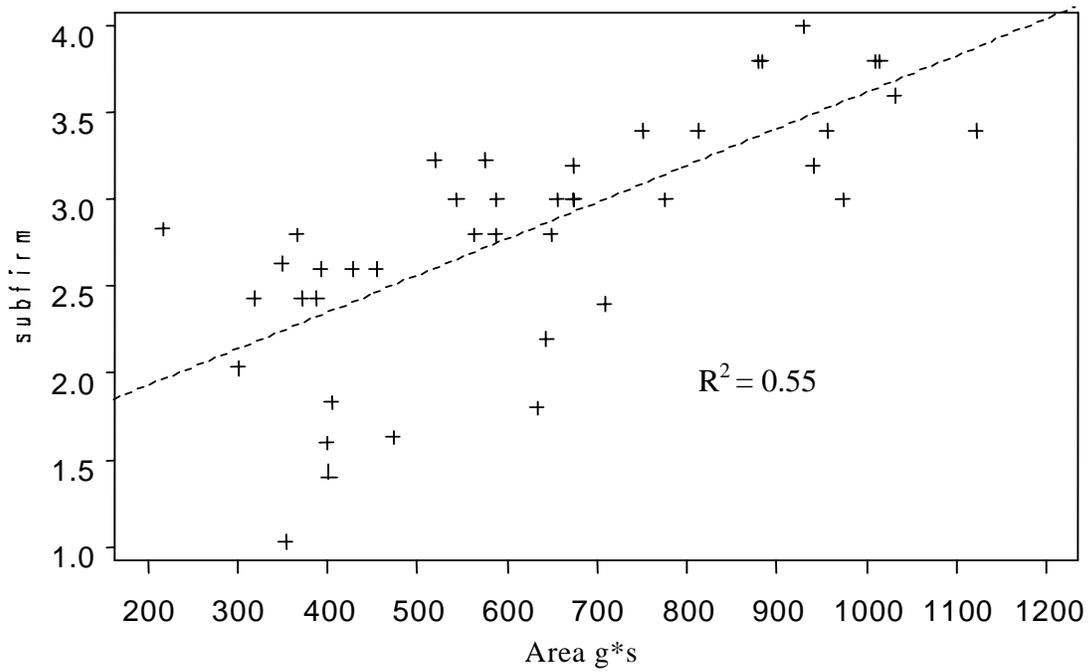


Figure 2. Plot of subjective firmness = height + area 1



Equation: subjective firmness = -1.1449 + 0.1239 height + 0.0015 area1

Figure 3. Plot of subjective firmness = height + area 4



Equation: subjective firmness = -0.9284 + 0.1119 height + 0.0018 area4

Figure 4. Histogram Displaying Subjective Firmness Scores of Panel.

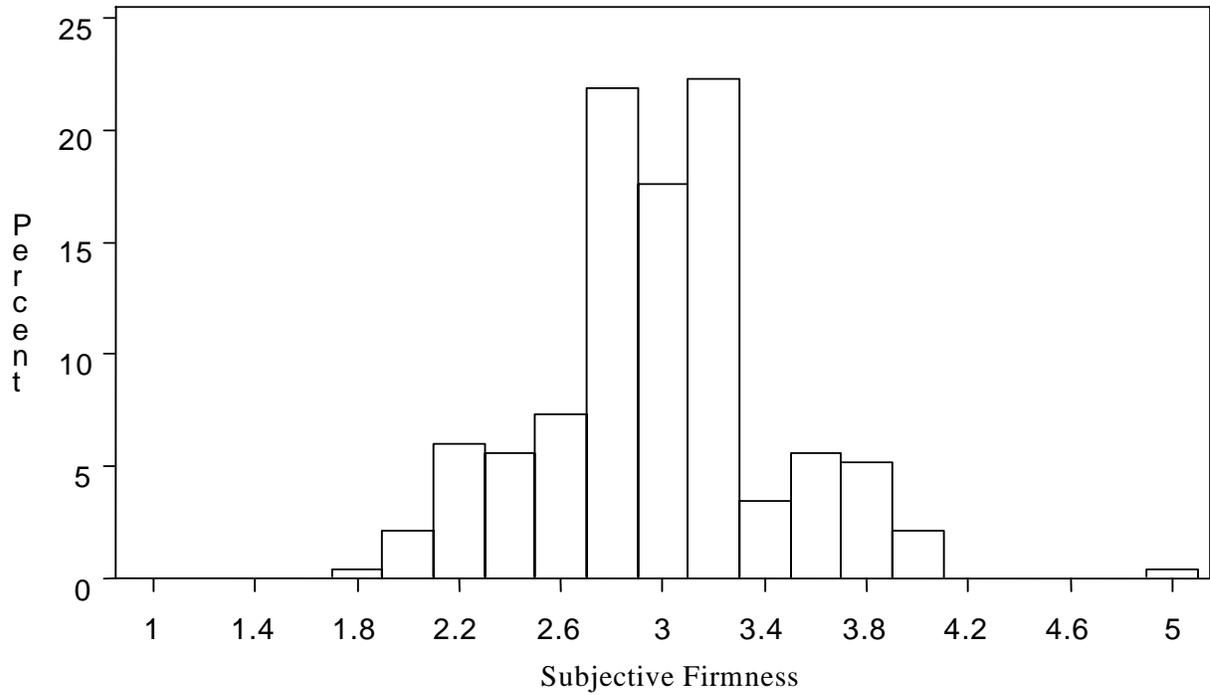


Figure 5. Histogram Displaying Predicted Firmness Scores from Area 1.

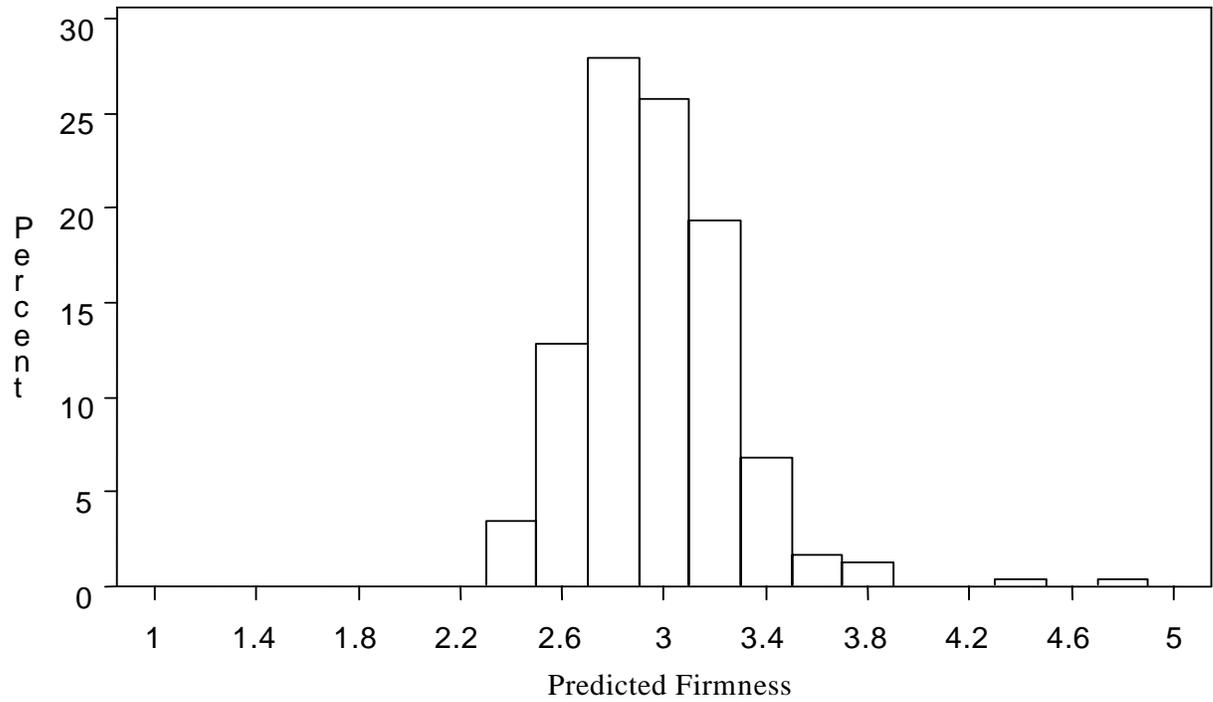


Figure 6. Histogram Displaying Predicted Firmness Scores from Area 4.

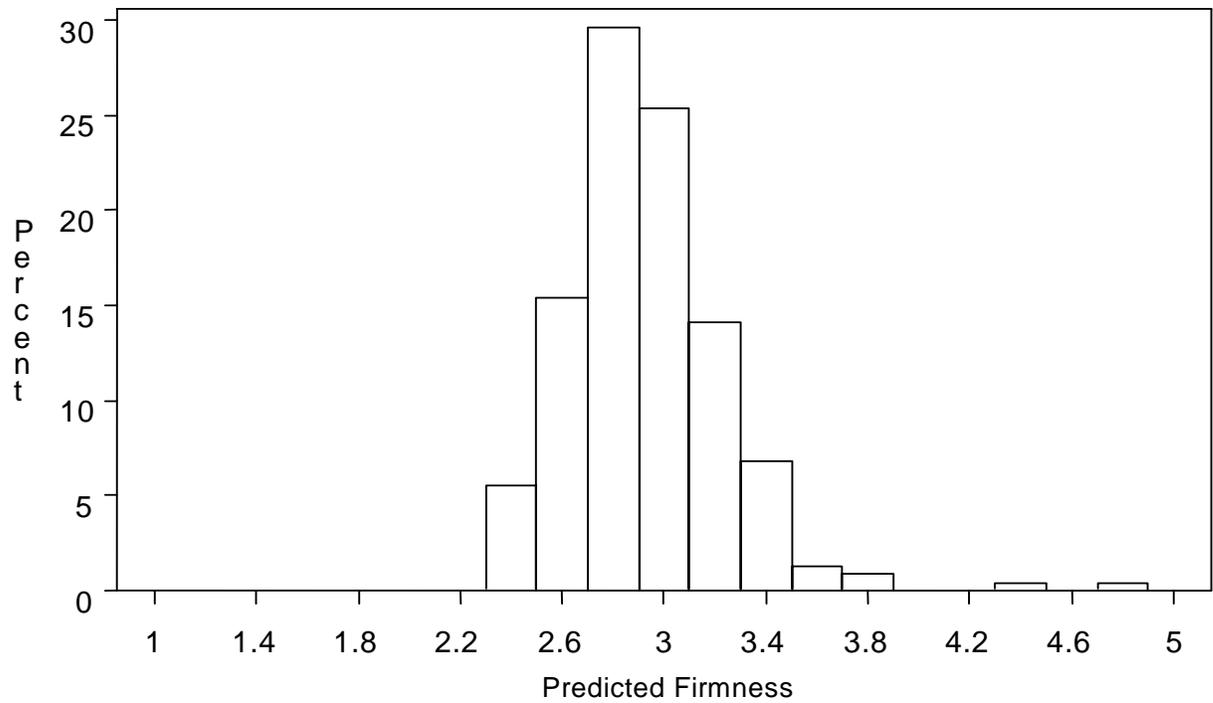
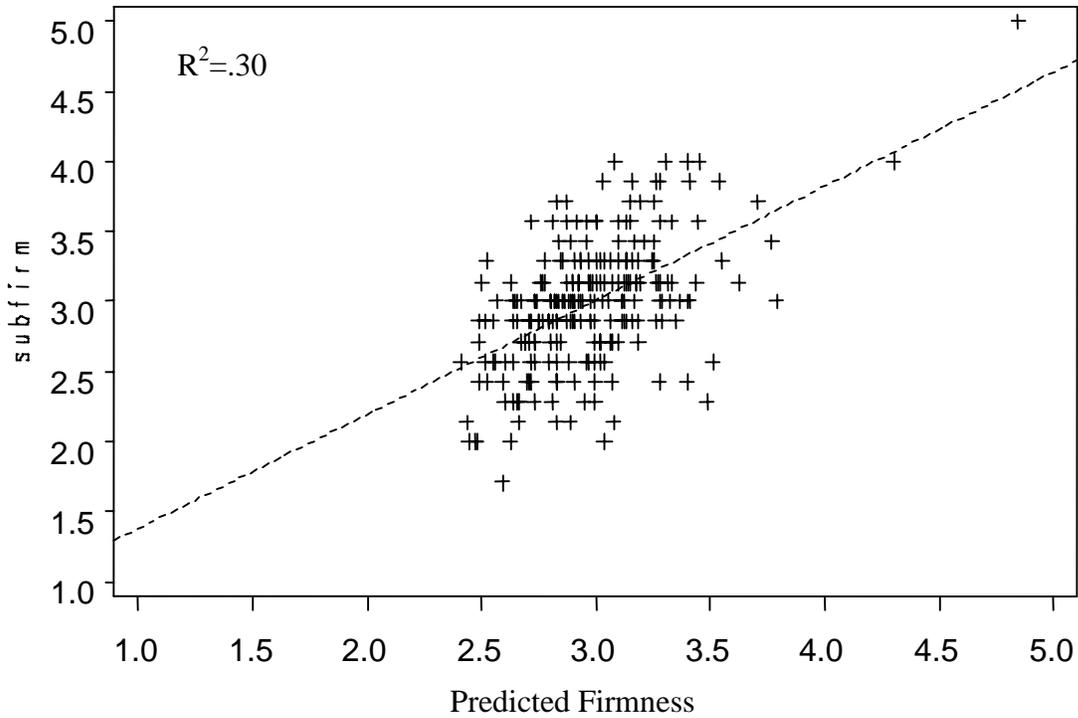
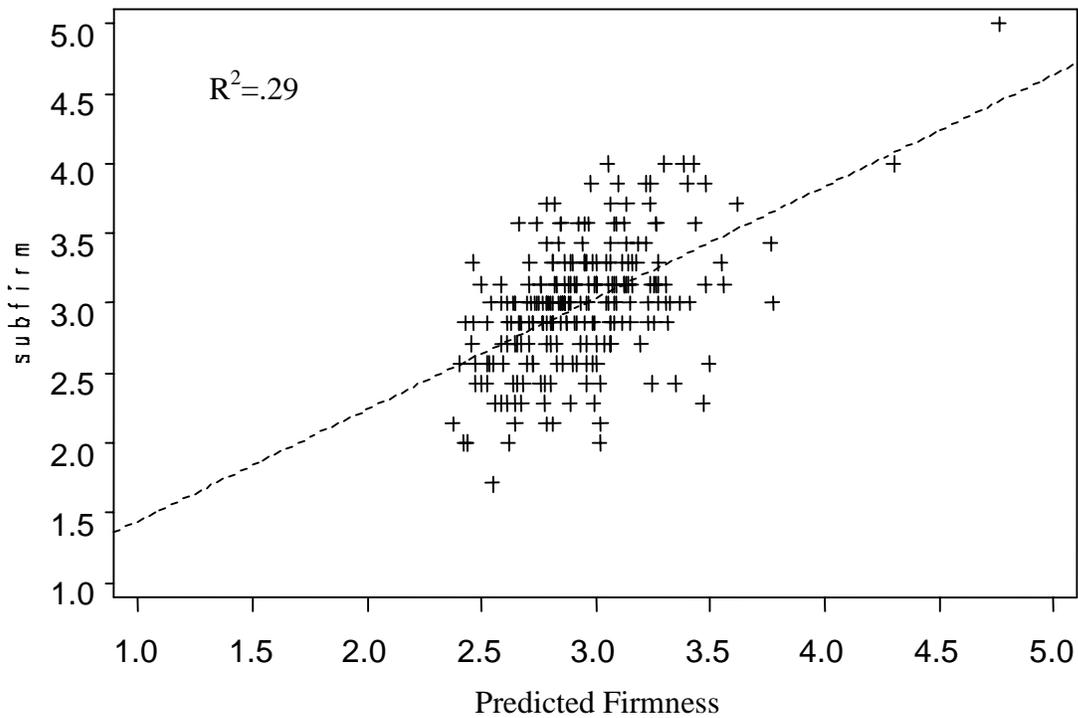


Figure 7. Regression Of Subjective Firmness By Predicted Firmness As Calculated From Initial Product Height Plus Area 1.



Equation: Subjective Firmness = 0.5653 + 0.8138 * Predicted Firmness

Figure 8. Regression Of Subjective Firmness By Predicted Firmness As Calculated From Initial Product Height Plus Area 4.



Equation: Subjective Firmness = 0.645 + 0.7973 * Predicted Firmness

AN INNOVATIVE APPROACH TO PREDICTING MEAT TENDERNESS USING BIOMECHANICAL PROPERTIES OF MEAT

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Key Words: tenderness, beef, compression, sensory, biomechanical

Introduction

Variation in beef tenderness is one of the largest problems facing the meat industry. Current techniques for predicting beef tenderness lack accuracy, are time consuming, destructive and require a cooked sample. The most utilized instrumental method for predicting tenderness is the Warner-Bratzler shear (WBS), but correlations of WBS with trained sensory panel are variable. Development of a non-destructive, objective method with superior accuracy, speed and repeatability would alleviate this problem.

This study was conducted to develop a non-destructive, more rapid and accurate tenderness assessment method using the technique and mathematical models developed by Spadaro (1996) and Spadaro et al. (2000), and verified by Marburger (1999). Initial studies by Spadaro (1996) used samples of raw, bovine *Longissimus dorsi* cubed (2.54 cm) to orient the fibers in a parallel and perpendicular direction to predict muscle tenderness. Cubed samples were compressed for 240 s using a flat platen. Spadaro (1996) and Spadaro et al. (2000) observed that biomechanical properties, stiffness and total energy dissipated, were highly correlated to overall sensory panel tenderness scores ($R^2 = 0.74$ and $R^2 = 0.83$, respectively). Marburger (1999) observed similar results for energy dissipated (parallel to fibers) and initial stiffness (perpendicular to fibers) ($R^2 = 0.73$ and $R^2 = 0.73$, respectively) and concluded these values to be effective predictors of sensory tenderness.

Objectives

The specific objectives of this study were to:

1. Evaluate the influence of muscle fiber orientation and sample temperature for predicting beef tenderness using the compressive cube method of Spadaro (1996).
2. Compare the cube (destructive) and probe compressive (non-destructive) methods at varying temperatures for predicting beef tenderness.

Methodology

Forty beef *Longissimus dorsi* muscles were randomly selected 24 hr post-slaughter from a commercial processing plant. Three steaks, 2.54 cm thick were cut from the anterior end of the loin. One 5.08 cm steak was removed for compressive testing using

the TA.XT2 Texture Analyzer (TA) (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). Steaks were assigned to the following analyses: 1 – chemical analysis; 2 – sensory evaluation; 3 – probe compression TA test; 4, 5 – cubed compression TA test. All analyses were performed on fresh (2 day post-mortem) samples.

Compressive Testing - Texture Analyzer (TA)

USDA Choice and Select quality grade samples were randomly distributed between measurement temperatures of -6.6° (n=14), 4.4° (n=13) and 10°C (n=13). Due to insufficient samples, not all loins were treated to every testing temperature.

Cubed Compressive Testing

A compressive test as described by Spadaro (1996) was performed on 2.54 cm cubed portions of the raw *Longissimus dorsi* samples at either -6.6°, 4.4° or 10°C of the sample temperature. Compressive measurements were performed perpendicular and parallel to the muscle fiber orientation using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) with samples being compressed for 240 s at 3% of sample height. Data were saved in ASCII file format for further analysis using the software package Matlab v4.2c1 (The MathWorks, Inc., Natick, MA). to obtain initial stiffness (ISTF), final stiffness (FSTF) and energy dissipated (ED) for parallel (PLC) and perpendicular (PPC) fiber orientation values for all samples.

Probe Compressive Testing

A modified, non-destructive compressive test was also performed on intact samples equilibrated to either -6.6°, 4.4° or 10°C. A 2 mm diameter probe was compressed 0.635 cm into the sample and held for 0.25 s, with four compressions obtained at different locations on the surface of each sample. Data were saved in ASCII file format for further analysis using the software package Matlab v4.2c1 (The MathWorks, Inc., Natick, MA) to obtain initial stiffness (ISTFPR), final stiffness (FSTFPR) and energy dissipated (EDPR) for each compression reading.

Revised mathematical models were developed from the initial Spadaro (1996) and Spadaro et al. (2000) models to calculate stiffness (initial and final) and energy dissipated values using the Matlab v4.2c1 (The MathWorks, Inc., Natick, MA) software package.

Sensory Evaluation

Companion loin steaks were evaluated by a trained descriptive attribute sensory panel using an 8-point scale for juiciness (8=extremely juicy, 1=extremely dry), muscle fiber tenderness (8=extremely tender, 1=extremely tough), overall tenderness (8=extremely tender, 1=extremely tough) and connective tissue amount (8=none, 1=abundant) as defined by AMSA (1995).

Statistical Analysis

Data were analyzed using the PROC MIXED procedure as described by the Statistical Analysis System (SAS, 1985) to assess if sample temperature during compression had an effect on biomechanical properties. Data were then separated on the basis of compression temperature and regression models to predict overall sensory tenderness from biomechanical measurements were derived for each temperature using the PROC REG procedure. PROC REG was run with a selection option of STEPWISE and RSQUARE to help distinguish which variables contributed to prediction equations with higher R-square values. Once the most favorable prediction equations were selected, the model was run under PROC REG to derive the appropriate β -values.

Results & Discussion

The effect of sample temperature during compression on biomechanical properties was analyzed and no significant differences between temperature treatments were observed. A prediction equation using biomechanical values for energy dissipated perpendicular to fibers of the cubed sample (EDPPC) and final stiffness of probe compression (FSTFPR) had the greatest prediction potential for overall sensory tenderness ($R^2 = 0.3953$). This formula incorporates a destructive and non-destructive measurement. To evaluate the non-destructive method, a formula using the probe compression biomechanical variables of initial stiffness (ISTFPR), final stiffness (FSTFPR) and energy dissipated (EDPR) was evaluated and resulted in a less effective predictor of overall tenderness ($R^2 = 0.1417$) than the equation that utilized a cubed sample and probe compression measurements.

Although sample temperature was not significant, data were still segregated based on sample temperature at compression. TA measurements for each temperature were regressed against overall sensory tenderness scores (Tables 1 and 2).

Cubed Compressive Testing

For TA cubed compressions, samples compressed at higher temperatures produced better prediction equations than those samples compressed at lower temperatures (Table 1). As compression temperature decreased from 10°C to 4.4° to -6.6°C, so did predictability. At -2.2°C muscle freezes, steaks held at -6.6°C would have had a proportion of water in the frozen state that would have affected compression values. Interestingly, the prediction equations at 4.4°C and 10°C both used a parallel fiber and perpendicular fiber orientation variable. The 4.4°C prediction equation utilized EDPLC and EDPPC while the 10°C prediction equation used the relationship between FSTFPLC and EDPPC.

Probe Compressive Testing

Probe compression data was segregated based on sample temperature at compression and regression models predicting overall sensory tenderness were derived for each temperature. R-square values (Table 2) for equations using ISTFPR, FSTFPR and EDPR

were higher for samples compressed at 4.4° and 10°C than equations using the same TA variables and compressing at -6.6°C. These results indicate that compression at refrigerated temperatures (4.4° and 10°C) proved to produce better predictability of overall tenderness than lower temperatures (-6.6°C). This may be the result of ice crystal formation affecting compression values. Reducing the number of variables in the prediction equation was evaluated. An equation using ISTFPR and FSTFPR at 10°C proved to be an acceptable predictor of tenderness, but an equation incorporating the same variables for 4.4°C lacked the same predictability (Table 2). It was expected that prediction equations with identical variables would have similar intercept values, which was not observed in this study (Table 2). The intercepts and the coefficients for each variable were different at each testing temperature. Perhaps effective utilization of probe compressive testing on a large-scale production would have to include prediction equations over a common range of temperatures expected during the chilling process post-mortem.

Conclusions

The overall goal of this study was to develop a more rapid, accurate and non-destructive objective instrumental method for measuring the tenderness of raw beef loins using the compressive technique developed by Spadaro (1996) and Spadaro et al. (2000) and to verify this new technique. Spadaro's (1996) technique was adapted to produce a non-destructive objective tenderness method. The most effective predictive models for tenderness were derived when steaks were assessed at 4.4°C and 10°C, rather than -6.6°C, using either the destructive compression test or the non-destructive probe method. This study verified the use of biomechanical probe measurements to more rapidly and effectively predict overall sensory tenderness of raw steaks without sample destruction. This innovative technique could guarantee tenderness to consumers, be integrated into on-line grading systems and be utilized as a powerful research tool.

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Tables and Figures

TABLE 1. Prediction equations for overall sensory tenderness utilizing TA cubed sample compression values¹ at various compression temperatures.

Equation	β value	R^2	MSE
<u>-6.6°C</u>			
Intercept	9.15711	0.4769	0.66982
FSTFPPC	-0.00027935		
<u>4.4°C</u>			
Intercept	7.45088	0.5361	0.35166
EDPLC	-0.66424		
EDPPC	0.37355		
<u>10°C</u>			
Intercept	4.88366	0.7075	0.40263
FSTFPLC	-0.00021298		
EDPPC	0.91179		

¹ FSTFPLC = final stiffness of parallel fiber orientation in cubed samples; FSTFPPC = final stiffness of perpendicular fiber orientation in cubed samples; EDPLC = energy dissipated of parallel fiber orientation of cubed samples; EDPPC = energy dissipated of perpendicular fiber orientation of cubed samples; R^2 = R-square; MSE = Mean Square Error.

TABLE 2. Prediction equations for overall sensory tenderness utilizing TA probe compression values¹ at various compression temperatures.

Equation	β value	R ²	MSE
<u>-6.6°C</u>			
Intercept	-1.35095	0.2936	1.08535
ISTFPR	0.02317		
FSTFPR	-0.01307		
EDPR	634.61514		
<u>4.4°C</u>			
Intercept	15.60995	0.7073	0.22409
ISTFPR	0.14377		
FSTFPR	-0.20226		
EDPR	-427.06157		
Intercept	8.22752	0.4710	0.36447
ISTFPR	0.11554		
FSTFPR	-0.14687		
<u>10°C</u>			
Intercept	8.11712	0.7019	0.47954
ISTFPR	0.25924		
FSTFPR	-0.31144		
EDPR	-89.94746		
Intercept	6.65498	0.6912	0.44699
ISTFPR	0.26102		
FSTFPR	-0.30943		

¹ Variable abbreviation explanation: ISTFPR = initial stiffness for intact samples compressed with probe; FSTFPR = final stiffness for intact samples compressed with probe; EDPR = energy dissipated for intact samples compressed with probe; R² = R-square; MSE = Mean Square Error.

USING SERUM CHEMISTRY PROFILES TO PREDICT BEEF TENDERNESS FOR THE PURPOSE OF ON-LINE INSTRUMENT GRADING

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Key Words: Beef, Tenderness, Instrumentation, Grading, Serum

Introduction

United States Department of Agriculture (USDA) quality grades are designed to sort and assign beef carcasses into grades of expected eating quality, using evaluations of carcass maturity and marbling. The vast majority (97%) of carcasses within the fed steer and heifer population are classified into the "A-maturity" group (McKenna et al., 2002). Furthermore, McKenna et al., (2002) reported that 77% of all carcasses from the fed steer and heifer population had marbling scores of "small" or "slight". With the majority of the fed steer and heifer slaughter in a narrow maturity range and in a narrow marbling range, the USDA quality grades do not effectively segregate these carcasses into uniform palatability groups. Recent research by Shanks (2002) examined 24 constituents (minerals, enzymes, and hormones) in beef blood at the time of exsanguination and found that meat tenderness could be predicted using a combination of several of these blood components. However, the Shanks (2002) study utilized only 20 animals. To validate this technique, it needed to be tested on a large number of randomly-selected animals, similar to that which would be found under normal grading conditions in a typical beef packing plant.

Objective

To determine if serum chemistry profiles are useful for predicting beef tenderness for the purpose of on-line instrument grading.

Methodology

Data were obtained from 286 head of cattle on five different dates from cattle harvested at Cargill Meat Solutions facilities. Three collections occurred at the Schuyler, NE plant and two collections occurred at the Plainview, TX plant. Cattle were selected randomly at 5-min intervals on the production line.

Serum Analysis

Blood samples were collected immediately following exsanguination, chilled in an ice bath for 2-4 hours, and centrifuged (3400 rpm) for 15 min to separate serum. Serum

was then immediately transported to the South Dakota State University Animal Disease Research and Diagnostic Laboratory and analyzed using a COBAS MIRA (F. Hoffmann-La Roche Ltd., Basel, Switzerland) spectrophotometer chemistry analyzer for nineteen compounds (Table 1). Frozen serum samples were shipped on dry ice to LINCO Research, Inc. (St. Charles, MO) to be assayed for glucagon and cortisol. Cortisol was measured using a double antibody cortisol 125I-radioimmunoassay, and glucagon was measured utilizing a 125I-labeled glucagon and a glucagon antiserum to determine the level of glucagon by the double antibody/PEG technique.

Carcass Data

After a 24 hour chill, experienced evaluators determined USDA yield and quality grades. After a 90 min bloom time, muscle color was measured on the exposed longissimus at the 12th/13th rib interface using a HunterLabs MiniScan XE colorimeter (Hunter Associates Laboratory Inc., Reston, VA) with a D65 illuminant.

Slice Shear Force Determination

One 2.5-cm-thick steak was removed from the 13th rib location from each side of each carcass. Steaks were vacuum packaged individually and shipped to Cargill Meat Solutions Laboratory (Wichita, KS) where one steak from each carcass was aged for 2 days and frozen and the other steak for 14 days and frozen. Steaks were cooked on an impingement oven to an internal temperature of 70C. A 5.1-cm x 2.5-cm slice was removed from each steak and slice shear force was determined using a slice shear force apparatus.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS to calculate least squares means for data from tough versus tender carcasses. A threshold value of 20 kg for 14-d slice shear force (SSF14) was used to determine “tender” versus “tough”. This 20-kg threshold was lower than the 27-kg threshold reported by Wheeler et al. (2004), but was chosen because there were very few carcasses ($n = 9 / 286 = 3.1\%$) in our data set with SSF14 greater than 27 kg. Because this research was exploratory in nature, and not intended to finalize prediction equations for use in the field, a threshold which resulted in a substantial number of “tough” carcasses was deemed critical for a valid statistical analysis. Statistical models to classify carcasses as “tender” or “tough” were developed and tested using logistic regression (Khattree and Naik, 2000). The LOGISTIC procedure of SAS was used to test various models using the SELECTION=SCORE option. Using a response variable equal to 0 for a “tender” carcass and equal to 1 for a “tough” carcass, carcasses were classified as “tough” if the estimated probability of classifying an observation as “tough” was greater than 0.112 (32/286 because we had 32 “tough” carcasses out of 286 total carcasses; Seber, 1984).

Results & Discussion

Tender carcasses (SSF14 < 20kg) had a higher ($P < 0.05$) proportion of steer carcasses, higher ($P < 0.05$) hot carcass weights, USDA Quality Grades, and a^* values and lower ($P < 0.05$) dark cutting discount (DARK), pH and 2-d slice shear force (SSF2) values than tough carcasses (Table 2). Tender carcasses also had lower aspartate aminotransferase (AAT), calcium, non-esterified fatty acids (NEFA), phosphorus, potassium (K), and sodium (NA) levels and higher ($P < 0.05$) cortisol (CORT) levels than tough carcasses (Table 3).

Logistic regression models were developed to sort carcasses into either a “certified tender” or “tough” groups with a threshold value of 20 kg for SSF14 (Table 4). No sorting resulted in a 100% certification of carcasses with an 11.2% occurrence of tough steaks in “Certified Tender” carcasses. The first type of model used USDA Quality Grade as the sole variable, and resulted in 61.5% certification of carcasses with an 8.0% occurrence of tough steaks in “Certified Tender” carcasses. The second type of model used USDA Quality Grade factors as variables, with the model that resulted in the lowest percentage tough in “Certified Tender” carcasses utilizing DARK, lean maturity score (LMAT), and marbling score, which resulted in 68.2% certification of carcasses with a 7.7% occurrence of tough steaks in “Certified Tender” carcasses. The third type of model used all carcass traits as variables with the model resulting in the lowest percentage of tough steaks in “Certified Tender” carcasses utilizing a^* , DARK, and LMAT, which resulted in 69.0% certification of carcasses with a 5.1% occurrence of tough steaks in “Certified Tender” carcasses.

The fourth type of model used serum constituent values for variables. In our opinion, the model that best combined the lowest error in “Certified Tender” and the most ease of application was the model that utilized CORT, NEFA, and K, which resulted in 65.4% certification of carcasses with an occurrence of tough steaks in 5.3% of “Certified Tender” carcasses. The fifth type of model made use of carcass traits and serum constituent values for variables. In our opinion, the model that best combined the lowest error in “Certified Tender” and the most ease of application was the model that utilized a^* , DARK and LMAT, which resulted in 69.0% certification of carcasses with a 5.1% occurrence of tough steaks in “Certified Tender” carcasses. The seventh type of model employed carcass traits, serum constituent values and SSF2 for variables and as expected had the lowest occurrence of tough steaks in “Certified Tender” carcasses and the highest certification rates.

Predicting tenderness using blood chemistry was more accurate than predicting tenderness with USDA quality grades but not as accurate as SSF2. Predicting tenderness using blood chemistry was similar in accuracy to tenderness prediction using measures of muscle color.

Shanks (2002) showed that bovine serum profiles can be used as a method to predict tenderness and had a 5% error rate for certified tender beef in a study that only used 20 head. The present study’s model for serum constitutes presented a 5.3% error rate for “certified tender” beef with a certification rate of 65.4% as compared to the error rate of 8.0% and certification rate of 61.5% for the model that used USDA Quality Grades. The 5.3% error rate for the serum constitutes model compares favorably against the error rates of 6.0 and 4.9% (slice shear force), 7.0 and 6.5% (colorimeter), and 12.1 and 10.3%

(BeefCam) at 70% and 60% certification level, respectively, found by Wheeler et al., (2002).

Conclusions

Predicting tenderness using blood chemistry was more accurate than predicting tenderness with USDA quality grades but not as accurate as 2-day slice shear force. Predicting tenderness using blood chemistry was similar in accuracy to tenderness prediction using measures of muscle color. Muscle color measurement could probably be applied at a much lower cost than blood chemistry, while blood chemistry has the advantage of application on live animals.

Acknowledgements

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Tables and Figures

Table 1. Abbreviations for serum constituents and carcass traits

Albumin	ALB
Alkaline Phosphatase	AP
Amylase	AMY
Aspartate Aminotransferase	AAT
Beta-hydroxy Butyric Acid	BHBA
Calcium	CA
Chloride	CL
Cortisol	CORT
Creatinine	CREA
Creatine Phosphokinase	CPK
Gamma-Glutamyltransferase	GGLT
Globulin	GLOB
Glucose	GLCS
Glucagon	GLGN
Magnesium	MG
Non-Esterified Fatty Acids	NEFA
Phosphorus	P
Potassium	K
Sodium	NA
Total Bilirubin	BILI
Total Protein	PROT
Adjusted Fat Thickness	FAT
Hot Carcass Weight	HCW
Ribeye Area	REA
Kidney, Pelvic and Heart Fat	KPH
Marbling Score	MARB
Skeletal Maturity Score	SMAT
Lean Maturity Score	LMAT
Overall Maturity Score	OMAT
Dark Cutting Discount	DARK
L*	L
a*	A
b*	B
Gender	SEX
pH	PH
2-d Slice Shear Force	SSF2
14-d Slice Shear Force	SSF14

Table 2. Least squares means of carcass traits for Tender (SSF14 < 20kg) and Tough groups

Trait	Tender (n=254)	Tough (n=32)	P-value
SEX ^{a}	0.29 ± 0.03	0.50 ± 0.08	0.0165
FAT, cm	1.30 ± 0.03	1.27 ± 0.12	0.7374
REA, cm ^{2}	89.93 ± 0.77	91.10 ± 2.13	0.6142
HCW, kg	365.19 ± 2.64	344.24 ± 7.43	0.0083
KPH, %	2.39 ± 0.03	2.36 ± 0.09	0.7283
USDA Yield Grade	2.87 ± 0.07	2.59 ± 0.18	0.1559
MARB ^{b}	409.49 ± 4.81	381.56 ± 13.56	0.0533
SMAT ^{c}	152.81 ± 1.14	154.38 ± 3.22	0.6607
LMAT ^{c}	153.58 ± 0.89	158.75 ± 2.52	0.0540
OMAT ^{c}	153.27 ± 0.93	155.31 ± 2.62	0.4626
USDA Quality Grade ^{d}	686.02 ± 2.94	660.75 ± 8.28	0.0043
DARK, %	0.00 ± 0.01	0.13 ± 0.02	<0.0001
L	39.70 ± 0.20	38.82 ± 0.55	0.1369
A	24.18 ± 0.13	21.88 ± 0.36	<0.0001
B	20.53 ± 0.18	18.62 ± 0.50	0.0004
PH	5.40 ± 0.01	5.55 ± 0.02	<0.0001
2-d Cooking Shrink, %	16.18 ± 0.21	17.63 ± 0.74	0.0607
SSF2	21.14 ± 0.49	30.62 ± 1.70	<0.0001
14-d Cooking Shrink, %	15.50 ± 0.14	15.69 ± 0.39	0.6495
SSF14	14.25 ± 0.19	25.28 ± 0.54	<0.0001

^{a}Gender; 0 = Steer, 1 = Heifer

^{b}300 = Slight ^{00}, 400 = Small ^{00}, etc.

^{c}100 = A ^{00}, 200 = B ^{00}, etc.

^{d}600 = Select ^{00}, 700 = Choice ^{00}, etc.

Table 3. Least squares means of carcasses traits for Tender (SSF14 < 20kg) and Tough groups

Trait	Tender (n=254)	Tough (n=32)	P-value
ALB, g/dL	4.53 ± 0.04	4.64 ± 0.11	0.3281
AP, U/L	144.74 ± 3.42	148.53 ± 9.64	0.7112
AMY, U/L	18.95 ± 0.44	19.59 ± 1.23	0.6239
AAT, U/L	84.24 ± 1.58	94.97 ± 4.43	0.0232
BHBA, mg/dL	0.26 ± 0.01	0.28 ± 0.04	0.6274
CA, mg/dL	9.51 ± 0.09	10.09 ± 0.26	0.0390
CL, mmol/L	103.56 ± 0.49	106.26 ± 1.41	0.0723
CORT, µg/dL	6.43 ± 0.13	5.26 ± 0.36	0.0023
CPK, U/L	593.53 ± 33.01	611.28 ± 92.82	0.8571
CREA, mg/dL	1.78 ± 0.03	1.96 ± 0.10	0.0764
GGLT, u/L	33.01 ± 0.92	37.44 ± 2.59	0.1086
GLOB, g/dL	4.24 ± 0.07	4.66 ± 0.21	0.0627
GLGN, pg/mL	683.22 ± 44.65	761.06 ± 125.80	0.5603
GLCS, mg/dL	189.23 ± 5.56	214.56 ± 15.66	0.1285
MG, mEq/L	2.10 ± 0.02	2.20 ± 0.06	0.1447
NEFA, mEq/L	0.20 ± 0.01	0.25 ± 0.02	0.0077
P, mg/dL	7.35 ± 0.10	8.02 ± 0.27	0.0216
K, mmol/L	7.55 ± 0.07	8.11 ± 0.20	0.0097
NA, mmol/L	149.68 ± 0.54	153.33 ± 1.52	0.0249
BILI, mg/dL	0.45 ± 0.01	0.46 ± 0.03	0.7168
PROT, g/dL	8.81 ± 0.10	9.30 ± 0.27	0.0917

Table 4. Certification rate and percentage tough in certified and not-certified groups for various types of logistic regression models

Obs	#	Model Variables	Certification Rate	Percentage Tough	
				Of those Certified	Of those Not-Certified
<u>No Sorting</u>					
286	0	UNSORTED	100.0	11.2	NA
<u>USDA Quality Grade</u>					
286	1	USDA Quality Grade	61.5	8.0	16.4
<u>USDA Quality Grade Factors</u>					
286	1	DARK	99.0	10.2	100.0
286	2	DARK, LMAT	83.6	9.2	21.3
286	3	DARK, LMAT, MARB	68.2	7.7	18.7
286	4	DARK, LMAT, MARB, SMAT	67.5	7.8	18.3
<u>Carcass Traits</u>					
284	1	A	66.5	5.8	22.1
284	2	A, DARK	69.0	7.7	19.3
284	3	A, DARK, LMAT	69.0	5.1	25.0
284	4	A, DARK, LMAT, HCW	70.1	6.5	22.4
284	5	A, DARK, LMAT, HCW, MARB	73.6	5.7	26.7
284	6	A, DARK, LMAT, MARB, REA, SEX	74.3	6.2	26.0
284	7	A, DARK, LMAT, MARB, REA, SEX, B	72.5	6.8	23.1
<u>Serum Constituents</u>					
286	1	CORT	59.4	6.5	18.1
286	2	CORT, NEFA	61.2	7.4	17.1
286	3	CORT, NEFA, K	65.4	5.3	22.2
285	4	CORT, NEFA, K, AAT	67.0	4.7	24.5
283	5	CORT, NEFA, K, AAT, CL	67.1	4.7	23.7
283	6	CORT, NEFA, K, AAT, CL, ALB	67.8	5.7	22.0
283	7	CORT, NEFA, K, AAT, CL, ALB, BHBA	68.2	5.7	22.2
283	8	CORT, NEFA, K, AAT, CL, ALB, BHBA, P	68.2	5.7	22.2
<u>Carcass Traits and Serum Constituents</u>					
284	1	A	66.5	5.8	22.1
284	2	A, DARK	69.0	7.7	19.3
284	3	A, DARK, LMAT	69.0	5.1	25.0
284	4	A, DARK, CORT, K	72.9	7.2	22.1
284	5	A, DARK, LMAT, CORT, K	71.8	5.9	25.0
284	6	A, DARK, LMAT, CORT, K, NEFA	71.8	5.9	25.0
281	7	A, DARK, LMAT, CORT, NEFA, CL, BILI	72.2	5.9	24.4
281	8	A, DARK, LMAT, CORT, NEFA, CL, BILI, K	73.0	3.9	30.3
<u>Carcass Traits, Serum Constituents and SSF2 Values</u>					
232	1	SSF2	90.5	5.2	31.8
232	2	SSF2, DARK	90.5	4.8	36.4
230	3	SSF2, DARK, CL	86.5	4.0	29.0
230	4	SSF2, DARK, CL, CORT	87.0	4.5	26.7
228	5	SSF2, DARK, CL, CORT, A	84.6	3.6	28.6
227	6	SSF2, DARK, CL, CORT, A, BILI	85.0	3.6	29.4
227	7	SSF2, DARK, CL, CORT, A, BILI, ALB	85.0	3.1	32.4
227	8	SSF2, DARK, CL, CORT, A, BILI, ALB, SMAT	85.5	3.1	33.3

PREDICTION OF LAMB TENDERNESS USING COMBINED QUALITY PARAMETERS AND MEAT SURFACE CHARACTERISTICS

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Key Words: tenderness prediction, Image analysis, texture features, Co-occurrence matrix, Run length matrix, Grey level difference histogram, Artificial neural networks

Introduction

The production of tender red meat that satisfies the consumer needs is an important factor to ensure that consumers become repeat buyers which will contribute to a viable financial future for the meat industry. Several objective and subjective methods are currently in use to determine meat tenderness which are destructive, time consuming, costly or do not meet the industry needs in terms of early post mortem measurements and speed of evaluation.

Computer vision, as a robust and consistent system, has enormous potential for evaluating meat quality. The prospective of computer vision to meet the industry requirements is supported by significant technological developments in information technology and image analysis techniques. The use of texture models to characterize randomness within an image is an established technique in the field of computer vision. A variety of techniques for analysing image texture have been proposed and they are generally classified into three categories. They are statistical, structural and spectral (Gonzalez and Woods, 2002). Common statistical approaches include the gray level co-occurrence matrix (GLCM) (Haralick et al., 1973), the gray level difference method (GLDM) (Weszka, Dyer & Rosenfeld, 1976) and the gray level run length matrix (GLRM) (Galloway, 1975).

Computer vision technology utilizing meat texture features has been successful in classifying beef into tender and tough classes with a 83% success rate (Li et al., 2001). Attempts to predict meat tenderness using computer vision has resulted in coefficient of determination (R^2) of 0.70 and 0.746 for beef (Li et al., 1999) and lamb (Chandraratne et al., 2003), respectively. In these studies, additional meat quality parameters (e.g. marbling and colour) were used which improved the prediction level.

Meat pH is a key factor that regulates many components of meat quality. Minor variations in ultimate pH have been associated with large differences in meat characteristics (Pösö and Puolanne, 2005). Indeed, pH may be a good predictor of meat tenderness. Thus, in this study, we also investigated the inclusion of ultimate pH as a predictor of tenderness in lamb. In addition to the GLCM we reported earlier

(Chandraratne et al., 2003), there are other texture measurements techniques worth investigating (e.g. GLDM and GLRM).

Objectives

The present study investigates a) the effectiveness of GLRM and GLDM texture parameters in predicting the tenderness of lamb meat; and b) the combined effects of texture features, marbling and ultimate pH of fresh meat on the prediction of the tenderness in cooked lamb meat.

Methodology

Samples: Lamb mid loin chops were taken from 160 lamb carcasses. The carcasses (17.37 ± 1.48 kg) were chilled at 10°C for 2 hours and then removed to 2°C till sampling. The ultimate pH of the carcasses (range 5.54-6.49; 12 carcasses had a pH > 5.80) was measured at 24 hrs post-mortem, and then two sets of mid loin chops were removed from both sides of the carcass at 13th rib. One set of samples was used for 24 hrs analysis (imaging and tenderness evaluation) and the other set of samples was aged at 1^o C for three weeks post-mortem and then used for tenderness evaluation.

Imaging system, image capture and analysis: The imaging system and image capture were as reported earlier (Chandraratne et al., 2002). Image processing and analysis was accomplished using Image-Pro Plus (Media Cybernetics, USA). A total of 12 image geometric variables were measured (Chandraratne et al., 2003).

Textural Properties: The GLCM, the GLDM and the GLRM were used to extract texture features. The number of texture parameters calculated from GLCM was 90 (Chandraratne et al., 2003, Haralick et al., 1973; Unser, 1986).

We generated five scalar measurements (contrast, angular second moment, entropy, mean and inverse difference moment) from gray-level difference histogram as described by Connors and Harlow (1980). In addition, we defined four scalar measurements; variance, product moment, cluster shade and cluster prominence analogous to the GLCM features. These nine features were calculated from gray-level difference histograms in 0^o, 45^o, 90^o and 135^o directions. There were a total of 36 gray-level difference histograms features.

To obtain numerical texture measures from run length matrix, we calculated five functions; short runs emphasis, long runs emphasis, gray level non-uniformity, run length non-uniformity and run percentage (Galloway, 1975). These five features were calculated from run length matrices in 0^o and 90^o directions. There were a total of 10 run length texture variables.

Shear force measurement: Shear force of cooked lamb to internal temperature of 75°C was measured using MIRINZ tenderometer as described previously (Bickerstaffe et al., 2001; Chandraratne et al., 2003).

Data Analysis: Statistical analysis was performed with Minitab (release 14.1, Minitab Inc.) and SPSS (release 10.0.5, SPSS Inc.). Neural network analysis was performed with NeuroShell 2 (Ward Systems Group Inc., Frederick, MD).

Results & Discussion

The GLCM, one of the first methods used in the texture analysis, is a second-order statistical approach. The GLCM is based on the estimation of second-order joint conditional probability density functions. The GLDM calculates first-order statistics. In the GLDM, we compute the occurrence of absolute difference between pairs of gray levels separated by a specified distance in a specified direction. It, thus, generates a set of single variable probability distributions. In addition to the GLCM and GLDM, another popular method to extract texture descriptors is the GLRM. The GLRM, a higher-order statistical approach, estimates the length of gray level runs. A gray level run is a connected set of pixels in a specific direction having the same gray values. The length of the run is the number of pixel points within the run. Gray level runs can be used to characterize the spatial variation of pixel values in an image by: gray level of the run, the length of the run and the direction of the run.

For each image, 148 features (12 geometric, 90 co-occurrence texture, 36 gray-level difference histogram, and 10 run length texture) were calculated. The standardized data (mean of zero and variance of one) were then reduced using Principal Component Analysis (PCA). According to the results of PCA, the 12 geometric variables were condensed into six variables whilst retaining 96% of the total variance. In a similar way, 90 variables calculated from co-occurrence matrix were condensed to eight whilst retaining 99% of the total variance (Chandraratne et al., 2003).

The 36 GLDM features were condensed to four features; mean (from 450 direction matrix), IDM (from 00 direction matrix), variance (from 00 direction matrix) and product moment (from 1350 direction matrix) retaining 87 % of total variance. The 10 run length texture features were condensed to four features; short runs emphasis, long runs emphasis, gray level non-uniformity and run length non-uniformity (all from 00 direction matrix) retaining 98 % of total variance.

Both non-linear regression and neural network analyses were performed to predict lamb tenderness. Neural networks (NNs) are information processing systems which implement simplified models of their biological counterparts, biological neural networks. Neural network contains many simple processing elements (neurons) and are capable of learning from the environment in which they operate and adapting their responses according to the feedback that they receive.

In a previous study, we used multi-layer perceptron (MLP) neural network with back propagation algorithm to train the network (Chandraratne et al., 2003). But in this study, we used Group Method of Data Handling (GMDH) network or polynomial network of Neuroshell 2. The GMDH network builds successive layers with complex connections that are the individual terms of the polynomial (Pham and Liu, 1995). The first layer is created by computing regressions of the input variables and then choosing the best values for the algorithm. The second layer is created by computing regressions of the values in the first layer along with the input variables and then choosing the best values. This process of building layers continues until the best possible result is achieved. The resulting network is a complex polynomial.

In the regression analysis, the 22 features (six geometric, eight GLCM, four GLDM and four GLRM) selected from the original set of 148 features and the ultimate pH (24 hrs pH) were used as predictors. Table 1 shows the results of non-linear regression analysis. The R² of 0.44 was achieved with 14 variables (6 geometric and 8 GLCM). The

addition of the ultimate pH improved the R² value to 0.50. The addition of GLDM and GLRM texture features improved the R² to 0.55 and 0.48, respectively. The addition of both GLDM and GLRM texture features improved the R² to 0.60. The highest R² of 0.65 using non-linear regression was achieved with 6 geometric, 8 GLCM, 4 GLDM, 4 GLRM and pH.

In the neural network analysis, the 22 features (6 geometric, 8 GLCM, 4 GLDM and 4 GLRM) selected from the original set of 148 features and the ultimate pH (24 hrs pH) were used as input variables. Table 2 shows the results of neural network analysis. The R² of 0.74 was achieved with 14 variables (6 geometric and 8 GLCM). The addition of the ultimate pH resulted in R² value of 0.75, indicating that any effects for the pH on tenderness were already taken into account by GLCM texture features. The addition of GLDM and GLRM texture features improved the R² to 0.79 and 0.86, respectively. The addition of both GLDM and GLRM texture features improved the R² to 0.87. The highest R² of 0.91 using neural network was achieved with 6 geometric, 8 GLCM, 4 GLDM, 4 GLRM and pH.

Ohanian and Dubas (1992) showed improved performance by combining GLCM features with other texture features. Their study revealed the GLCM features' inability to capture all texture information and the fact that it only captures second-order texture information. This may explain the higher predictability in the present study compared with the study of Chandraratne et al. (2003).

The general principle of a GMDH network is to fit a polynomial to a non-linear mapping. By splitting the experimental data into training and selection sets, over fitting can be avoided. The growth and selection strategies of the GMDH algorithm produce a network with a flexible structure. This flexible structure always grows in the direction of decreasing errors.

Conclusions

The PCA was used to reduce the original set of 148 geometric and texture features to 22 geometric and texture features. The prediction showed encouraging results indicating that the raw meat surface has some information regarding the cooked meat tenderness. The greatest predictability ($R^2 = 0.91$) was achieved using six geometric, eight co-occurrence, four GLDM, four run length features and the ultimate pH. The cumulative effect of different texture analysis techniques together with pH proved to be effective in predicting cooked lamb tenderness.

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Tables and Figures

Table 1. Results of regression models

	Variables	Coefficient of determination (R^2)
1	6 geometric + 8 GLCM	0.44
2	6 geometric + 8 GLCM+ pH	0.50
3	6 geometric + 8 GLCM + 4 GLDM	0.55
4	6 geometric + 8 GLCM + 4 GLRM	0.48
5	6 geometric + 8 GLCM + 4 GLDM + 4 GLRM	0.60
6	6 geometric + 8 GLCM + 4 GLDM + 4 GLRM +pH	0.65

Table 2. Results of neural network models

	Variables	Coefficient of determination (R^2)
1	6 geometric + 8 GLCM	0.74
2	6 geometric + 8 GLCM+ pH	0.75
3	6 geometric + 8 GLCM + 4 GLDM	0.79
4	6 geometric + 8 GLCM + 4 GLRM	0.86
5	6 geometric + 8 GLCM + 4 GLDM + 4 GLRM	0.87
6	6 geometric + 8 GLCM + 4 GLDM + 4 GLRM +pH	0.91

MESOSTRUCTURE ASSESSED BY ALTERNATING CURRENT SPECTROSCOPY DURING MEAT AGEING

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Key Words: beef ageing; electrical conduction; biomathematics; inverse modeling; dielectrical properties; membrane.

Introduction

Since different types of tissues exhibit different conductivity parameters, numerous studies have been carried out for many years to characterize biological tissues by means of their electrical properties. Impedance measurements observed with increasing frequencies are mainly attributed to changes in membrane conductivity and ion and charged molecules mobility (mainly ions Na^+ , K^+ , Cl^-). Equivalent circuits with passive electrical components are frequently used as a support model for presentation and analyses of the behavior of tissue submitted to electrical fields. An attempt to describe electrical model is the Fricke's model. In this model the elements are resistive and capacitive. It is composed of a resistive element (R_p), corresponding to the extracellular electrolyte, placed in parallel with a capacitive element (C_s), corresponding to insulating membranes in series, and a resistive element (R_s) corresponding to intracellular electrolytes (Fig. 1). Impedance measurements can be explained using this model: most of the current flows around the cell at lower frequencies without being able to penetrate into the cell, at higher frequencies the membrane is no longer an impediment and the

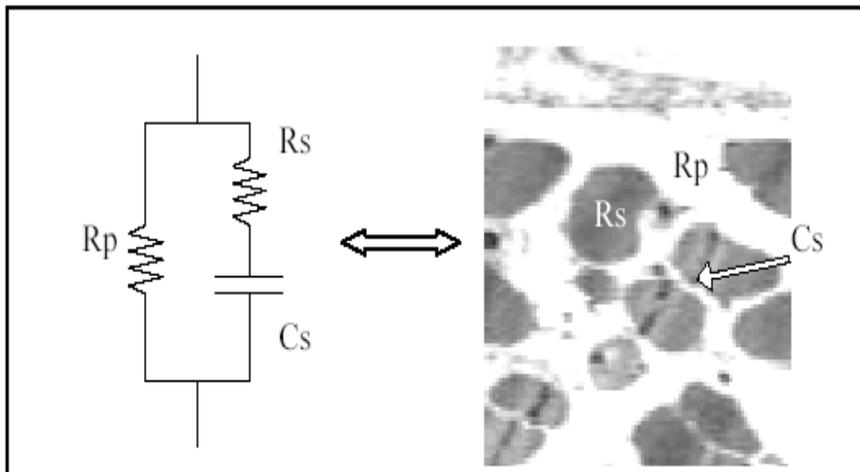


FIGURE 1 Intra- and extracellular conduction, R_s , R_p and membrane capacitance C_s with the equivalent electrical Fricke's model.

current flows both through the extra- and intracellular compartments. Since ageing induces structural change, particularly on membrane integrity, the insulating properties of membranes decrease, intra- and extracellular electrolytes are mixed which modify electrical properties. A method presented here has been developed to monitor and explain the change in tissue conductivity in preferential directions.

Objectives

The main objective of this study is to evaluate the optimal meat ageing duration by means of electrical measurements. In this study, we tried to use equivalent electrical circuit parameters to characterize change in beef meat mesostructure. In previous study, we observed that meat electrical parameters are anisotropic and depend of meat fiber orientation [Damez et al., 2000] and so strongly correlate to meat ageing. Herein we try to replace the traditional rheological texture measurement with a non-invasive electrical measurement. In this study we established a model that permitted to calculate parameters corresponding to structural components at the cell level.

Methodology

Impedance measurements were made with a probe composed of 2 electrodes spaced 5cm ($\phi=0.6$ mm; $L=5$ mm) allowing measurements longitudinally and transversally to the fiber direction. Measurements were made with a HP 4194A Impedance/Gain-Phase analyzer at a frequency range between 1 KHz and 1.5 MHz. The meat samples were 3 types of beef muscle, Rectus abdominis (RA), Semimembranosus (SM) and Semitendinosus (ST) from 7 cows at different post-mortem times (PM+days), 2, 3, 6, 9, 14, 20 and 29 days of ageing.

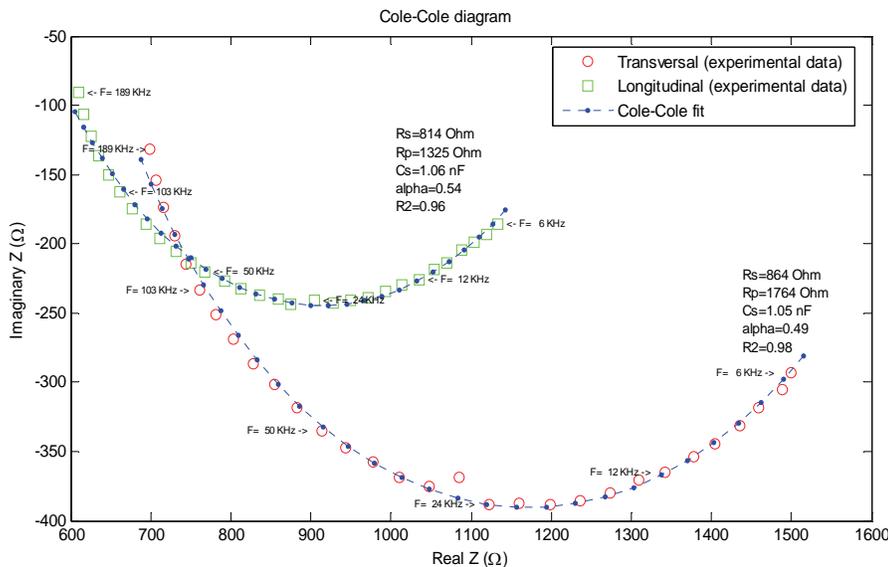


FIGURE 2 Typical Cole-Cole diagram and calculated conduction parameters on two orthogonal fiber directions.

Resistive and capacitive electrical properties are modeled using an adapted Cole-Cole relaxation equation (Fig. 2)[Cole & Cole, 1941][Foster & Schwan, 1989].

The impedance Z is a complex function of alternating current frequency f so that

$$Z = R_{\infty} + \frac{(R_0 - R_{\infty})}{1 + (i\omega\tau)^{1-\alpha}} \quad \text{where, } \omega = 2\pi f, R_0 \text{ and } R_{\infty} \text{ are respectively the impedance at}$$

very low and very high frequency, and the dimensionless exponent α a constant correcting the non strict capacitive compartment of membranes due to dielectric losses.

$$\text{From the Fricke's model, } \tau = (R_s + R_p)C_s, R_0 = R_p \text{ and } R_{\infty} = \frac{R_p R_s}{R_p + R_s}.$$

For fitting, we have implemented an improved algorithm derivate from the *fminsearch* function of Matlab R14 based on Nelder-Mead simplex method. This fitting gives a very good result as it gives a fit on each data point. Ageing is characterized with a rheological compression test using the method described by Lepetit and Buiere (1995).

Results & Discussion

Conduction parameters are obtained by the data fitting method using Fricke's model. Dielectric calculated parameters are summarized in Table 1. It is important to note that parameters in longitudinal and transversal fibers directions are highly different. Resistance of extracellular space in the transversal direction (R_pT) is higher than resistance in the longitudinal direction (R_pL), which can be explained by the longer path of current flow. Capacitive element C_s is almost the same in both directions and decreases with ageing that is in agreement with the rupture of cellular membrane. Resistance of intracellular spaces (R_s) is almost the same in transversal and longitudinal directions and increase with ageing denoting that intracellular space becomes less conductive.

Conclusions

The method presented here has been developed to monitor and explain the changes in tissue conductivity in preferential directions during the ageing process. Correlations between calculated electrical parameters and compression strain remain low, but calculated parameters agreed with the behavior of the meat structure and could be useful to determine cell membrane state and intra- and extracellular state at different postmortem stages.

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Tables and Figures

TABLE 1 Calculated dielectric parameters of meat samples from different muscles, Rectus abdominis (RA), Semimembranosus (SM) and Semitendinosus (ST) at different post-mortem times (PM+days).

	Longitudinal impedance			Transversal impedance			Compression strain
	Rs (Ω)	Rp (Ω)	Cs (nF)	Rs (Ω)	Rp (Ω)	Cs (nF)	20% (N/cm ²)
RA PM+02	813	1881	2.09	878	2302	2.24	26.56
RA PM+02	1039	2325	1.96	820	2980	2.34	49.19
RA PM+03	714	2012	2.25	712	2561	2.61	20.19
RA PM+03	979	2216	1.78	1399	3550	1.49	34.06
RA PM+06	1002	1291	1.65	808	1410	1.94	11.28
RA PM+14	817	1311	1.94	765	1452	2.05	6.03
RA PM+20	1721	683	0.36	1544	746	0.38	18.86
RA PM+29	3755	636	0.29	2998	724	0.35	20.29
SM PM+02	1745	601	0.37	1843	603	0.30	18.12
SM PM+02	1395	664	0.74	1255	643	0.37	13.46
SM PM+03	2019	687	0.36	2103	694	0.34	18.90
SM PM+03	4508	497	0.50	4772	534	0.56	16.65
SM PM+06	3413	547	0.59	3447	574	0.40	4.74
SM PM+06	3427	617	0.25	1918	655	0.92	5.18
SM PM+06	3589	599	0.18	2827	684	0.75	4.84
SM PM+14	2800	667	0.29	2586	685	0.29	7.40
SM PM+14	4107	724	0.69	2064	710	0.36	5.16
ST PM+02	914	814	1.20	916	768	2.22	27.76
ST PM+06	1052	1378	1.11	1027	1476	1.29	7.41
ST PM+14	1901	763	1.68	1783	734	1.95	5.86

IDENTIFICATION OF TENDERNESS THRESHOLD FOR WARNER-BRATZLER SHEAR FORCE IN VENEZUELAN BEEF

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Key Words: Beef, Tenderness, Threshold, Warner-Bratzler shear

Background

In Venezuela, tenderness is also the most important factor influencing consumer satisfaction for beef palatability (Huerta and Rodas, 1998) and consumers demand for consistently tender meat is increasing. Regrettably, most of the beef production comes from mature cattle, particularly entire males (bulls), grass feeding, with high predominance of *Bos indicus* types. These conditions are largely responsible for the high variation observed in beef palatability in Venezuelan (Huerta *et al.*, 2004) and make more difficult to target consistency in tenderness.

Years ago, it was thought that a means of segregate tender beef would be through the beef carcass grading system. Currently, Venezuelan grading systems for quality (Decree 181, 1994; Decree 1896, 1997) have not provided an accurately mean to segregate carcasses into expected palatability groups (Huerta *et al.*, 1996; Malaver *et al.*, 2000). Even though, in the United States with a vast experience in the grading of carcass for quality have revealed the same kind of inconsistency (Shackelford *et al.*, 1997).

Due to the deficiencies of the quality grading systems, different groups of investigators have tried to locate the separation line between tender and tough meats (Shackelford *et al.*, 1991; Tatum *et al.*, 1996; Shackelford, *et al.*, 1997; Carr *et al.*, 1998;) to generate more accurate classification for tenderness. The establishment of tenderness thresholds serves as quality control with more precision to guarantee tender meats and to assure consumer acceptability.

Objectives

To obtain a tenderness threshold classes by relating trained panel data to Warner Bratzler shear force in beef.

Materials and methods

Animals: Cattle (n=738) originated from the main beef producing regions of Venezuela representing different breeds (crossbred *Bos indicus* x *Bos taurus*), sex (bulls, steers, heifers and cows) and age (1-11 years). All cattle were slaughtered at a commercial packing plant. At 24 h *postmortem*, carcasses were ribbed and evaluated by USDA quality grade (USDA, 1989) and by Venezuelan grading system (Decree 1896,

1997). At 48 h postmortem, four steaks (2.54-cm thick) were removed from the *longissimus dorsi thoracis* muscle (ribeye) from the right side of each carcass and vacuum-packaged for sensory panel and Warner Bratzler shear force (WBS) evaluation. These steaks were frozen at -30°C immediately (2 d postmortem) until the date of their evaluation.

Steaks were thawed at 4 °C for 24 h prior to sensory or shear tests. Preparation and cooking of samples for sensory and shear force evaluation were carried out following the general recommendations of the American Meat Science Association (AMSA, 1995). The steaks were cooked on an electric grill, which was preheated (approximately at 165 °C). Steaks were turned once during broiling and removed from the grill when they reached the desired internal temperature (70 °C).

The taste panel was comprised of eight highly trained judges (Jerez *et al.*, 1994) from both sexes with different education levels, age between 25 and 45, that tasted a maximum of 12 samples (in two sessions) per day. Two or three, cubed samples taken from steaks of each animal were served warm to each judge. Judges scored the samples for muscle fiber tenderness, overall tenderness, juiciness, amount of connective tissue and flavor intensity using an 8-point structured rating scale for each attribute (where 1 = extremely tough, extremely tough, extremely dry, an abundant amount of connective tissue, extremely bland, respectively, and 8= extremely tender, extremely tender, extremely juicy, no connective tissue, extremely intense, respectively)(AMSA, 1995, Jerez *et al.*, 1997).

Cooked rib steaks for shear evaluations were allowed to cool down to room temperature and four to ten core samples (1.27cm in diameter) depending on the area of the *longissimus* muscle, were removed parallel to the muscle fiber orientation, taking care not to include pieces of fat or chunks of connective tissue in the core. Each core was sheared once using a Warner-Bratzler shear machine (G-R Elec. Mfg. Co, Manhattan, KS). The four to ten Warner-Bratzler Shear force (WBS) values were recorded and averaged to obtain a single shear force value for each steak.

Simple descriptive statistics were computed for carcass traits using PROC MEANS (SAS, 1996) to characterize the animals from database. To establish the tenderness threshold, a simple linear regression of the WBS values on the overall tenderness score of sensory panel was made using the SAS statement PROC REG (SAS, 1996).

Results and Discussion

Means, standard deviations (SD), and minimum/maximum values for carcass characteristics and WBS values are presented in Table 1. Since the carcass sampled were representative of the Venezuelan beef herds, a wide range in each of the carcass traits were observed. The chronological age revealed a high variation (12 to 132 mo.) corresponding to the high variation observed in skeletal and lean maturity indicators (A to D). Furthermore, fat external finish showed a high variation (very abundant to devoid), but such variation does not correspond to marbling score, that ranged between “small” and “practically devoid”. According to the Venezuelan grading criteria (Decree 1896, 1997) 60.11% of the carcasses was graded in the “A” category (also named “Excelent”) followed by the “B” category (29.64 %, or “Select”). According to USDA grading system, (USDA, 1989) 88.65% of the carcasses were categorized as “Standard”.

Lineal regression analysis of WBS on overall tenderness ratings (with trained panel) in our laboratory indicates that a sample rated “slightly tender” correspond to the WBS value 3.86 kg. A sample was classified as “tender” if its shear value was less than 3.86 kg. The following procedure was to separate the group of “tough” meats in some of “intermediate” meat (which tenderness rating and acceptance could be improved with ageing) and other frankly “tough” ones (showing off their doubtful acceptance, being even ageing). The separation line between “intermediate” and “tough” meat, correspond to the sensory panel description of "slightly tough" with WBS values greater than 4.98 kg. Therefore, the interval of meat with “intermediate” toughness was set between 3.86 and 4.98 kg, so a sample was classified as “tough” if its shear value was higher than 4.98 kg.

There are several thresholds reported in the literature. They defined meat as “tender” when they have less than 6 kg. (Shackelford et al., 1997), 4.6 kg. (Shackelford et al., 1995), 3.85 kg. (Tatum et al., 1996) or 3.0 kg. (Huffman et. al., 1996; Wheeler et al., 1997) of WBS. These differences among research centers in the tenderness threshold may be due to differences in the sensory panel training procedures, differences in shear force assessment, or both (Wheeler et al., 1997). However, our threshold is similar to the one reported by Tatum et al. (1996). Some researcher have reported (Shackelford et al., 1991; Miller et al., 1995; Huffman et al., 1996) that consumer are able to distinguish differences in beef tenderness that have been classify based on Warner-Bratzler shear force; consequently, in necessary evaluate our tenderness threshold by Venezuelan consumer.

Conclusions

The tenderness threshold obtained in this study allows the definition of three categories: WBS values <3.86 kg. for tender meats, >3.86 <4.98 kg. for intermediate meats and >4.98 kg. for tough meats. This threshold was obtained based on trained sensory panel, and it lacks of the final consumer's opinion. Further studies that include consumer acceptability are necessary to establish the true value of tenderness.

The generation of these thresholds will be useful in the formulation of programs to generate commercial marks that allow the certification of quality beef in the market of demanding consumers.

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TABLE 1.
STATISTICS FOR CARCASS TRAITS AND SHEAR FORCE.

Trait	Mean	SD	Min.	Max.
Leg muscle profile ^a	3.09	0.79	1.00	5.00
Fat external finish ^b	3.39	0.71	1.00	5.00
Back fat thickness, mm ^c	2.59	2.24	0.00	19.00
Marbling score ^d	4.95	0.23	3	5
Ribeye area, cm ²	73.66	12.70	36.12	121.93
Bone maturity ^e	B ¹⁹	53.96	A ³⁰	D ⁵⁰
Lean maturity ^e	B ⁰⁴	49.88	A ²⁰	D ⁰⁰
Adipose maturity ^f	2.70	0.49	2.00	4.00
Final maturity ^g	B ¹¹	44.32	A ⁴⁷	D ³⁰
Carcass weight, kg	268.90	37.28	149.00	465.00
Chronological age, mo.	36.45	10.64	12.00	132.00
Venezuelan grade, % ^h	AA: 2.49 A: 60.11 B: 29.64 C: 7.76			
USDA quality grade, %	Choice: 0.27 Select: 1.50 Standard: 88.65 Utility: 9.30 Cutter 0.27			
Shear force at 2 d postmortem, kg.	5.17	1.89	1.87	11.88

SD: standard deviation

^a According to Presidential Decree No. 1896 (1997): where 1 = very convex, 2 = convex, 3 = straight, 4 = concave and 5 = very concave.

^b According to Presidential Decree No. 1896 (1997): where 1 = very abundant, 2 = abundant, 3 = moderate, 4 = slight and 5 = devoid

^c Measured at the 12th/13th rib interface.

^d Marbling scores according to Presidential Decree No. 181 (1997): where 3 = Small; 4= Slight; 5=traces and Practically devoid.

^e Maturity scores according to USDA (1989). Where A= younger maturity; D=older maturity; degrees 0-99.

^f Adipose tissue maturity based on fat color, according to Presidential Decree No. 1896 (1997); where 1 = ivory white, 2= creamy white, 3 = yellowish, 4 = yellow, and 5 = orange.

^g According to Presidential Decree No. 1896 (1997).

^h Carcass grade according to Presidential Decree No. 1896 (1997); where A=Excellent; B=Select.

CONSUMER'S ABILITY TO DETECT TENDER MEAT USING VENEZUELAN TENDERNESS THRESHOLD

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Key Words: Beef, Tenderness, Threshold, Warner-Bratzler shear, acceptability.

Background

The tenderness thresholds have been determined through the relationship between the sensory trained panels and Warner Bratzler shear force (WBS) evaluation (Shackelford *et al.*, 1991; Tatum *et al.*, 1996; Shackelford, *et al.*, 1997; Rodas *et al.*, 2005). The tenderness thresholds could serve as quality control to guarantee tender meats and to assure consumer acceptability, but these tenderness thresholds not always have the same meaning to consumers, because consumers consider besides of tenderness, juiciness and flavor to the overall acceptability of beef steaks (Carr *et al.*, 1998).

Our group developed a tenderness threshold based on trained sensory panel and WBS values (Rodas *et al.*, 2005). A following study that includes consumer acceptability is necessary to establish the true value of tenderness in Venezuelan beef.

Objectives

To assess the consumer's ability to detect three categories of tenderness from steaks classified according to WBS values: <3.86 kg as "tender", 3.86 to 4.98 "intermediate" and >4.98 tough meat.

Materials and methods

Data from three experiments with consumer (untrained) panel were chosen and polled. Experiment 1 and 2 were composed by Crossbred steers and bullocks (n=34) (F1 *Bos indicus* x *Bos taurus*), and experiment 3 by Criollo Limonero steers (*Bos taurus* tropically adapted) (n=23), with different ages (19 mo to 40 mo). Animals were slaughtered at a commercial packing plant. At 24 h *postmortem*, carcasses were ribbed and evaluated by USDA quality grade (USDA, 1989) and by Venezuelan grading system (Decree 1896, 1997). At 48 h *postmortem*, four steaks (2.54-cm thick) were removed from the *longissimus dorsi thoracis* muscle (ribeye) from the right side of each carcass and vacuum-packaged for consumer panel and Warner Bratzler shear force (WBS) evaluation. These steaks were frozen at -30°C immediately (2 d *postmortem*) until the date of their evaluation.

Steaks were thawed at 4 °C for 24 h prior to sensory or shear tests. Preparation and cooking of samples for sensory and shear force evaluation were carried out following the

general recommendations of the American Meat Science Association (AMSA, 1995). The steaks were cooked on an electric grill, which was preheated (approximately at 165 °C). Steaks were turned once during broiling and removed from the grill when they reached the desired internal temperature (70 °C).

The total consumer panelists were comprised of 302 persons from both sexes with different education levels, income between \$46 and \$ 651 and more, age between 18 and 60, which tasted a maximum of 4 to 5 samples in one session per day (1 d for experiment one and two; and 2 d for the experiment three). Two or three samples taken from steaks of each animal were served warm to each untrained judges. Consumers scored the samples through hedonic scale from 1 to 9 (1=dislike extremely; 9=like extremely) for each of the attributes: overall impression, flavor, flavor intensity and tenderness (AMSA, 1995).

Cooked rib steaks for shear evaluations were allowed to cool down to room temperature and four to ten core samples (1.27cm in diameter) depending on the area of the *longissimus* muscle. Cores were removed parallel to the muscle fiber orientation, taking care not to include pieces of fat or chunks of connective tissue in the core. Each core was sheared once using a Warner-Bratzler shear machine (G-R Elec. Mfg. Co, Manhattan, KS). The four to ten Warner-Bratzler Shear force (WBS) values were recorded and averaged to obtain a single shear force value for each steak.

Data collected were analyzed as a RBD using ANOVA by SAS (SAS, 1996) using tenderness categories as main effect (according to WBS, <3.88 kg="tender"; 3.86 to 4.98kg= "intermediate"; >4.98 kg= "tough" meats), and consumer panelist as block. When a significant effect was detected ($P<.05$) the least square mean were compared using LSD test. Additionally, for each tenderness categories, a frequency analysis of the observations allowed to describe the proportion of consumer's acceptability; taking as acceptable when cooked meat had equal or higher 6 points of the hedonic scale (6=Like slightly).

Results and Discussion

Some researcher have reported (Shackelford et al., 1991; Miller et al., 1995; Huffman et al., 1996, Miller et al., 2001) that consumer are able to distinguish differences in beef tenderness that have been classify based on Warner-Bratzler shear force. Tenderness classes evaluated in this study were obtained by lineal regression analysis of WBS on overall tenderness ratings (Rodas-Gonzalez et al., 2005, this congress). Result from that study allowed to classify as "tender" meat if its WBS value was less than 3.86 kg.; an intermediate meat (which tenderness rating and acceptance could be improved with ageing) if its WBS values is between 3.86 and 4.98 kg., and "tough" meat (showing off their doubtful acceptance, being even ageing), if its shear value is higher than 4.98 kg. Considering that the next step after getting our tenderness threshold, was to evaluate our tenderness threshold with a consumer panel.

The effect of tenderness class on consumer sensory traits is shown on Table 1. Flavor and flavor intensity ratings were not different ($P>.10$) among the tenderness classes. Even though, significant differences ($P<.05$) were not detected by ANOVA for overall impression and tenderness when comparing tenderness categories the results revealed a

trend that shows that overall impression and tenderness ratings are greater in meat classify as “tender” than in meat classify as “tough”, but statistically ($P>0.10$) similar to the ones classify as “intermediate”. Consumer were able to differentiate between “tender” and tough” meat in overall impression and tenderness ($P=0.08$ and $P=0.10$, respectively), but these differences were not highly significant.

The ability of consumers to differentiate the degree of tenderness is important for the beef industry and its attempt to implement a tenderness threshold classification system that would allow segregating tender from tough meat. The overall acceptability ratings are shown in Table 2. In general, consumer tenderness acceptability increased as WBS values decreased. However, only 53.6 % of consumer found meat with WBS force <3.86 kg acceptable. Miller et al. (2001) suggested that consumer WBS tenderness values of 4.0 kg. would result in 94 % of customer satisfaction for beef tenderness. These differences could be due to variation in tenderness perception, consumer may not require the same tenderness in beef to be satisfied and that either beef flavor o juiciness may influence their perception of tenderness and their overall acceptability ratings.

Conclusions

Results from present study show that consumer can segregate differences between “tender” and “tough” meats. The low percent of consumer acceptability for “tender” meat could be explained by the individual perception of tenderness.

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Table 1. Least squares means \pm standard error for consumer panelist trait by tenderness categories

Trait	Tenderness Categories*			P-value
	Tender	Intermediate	Tough	
Overall impression	6.43 \pm 0.26 ^a	5.5. \pm 0.44 ^{a,b}	5.21 \pm 0.53 ^b	0.08
Flavor	6.07 \pm 0.29	5.41 \pm 0.50	6.09 \pm 0.619	>0.10
Flavor intensity	5.34 \pm 0.45	5.79 \pm 0.41	5.07 \pm 0.42	>0.10
Tenderness	6.60 \pm 0.32 ^a	6.11 \pm 0.30 ^{a,b}	5.72 \pm 0.30 ^b	0.10

*Tenderness categories obtained by Rodas-Gonzalez *et al.*, 2005: Tender (WBS values <3.86 kg.), Intermediate (WBS values >3.86 <4.98 kg.) and Tough (WBS values >4.98 kg).

Table 2. Frequency of overall acceptability by tenderness categories

Trait	Tenderness Categories*		
	Tender	Intermediate	Tough
Acceptability**	53.64 %	24.09 %	22.27 %

*Tenderness categories obtained by Rodas-Gonzalez *et al.*, 2005: Tender (WBS values <3.86 kg.), Intermediate (WBS values >3.86 <4.98 kg.) and Tough (WBS values >4.98 kg).

**Acceptable when cooked meat had equal or higher 6 points of the hedonic scale (6=Like slightly).

A METHOD TO EVALUATE SHEAR FORCE MEASUREMENTS FROM VARIOUS INSTRUMENTS

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Key Words: shear force, texture

Introduction

Aside from calibration and validation of individual texture instruments, there is no method for evaluating the whether shear force results are comparable among instruments or laboratories. Wheeler et al. (1997) cited failure to adhere closely to protocol and instrument variation contributing to differences in shear force values among laboratories, making comparisons of data invalid. Eastridge and Solomon (1995, 1996) identified that some of the variability in the meat shear test was due to the shear cell attachment used, the coring tool and how at closely to perpendicular the sample is sheared. In a comparison of meat shear cells all from the same manufacturer (six new and one > 7 years), Eastridge and Solomon (1995) detected significant differences among the meat shear cells. Further, shear force values from the old cell were, on average, double that of the newly manufactured ones. Variations in endpoint temperature and corers used contributed up to 2 mm variation in the diameter of the cores for shear force analysis (Eastridge and Solomon 1996).

Texture quality grades of some foods change annually based on the first harvest of the crop, for example, peas. Standardization of texture used for grading peas is accomplished by testing every shear attachment at a centralized laboratory using one instrument and calculating a calibration factor that is valid for one year. In the meat industry, there is no test or standardization of meat texture measuring systems. Although a number of products have been suggested for use as a standard material (Eastridge and Solomon, 1995), they are limited by being a single-point measurement and by changes in the material with increasing storage time.

Texture, in particular tenderness, is considered the most important quality trait that determines satisfaction with and decision to repurchase meat. Variable and inconsistent tenderness of meat can be influenced by ante- and postmortem factors including breed, age, sex, nutrition, carcass treatments, chilling regimes, type of muscle, etc., and their interactions. The inherent variability of meat (Dransfield and MacFie 1980; Kerth et al. 2002; Reuter et al. 2002; Belew et al. 2003) makes it unsuitable to use for checking repeatability and accuracy of the shear test for tenderness. Lyon and Lyon (1998) evaluated three shear test configurations to see if chicken breast shear force values obtained in a similar manner could be used interchangeably. The V-notched Warner-Bratzler (WB) shear blade was used on two instruments, the WB and TA.XT2 apparatus, and only the TA.XT2 was used compared two blade types, the V-notched WB blade versus a 45° chisel-end blade. Chicken breast shear force values were similar when using the WB blade on either apparatus; however, the change in blade configuration resulted in different shear force means. These types of comparisons are based on a single point and

are not adequate to determine similarity of shear values over a range of conditions. Jones et al. (2003) prepared gelatin gels of different strengths that had WB shear force ranging from 1.9 to 6.8 kgf for training sensory panelists to distinguish between three levels of meat tenderness. Plotting the shear force values for various gel concentrations showed a strong linear relationship. A similar approach using gelatin gels could be employed for evaluating whether shear force measurements could be compared among meat texture instruments rather than relying on whether shear force means are similar.

Objectives

It was the objective of this experiment, then, to evaluate the shear force of gels varying in gelatin concentration to determine whether texture measurements are comparable among laboratories and instruments.

Methodology

Gel formulations of Jones et al. (2003) were adapted to give shear values in the range likely for cooked fresh meat. Eleven batches of gels, each with five concentrations of gelatin, were prepared and shear force determined on six texture measuring systems. Due to difficulties in handling and transferring, gelatin (type A from porcine skin, 300 bloom) was weighed directly into zip lock plastic bags and mixed with a plasticizer solution (30% w/v sorbitol, 10% v/v glycerol) to make 200 mL amount. Gelatin concentrations used were 12, 15, 17, 18, 20, 22, 25, 27, 30, 32, 35, 37 and 38% (w/v) concentration. After 20 min hydration at room temperature, gels were dissolved in a water bath at 68 °C with the aid of sonication to eliminate foam formation. The bags were kept free of air as much as possible. When the gels were completely dissolved and clear, the bags were clamped in a form (15 × 17 × 1 cm) and stored at 4 °C for 18 hr for maximum gel strength to form. Each gel was removed and the edges were trimmed before cutting into as many 1 × 1 cm strips as possible. Due to the difficulty of obtaining uniform cores, only strips were used. Strips were vacuum packaged, three randomly selected per bag, then refrigerated until tested. During the shear test, strips were kept on a cold plate so that the temperature at shearing did not exceed 10 °C. One package was used for each instrument, and each strip was sheared five times for n=15 values per gel concentration. The shear cell attachment on five instruments met the specifications for blade thickness and an inverted-V cutout of the Warner-Bratzler shear test, while the sixth instrument sheared using the unsharpened edge of a disposable microtome knife mounted in a razor blade jig. Instruments used were: TMS-90 and TMS-2000 (Food Technology Corp., Sterling, VA), TA.XT2i (Texture Technologies Corp., Scarsdale, NY), Instron 4400 with Series IX software (Instron Corp., Canton, MA), Shimadzu EZ Texture Analyzer (Shimadzu Scientific Instruments, Columbia, MD), and WBS2000 digital (G. R. Manufacturing Co., Manhattan, KS). Among the instruments, two were >12 yr and the remainder were recently acquired. Since the objectives were to evaluate shear results rather than instruments, they were randomly assigned letters A, B, C, D, E or F. Regression analysis was performed (SAS v 9.13, 2000-2003, SAS Institute, Cary, NC) to determine intercept, slope and R²-values for each batch and instrument and correlations tested. Differences in

these parameters were tested in a mixed model using instrument as a fixed effect and batch as a repeated measures random effect.

Results & Discussion

The gels used varied in gelatin concentrations in order to produce shear force ranging from approximately 1 to 10 kgf. The correlation of gel concentration to shear force was highly significant ($p > 0.0001$). Table 1 presents the regression parameters for the relationship of gel strength and shear force. The linearity was highly significant and R^2 values ranged from .91 to .97. There were no differences ($p > 0.05$) in R^2 for instruments A-E; although, F had significantly lower R^2 and the adjusted R^2 was not different ($p > 0.05$) for any instrument. Batch differences in regression line parameters (data not shown) were related to gelatin manufacturer lot used. The bloom value for gelatin purity, and consequently strength, is somewhat subjective (Gelatin Manufacturers Institute, 2003), thus it is not surprising that there were differences were detected related to the lot. Preliminary testing confirmed that gels dissolved at 60 °C had higher and more consistent shear force than those dissolved at 90 °C and allowed using a lower gelatin concentration to achieve the higher shear force values. The 68 °C temperature was used so that the higher concentration gels would dissolve in < 3 hr. Batches also varied slight by the gelatin concentrations used, ranging from 12 to 38%. Five concentrations were used for each batch and were modified to obtain equal differences in shear force between concentrations. This resulted in the first four batches having different concentrations of gels. The later batches of gels had concentrations 12, 17, 22, 27 and 32% that was judged to be easiest and most consistent to prepare. Higher concentrations of gelatin took longer to dissolve and were less uniform. Overall, results show a good linear relationship between gelatin concentrations and shear force measurements, supporting use of gels for determining compatibility of shear test results.

The slope and intercept parameters were used to identify similarities and differences among instruments. Shear force values obtained from instruments B, C, D and E were compatible, that is, their slopes were not different ($p > 0.05$); although their intercepts differed ($p < 0.01$). Shear values from these instruments could be adjusted by their intercept values for combining or comparing test results. Jones et al. (2003) evaluated gels having shear force in the range of 1.5 to 6.8 kgf on the WB instrument to results using the TA.XT2i. Although shear force values were highly correlated between the two instruments, slopes of the regression lines were not equal and shear forces differences increased as the values increased. Regression line from A had a significantly higher slope, and the resulting shear force was different from all other instruments. Instrument A was one of the two older instruments in use, and was distinguished from the other because the shear cell attachment had never been replaced. In contrast, the shear cell attachment was replaced < 4 yr previously on the second instrument. The higher shear force values obtained with A are consistent with Eastridge and Solomon (1995) report where the older shear cell attachment gave significantly higher shear values compared to six new attachments. It is likely that the shear blade edges become flatter or dulled with long term use and other changes increase friction as the blade moves through the slot, resulting in higher shear values. The lowest slope was for F which differed from all other instruments except for B. A possible explanation is that this instrument was acquired after

several batches of gels had been tested. The differences may be related to fewer batches analyzed.

Conclusions

We used gels representing ranges of equally spaced shear force values to evaluate whether shear force test results could be compared or adjusted to be equivalent to other instruments. The gels used produced highly significant linear response that was useful for identifying which instruments were similar based on equal slopes of the regression lines. While this method does not allow comparisons across time, it is a tool that can be used to determine compatibility of shear force data for projects involving multiple laboratories.

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Tables and Figures

Table 1. Regression of gel concentration and shear force parameters to test compatibility of shear force results obtained using six instruments

Instrument	Intercept	Slope	R ²	Adjusted R ²
A	-7.996 ^d	.6457 ^b	.9718 ^b	.9713
B	-4.738 ^b	.4104 ^{cd}	.9621 ^b	.9613
C	-6.044 ^c	.4580 ^c	.9556 ^b	.9354
D	-5.871 ^c	.4626 ^c	.9540 ^b	.9506
E	-6.618 ^c	.4653 ^c	.9569 ^b	.9559
F	-4.766 ^{bc}	.3694 ^d	.9100 ^c	.9079

^a Instruments used were manufactured by Food Technology Corp. (Sterling, VA), G. R. Manufacturing Co. (Manhattan, KS), Instron Corp. (Canton, MA), Shimadzu Scientific Instruments (Columbia, MD) and Texture Technologies Corp. (Scarsdale, NY) and are identified by a randomly assigned letter.

^{bcd} Within a column, means with different superscripts are different ($p < 0.01$).

THE EFFECT OF RACTOPAMINE AND INTRAMUSCULAR FAT CONTENT ON SENSORY ATTRIBUTES IN PORK FROM PIGS WITH SIMILAR GENETICS

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Key Words: Marbling, Pork, Ractopamine

Introduction

The impact of intramuscular fat level, or marbling, on flavor and tenderness of fresh pork has been of great interest recently in the pork industry. With increasing demand for pork with higher marbling by the export market as well as food service and branded retail products, it would be beneficial to better understand the effect of marbling on sensory characteristics. Additionally, the prevalence of ractopamine use in the industry warrants the investigation of any effects on sensory characteristics concerning ractopamine and intramuscular fat content.

Objectives

The objective of this study was to characterize the effects of ractopamine and amount of intramuscular fat on sensory characteristics using pigs from the same genetic line. Utilizing one genetic line should minimize any differences due to genotype or breed differences.

Methodology

Fresh boneless pork loins were collected from 233 barrows of the same genetic line over a two-day period from a commercial slaughter facility. Live treatments included a control diet and the control diet with the addition of ractopamine (RAC) fed at 5 ppm for 28 days. Loins were vacuum packaged, transported to the Meat Science Lab at the University of Illinois and held at 4 °C until they were opened over a two-day period at 7 days post mortem (loins collected on day one at the plant were opened on day one at the Meat Science Lab and likewise for day two). Ultimate pH (pH_u) was collected at 7 days post mortem using the pH star (SFK Technologies) calibrated with two buffers of pH 4.0 and 7.0. Objective color measurements (L*, a*, and b*) were collected using a Minolta Chromameter CR-300 (Minolta Camera Co.). Multiple 2.54 cm chops were cut for use in determination of proximate composition, cook loss, Warner-Bratzler shear force, and sensory analysis. Sensory analysis was performed by a trained 6-member panel using an anchored, unstructured 15 cm line scale and including tenderness, juiciness, and pork

flavor as evaluation parameters. Initial statistical analysis of main effects was performed utilizing the Mixed Procedure of SAS including treatment and blocking by date of harvest in the random statement. Subsequent analysis for determining the relationship between intramuscular fat content and flavor was performed using the REG procedure of SAS (SAS Institute, Inc. version 8.2).

Results & Discussion

The effect of RAC on analyzed variables was minimal as displayed in Table 1. Numerically small, but statistically significant differences were identified for pH_u (5.62 vs. 5.70), Minolta a* (7.53 vs. 6.86), and Minolta b* (3.87 vs. 3.46) for control vs. RAC treatments, respectively. Due to the lack of significant differences concerning extractable lipid and sensory characteristics, all data were pooled for analysis by regression. Percent lipid and pH_u were regressed against sensory panel tenderness, juiciness, and pork flavor both independently and together.

Figure 1 displays the distribution of extractable lipid from the population of loins. Although it was not a uniform distribution, there was a wide range of extractable lipid, which should allow for regression against sensory characteristics. The pH distribution was relatively small with the average and standard deviation being 5.65 ± 0.15 units. One would not expect this to regress well due to the narrow range, however it could be used to regress in conjunction with extractable lipid to help explain some of the variation.

Results from regression of extractable lipid with tenderness, juiciness, and flavor as determined by the sensory panel can be seen in Figures 2, 3, and 4. Extractable lipid was unable to explain much variation in any of the sensory categories. Even when coupled with pH and regressed against the sensory characteristics, the R² achieved by extractable lipid did not exceed 0.10.

Conclusions

Results from this study indicate that RAC did not have any significant effects on quality or sensory characteristics. They also indicate that for this particular genetic line, extractable lipid was not a good indicator of sensory properties as determined by a trained sensory panel.

Tables and Figures

Table 1. Effects of RAC on Loin Characteristics

	Control	RAC	SEM	P-Value
Moisture (%)	73.84	73.71	0.09	0.21
Extractable Lipid (%)	2.89	2.75	0.09	0.27
Cook Loss (%)	21.34	21.04	0.45	0.64
Shear Force (kg)	2.86	2.79	0.10	0.23
Juiciness	7.52	7.44	0.17	0.59
Tenderness	7.71	7.68	0.22	0.86
Pork Flavor	6.38	6.37	0.43	0.95
NPPC Color	3.16	3.25	0.42	0.16
NPPC Firmness	2.32	2.40	0.05	0.23
NPPC Marbling	1.71	1.70	0.08	0.94
Ultimate pH	5.62	5.70	0.04	< 0.01
Minolta L*	47.52	46.86	0.26	0.07
Minolta a*	7.53	6.86	0.13	< 0.01
Minolta b*	3.87	3.46	0.28	< 0.01

Figure 1. Distribution of Extractable Lipid

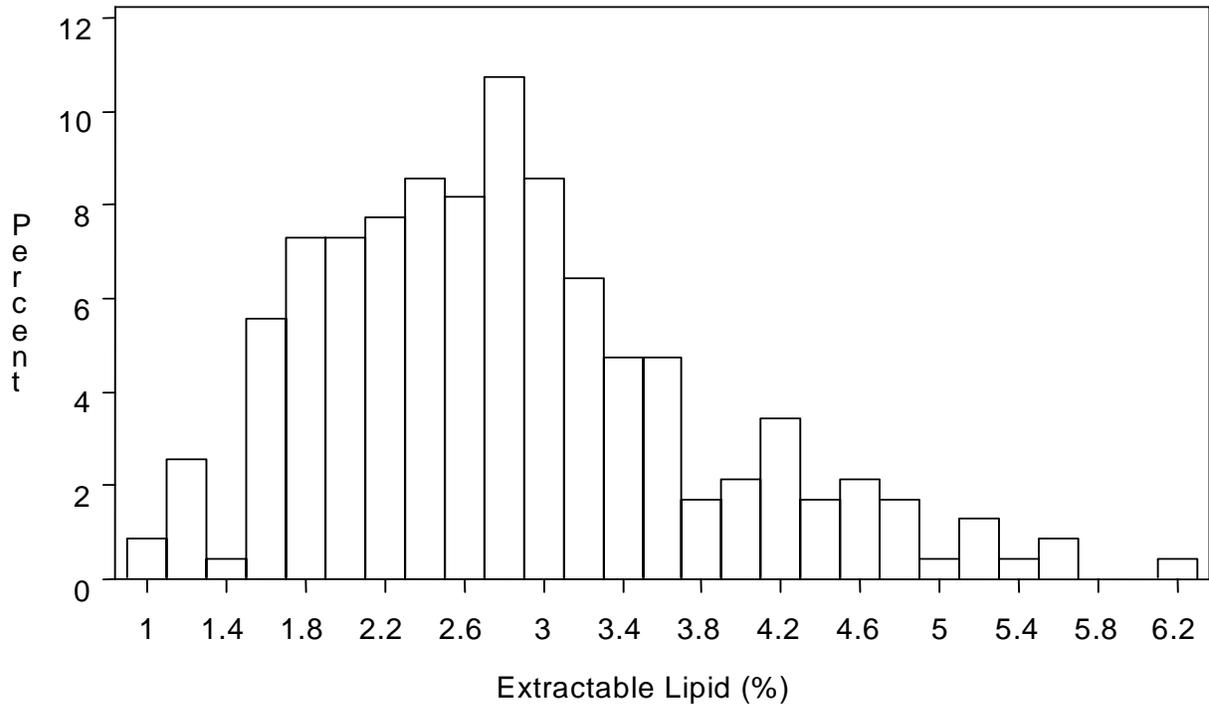


Figure 2. Regression of Extractable Lipid * Sensory Panel Tenderness

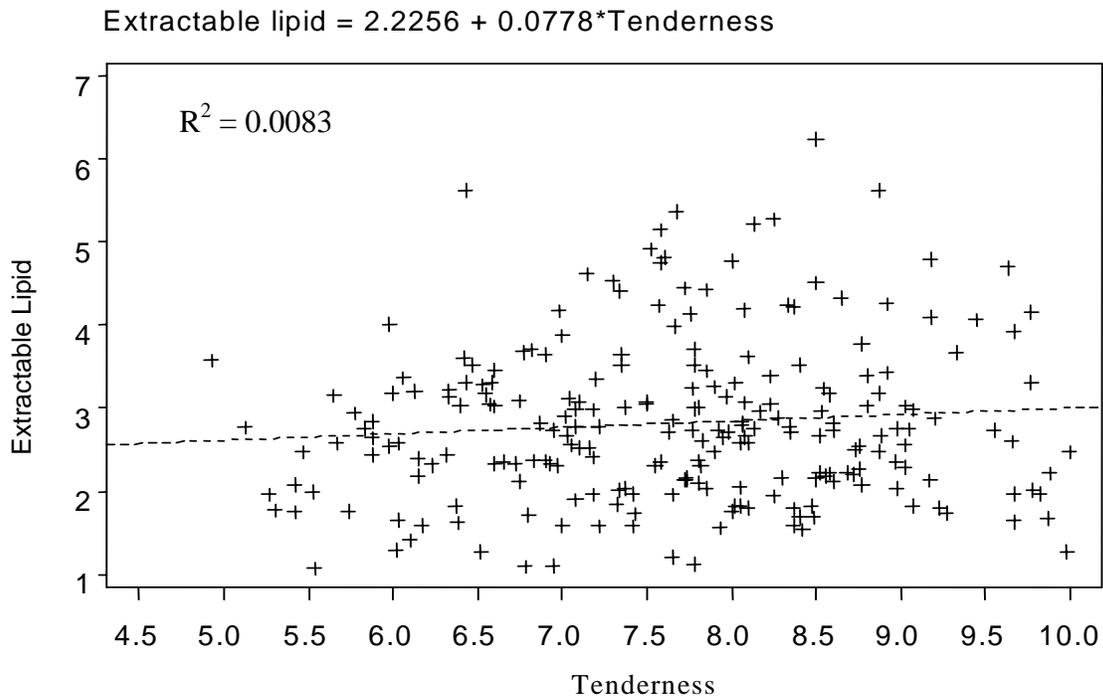


Figure 3. Regression of Extractable Lipid * Sensory Panel Juiciness

$$\text{Extractable Lipid} = 1.8921 + 0.1245 * \text{Juiciness}$$

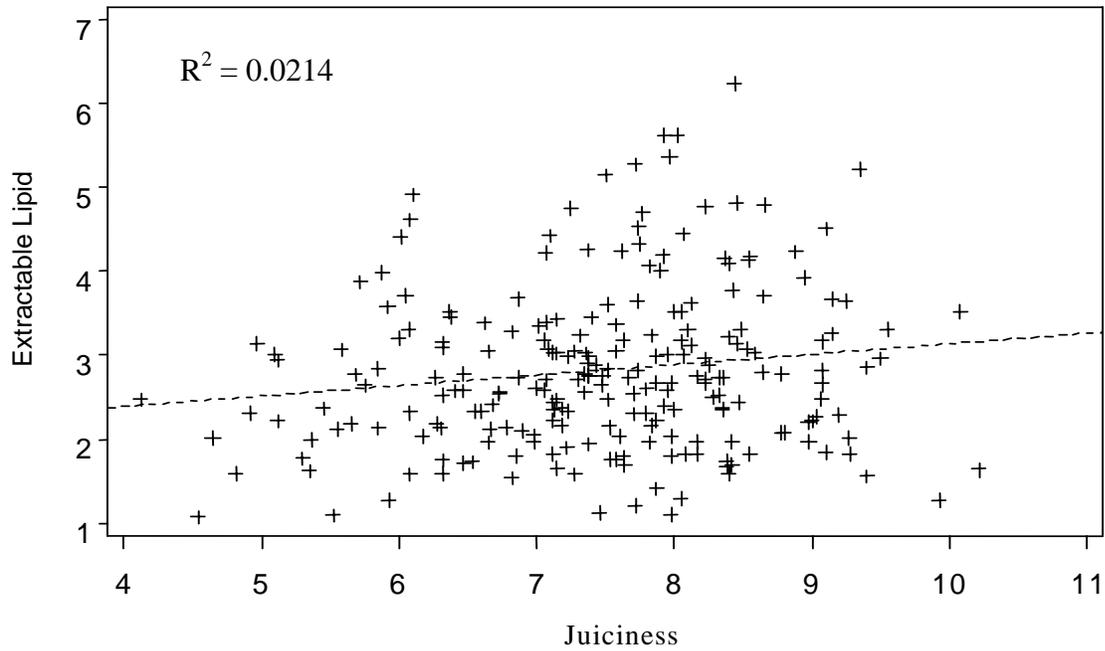
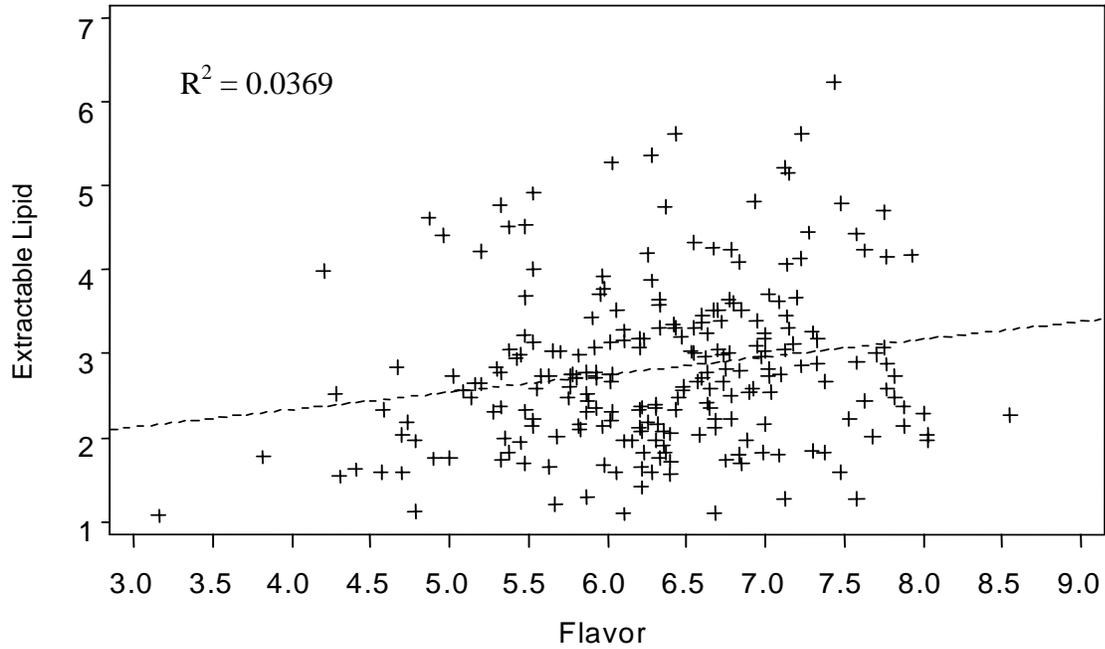


Figure 4. Regression of Extractable Lipid * Sensory Panel Pork Flavor

$$\text{Extractable Lipid} = 1.4957 + 0.2096 * \text{Flavor}$$



RETAIL CUTTING CHARACTERISTICS FOR RIB AND LOIN SUBPRIMALS FROM TWO GRADE GROUPS

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Key Words: Beef; Retail cutting test; Retail merchandising; Time allocation; Yield

Introduction

CARDS (Computer Assisted Retail Decision Support) is a computer software program that was developed so retailers could evaluate the price/value relationship of beef subprimals (Garrett et al., 1991). This program continues to serve as a valuable reference to assist retailers in the process of making meat purchasing and merchandising decisions.

Research previously conducted on beef retail yield and fabrication times (Garrett et al., 1991) led to research on pork (Lorenzen, Griffin, Dockerty, Walter, Johnson, & Savell, 1996a; Lorenzen, Walter, Dockerty, Griffin, Johnson, & Savell, 1996b), lamb (Lorenzen et al., 1997), and veal (McNeill, Griffin, Dockerty, Walter, Johnson, & Savell, 1998), and allowed for CARDS program development for these species. CARDS was designed originally for the retail sector; however, the foodservice industry later requested similar information. Weatherly et al. (2001) determined cutting yields and time requirements for beef subprimals as they were portioned into ready-to-cook foodservice cuts.

We undertook the process of updating the database with cuts not previously evaluated, because it had been more than a decade since the original data were generated for the Beef CARDS program. Because of the extensive number of cutting tests conducted and the need to narrow the scope to be reported, the information presented herein encompasses the rib and loin cuts.

Objectives

The objective of this study was to perform a thorough evaluation of rib and loin cuts included in the present version of Beef CARDS in order to pinpoint deficiencies and/or inconsistencies in the data and to obtain current yield and time data for a new updated list of subprimals. Another objective of this research was to improve and expand the existing Beef CARDS for the exiting Beef CARDS program for the benefit of the retail and foodservice industry.

Methodology

Product selection

Beef subprimals (n = 120) from the rib and loin (Table 1), representing USDA Choice and Select grades, were obtained from a major beef processor and shipped to the Rosenthal Meat Science and Technology Center at Texas A&M University. Selected subprimals represented the normal weight variation and standard packer fat trim levels associated with commodity boxed beef. Specifications for all subprimals complied (within packer variations) with Institutional Meat Purchase Specifications (IMPS) as described by USDA (1996) and NAMP (2003).

Table 1
USDA (1996) Institutional Meat Purchase Specifications (IMPS) descriptions of rib and loin subprimals used for retail cutting tests

IMPS #	Subprimal
109E	Beef Rib, Ribeye Roll, Lip-On ^a , Bone-In
112	Beef Rib, Ribeye Roll
112A	Beef Rib, Ribeye, Lip-On ^a
112A Modified	Beef Rib, Ribeye, Lip-On ^a Modified (1 × 1) ^c
175	Beef Loin, Strip Loin (1 × 0) ^d
180	Beef Loin, Strip Loin, Boneless (1 × 0) ^d
184E	Beef Loin, Top Sirloin, Boneless, 2 Pc ^e

^aLip = *M. serratus dorsalis*, *M. longissimus costarum* and related intermuscular fat lateral to the *M. longissimus thoracis* (USDA, 1996).

^b(1 × 1) = Lip does not exceed 2.54 cm.

^cIM = Individual muscle.

^d(1 × 0) = The flank side shall be lateral to, but not more than 2.54 cm from, the *M. longissimus lumborum* at the rib end to a point on the sirloin end immediately lateral to the *M. longissimus lumborum* (USDA, 1996).

^ePc = Piece.

Cutting tests

A refrigerated cutting room in the Rosenthal Meat Science and Technology Center was modified to simulate a retail market environment for the purpose of conducting controlled retail yield tests. Meat merchandisers from different regions of the United States and with extensive retail meat industry cutting experience were enlisted for this study. Discussion between meat merchandisers and investigators resulted in the development of merchandising schemes for each subprimal to represent best current industry practices. Universal Product Code (UPC) descriptions (Industry-Wide Cooperative Meat Identification Standards Committee, 2003) were used as the naming convention for retail cuts.

The cutting tests were conducted as follows. Vacuum packaged subprimals were weighed before and after opening, and purge loss was determined. Subprimals were cut

following defined merchandising schemes, retail cuts, unless otherwise specified, were trimmed not to exceed 0.32 cm of fat, and when trimmings were generated, the targeted visual lean percentage was 90%. Processing times were recorded as an estimate of labor requirements for each merchandising scheme. Timed activities for each cutting test included two major phases: opening (retrieval of the subprimal from vacuum-packaged bag) and cutting (removal of external and seam fat, connective tissue, and separation of individual muscles, as well as producing tray-ready retail cuts as applicable). The two phases were combined for total processing time. After each cutting test, technicians recorded weights of all cuts, lean trimmings, fat trim, and bone ensuring at least 99% recovery of subprimal weight.

Ribeye Rolls, Lip-On, Bone In (IMPS #109E) were merchandised two ways. One style consisted of cutting the bone-in ribeye rolls into three Ribeye Roasts, Lip-on, Bone In (UPC 1193): the 6th and 7th rib section, the 8th and 9th rib section, and the 10th through 12th rib section. For the second style, the ribeye roll was cut into 3.2 cm Ribeye Steaks, Lip-On, Bone In (UPC 1197).

Ribeye Rolls (0 × 0) (IMPS #112) were knife-cut end-to-end into 2.54 cm Ribeye Steaks (UPC 1209). Ribeye Rolls, Lip-On (2 × 2) (IMPS #112A) and (1 × 1) (IMPS #112A Modified) were merchandised two ways. One style consisted of the subprimal being cut into 2.54 cm Ribeye Steaks, Lip-On, Boneless (UPC 1203) and the second style consisted of cutting 2.54 cm Ribeye Steaks, Lip-On, Boneless (UPC 1203) throughout the small (posterior) end with the large (anterior) end remaining intact as a Ribeye Roast, Lip-On, Boneless (UPC 1194).

Strip Loins (IMPS #175) and Strip Loins, Boneless (IMPS #180) were cut into 2.54 cm Top Loin Steaks (UPC 1398) and Top Loin Steaks, Boneless (UPC 1404), respectively. Center-cut strip steaks and vein steaks (steaks that had *M. gluteus medius* on both sides of the cut) were recorded separately.

Top Sirloins, Boneless, 2 Pc (IMPS #184E) were vacuum packaged together. Bag opening time, initial weight, bag weight, and purge weight were collectively measured before separate cutting tests were performed on the Beef Loin, Top Sirloin Butt, Center-Cut, Boneless (IM) (IMPS #184B) and the Beef Loin, Top Sirloin, Cap (IM) (IMPS #184D). Top Sirloin Steaks, Boneless, Cap Off (UPC 1426) and Top Sirloin Cap Steaks, Boneless (UPC 1421) were cut 2.54 cm thick and perpendicular to muscle fiber orientation.

Statistical analysis

The experiment was planned as a completely randomized design. Data were analyzed, by subprimal, using SAS (SAS Institute, Inc., Cary, NC) PROC GLM with quality grade tested as the main effect. Least squares means were generated, and when an alpha-level of $P < 0.05$ was found, least squares means were separated with the PDIF option.

Results & Discussion

Retail yields and processing times for the rib and loin subprimals cut are reported in Tables 2-11. These cutting tests will be useful to beef merchandisers in making informed purchase and cutting decisions to optimize value of closely-trimmed beef subprimals. Having standardized cutting tests, including time requirements to perform various tasks,

allow the beef industry to have benchmark information not previously available in the public domain.

Roast percentage and total saleable yield was very similar between U.S. Select and U.S. Choice bone-in ribeye rolls cut into roasts (IMPS #109E) (Table 2). U.S. Choice bone-in ribeye rolls portioned into steaks produced a greater amount of trimmed fat and purge than the U.S. Select bone-in ribeyes resulting in cutting and total time to be significantly higher in U.S. Choice bone-in ribeye rolls (Table 3). Total saleable yield was higher in the initial cutting style fabricated into roasts (95%) compared to subprimals cut into steaks (90-95%).

Table 2
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Rib, Ribeye Roll, Lip-On, Bone In (IMPS #109E), cut into roasts from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		7.80	8.33	0.20	0.11
<i>Retail yield</i>		% —————			
Ribeye roast, lip-on, bone in	1193				
6 th – 7 th rib roast		24.20	25.74	0.57	0.09
8 th – 9 th rib roast		27.06	27.27	0.52	0.78
10 th – 12 th rib roast		43.62	42.03	0.66	0.12
Lean trimmings (90% lean)	1653	0.75	0.47	0.31	0.54
Fat		4.09	4.32	0.65	0.81
Purge		0.29	0.19	0.08	0.42
Cutting loss		0.01	0.00	0.03	0.63
Total saleable yield		95.62	95.51	0.63	0.90
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		11.4	17.8	1.2	0.004
Trimming/cutting time		120.7	126.0	24.1	0.88
Total time		132.1	143.9	24.3	0.74

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

U.S. Choice boneless ribeye rolls (0 × 0) (IMPS #112) displayed a slight increase in the amount of fat produced, as well as the time necessary to cut boneless ribeye steaks and U.S. Select ribeye rolls produced a higher percentage of trimmed ribeye steaks (Table 4). U.S. Select boneless ribeye rolls (2 × 2) (5.08 × 5.08) (IMPS #112A) cut only into steaks required a significantly longer cutting time, thus resulting in a significantly longer total processing time (Table 5) when compared to U.S. Choice ribeye rolls. U.S. Choice ribeye rolls tended to be fatter and U.S. Select ribeye rolls produced a higher percentage of total saleable product (Table 5 and 6). In the second cutting style, including steaks and roasts, total saleable yield was very similar between grade groups (Table 6). When comparing cutting styles, a greater percentage of fat and lean trim was produced from the cutting style containing all steaks.

Table 3
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Rib, Ribeye Roll, Lip-On, Bone In (IMPS #109E), cut into steaks, from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		7.50	6.40	0.78	0.5
<i>Retail yield</i>		% —————			
Ribeye steak, lip-on, bone in	1197	87.52	90.13	0.63	0.08
Lean trimmings (90% lean)	1653	2.99	2.35	0.64	0.50
Fat		8.08	6.33	0.84	0.18
Purge		0.28	0.17	0.09	0.41
Cutting loss		1.12	0.96	0.07	0.13
Total saleable yield		90.51	92.48	0.86	0.14
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		12.8	12.7	1.6	0.95
Trimming/cutting time		319.8	264.4	16.9	0.05
Total time		322.7	277.1	17.3	0.05

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

Table 4
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Rib, Ribeye Roll (IMPS #112), from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		4.44	3.67	0.32	0.23
<i>Retail yield</i>		% —————			
Ribeye steak	1209	95.18	97.24	1.02	0.29
Lean trimmings (90% lean)	1653	1.88	0.83	1.03	0.55
Fat		1.81	1.31	0.29	0.34
Purge		1.19	0.68	0.45	0.51
Cutting Loss		0.00	0.00	0.00	0.00
Total saleable yield		97.06	98.07	0.24	0.10
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		8.1	6.6	1.0	0.42
Trimming/cutting time		102.1	69.0	7.3	0.08
Total time		110.2	75.6	8.2	0.10

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

Table 5
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Rib, Ribeye Roll, Lip-On (IMPS #112A) cut to include steaks from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		6.34	6.85	0.21	0.11
<i>Retail yield</i>		% —————			
Ribeye steak, lip on, boneless	1203	84.08	84.91	0.76	0.44
Lean trimmings (90% lean)	1653	3.78	4.28	0.47	0.47
Fat		11.13	9.89	0.89	0.33
Purge		0.95	0.84	0.23	0.72
Cutting loss		0.06	0.11	0.06	0.55
Total saleable yield		87.87	89.19	0.91	0.32
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		11.7	11.3	1.2	0.85
Trimming/cutting time		221.2	272.3	16.5	0.05
Total time		232.7	283.6	16.3	0.05

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

Table 6
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Rib, Ribeye Roll, Lip-On (IMPS #112A), cut into steaks and roasts from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		7.21	6.61	0.25	0.12
<i>Retail yield</i>		% —————			
Ribeye steak, lip-on, boneless	1203	43.54	49.08	2.96	0.21
Ribeye roast, lip-on, boneless	1194	44.19	38.00	2.66	0.12
Lean trimmings (90% lean)	1653	2.97	3.89	0.46	0.18
Fat		8.47	8.02	0.91	0.73
Purge		0.80	0.89	0.21	0.78
Cutting loss		0.03	0.13	0.08	0.39
Total saleable yield		90.70	90.97	0.92	0.83
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		11.8	10.9	1.1	0.61
Trimming/cutting time		198.4	213.6	22.0	0.63
Total time		210.2	224.5	21.2	0.63

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

U.S. Choice boneless ribeye rolls (1 × 1) (2.54 × 2.54) (IMPS #112A modified) required significantly more fat trimming than U.S. Select ribeye rolls (Table 7). U.S. Select ribeye rolls also produced a greater amount of purge, as well as 3.2 % more ribeye steaks.

U.S. Select bone-in strip loins (IMPS #175) tended to have a greater percentage of center top loin steaks and consequently a higher percentage of saleable yield when compared to U.S. Choice bone-in strip loins (Table 8). Retail yields and processing times for boneless strip loins (IMPS #180) are presented in Table 9. U.S. Select strip loins had a significantly greater percentage of purge loss when compared to the U.S. Choice strip loins. The retail cutting percentages are relatively similar between U.S. Choice and U.S. Select grade strip loins; however, there does appear to be a slight increase in the percentage of U.S. Select lean trim.

U.S. Choice center-cut top butts had a greater percentage of trimmable fat ($P < 0.04$), as well as a greater amount of lean trim, thus resulting in a requirement for a longer cutting time (Table 10). U.S. Select center-cut top butts also tended to produce a slightly higher percentage of steaks thus resulting in a higher saleable yield. U.S. Choice top sirloin caps (IMPS #184D) had a higher percentage of steaks when compared to the U.S. Select top sirloin caps (Table 11). U.S. Select top sirloin caps possessed a higher percentage of purge loss when compared to their U.S. Choice counterparts. Saleable yield for U.S. Choice top sirloin caps (98%) was higher than Weatherly et al. (2001) findings, which reported yields to be between 94 and 96%.

Table 7
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Rib, Ribeye Roll, Lip-On, Modified 1 × 1 (2.54 × 2.54 cm) (IMPS #112A modified), from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		4.26	6.25	2.19	0.26
<i>Retail yield</i>		% —————			
Ribeye steak, lip-on, boneless	1203	40.98	44.18	2.16	0.37
Ribeye roast, lip-on, boneless	1194	47.11	45.97	2.41	0.75
Lean trimmings (90% lean)	1653	2.51	2.28	1.14	0.89
Fat		9.40	6.33	0.72	0.02
Purge		0.00	1.06	0.95	0.41
Cutting loss		0.22	0.19	0.14	0.90
Total saleable yield		90.60	92.43	1.40	0.42
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		7.3	11.9	0.3	0.01
Trimming/cutting time		140.1	149.3	15.8	0.70
Total time		147.4	161.2	15.7	0.57

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

Table 8
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Loin, Strip Loin (IMPS #175) from different USDA quality grades

Item	UPC ^a	U.S. Choice	U.S. Select	SEM ^b	P-value
		(n=6)	(n=6)		
Net weight, kg		6.36	6.00	0.60	0.38
<i>Retail yield</i>		% -----			
Top loin steak, bone in (center)	1398	60.94	66.75	2.40	0.14
Top loin steak, bone in (vein) ^c	1398	17.62	15.44	1.53	0.35
Lean trimmings (90% lean)	1653	4.36	3.62	0.54	0.37
Fat		12.45	12.22	1.28	0.91
Purge		0.37	0.29	0.11	0.62
Cutting loss		1.36	1.25	0.11	0.48
Total saleable yield		82.93	85.81	1.60	0.25
<i>Processing time, per subprimal</i>		s -----			
Bag opening time		13.5	12.8	2.4	0.84
Trimming/cutting time		435.8	427.1	12.5	0.64
Total time		449.3	440.0	13.8	0.65

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

^c Steaks with the *M. gluteus medius* present on both cut surfaces.

Table 9
Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Loin, Strip Loin, Boneless (IMPS #180) from different USDA quality grades

Item	UPC ^a	U.S. Choice	U.S. Select	SEM ^b	P-value
		(n=6)	(n=6)		
Net weight, kg		4.87	4.71	0.32	0.73
<i>Retail yield</i>		% -----			
Top loin steak, boneless (center)	1404	67.39	66.88	1.43	0.81
Top loin steak, boneless (vein) ^c	1404	17.92	17.04	1.58	0.70
Lean trimmings (90% lean)	1653	1.88	2.35	0.42	0.50
Fat		11.26	11.21	0.89	0.97
Purge		1.50	2.36	0.08	<0.001
Cutting loss		0.04	0.16	0.08	0.30
Total saleable yield		87.20	86.27	0.82	0.45
<i>Processing time, per subprimal</i>		s -----			
Bag opening time		8.5	8.5	0.6	0.99
Trimming/cutting time		223.2	227.2	9.9	0.78
Total time		231.7	235.7	10.1	0.79

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

^c Steaks with the *M. gluteus medius* present on both cut surfaces

Table 10

Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Loin, Top Sirloin Butt, Center-Cut, Boneless, (IM) (IMPS #184B) from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		3.65	4.21	0.07	0.04
<i>Retail yield</i>		% —————			
Top sirloin steak, boneless, cap off	1426	79.39	82.72	1.41	0.17
Lean trimmings (90% lean)	1653	10.26	9.32	0.96	0.53
Fat		8.82	6.03	0.68	0.04
Purge		0.12	0.25	0.04	0.14
Cutting loss		0.00	0.00	0.01	0.86
Total saleable yield		89.65	92.05	1.21	0.24
<i>Processing time, per subprimal</i>		s —————			
Trimming/cutting time		147.7	133.2	11.8	0.43

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

Table 11

Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Loin, Top Sirloin, Cap (IM) (IMPS #184D) from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		0.66	0.82	0.06	0.08
<i>Retail yield</i>		% —————			
Top sirloin cap steak, boneless	1421	75.06	73.23	1.98	0.55
Lean trimmings (90% lean)	1653	23.17	24.84	2.14	0.61
Fat		0.00	0.00	0.00	-----
Purge		1.56	2.66	0.53	0.24
Cutting loss		0.22	0.23	0.44	0.98
Total saleable yield		98.22	98.07	0.80	0.90
<i>Processing time, per subprimal</i>		s —————			
Trimming/cutting time		9.5	9.3	1.3	0.90

^a UPC = Universal product code.

^b SEM is the standard error of the least squares means.

Conclusions

In addition to providing these cutting tests for comparative purposes, there are several key points to be made based on our findings. USDA quality grade had little or no effect on retail cutting yields and processing times for subprimals from the rib and loin. Some historical differences in retail yield between USDA quality grades may have been due to the differences in trimmable fat now being removed at the packer level. Therefore, beef merchandisers may choose to utilize either U.S. Choice or U.S. Select rib and loin subprimals based on parameters other than yield.

Even though many of the cutting tests revealed relatively high retail yields (ranging from 80.27% to 98.22%), there still are missed yield opportunities because of fat trim, purge and cutting losses. To further increase retail yields from the rib and loin subprimals, efforts can not be focused exclusively on reducing fat trim specifications, but may need to include methods of minimizing purge and increasing cutting efficiencies. Retail yields for a class of subprimals are based on two key factors: purchase specifications and merchandising schemes. Purchase specifications allow retailers to select from a variety of products to find those that closely match how products should be merchandised based on historic consumer preference and seasonal demand. Evidence of how purchase specification and merchandising scheme impacts retail yields is best demonstrated by our ribeye cutting information.

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RETAIL CUTTING CHARACTERISTICS FOR CHUCK AND ROUND SUBPRIMALS FROM TWO GRADE GROUPS

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Introduction

Merchandising chuck and round subprimals can often be challenging and require reduced prices. This creates a problem because these subprimals represent about 38% of the weight of an average beef carcass (Griffin, Savell, Morgan, Garrett, & Cross, 1992). Through efforts to optimize value from these areas, it has been found that merchandising single-muscle cuts allows the industry to provide a more consistent, higher quality product to consumers (NCBA, 2001). With the shift of consumers toward smaller, single-muscle cuts, McKenna, Griffin, Johnson, Covington, and Savell (2003) found that percentage retail yields decreased and processing times increased with this type of cutting style. In addition, Beef Value Cuts have been shown to have desirable characteristics in terms of palatability and visual appeal, but there is a lack of standardized information regarding cut out yields and labor requirements of subprimals fabricated to this endpoint (McKenna et al., 2003).

In order for retailers to evaluate the price/value relationship of beef subprimals, the CARDS (Computer Assisted Retail Decision Support) software was developed (Garrett et al., 1991). This program serves as a valuable reference to assist retailers in the process of making decisions regarding meat purchasing and merchandising. This project was designed to evaluate cuts not previously included for the program and to update the Beef CARDS database with these cuts. Emphasis was put on providing the retail industry of processing yields and time allocations in the expanded database. Because of the extensive number of cutting tests conducted and the need to narrow the scope to be reported, the information presented herein encompasses the chuck and round cuts.

Objectives

The objective of this study was to perform a thorough evaluation of chuck and round cuts included in the present version of Beef CARDS in order to pinpoint deficiencies and/or inconsistencies in the data, while obtaining current yield and time data for a new updated list of subprimals. This should allow for improvement and expansion of the existing Beef CARDS program for the benefit of the retail and foodservice industry.

Methodology

Product Selection

Beef subprimals (n = 116) from the chuck and round (Table 1), representing USDA Choice and Select grades, were obtained from a major beef processor and shipped to the Rosenthal Meat Science and Technology Center at Texas A&M University. Specifications for all subprimals complied (within packer variations) with Institutional Meat Purchase Specifications (IMPS) as described by USDA (1996) and NAMP (2003).

Table 1
USDA (1996) Institutional Meat Purchase Specifications (IMPS) descriptions of chuck and round subprimals used for retail cutting tests

IMPS #	Subprimal
114C	Beef Chuck, Shoulder Clod, Trimmed
114D	Beef Chuck, Shoulder Clod, Top Blade, Roast
116A	Beef Chuck, Chuck Roll
168	Beef Round, Top (Inside)
171B	Beef Round, Outside Round (Flat)
171C	Beef Round, Eye of Round (IM ^a)

^a IM = Individual muscle.

Cutting tests

Controlled retail cutting tests were conducted as described in Voges (2004). A refrigerated cutting room in the Rosenthal Meat Science and Technology Center was modified to simulate a retail market environment, and experienced meat merchandisers were enlisted to perform cutting yield tests. Universal Product Code (UPC) descriptions (Industry-Wide Cooperative Meat Identification Standards Committee, 2003) were used as the naming convention for retail cuts.

Trimmed shoulder clods (IMPS #114C) were cut initially by removing accessory muscles from the *Mm. triceps brachii* and converting them into Beef for Stew (UPC 1727). The *M. triceps brachii caput longum* then was cut into 2.54 cm Shoulder Center Steaks (UPC 1162) and the *M. triceps brachii caput laterale* was cut into 2.54 cm Shoulder Top Steaks (UPC 1163). Top blade roasts (IMPS #114D) were trimmed of all fat and connective tissue. The *M. infraspinatus* was filleted horizontally into two separate flat pieces with the heavy connective tissue removed before portioning into Shoulder Top Blade Steaks (UPC 1166).

Chuck rolls (IMPS #116A) were cut initially by removing the *M. trapezius* and *M. latissimus dorsi*. The *M. serratus ventralis* was removed and designated as a Chuck Eye Edge Pot Roast (UPC 1092). The remaining pieces of the *M. serratus ventralis* were cut into boneless short ribs. Chuck steaks then were cut from the posterior end of the

remaining chuck roll section until seam fat was no longer present between the *M. longissimus thoracis* and the *M. rhomboideus thoracis*. Chuck Eye Steaks (UPC 1102), were separated from the Underblade Steaks, Boneless (UPC 1158). Chuck Eye Roasts (UPC 1095) then were cut into 5.08 cm portions from the remainder of the chuck roll. The remaining anterior end of the chuck roll was separated into Beef for Stew (UPC 1727) or Lean Trimmings (UPC 1653).

For the Top (Inside) Rounds, Untrimmed (IMPS #168), the *M. gracilis*, *M. pectineus*, and *M. sartorius* were removed and portioned into pieces for Beef Round for Cubed Steak (UPC 1577). The Top Round Steak, 1st Cut (UPC 1556) was cut 3.81 cm thick from the proximal edge of the *M. semimembranosus* and *M. adductor*. Subsequent Top Round Steaks (UPC 1553) were cut 1.27 cm thick until the remaining distal portion was deemed not suitable for steaks. This portion, after trimming, was merchandised as a Top Round Roast, Cap Off (UPC 1454).

Outside rounds (flat) (IMPS #171B) were cut two ways. The initial cutting style consisted of removing the ishiatic head of the *M. gluteobiceps*, trimming all heavy connective tissue, and preparing it as a Bottom Round Roast (UPC 1464). The remainder of the *M. gluteobiceps* was portioned into 3.81 cm Bottom Round Steaks (UPC 1466) by cutting perpendicular to the muscle fiber orientation with remaining product merchandised as material for Beef Round for Cubed Steak (UPC 1577). The second style consisted of removal of the ishiatic head and the distal portion of the *M. gluteobiceps* producing two Bottom Round Roasts (UPC 1464). Two or three subsequent 3.81 cm Bottom Round Steaks (UPC 1466) were cut, and the remaining proximal portion of the *M. gluteobiceps* was designated as a Bottom Round Rump Roast (UPC 1519).

Eye of rounds (IM) (IMPS #171C) were cut three ways. Initially, all styles were trimmed practically free of fat and connective tissue. The first style consisted of cutting the *M. semitendinosus* in half with one portion cut into 1.27 cm to 1.91 cm Eye of Round Steaks (UPC 1481) and the other left intact as an Eye of Round Roast (UPC 1480). In the second style, the subprimal was cut in half to make two Eye of Round Roasts (UPC 1480). The third style merchandised the entire muscle as an Eye of Round Roast (UPC 1480).

Statistical analysis

The experiment was planned as a completely randomized design. Data were analyzed, by subprimal, using SAS (SAS Institute, Inc., Cary, NC) PROC GLM with quality grade tested as the main effect. Least squares means were generated, and when an alpha-level of $P < 0.05$ was found, least squares means were separated with the PDIFF option.

Results & Discussion

Retail yields and processing times for the chuck and round subprimal cut are reported in Tables 2-9. These cutting tests will be useful to beef merchandisers in making informed purchase and cutting decisions to optimize value of closely-trimmed beef subprimals. Having standardized cutting tests and associated time requirements allows the beef industry to have benchmark information not previously available.

U.S. Select shoulder clods (IMPS #114C) had a higher percentage of shoulder top steaks ($P < 0.01$) and boneless shoulder pot roasts ($P < 0.03$), thus allowing them to

produce a higher percentage (3.5%) of total saleable yield similar to the findings of Garrett et al. (1991) and McKenna et al. (2003). The U.S. Choice shoulder clods possessed more trimmable fat, and required a longer amount of time to process (Table 2). Retail yields for shoulder clods were higher (85-88%) than those found by McKenna et al. (2003) (73-78%), but lower than the retail yield reported by Garrett et al. (1991) using a traditional fabrication style.

Table 2. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Chuck, Shoulder Clod, Trimmed (IMPS #114C), from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=9)	U.S. Select (n=9)	SEM	P-value
Net weight, kg		5.92	5.60	0.35	0.53
<i>Retail yield</i>		% —————			
Shoulder center steak	1162	19.80	16.82	1.12	0.11
Shoulder top steak	1163	8.87	14.53	1.12	0.01
Shoulder pot roast, boneless	1132	5.52	10.69	1.4	0.03
Beef for stew	1727	23.60	23.54	0.67	0.95
Lean trimmings (90% lean)	1653	27.91	30.37	1.31	0.22
Fat		13.76	11.93	1.35	0.37
Purge		0.89	0.76	0.21	0.68
Cutting loss		0.10	0.00	0.11	0.30
Total saleable yield		85.32	88.82	1.69	0.19
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		8.9	7.8	0.8	0.36
Trimming/cutting time		514.7	500.2	37.1	0.79
Total time		523.6	508.0	37.2	0.78

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

Contrary to the shoulder clod, U.S. Select top blade roasts (IMPS #114D) yielded a greater percentage of fat, while U.S. Choice top blade roasts produced a greater amount of purge (Table 3). No difference ($P < 0.05$) was found in saleable yield between U.S. Choice and U.S. Select supporting McKenna et al. (2003) findings. McKenna et al. (2003) also reported slightly higher saleable yields, mainly due to the increase of fat in the present study.

Table 3. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Chuck, Shoulder Clod, Top Blade, Roast (IMPS #114D), from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=9)	U.S. Select (n=9)	SEM ^b	P-value
Net weight, kg		2.25	2.12	0.15	0.57
<i>Retail yield</i>		% —————			
Shoulder top blade steak (flat iron)	1166	50.55	48.86	1.58	0.46
Lean trimmings (90% lean)	1653	31.43	32.01	1.67	0.81
Fat		17.35	18.81	1.25	0.42
Purge		0.71	0.40	0.20	0.29
Cutting loss		0.00	0.00	0.07	0.60
Total saleable yield		81.98	80.87	1.21	0.53
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		7.0	7.1	0.6	0.83
Trimming/cutting time		280.3	262.7	25.5	0.63
Total time		287.3	269.8	25.5	0.63

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

U.S. Choice chuck rolls (IMPS #116A) tended to possess a greater percentage of underblade steaks and fat. U.S. Select chuck rolls had higher yield percentages for lean trimmings and beef for stew when compared to U.S. Choice chuck rolls (not in tabular form).

U.S. Choice inside rounds (IMPS #168) had a higher percentage of fat ($P < 0.001$), thus resulting in a greater amount of cutting ($P < 0.03$) and total time ($P < 0.04$) required when compared to U.S. Select inside rounds. U.S. Select inside rounds displayed a significantly higher percentage of roasts and purge when compared with U.S. Choice rounds (Table 4).

Table 4. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Round, Top (Inside) (IMPS #168), from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=9)	U.S. Select (n=9)	SEM ^b	P-value
Net weight, kg		11.0	9.10	0.48	0.01
<i>Retail yield</i>		----- % -----			
Top round roast, cap off	1454	23.79	28.92	1.32	0.02
Top round steak	1553	21.11	20.81	0.99	0.83
Top round steak, 1 st cut (London Broil)	1556	7.09	8.29	1.38	0.55
Cap		6.38	7.44	0.40	0.08
Beef round for cubed steak	1577	5.05	6.73	0.66	0.09
Lean trimmings (90% lean)	1653	16.71	15.15	0.90	0.24
Fat		18.93	10.94	1.09	<0.001
Purge		0.90	1.75	0.22	0.02
Cutting loss		0.04	0.00	0.04	0.24
Total saleable yield		80.13	87.34	1.13	0.004
<i>Processing time, per subprimal</i>		----- s -----			
Bag opening time		11.4	15.7	1.4	0.04
Trimming/cutting time		606.0	509.5	28.7	0.03
Total time		617.3	525.2	28.8	0.04

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

Retail yield cutting percentages and times for the initial cutting style of outside rounds (IMPS #171B) consisting of steaks, bottom round roasts, and cubed steaks are reported in Table 5. U.S. Select outside rounds displayed a higher percentage of steaks ($P < 0.02$) and U.S. Choice outside rounds had a three-percentage points decrease ($P < 0.05$) in saleable yield than U.S. Select outside rounds, with most of the difference accounted for by more ($P < 0.05$) trimmable fat when compared to U.S. Choice rounds. Additionally, purge and cutting loss was significantly greater for U.S. Select rounds preventing an even larger difference in saleable yield when compared to U.S. Choice rounds. The second outside round cutting style including steaks, rump roasts, and bottom round roasts, displayed significant differences between U.S. Choice and U.S. Select (Table 6), with U.S. Select outside rounds yielded a higher percentage of bottom round roasts ($P < 0.001$) and having greater amount of purge loss when compared with U.S. Choice rounds. The U.S. Choice outside rounds had a significantly higher percentage of lean trim and trimmable fat, and required a greater amount of time for cutting ($P < 0.03$) and total time ($P < 0.04$). The total saleable yield is very similar to the data found by Garrett et al. (1991) (92%) and by McKenna et al. (2003) (91%). The second cutting style had a saleable yield of 89-92%, which is greater than the initial cutting style's saleable yields of 87-90%. This is most likely due to the greater amount of fat trim in the initial style. Less lean trim between styles primarily caused the second cutting style to produce a

greater percentage (78-86%) of roasts and steaks when compared to the initial cutting style (65-73%).

Table 5. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Round, Outside Round (IMPS #171B) cut to include roasts, steaks, and cubed steak from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		5.60	6.44	0.22	0.02
<i>Retail yield</i>		% -----			
Bottom round steak	1466	47.05	49.88	0.72	0.02
Bottom round roast	1464	12.33	13.33	0.55	0.22
Beef round for cubed steak	1577	5.89	9.87	1.81	0.14
Lean trimmings (90% lean)	1653	22.34	17.20	2.13	0.11
Fat		10.82	6.03	0.73	0.001
Purge		1.44	3.38	0.51	0.02
Cutting loss		0.13	0.31	0.04	0.01
Total saleable yield		87.61	90.28	0.84	0.05
<i>Processing time, per subprimal</i>		s -----			
Bag opening time		14.3	12.8	1.0	0.30
Trimming/cutting time		405.8	306.1	33.4	0.06
Total time		420.1	318.9	33.9	0.06

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

Table 6. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Round, Outside Round (IMPS #171B) cut to include steaks and roasts from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		5.86	6.27	0.37	0.44
<i>Retail yield</i>		% -----			
Bottom round steak	1466	6.88	7.59	1.41	0.72
Bottom round rump roast	1519	30.83	31.58	0.78	0.50
Bottom round roast	1464	40.48	46.83	0.95	<0.001
Lean trimmings (90% lean)	1653	11.31	6.09	1.15	0.01
Fat		8.73	4.33	1.08	0.02
Purge		1.54	3.57	0.53	0.02
Cutting loss		0.25	0.007	0.09	0.09
Total saleable yield		89.49	92.09	1.22	0.16
<i>Processing time, per subprimal</i>		s -----			
Bag opening time		10.9	12.8	15.0	1.87

Trimming/cutting time	337.3	240.4	27.3	0.03
Total time	348.3	255.4	27.7	0.04

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

U.S. Select eye of rounds (IMPS #171C), cut to include steaks and a roast, had a significantly larger percentage of purge loss when compared to U.S. Choice eye of rounds that displayed a significantly larger cutting loss percentage (Table 7). U.S. Choice eye of rounds cut to include two roasts appeared to yield a higher percentage of roasts when compared to fatter U.S. Select eye of rounds (Table 8). Retail yields and processing times for eye of rounds left as intact roasts are presented in Table 9. U.S. Choice eye of rounds tended to have a greater percentage of roast weight thus resulting in a higher percentage of total saleable product when compared to fatter U.S. Select eye of rounds. U.S. Select eye of rounds had a higher percentage of trimmable fat. The initial cutting style of steaks and roast produced a greater percentage of lean trim and required a longer processing time when compared with the cutting styles containing only roasts. McNeill et al. (1998) and Weatherly et al. (2001) found similar results, observing an increase in total processing time as the number of retail cuts from subprimals increased.

Table 7. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Round, Eye of Round (IMPS #171C) cut to include steaks and a roast from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		2.60	2.99	.037	0.14
<i>Retail yield</i>		% —————			
Eye of round steak	1481	38.84	37.27	1.78	0.55
Eye of round roast	1480	52.65	52.62	2.26	0.99
Lean trimmings (90% lean)	1653	3.56	3.80	0.63	0.80
Fat		3.63	4.49	1.02	0.57
Purge		0.87	1.72	0.17	0.006
Cutting loss		0.44	0.10	0.06	0.002
Total saleable yield		95.06	93.69	1.07	0.39
<i>Processing time, per subprimal</i>		s —————			
Bag Opening time		9.16	7.74	0.89	0.29
Trimming/cutting time		100.41	90.81	10.77	0.54
Total time		109.57	98.55	11.25	0.51

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

Table 8. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Round, Eye of Round (IMPS #171C) cut into two roasts from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		2.35	2.47	0.10	0.38
<i>Retail yield</i>		% —————			
Eye of round roast	1480	93.50	92.61	0.87	0.49
Lean trimmings (90% lean)	1653	1.41	2.76	0.58	0.20
Fat		3.84	4.48	0.85	0.61
Purge		1.63	1.74	0.34	0.83
Cutting loss		0.45	0.08	0.16	0.20
Total saleable yield		93.31	92.17	1.15	0.54
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		6.96	6.33	0.66	0.52
Trimming/cutting time		55.32	59.16	7.27	0.72
Total time		62.28	65.49	7.64	0.77

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

Table 9. Least squares means of retail yields (%) and processing times (s) for fabrication of Beef Round, Eye of Round (IMPS #171C) cut into one roast from different USDA quality grades

Item	UPC ^a	U.S. Choice (n=6)	U.S. Select (n=6)	SEM ^b	P-value
Net weight, kg		2.31	2.47	0.32	0.52
<i>Retail yield</i>		% —————			
Eye of round roast	1480	92.01	89.76	1.63	0.36
Lean trimmings (90% lean)	1653	2.52	2.24	0.73	0.79
Fat		4.03	6.84	1.33	0.17
Purge		1.24	1.39	0.29	0.70
Cutting loss		0.20	0.09	0.13	0.56
Total saleable yield		94.53	91.16	1.36	0.12
<i>Processing time, per subprimal</i>		s —————			
Bag opening time		6.51	6.89	0.28	0.55
Trimming/cutting time		74.18	67.07	9.65	0.61
Total time		80.69	73.93	9.90	0.64

^aUPC = Universal product code.

^bSEM is the standard error of the least squares means.

Conclusions

In addition to providing these cutting tests for comparative purposes, there are several key points to be made based on our findings. Only total saleable yields of the inside

round and outside round were affected by USDA quality grade, where U.S. Select rounds had higher ($P < 0.05$) yields than U.S. Choice rounds. This difference was driven by a higher percentage of fat trimmed from the U.S. Choice rounds compared to the U.S. Select rounds. Voges (2004) found little or no effect for USDA quality grade on total saleable yield from the rib and loin subprimals.

It was also found that several U.S. Select subprimals had less fat than the same U.S. Choice cuts and greater purge losses. Specifically, greater ($P < 0.05$) purge losses were found for the U.S. Select inside round, outside round, and eye of round compared to U.S. Choice round subprimals.

Finally, a challenge that retailers face when merchandising cuts from the round and chuck is the variety of retail cuts that are generated, such as steaks and roasts, beef for stew, lean trimmings, etc. This variety could cause processing times to be quite long, which could add to the labor requirements. This elevated requirement is often reflected in increased retail prices or limited merchandising options.

These cutting test data will be used to update the Beef CARDS software program. By incorporating new information into this dynamic decision-making program, users will be able to evaluate pricing and labor costs to determine how purchase and merchandising factors affect profitability.

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AN ALTERNATIVE CARCASS FABRICATION METHOD TO OPTIMIZE BEEF VALUE

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Introduction

Traditionally, the beef industry has changed little to manipulate the fabrication of beef carcasses into more efficiently used primals and subprimals. In the past, most of the breaking points had been done out of tradition rather than optimization of the muscles. Cuts even as major as the separation of the chuck and round from the rib and loin should be questioned since multiple muscles and muscle groups are bisected. With current retail trends moving towards merchandising single muscles and/or muscle groups, there is a growing need to better utilize beef carcasses.

With this trend in mind, the Beef Value Cuts Program (NCBA, 2001) and the Muscle Profiling and Bovine Myology studies (Jones, Burson, & Calkins, 2001) began. The goal of these studies was to increase utilization of individual muscles instead of marketing beef in the traditional multi-muscle cuts. Using these concepts, we used an innovative fabrication method to improve yields and value of beef carcasses.

Objectives

To examine alternative styles of carcass fabrication and how they would: (1) impact subprimal and total yields, and (2) impact the overall carcass value.

Methodology

Carcass selection

Beef carcasses (n = 30) were selected from a commercial packing facility and transported to Texas A&M University for subsequent fabrication. Carcasses were selected by trained evaluators to obtain an equal mix of USDA (1997) Choice and Select, yield grade 2 and 3 carcasses. Additional criteria included: sex (steer), approximate weight range (325 to 390 kg), and minimal slaughter/dressing defects (e.g., incorrect carcass splits, major fat tears, large bruises, excess trimming of lean and/or fat).

Carcass fabrication

Carcasses were separated into beef quarters and transported by refrigerated carrier. Comparisons were made by fabricating one side of each carcass in a conventional manner, whereas the opposite side was fabricated by an innovative method.

Conventional style

Carcasses were fabricated into boneless and semi-boneless, closely-trimmed subprimal cuts by Texas A&M personnel. Carcasses were fabricated to produce cuts (Table 1) following the Institutional Meat Purchase Specifications (IMPS) described by NAMP (2003) and USDA (1996).

Table 1. USDA (1996) Institutional Meat Purchase Specifications (IMPS) descriptions of subprimals used in conventional fabrication

IMPS #	Subprimal
112A	Beef Rib, Ribeye, Lip-On ^a
114	Beef Chuck, Shoulder Clod
116	Beef Chuck, Chuck Roll
120	Beef Brisket, Deckle-Off, Boneless
121C	Beef Plate, Outside Skirt (IM) ^b
121D	Beef Plate, Inside Skirt (IM) ^b
124	Beef Rib, Back Ribs
130	Beef Chuck, Short Ribs
167A	Beef Round, Tip (Knuckle), Peeled
168	Beef Round, Top (Inside), Untrimmed
171B	Beef Round, Outside Round (Flat)
171C	Beef Round, Eye of Round (IM) ^b
180A	Beef Loin, Strip Loin, Boneless (1 × 0) ^c
184	Beef Loin, Top Sirloin Butt, Boneless
185A	Beef Loin, Bottom Sirloin Butt, Flap, Boneless (IM) ^b
185B	Beef Loin, Bottom Sirloin Butt, Ball Tip, Boneless
185C	Beef Loin, Bottom Sirloin Butt, Tri-Tip, Boneless (IM) ^b
189A	Beef Loin, Tenderloin, Full, Side Muscle On, Defatted
193	Beef Flank, Flank Steak (IM) ^b

^a Lip = *M. serratus dorsalis*, *M. longissimus costarum* and related intermuscular fat lateral to the *M. longissimus thoracis* (USDA, 1996).

^b IM = Individual muscle.

^c (1 × 0) = The flank side shall be lateral to, but not more than 2.54 cm from, the *M. longissimus lumborum* at the rib end to a point on the sirloin end immediately lateral to the *M. longissimus lumborum* (USDA, 1996).

Additionally, special trim items including cap and wedge meat, chuck tender, pectoral muscle and flank muscle were removed intact from each carcass. Finally, lean trimmings (approximately 80% lean determined visually), fat, and bone were obtained from each carcass. Further details of conventional fabrication can be found in Pfeiffer (2004).

Innovative style

The following describes the fabrication of the innovative forequarter, which was initiated with the quarter hung on the rail. The inside and outside skirt muscles were removed, and all major connective tissue and fat was trimmed in preparation. The *M. rhomboideus*, *M. trapezius*, and the *M. latissimus dorsi* were removed, leaving the *M. serratus ventralis* exposed. Collectively, the muscles were trimmed practically free of fat and weighed as blade meat. The shoulder was removed through the natural seam. The *M. serratus ventralis* then was removed intact from the carcass. The thoracic limb was hung by the foreshank and a subprimal similar to the IMPS #114 Outside Shoulder Clod was removed and trimmed practically free of fat. The IMPS #116B Chuck (Mock) Tender was fabricated by removal from the scapula. Innovative brisket fabrication included removal of the *M. pectoralis profundus* from the ventral edge of the forequarter. The deckle and hard fat, along the ventral edge, were trimmed flush with the lean surface.

The rib/chuck separation was made by a saw cut between the fourth and fifth ribs, instead of the conventional fifth/sixth rib separation. The rib/plate separation was made by a saw cut 10.16 cm from the ventral edge of the *M. longissimus thoracis* on the anterior end and 7.62 cm from the ventral edge of the *M. longissimus thoracis* on the posterior end. The back ribs (similar to the IMPS #124 Back Ribs) were removed. The tail was reduced to 2.54 cm on both ends. A saw cut between the fifth and sixth cervical vertebra was made to separate the neck from the chuck. The sternum and associated ribs were separated from the chuck by a saw cut immediately ventral to the vertebral column. All bones and *ligamentum nuchae* were removed in fabrication of the chuck roll, and the tail was reduced to 2.54 cm on both ends, creating a subprimal similar to the IMPS #116D Chuck Eye Roll. The remaining neck, plate, foreshank, and rib portions were separated into lean trimmings, excess fat, and bone components.

The following describes the fabrication of the innovative hindquarter which was initiated with the quarter hung on the rail. The IMPS #193 Flank Steak was fabricated by removal from carcass and trimming all fat and heavy connective tissue. Kidney and pelvic fat then was removed. The *M. tensor fasciae latae* was removed and trimmed practically free of fat to create the IMPS #185C Bottom Sirloin Butt, Tri-Tip, Boneless, Defatted. The bottom sirloin flap was removed and trimmed practically free of fat. Starting at the patella, the entire *M. quadriceps femoris* was removed through the natural seam and trimmed of any bone (patella) and fat. The entire *M. gluteobiceps* was removed, as suggested by Reuter, Wulf, and Maddock (2002a), resulting in a subprimal similar to the IMPS #184D Top Sirloin, Cap. The top sirloin cap was completely trimmed of fat and connective tissue. The remainder of the *M. gluteobiceps* was trimmed, and the heavy connective tissue ("silver skin") was completely removed creating a subprimal similar to the IMPS #171B Outside Round (Flat). The IMPS #168 Top (Inside) Round was removed through the natural seam and was trimmed practically free of fat. The IMPS #189A Tenderloin, Full, Side Muscle On, Defatted was fabricated from the full loin. The sirloin-short loin separation was made by a saw cut immediately anterior to the tuber coxae (hip bone) and the IMPS #184B Top Sirloin Butt, Center-Cut, Boneless, Cap Off was fabricated. In preparation of the IMPS #180A Strip Loin, the body of the vertebrae and all other bones were removed from the short loin. In addition, the tail was reduced to 2.54 cm from the ventral edge of the *M. longissimus thoracis* on the anterior end and 0.00 cm from the ventral edge of the *M. longissimus lumborum* on the posterior end. The

hindshank (including the aitch bone) was separated into lean trimmings, excess fat, and bone components.

Carcass value

Data collected during carcass fabrication were used in determination of value differences that may have occurred between cutting styles. Subprimal and component prices used in the analysis were obtained from the United States Department of Agriculture, Agricultural Marketing Service (USDA, 2001; USDA, 2002; USDA, 2003). Subprimal and component prices were averaged over the three-year period to minimize any seasonal or annual price biases.

Many subprimals generated from the innovative side were not identical to that of the conventional style, thus were not identical in terms of IMPS numbers and would most likely be priced independently by the market. For comparative purposes, the innovative brisket, shoulder clod, chuck roll, ribeye roll, back ribs, blade meat, 2-piece top sirloin butt, round tip, and outside round flat from this study were priced identical to their conventional counterparts. The *M. serratus ventralis* was priced using the reported prices for the IMPS # 109B Rib, Blade Meat.

Statistical analysis

Subprimal weights, percentages, and values were analyzed using the MIXED procedure of SAS (Version 9, SAS Institute, Inc., Cary, NC). Models included cutting style and quality grade as main effects, and carcass number was included as a randomized effect. Least squares means were generated, and when an alpha-level of $P < 0.05$ was found, they were separated using the PDIF option.

Results & Discussion

Carcass fabrication

Forequarter wholesale cuts and carcass component percentages were analyzed by cutting style and reported in Table 2. The innovative brisket comprised a greater ($P < 0.001$) percentage of the beef forequarter than the conventional brisket. Inclusion of the pectoral meat with the conventional brisket still resulted in a lighter combined subprimal weight than the innovative brisket.

The conventional shoulder clod was higher ($P < 0.001$) yielding than the innovative shoulder clod. This was expected due to the portions of the *M. trapezius* and *M. latissimus dorsi* that remained on the conventional shoulder clod, but were removed from the innovative shoulder clod and were included as a portion of the blade meat.

Reuter, Wulf, Shanks, and Maddock (2002b) concluded that a rib/chuck separation between the fourth and fifth rib could result in merchandizing four additional ribeye steaks per carcass without decreasing tenderness or consumer acceptance of these steaks. Based on their conclusions, the innovative rib/chuck separation was made between the fourth and fifth ribs instead of the conventional fifth/sixth rib separation. Consequently,

the innovative ribeye represented a greater ($P < 0.001$) percentage of the forequarter, and the innovative chuck roll was a lesser ($P < 0.001$) percentage.

Table 2. Least-squares means for forequarter subprimal and component percentages stratified by conventional (CONV) and innovative (INNOV) cutting styles

Subprimal	CONV	INNOV	SEM ^a	$P > F$
	%			
Brisket	5.77	8.41	0.12	<0.001
Shoulder clod	9.70	8.60	0.08	<0.001
Chuck tender	1.57	1.57	0.02	0.81
Pectoral meat	1.16	-	0.04	-
Chuck roll	8.01	3.02	0.10	<0.001
<i>M. Serratus ventralis</i>	-	4.69	0.09	-
Chuck short rib	1.65	-	0.04	-
Inside skirt	1.22	1.25	0.03	0.29
Outside skirt	0.68	0.68	0.02	1.00
Ribeye roll	5.53	6.19	0.08	<0.001
Back ribs	1.75	1.96	0.03	<0.001
Blade meat	1.85	4.19	0.09	<0.001
Subprimal total	38.86	40.55	0.29	<0.001
Lean trimmings (85% lean)	27.23	26.12	0.35	<0.001
Fat	15.75	15.39	0.47	0.27
Bone	18.16	17.94	0.23	0.27

^aSEM is the standard error of the least-squares mean.

It was a priority to remove the three extrinsic muscles of the forelimb (*M. rhomboideus*, *M. trapezius*, and *M. latissimus dorsi*) in their entirety. Conventionally, these muscles are portioned throughout several subprimals and are primarily merchandized as lean trimmings. The innovative fabrication style optimized the merchandizing potential of these individual muscles by removing them as whole muscles. Blade meat fabricated from the innovative side was heavier (3.82 vs. 1.65 kg), comprising a greater ($P < 0.001$) forequarter percentage than the conventional blade meat. The chuck tender, inside skirt, and outside skirt were not affected ($P > 0.05$) by fabrication style. The combined forequarter subprimal yield of the innovative fabrication style was greater ($P < 0.001$) than the combined yield of the conventional cuts, and less ($P < 0.001$) lean trimmings were generated by the innovative fabrication style.

Hindquarter wholesale cuts and carcass component percentages were analyzed by cutting style and reported in Table 3. The tenderloin fabricated from the innovative side was heavier ($P < 0.001$) than the conventional tenderloin because it contained the most posterior portion of the *M. iliopsoas*, which is typically excluded from the tenderloin by the conventional round/loin break.

Table 3. Least-squares means for hindquarter subprimal and component percentages stratified by conventional (CONV) and innovative (INNOV) cutting styles

Subprimal	CONV	INNOV	SEM ^a	<i>P</i> > <i>F</i>
	%			
Flank Steak	1.08	1.06	0.02	0.34
Tenderloin	3.30	3.46	0.04	<0.001
Bottom sirloin flap	1.94	2.06	0.04	0.01
Strip loin	6.19	6.10	0.09	0.34
Flank muscle	1.24	1.17	0.05	0.15
Center-cut top sirloin	4.73	4.64	0.08	0.30
Top sirloin cap	0.96	1.43	0.03	<0.001
Bottom sirloin ball tip	1.15	-	0.07	-
Bottom sirloin tri-tip	2.28	2.63	0.05	<0.001
Round tip	5.67	6.68	0.09	<0.001
Top round	11.75	11.83	0.12	0.46
Eye of round	3.31	3.24	0.05	<0.01
Bottom round flat	7.70	6.94	0.09	<0.001
Subprimal total	50.32	50.10	0.34	0.38
Lean trimmings (85% lean)	10.72	10.76	0.17	0.86
Fat	24.27	24.53	0.43	0.42
Bone	14.68	14.62	0.19	0.73

^aSEM is the standard error of the least-squares mean.

The top sirloin cap (coulotte) was separated at a point immediately anterior to its caudal origin at the lateral tuberosity of the tuber ischiadicum, which is the point of separation recommended by Reuter et al. (2002a). Their study showed that the *M. gluteobiceps* was most tender at the origin (sirloin section) and was tougher 7 to 10 cm posterior to the conventional round/loin break. In addition, the authors explained that the conventional round/loin separation bisected the most tender portion of the *M. gluteobiceps*. In our study, the innovative top sirloin cap (coulotte) was higher ($P < 0.001$) yielding than the conventional cut, which was a direct result of how the *M. gluteobiceps* was fabricated. This cutting style may provide the beef industry with an opportunity to better utilize the proximal portion of the *M. gluteobiceps*.

The innovative bottom sirloin tri-tip was higher ($P < 0.001$) yielding than its conventional counterpart, because this style included removal of the *M. tensor fasciae latae*, including the distal tip of the muscle that is normally excluded by the round/loin separation. The round tip (*M. quadriceps femoris*) of the innovative style comprised a greater ($P < 0.001$) percentage of the hindquarter due to the inclusion of the bottom sirloin ball tip in the innovative round tip.

Due to the separation that created a larger top sirloin cap (coulotte) from the innovative side, the bottom round flat was lower ($P < 0.001$) yielding in comparison to the conventional bottom round flat. The flank steak, strip loin, special trim flank muscle, center-cut top sirloin, top round, lean trim, bone, and fat were not affected ($P > 0.05$) by cutting style.

Carcass value

Forequarter value comparisons were made between cutting styles and reported in Table 4. Value differences parallel weight and yield differences between cutting styles. Due to the weight differential created by fabricating the whole *M. pectoralis profundus*, the innovative brisket was more ($P < 0.001$) valuable than the conventional brisket, whereas the innovative chuck roll generated less ($P < 0.001$) value due to extreme weight differences. The conventional shoulder clod was more ($P < 0.001$) valuable than the innovative shoulder clod, though by excluding the *M. trapezius* and *M. latissimus dorsi*, the innovative shoulder clod should realistically command a higher market price. The innovative ribeye roll was more ($P < 0.001$) valuable, though in a market setting, this cut may not realize the same unit price as the conventional ribeye roll. Overall, total subprimal, saleable yield, and forequarter values were higher ($P < 0.001$) for the innovative style.

Table 4. Least-squares means for forequarter subprimal and component values (U.S. \$) stratified by conventional (CONV) and innovative (INNOV) cutting styles

Subprimal	CONV	INNOV	SEM ^a	P > F
	U.S. \$			
Brisket	10.97	15.97	0.25	<0.001
Shoulder clod	24.48	21.70	0.34	<0.001
Chuck tender	4.23	4.24	0.07	0.71
Pectoral meat	3.74	-	0.13	-
Chuck roll	22.13	8.34	0.37	<0.001
<i>M. Serratus ventralis</i>	-	14.36	0.33	-
Chuck short rib	5.45	-	0.15	-
Inside skirt	5.28	5.39	0.14	0.25
Outside skirt	2.93	2.93	0.09	0.99
Ribeye roll	46.64	52.27	0.71	<0.001
Back ribs	4.32	4.85	0.08	<0.001
Blade meat	5.55	12.88	0.34	<0.001
Lean trimmings (85% lean)	57.14	54.84	1.03	<0.001
Fat	3.46	3.38	0.10	0.28
Bone	1.82	1.80	0.03	0.37
Subprimal total value	135.72	142.94	1.68	<0.001
Forequarter total value	198.13	202.96	2.38	<0.001

^aSEM is the standard error of the least-squares mean.

Hindquarter value comparisons were made between cutting style and reported in Table 5. The increased weight of the innovative tenderloin, 2-piece top sirloin butt, and bottom sirloin tri-tip resulted in greater ($P < 0.001$) value, as did the innovative bottom sirloin flap ($P < 0.005$). As was found for the forequarter, total subprimal, saleable yield, and hindquarter values were greater ($P < 0.05$) for the innovative style.

Table 5. Least-squares means for hindquarter subprimal and component values (U.S. \$) stratified by conventional (CONV) and innovative (INNOV) cutting styles

Subprimal	CONV	INNOV	SEM	<i>P</i> > <i>F</i>
	U.S. \$			
Flank Steak	6.02	5.88	0.16	0.37
Tenderloin	42.77	44.93	0.66	<0.001
Bottom sirloin flap	8.20	8.70	0.21	0.005
Strip loin	43.80	43.27	0.71	0.39
Flank muscle	3.34	3.15	0.13	0.15
2-piece top sirloin butt	29.83	31.82	0.55	<0.001
Bottom sirloin ball tip	3.44	-	0.23	-
Bottom sirloin tri-tip	4.73	5.46	0.11	<0.001
Round tip	14.91	17.61	0.30	<0.001
Top round	30.02	30.31	0.44	0.28
Eye of round	9.66	9.48	0.17	0.002
Bottom round flat	18.27	16.50	0.27	<0.001
Lean trimmings (85% lean)	19.79	19.91	0.41	0.71
Fat	4.69	4.74	0.08	0.46
Bone	1.29	1.29	0.02	0.84
Subprimal total value	214.98	217.12	2.54	0.04
Hindquarter total value	240.75	243.07	2.79	0.01

^aSEM is the standard error of the least-squares mean.

Conclusions

Our focus was to optimize beef carcass value through exploring innovative fabrication styles. Although labor requirements were not measured, in this demonstration, innovative fabrication increased subprimal yield and beef carcass value of approximately U.S. \$14. In general, innovative subprimals produced similar steak/roast and saleable yields as compared to conventional subprimals.

As the industry changes with consumer demands, traditional cutting methods will begin to fade away. In their place, new and innovative styles will begin to form as muscles are merchandized individually, creating a more valuable and consistent retail cut for the industry and consumer.

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**PROXIMATE COMPOSITION AND FAT RETENTION OF BEEF STEAKS AS
INFLUENCED BY TISSUE COMPOSITION, USDA QUALITY GRADE, AND
COOKING METHOD**

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Key Words: Broiling; Seam fat; Oven broiling; Separable lean; Surface fat; Waste

Introduction

Various factors are known to affect lipid content, and consequently, the overall proximate composition of meat. Some of these factors include retail cut (Jones et al., 1992b; Wahrmund-Wyle et al., 2000b), cooking method (Jones et al., 1992b; Renk et al., 1985, Morgan et al., 1988), USDA quality grade (Jones et al., 1992b; Wahrmund-Wyle et al., 2000b), and the extent of fat trimming and fat retention (Coleman et al., 1988; Jones et al., 1992b). While several researchers have reported that increasing amounts of surface fat left on retail cuts before cooking increases fat retention (Coleman et al., 1988; Morgan et al., 1988; Jones et al., 1992c) and the corresponding fat concentration of the separable lean (Jones et al., 1992c; Coleman et al., 1988; Morgan et al., 1988), others have disregarded such claims (Novakofski et al., 1989) or found mixed results (Goihl et al., 1992; Wahrmund-Wyle et al., 2000a) in beef cuts. Evidenced by the relatively high amount of chemical fat retained in some retail cuts trimmed of surface fat, it has been suggested that fat migration can be also attributed to the seam fat (Jones et al., 1992c). Therefore, further studies are needed to elucidate the effect of the relative proportion of separable fat depots left in retail cuts on fat retention and nutrient content of the edible portion across cooking methods and quality grades.

Objectives

a) To examine the variation in raw tissue composition, cooking yield, fat retention, and proximate composition of the edible portion of four retail cuts differing in seam and surface fat. b) To identify the influence of cooking method and quality grade on the same response variables.

Methodology

Carcass selection and fabrication

The right side of six USDA Choice and six USDA Select carcasses was fabricated into the following four subprimals as described by their USDA (1996) Institutional Meat Purchase Specifications (IMPS) Number: Ribeye (IMPS# 112); Shoulder Clod (IMPS# 114); Top round (IMPS# 168); and Tenderloin (IMPS# 190). Steaks, 2.54 cm thick, were cut from each listed subprimal, assigned a cooking method, vacuum packaged, and frozen for subsequent cooking and proximate composition analysis. Ribeye steaks were trimmed of any surface fat while top round and shoulder clod steaks were trimmed to 0.32 cm surface fat. Tenderloins were defatted (no surface fat present) but not trimmed of any seam fat.

Dissection and Cooking

All retail cuts were vacuum packaged, grouped according to cooking method, and stored frozen at -23°C until needed for cooking and dissection. Steaks were analyzed as a broiled, oven broiled, or raw sample. Samples that were treated as raw were thawed and dissected into separable tissue components (separable lean, surface fat, seam fat, and waste) that were weighed and converted to percentages. Broiled steaks were cooked to an internal temperature of 70°C in an electric Farberware Open-Hearth Broiler. Steaks designated as oven broiled were cooked to an internal temperature of 65°C in a preheated broiler. Raw, cooked, and cold weights (used to determine percentage yield) along with on and off temperatures were recorded. Cooked weights of beef retail cuts taken after cuts were chilled and were used to calculate cooking yields as follows:

$$\text{Percentage cooking yield} = (\text{cooked weight})/(\text{raw weight}) \times 100$$

Proximate composition

Separable lean from raw and cooked steaks was homogenized in a Cuisinart® food processor. Protein, moisture, and ash analyses were conducted by AOAC methods (AOAC, 1990). Fat analysis was determined by the Modified Folch Method (Folch et al., 1957).

Fat retention

Fat retention values were determined for each sample using raw versus cooked data as described by Jones et al. (1992c), and expressed as follows:

$$\text{Percentage fat retention} = (\% \text{ fat in cooked lean})/(\% \text{ fat in raw lean}) \times \% \text{ cooking yield}$$

Statistical analysis

Data were analyzed using SAS (2002) PROC GLM. Main effects and interactions were analyzed. Independent variables not significant for the interactions were pooled into the error term. A significance level of $P < 0.05$ was used, and means were separated using the PDIF option of SAS.

Results & Discussion

Table 1
Least squares means and standard errors for percentages of raw tissues components of four retail steaks

	Retail cut			
	Ribeye steaks (n=12)	Tenderloin Steaks (n=12)	Shoulder clod steaks (n=10)	Top round steaks (n=12)
Separable lean, %	77.5±1.8a	82.1±1.8ab	77.8±2.0a	85.9±1.8b
Surface fat, %	0.0±1.1a	0.0±1.1a	4.4±1.2b	6.0±1.1b
Seam fat, %	15.2±1.8a	12.0±1.8ab	10.5±1.9ab	2.9±1.8c
Waste, %	6.7±1.1	4.9±1.1	6.9±1.2	4.6±1.1

Means in the same row lacking a common letter differ ($P < 0.05$)

Raw tissue composition

Percentage waste was the only dissectible component affected ($P < 0.01$) by USDA quality grade (results not shown in tabular form). Choice steaks yielded 3% more ($P < 0.05$) waste than their Select counterparts (percentage LS Means and SE were 7.4 ± 0.8 vs. 4.3 ± 0.8 , respectively). Conversely, Wahrmond-Wyle et al. (2000a) did not find any differences in percent waste between USDA Select and Choice.

Retail cuts varied significantly in tissue components (Table 1). Ribeye and shoulder clod steaks produced the lower amounts of separable lean ($P < 0.05$) whereas top round steaks ranked highest in lean yield.

Cooking yield

USDA grade or cooking method did not affect cooking yield ($P > 0.05$). Lack of variation in cooking yield due to USDA grade has been reported previously (Jones et al., 1992c; Wahrmond-Wyle et al., 2000b). Retail cuts trimmed to zero surface fat (i.e., ribeye and tenderloin) had lower ($P < 0.05$) cooking losses and exhibited the higher yields (Table 2). This finding contradicts that of Jones et al. (1992c) where cooking yields were not significantly affected by surface trim levels and an inverse trend to our results was observed for most cuts (i.e., lower numerical values for predicted cooking yields were found in those retail cuts trimmed to zero before cooking).

Table 2
Least squares means and standard errors for cooking yield and related variables of four retail cuts

	Retail cut			
	Ribeye steaks	Tenderloin	Shoulder clod	Top round

	(n=24)	steak (n=24)	steak (n=23)	steak (n=24)
Raw weight, g	263.5 ± 12.4a	188.1 ± 12.4b	515.3 ± 12.6c	333.8 ± 12.4d
Cooked weight, g	209.8 ± 10.4a	146.9 ± 10.4b	394.5 ± 10.7c	251.5 ± 10.4d
Cook loss, g	42.7 ± 2.9a	34.8 ± 2.9a	79.4 ± 3.0b	62.3 ± 2.9c
Cook yield, %	75.9 ± 0.8a	74.3 ± 0.8a,b	73.5 ± 0.8b	72.7 ± 0.8b

Means in the same row lacking a common letter differ ($P < 0.05$)

Proximate composition and fat retention of the separable lean

Chemical fat. Cooked samples had higher ($P < 0.05$) fat contents (7.6% and 6.8% for broiled and oven broiled steaks, respectively) than their raw (4.5%) counterparts (Figure 1). The higher fat extraction in cooked samples is widely supported (Renk et al., 1985; Smith et al., 1989; Goihl et al., 1992; Jones et al., 1992c), but Wahrmond-Wyle et al. (2000b) found the opposite. The significant variation in fat content due to broiling method was not found by Renk et al. (1985) in pork samples.

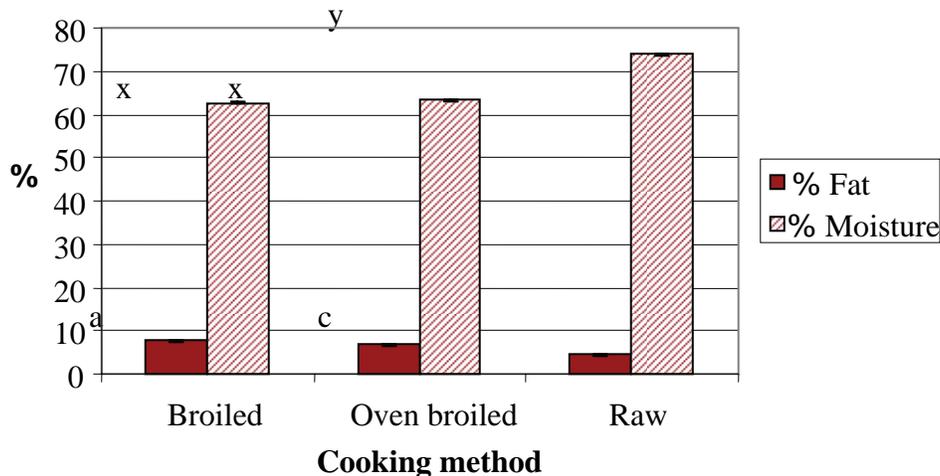


Figure 1. Least squares means and standard errors for percentages of chemical fat and moisture from proximate analysis of broiled, oven broiled and raw retail cuts. Columns with different letters differ ($P < 0.05$).

The general trend reported previously by Wahrmond-Wyle et al., (2000b) and Jones et al. (1992b) was that Choice steaks had significantly more percentage chemical fat than their Select counterparts. However, there was an interaction ($P < 0.0001$) between retail cut and USDA quality grade (Figure 2). Whereas fat content progressively decreased as seam fat proportion in Choice retail cuts decreased (in the order: Ribeye > Tenderloin > Shoulder clod > Top round), an irregular pattern in fat content was observed among retail cuts of the Select grade.

Moisture. Raw samples had higher ($P < 0.05$) moisture contents compared to counterparts of broiled or oven broiled steaks (Figure 3). The lower percentage moisture in the cooked steaks has been reported previously (Smith et al., 1989; Goihl et al., 1992; Jones et al., 1992b). Comparison of quality grades shows a similar tendency to that reported by Wahrmund-Wyle et al. (2000b) and Jones et al. (1992b) in that separable lean of Select steaks had more moisture than their Choice counterparts. However, there was an interaction ($P = 0.0028$) between retail cut and USDA quality grade (Figure 3) that shows an opposite trend to that described for the fat content. Whereas moisture content progressively increased as seam fat in Choice retail cuts decreased (in the order: Ribeye > Tenderloin > Shoulder clod > Top round), an irregular pattern in moisture content was observed among retail cuts of the Select grade.

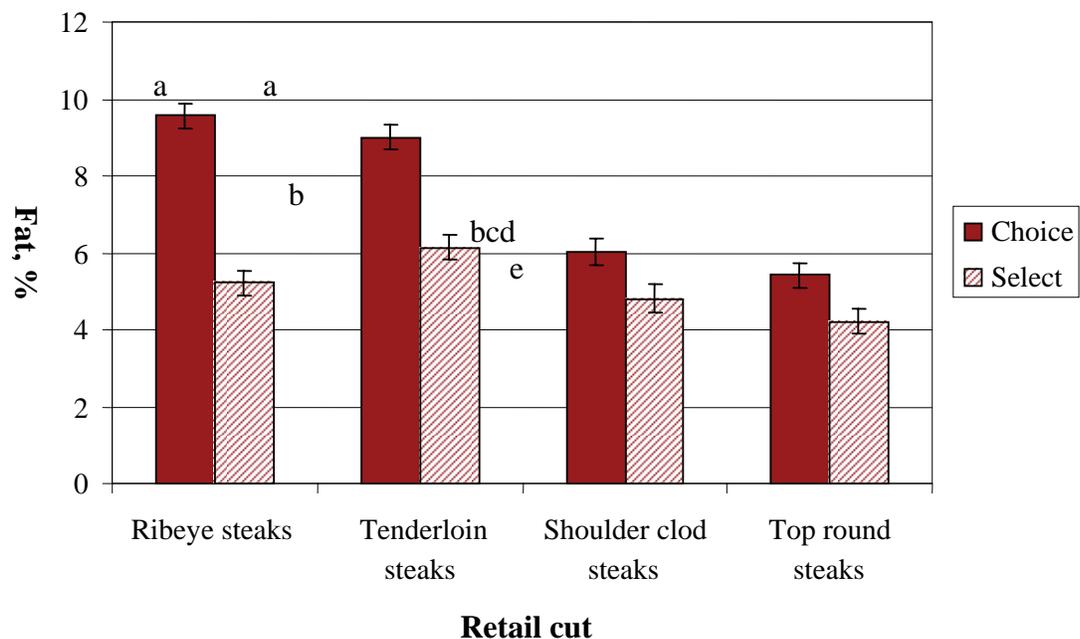


Figure 2. Least squares means and standard errors for percentages of fat from proximate analysis as affected by retail cut and USDA quality grade. Columns with different letters differ ($P < 0.05$).

Protein. There were two significant two-way interactions for protein: cooking method \times USDA quality grade ($P = 0.0217$) and retail cut \times cooking method ($P = 0.0043$).

Figure 4 shows that broiled steaks of the Select grade had more ($P < 0.05$) protein than their Choice counterparts ($P < 0.05$) but such differences (if any) in grade were of lesser magnitude and not significant for oven broiled or raw steaks (Figure 5). Cooked steaks did not differ in percent protein within the same USDA quality grade whereas protein contents of raw steaks regardless of USDA grade were lower than Choice and Select cooked steaks ($P < 0.05$).

Figure 5 depicts the relationship between retail cut and cooking method, showing that variations in protein content due to cooking treatments were of different magnitudes across retail cuts although the protein content was always lowest in the raw samples.

Ash. Percent ash of the separable lean was only affected by retail cut ($P = 0.0109$), but differences were minor among cuts (results not shown in tabular form). Top round steaks exhibited the highest ash percentage (1.09 ± 0.03), different ($P < 0.05$) from ribeye (0.96 ± 0.03) and tenderloin (0.99 ± 0.03) steaks but similar to shoulder clod (1.01 ± 0.03) steaks ($P = 0.0554$).

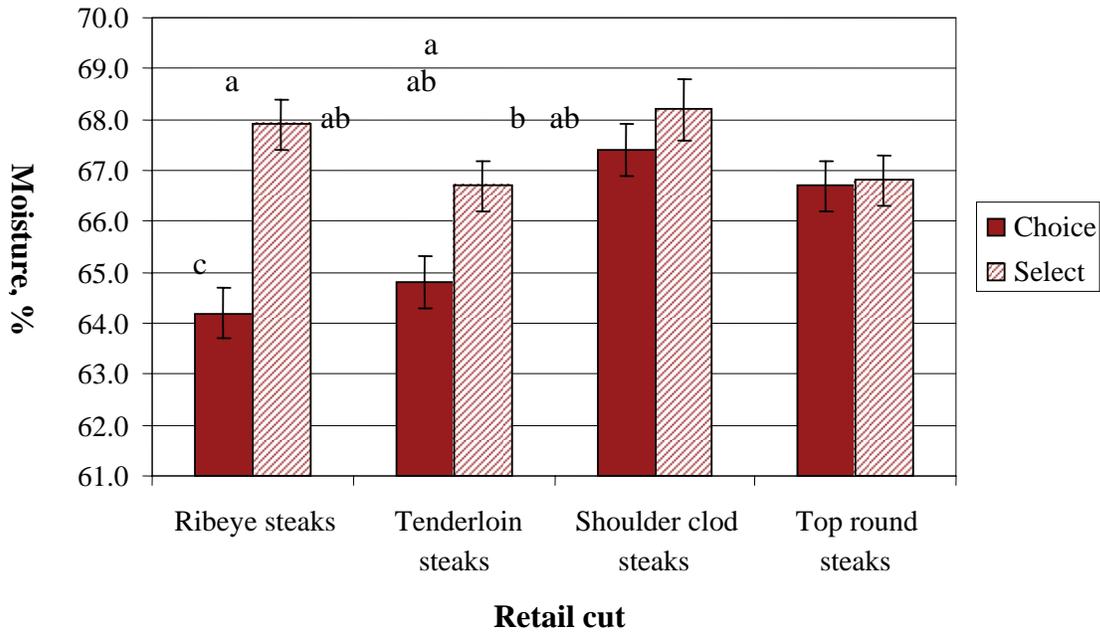


Figure 3. Least squares means and standard errors for percentages of moisture as affected by retail cut and USDA quality grade. Columns with different letters differ ($P < 0.05$).

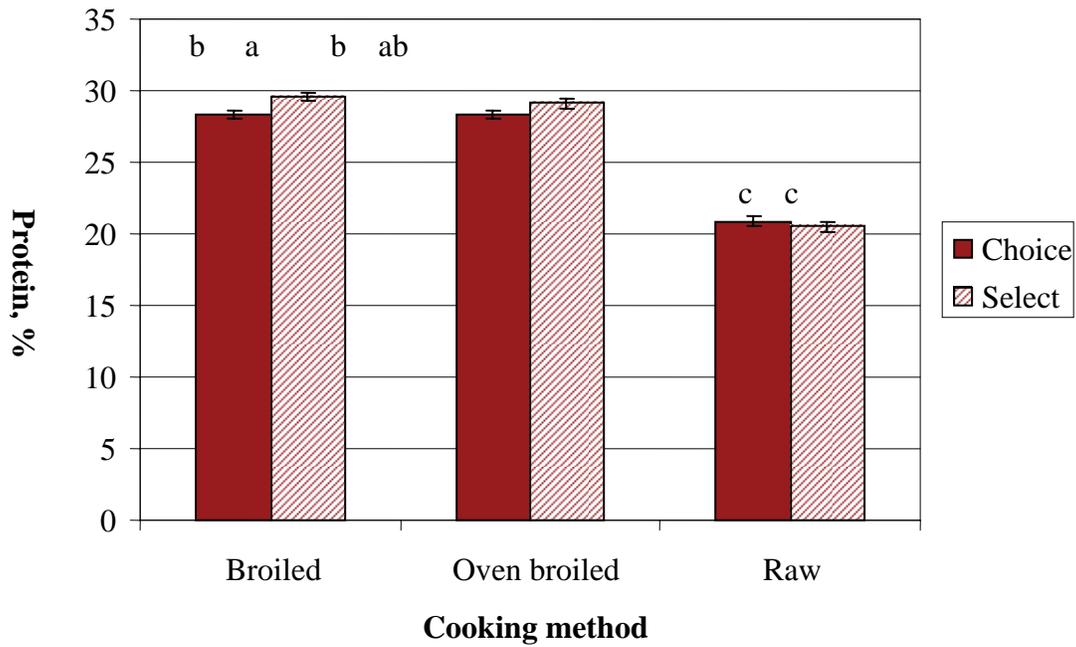


Figure 4. Least squares means and standard errors for percentages of protein as affected by USDA quality grade and cooking method. Columns with different letters differ ($P < 0.05$).

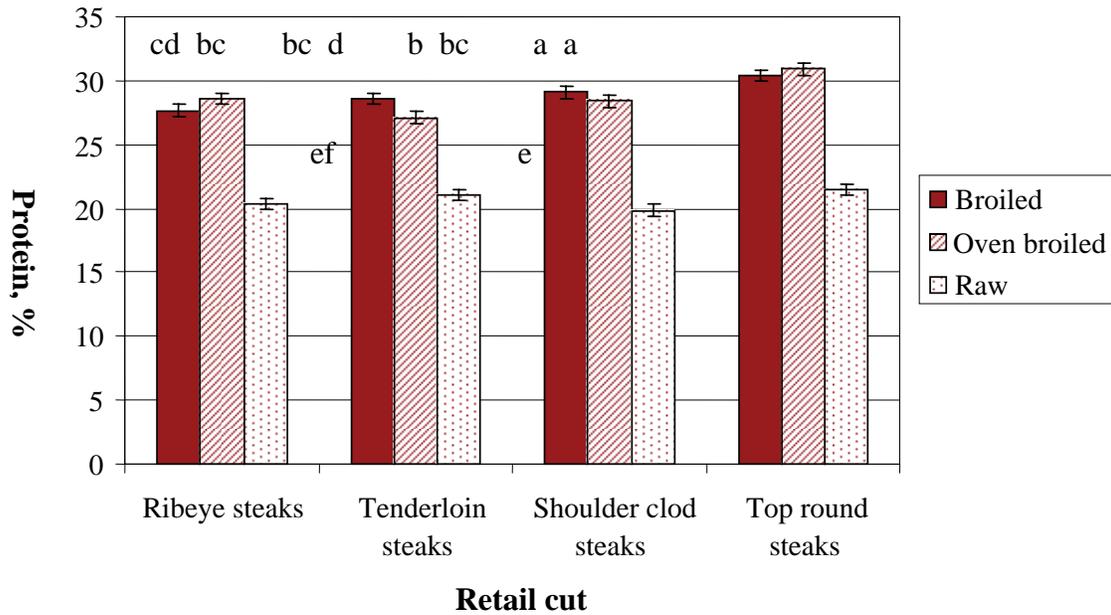


Figure 5. Least squares means and standard errors for percentages of protein as affected by retail cut and cooking method. Columns with different letters differ ($P < 0.05$).

Fat retention. Retention of fat within the lean after cooking was only affected significantly by USDA quality grade ($P = 0.0128$) and cooking method ($P = 0.0053$). The non-significant variation in fat retention due to retail cut (Figure 6) does not agree with the findings of Morgan et al. (1988) and Jones et al. (1992c). It also contradicts our hypothesis that fat retention would be affected by retail cut due to the wide range of seam and surface fat present in the selected retail cuts. Adjusted means for fat retention in the lean portion exceeded 100% in all selected retail cuts (Figure 6), supporting the common suspicion that lipids from other depots migrated into the lean. However, lack of significant variation in fat retention among retail cuts differing in proportion or seam fat does not contribute to support the influence of rendered lipids migrating from the seam fat depots into the lean edible portion as suggested by Jones et al. (1992c) or Renk et al. (1985).

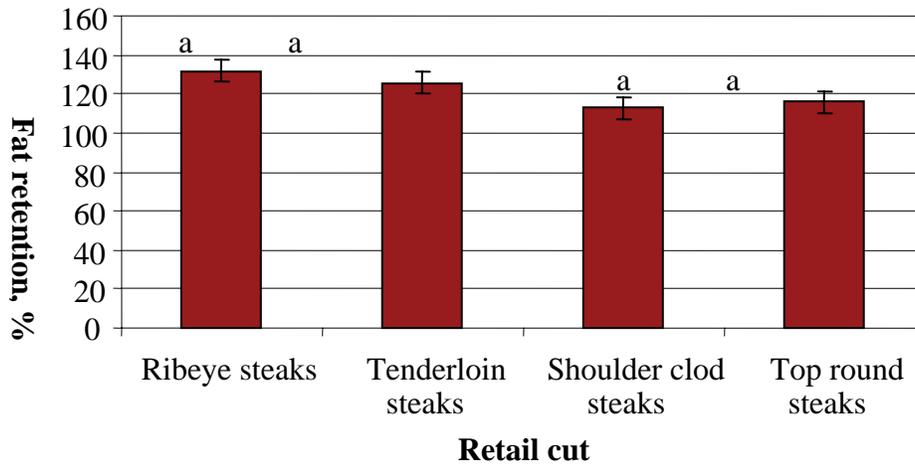


Figure 6. Least squares means and standard errors for fat retention percentages of ribeye, tenderloin, shoulder clod, and top round steaks. Columns with different letters differ ($P < 0.05$).

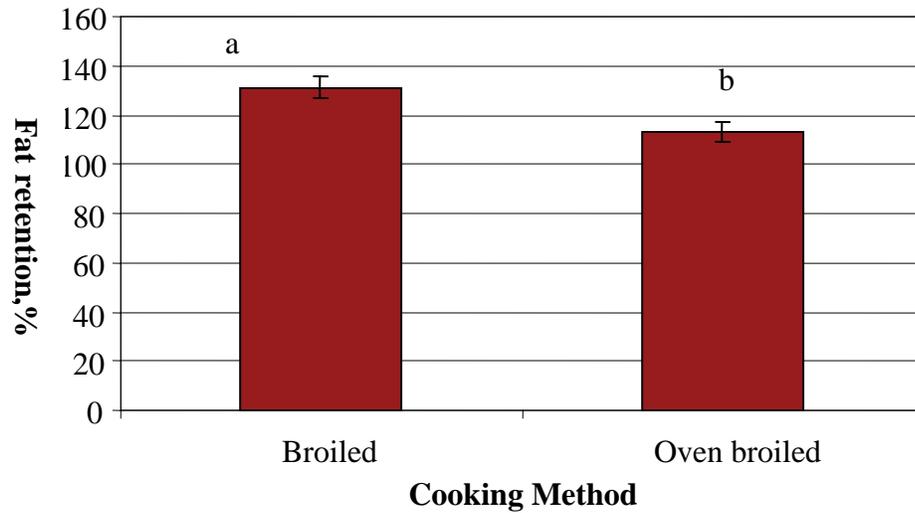


Figure 7. Least squares means and standard errors for percentage of fat retention of broiled and oven broiled steaks. Columns with different letters differ ($P < 0.05$).

Percentage fat retention was higher ($P = 0.0026$) for broiled steaks (Figure 7). Conversely, Renk et al. (1985) reported that cooking method did not affect fat retention. Fat retention was higher in separable lean of Select steaks than in Choice steaks (Figure 8). However, Renk et al. (1985) reported that degree of marbling did not have an effect on fat retention.

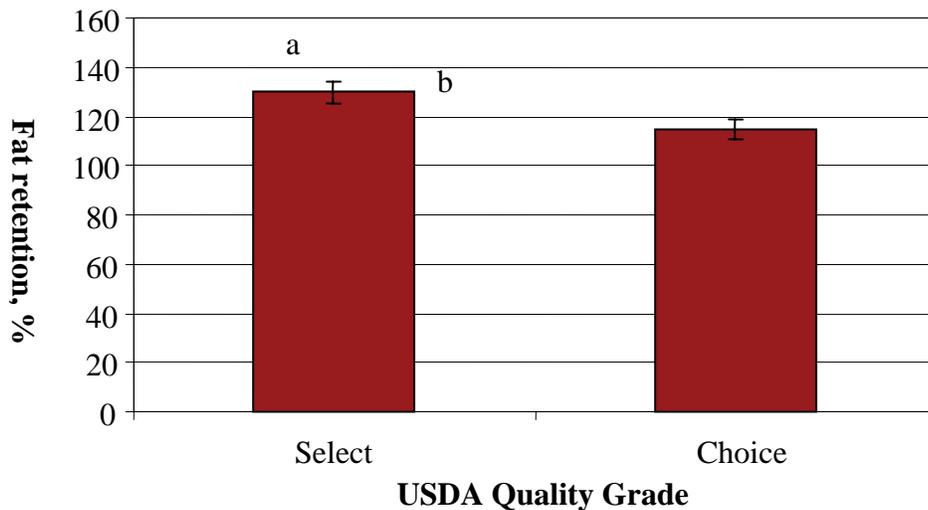


Figure 8. Least squares means and standard errors for fat retention percentages of Select and Choice steaks. Columns with different letters differ ($P < 0.05$).

Conclusions

Retail cuts, due to their anatomical location, are physically composed of different percentages of lean, surface fat, and seam fat. Retail cut had a direct effect on dissection yields and an indirect effect on proximate composition values simply because of the amount of fat deposition and fat trimming level. Cooking method did alter the percentage of proximate composition with regards to percentage chemical fat and moisture. Migration of fat throughout the lean is affected by cooking method. However, the question regarding the original source of these migrating lipids remains unresolved.

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BIOCHEMICAL AND PHYSICAL PROPERTIES ASSOCIATED WITH MEAT COLOR OF BEEF MUSCLES TARGETED FOR FUTURE MARKET PENETRATION

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Key Words: Beef muscles, Meat color, Oxygen consumption ability, Anaerobic reducing ability, Shelf life, Carbon monoxide

Introduction

Meat color significantly influences consumer preferences and the likelihood they will purchase beef (Carpenter et al., 2001). The pigment responsible for the bright red color is oxymyoglobin. Muscles can have different biochemical and physical properties that dramatically affect their ability to produce and maintain a desirable bright red color (Arnold et al., 1992; Reddy and Carpenter, 1991). Reducing conditions, oxygen penetration depth, oxygen consumption rate, metmyoglobin accumulation, pH, and light all play a role in influencing beef color (Claus et al., 1984; O'Keefe and Hood, 1982; Reddy and Carpenter, 1991).

New approaches to stabilizing the fresh color may be critical to introducing muscles that traditionally have not been marketed at the retail level as steaks. Carbon monoxide is known to effectively bind to myoglobin to form a more stable, bright red color (Brewer et al., 1994). Carbon monoxide at levels below 1% is considered safe (Sorheim et al., 1997). One concern with the use of carbon monoxide is the potential to stabilize the bright red color beyond the microbiological shelf life of the meat.

The National Cattlemen's Beef Association identified ten muscles for future market penetration. Information is lacking on the meat color properties of these muscles.

Objectives

- 1) Determine the differences in muscle oxygen penetration, oxygen consumption ability, and reducing ability.
- 2) Establish the color display shelf life.
- 3) Determine the ability of a modified atmosphere packaging containing carbon monoxide to extend the refrigerated color display shelf life.

Methodology

Cattle (n=6) in the USDA Standard to Choice were used. Carcasses (no dark cutters or PSE) were chilled for 2 days before the muscles were excised. Muscles evaluated were: *Adductor (AD)*, *Complexus (CM)*, *Gracilis (GR)*, *Pectineus (PE)*, *Romboideus*

(RH), Sartorius (SA), Superficial pectoral (SP), Serratus ventralis (SV), Vastus intermedius (VI) and Vastus lateralis (VL). Muscles were vacuum packaged and aged (3°C, 7 days postmortem).

pH Determination and Muscle Oxygen Penetration. pH was measured 24 hours after preparing the steaks. Oxygen penetration was measured at 1 and 24 hr (3°C) after cutting the steaks.

Oxygen Consumption Ability (OCA). Sample slices from the muscles were vacuum packaged and allowed to deoxygenate for 24 hours (3°C). Steaks were bloomed for 75 min (3°C). Steaks were then vacuum packaged and immediately measured (reflectance, 400 to 700 nm, time 0 min.). Measurements were repeated every 5 min for an hour.

Anaerobic Reducing Ability. Muscles were cut into steaks and allowed to bloom (3°C, 24 hr). These samples were then vacuum packaged, immediately inflated with a modified atmosphere (1% oxygen, 99% nitrogen), and stored (3°C, 48 hr). Samples were removed from the modified atmosphere, vacuum packaged, and immediately measured (time 0) using a reflectance spectrophotometer and a chromameter. Measurements were repeated every hour for 8 consecutive hr and then at 24 and 48 hr.

Display Shelf Life. On the day of sample preparation, a steak from each muscle was taken for each display conditions (atmospheric air; carbon monoxide modified atmosphere). One steak was over-wrapped in PVC and displayed (3°C). The other steak was vacuum packaged and inflated with a gas mixture (0.4% carbon monoxide, 30% carbon dioxide, and balance nitrogen) prior to being displayed. Steaks were displayed (3°C) continuously according to AMSA (1991) guidelines. Color measurements were taken day 0, 3, 5 and 7.

Meat color measurements. A chromameter (model CR-310, Minolta Camera Co.) was used for measuring the color of samples in the anaerobic reducing ability and shelf life experiments. The instrument was calibrated with a standard white plate. A spectrophotometer (model UV-2401 PC) fitted with a multipurpose large sample compartment (model MPC-2200, Shimadzu Corp.) calibrated against a barium sulphate plate was used. Chemical states of myoglobin were estimated as follows: estimate of oxymyoglobin (Est.OMb: %R610 nm / %R 525 nm, %R= percentage reflectance), estimate of redness (Est.Red: %R630nm-%R580), estimates of deoxymyoglobin (Est.DMb: %R474 nm / %R525 nm), and estimates of metmyoglobin (Est.MMb: %R572 nm / %R525 nm; MMb%: metmyoglobin percentage, determined based on results of Stewart et al., 1965) as recommended by AMSA (1991).

Results & Discussion

pH. The ultimate pH of the muscles ranged from 5.50 to 5.79. There was significant difference among muscles. Several workers reported that within the pH range of 5.68±0.12, pH had limited influence on metmyoglobin formation and display color stability of beef muscles (Ledward, 1985; Hood, 1980).

Oxygen penetration depth. The average depth of oxygen penetration over the period of 24 hr was different among muscles. At 1 and 24 hr the AD showed one of the deepest oxygen penetrations while the SV was the lowest. Earlier research has shown that at slaughter and 10 days post-slaughter the oxygen penetration depth in the *M. Longissimus*

dorsi and *M. Psoas major* was different. But on the second day of post-slaughter they found no difference between these two muscles (O'Keefe and Hood, 1982)

Oxygen consumption ability. The initial level of OMB (Est. OMB at time 0) was not different indicating they were uniformly oxygenated. The Est. OMB pooled over the entire measurement period indicated some differences among the means. However, there were not any differences among the muscles in the absolute reduction in Est. OMB nor the relative OCA. The time of oxygen consumption_{1/2} did have differences among the muscles. The SP took 20 minutes to reduce the Est. OMB to half of its initial level whereas the AD and VL took 36 to 37 minutes.

Anaerobic reducing ability. Muscles had a significant influence on anaerobic reducing ability (An.RA, Table 1). SP had the highest mean CIE a* value over the entire period indicating its ability to withstand the oxidizing effect of lower concentrations of oxygen. AD, GR, PE, and SA tended to have lower CIE a* values. However, chroma C* values found the SP, AD, SV were higher than all other muscles. The GR and SA had the lowest chroma C* values. Means for MMB% at time 0 indicated that the AD, SV, and PE were least able to minimize the oxidizing effects of the 1% O₂ atmosphere, whereas the RH, GR, and SA were some of the most effective at preventing the brown pigment from forming (Table 1). The absolute MMB% reduction was calculated over three time periods (8, 24, and 48 hr). The SP, SV, VI, and VL generally had the largest absolute reduction in the MMB%, regardless of the time period. The SP had the highest relative An.RA%.

Display shelf life. In terms of main effects, the PE was the lightest (CIE L*) and the GR the darkest. The SP tended to be the most red (CIE a*). The SA and GR were the least yellow (CIE b*).

CO packaging produced lighter steaks from the CM, GR, RH, SA, SV, and VI than when PVC packaged. The other muscles were not different. For CIE a*, the AD, GR, PE, SP, SV, and VI were more red under CO packaging (Table 2). No differences in yellowness were found. Chroma C* was higher for AD, PE, SP in CO but lower for the GR and RH. It is unknown as to why the RH did not respond favorably to CO packaging. The GR had one of the highest MMB% which would negatively influence the chroma C*.

Except on day 1, CIE L* and a* values were consistently higher for CO steaks than PVC steaks. Day 1 CIE a* values were lower for CO than PVC (Table 3). Most likely this was associated with the transition from an aerobic environment to an anaerobic environment yielding low levels of oxygen known to favor the formation of metmyoglobin. As with CIE a*, chroma C* and Est. Red values were lower on day 1 for the CO packaged steaks. After day 3, CO packaged steaks had higher chroma C* values than PVC packaged steaks. CO packaged steaks maintained chroma C* values above 16 (acceptability threshold) whereas the PVC steaks were less than 16 on day 7. In support of the lower CIE a* values on day 1 for the CO steaks, MMB% was higher on day 1 than PVC steaks. Day 5 and 7 exhibited lower MMB% in the CO steaks.

Sorheim et al. (1997) reported a longer shelf life of meat packaged in the 0.4% CO mixture than that of meat packaged in the commonly used atmospheres with high oxygen. They also reported that the consumption of meat that has been packaged in a CO mixture would result in only negligible levels of carboxyhaemoglobin in the blood. But another work by Sorheim et al. (1999) revealed that meat stored in 0.4% CO/60% CO₂/40% N₂ had a stable bright red color that lasted beyond the time of spoilage. Consumers use beef color as an indicator of freshness and will make a no-purchase decision when the surface

metmyoglobin exceeds 30 to 40% (Greene et al., 1971). Therefore, CO packaging in our research provided at least two additional days of retail display life over PVC.

Conclusions

The ten targeted beef muscles for future market penetration displayed some differences in the various physical and biochemical properties. Nevertheless, all muscles appeared to have an adequate ability to allow sufficient oxygen penetration. Limited differences were found among the muscles in terms of oxygen consumption ability. However, some muscles were better at handling exposure to oxidizing conditions than others and therefore, appropriate packaging and storage will aid in their merchandizing value. Not all muscles will benefit from CO packaging. However, CO packaging will typically extend the display life of the beef steaks.

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Table 1. Main effect means for the anaerobic reducing ability of ten targeted beef muscles.

Muscle	Dependent variable ¹			
	MMb% at time 0	Absolute MMb% reduction 48 hr	Overall MMb%	Relative An.RA%
AD	80.6 ^a	45.5 ^d	69.37 ^a	26.62 ^{cd}
CM	65.1 ^{bcd}	62.0 ^{bc}	52.38 ^b	20.32 ^d
GR	55.7 ^{de}	53.9 ^{cd}	44.11 ^d	33.04 ^{bcd}
PE	77.7 ^{ab}	60.3 ^c	64.91 ^a	22.11 ^d
RH	51.5 ^e	52.4 ^{cd}	43.37 ^d	24.71 ^d
SA	58.5 ^{cde}	51.8 ^{cd}	49.50 ^{bc}	25.01 ^d
SP	58.8 ^{cde}	56.2 ^{cd}	28.61 ^e	81.21 ^a
SV	80.4 ^a	81.4 ^a	52.71 ^b	44.21 ^{bcd}
VI	65.0 ^{bcd}	55.2 ^{cd}	43.86 ^d	55.64 ^{bc}
VL	68.9 ^{abc}	74.7 ^{ab}	44.78 ^{cd}	56.50 ^b
Std. Error	6.38	6.64	4.10	11.54

^{a-e}Means within a column with unlike superscripts are different (P<0.05). Means pooled across time (0-48 hr).

¹Dependent variable: Relative An.RA% - the relative reduction of surface metmyoglobin (MMb%) under anaerobic conditions from 0 to 8 hours (higher value, greater ability to reduce surface metmyoglobin).

Table 2. Interaction means (muscle by packaging¹) for the display shelf life of ten targeted beef muscles.

Muscle	Dependent variable ²							
	CIE L*		CIE a*		Chroma C*		MMb%	
	PVC	CO	PVC	CO	PVC	CO	PVC	CO
AD	42.79 ^a	42.92 ^a	16.56 ^b	20.71 ^a	19.01 ^b	21.59 ^a	32.3 ^a	20.3 ^b
CM	41.73 ^b	43.82 ^a	17.04 ^a	18.42 ^a	19.27 ^a	19.47 ^a	31.07 ^a	26.64 ^a
GR	39.79 ^b	40.87 ^a	17.28 ^a	14.93 ^b	19.01 ^a	15.55 ^b	26.68 ^a	31.16 ^a
PE	45.61 ^a	46.28 ^a	16.20 ^b	20.23 ^a	19.19 ^b	21.35 ^a	39.80 ^a	25.58 ^b
RH	42.92 ^b	44.31 ^a	19.10 ^a	18.74 ^a	21.20 ^a	19.44 ^b	17.62 ^a	22.32 ^a
SA	42.79 ^b	44.75 ^a	16.57 ^a	17.78 ^a	18.44 ^a	18.33 ^a	25.11 ^a	22.17 ^a
SP	43.99 ^a	44.82 ^a	17.93 ^b	21.44 ^a	20.44 ^b	22.37 ^a	26.54 ^a	24.43 ^a
SV	42.64 ^b	45.32 ^a	16.94 ^b	20.03 ^a	19.58 ^a	21.10 ^a	38.63 ^a	29.84 ^b
VI	40.21 ^b	42.49 ^a	16.79 ^b	18.57 ^a	19.15 ^a	19.63 ^a	37.63 ^a	30.11 ^a
VL	42.25 ^a	42.96 ^a	16.98 ^a	17.28 ^a	19.29 ^a	18.17 ^a	29.33 ^a	22.86 ^a

^{ab}Means within a row and dependent variable with unlike superscripts are different (P<0.05). Standard errors: CIE L* (0.54), CIE a* (0.87), Chroma C* (0.85), MMb% (3.90).

¹Packaging: PVC= polyvinylchloride film; CO= 0.4% carbon monoxide modified atmosphere packaging.

²Dependent variable: CIE L*-lightness (higher number lighter); CIE a*-redness (higher number more red); Chroma C* (values above 16 indicate acceptable meat color); MMb%-percentage metmyoglobin.

Table 3. Interaction means (packaging¹ by day) for the display shelf life of ten targeted beef muscles.

Day	Dependent variable ²					
	CIE a*		Chroma C*		MMb%	
	PVC	CO	PVC	CO	PVC	CO
1	22.58 ^a	18.58 ^b	24.87 ^a	19.03 ^b	10.91 ^b	22.38 ^a
3	18.37 ^b	19.74 ^a	20.37 ^a	20.61 ^a	20.25 ^a	22.65 ^a
5	15.42 ^b	18.84 ^a	17.63 ^b	19.86 ^a	33.67 ^a	25.62 ^b
7	12.19 ^b	18.10 ^a	14.95 ^b	19.30 ^a	57.06 ^a	31.51 ^b

^{ab}Means within a row and dependent variable with unlike superscripts are different (P<0.05). Standard errors: CIE a* (0.55), Chroma C* (0.54), MMb% (2.47).

¹Packaging: PVC= polyvinylchloride film; CO= 0.4% carbon monoxide modified atmosphere packaging.

²Dependent variable: CIE a*-redness (higher number more red); Chroma C* (values above 16 indicate acceptable meat color); MMb%-percentage metmyoglobin.

PHYSICAL, CHEMICAL, AND HISTOLOGICAL CHARACTERISTICS OF 18 LAMB MUSCLES

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Introduction

Consumer demand for meat products is determined by palatability, appearance, fat content, economic value, ease of preparation, and convenience (Ward, Trent, & Hildebrand, 1995). The demand for lamb products has declined over recent years in comparison to other meat sources, probably because lamb has not met these consumer driven criteria. Studies detailing the characteristics of individual muscles in beef and pork have identified muscles that can be marketed more effectively on an individual basis (Jones, Burson, & Calkins, 2001; Jones, Burson, Devine, Schafer, & Poday, 2000). The identification of lamb muscles that can be marketed this way could increase the demand for lamb products by improving the consistency of products and allowing processing technologies to be targeted toward maximum effectiveness, both of which could increase carcass value. Furthermore, marketing muscles in this manner allows the removal of seam fat, producing more attractive cuts with greater nutritional quality.

Objective

To quantify of factors affecting palatability and appearance of individual lamb muscles, with specific interest in identifying muscles suitable for use in individual muscle applications.

Methodology

Carcass selection and dissection

Lamb carcasses (n = 20) were selected to represent the commercially produced population, and were shipped to the Rosenthal Meat Science and Technology Center at Texas A&M University. Carcasses were selected from those with unchilled weights between 30.5 and 32.7 kg, and were ribbed at the 12th-13th rib interface with the following information obtained by Texas A&M personnel: fat thickness, adjusted fat

thickness, body wall thickness, ribeye area, leg conformation score, maturity score, and flank streaking score (USDA, 1992; Table 1).

Carcasses were dissected, and the following muscles were selected from both sides for further analysis: *M. adductor*, *M. gluteobiceps*, *M. gluteus medius*, *M. infraspinatus*, *M. latissimus dorsi*, *M. longissimus lumborum*, *M. longissimus thoracis*, *M. pectoralis profundus*, *M. psoas major*, *M. rectus femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. supraspinatus*, *M. serratus ventralis*, *M. triceps brachii*, *M. tensor fasciae latae*, *M. teres major*, and *M. vastus lateralis*. The *M. gluteobiceps* was further separated into the proximal and distal portions. Following dissection, muscles from the left side of the carcass were denuded, individually vacuum packaged, and aged approximately 7 d in a $2 \pm 2^\circ\text{C}$ cooler.

Table 1

Simple statistics for carcass traits

Trait	Mean	SD	Minimum	Maximum
Carcass weight (kg)	30.3	0.8	28.7	31.4
Fat thickness at 12th rib (mm)	4.8	1.5	2.0	7.6
Body wall (mm)	23.4	3.8	17.7	31.6
<i>M. longissimus thoracis</i> area (cm ²)	17.6	1.6	15.2	20.7
Lean maturity ^a	149.5	10.5	140	170
Skeletal maturity ^a	157.0	9.8	140	180
Flank streaking ^b	11.4	0.8	10	13
Quality score ^b	11.4	0.8	10	13
Confirmation score ^b	12.3	0.8	11	14
Quality grade ^b	11.7	0.7	10	13

^aA⁴⁰ = 140, A⁵⁰ = 150, A⁶⁰ = 160, A⁷⁰ = 170, A⁸⁰ = 180.

^bChoice⁻ = 10, Choice^o = 11, Choice⁺ = 12, Prime⁻ = 13, Prime^o = 14.

Muscle dimensions

Weights and dimensions, including length, width, minimum thickness, and maximum thickness, were recorded on individual muscles from the left sides of carcasses. Weights were taken using an analytical scale (Model PB3002-S; Mettler Toledo, Switzerland), and minimum and maximum thickness measurements were taken from the thinnest and thickest portion of the muscle, respectively, using electronic digital calipers (Traceable Model 14-648-17; Control Company, Friendswood, TX). All other dimensions were measured with a metal ruler. Length was determined to be the longer of the two dimensions, and both length and width were taken across the longest line, or diagonal, of the muscle.

Warner-Bratzler shear force

Following aging, raw weights were recorded on individual muscles. Whole muscle roasts were cooked in a preheated (177°C for 20 min), forced-air convection oven (Model DNO97; Hobart Corp., Troy, OH) to an internal temperature of 70°C. Muscles were

grouped so that those of similar size were cooked together. Internal muscle temperatures were monitored using an Omega HH501BT thermometer (Omega Engineering, Inc., Stamford, CT). When muscles reached 70°C, they were removed from the oven and allowed to rest at room temperature for 10 min. Muscles then were weighed, wrapped in plastic film, and chilled at 4°C for 18 hours.

Chilled muscles were allowed to equilibrate to room temperature before being cut into 2.54 cm-thick slices. Four to six, 1.27 cm cores were removed parallel to muscle fiber orientation, and sheared once with an Instron Universal Testing Machine (Model 1011; Instron Corp., Canton, MA) equipped with a standard Warner-Bratzler attachment. Warner-Bratzler shear force reported is the mean force required to shear the cores from each muscle.

Color

Defatted individual muscles dissected from the right side of the carcass were cut into 2.54 cm-thick slices. The *M. serratus ventralis*, *M. latissimus dorsi*, *M. pectoralis profundus*, *M. teres major*, and *M. tensor faciae latae* muscles were left intact. Muscles were allowed to bloom for 15 min, and objective color measurements (L^* , lightness; a^* , redness; b^* , yellowness values) were taken using a Minolta Colorimeter (Model CR-200 Chroma Meter, Illuminant D65, 2° observer; Minolta Corp., Ramsey, NJ) on three chops selected at random. Thin muscles (i.e. *M. latissimus dorsi*) were left intact, and color measurements were taken from three different surface locations.

Expressible moisture and sarcomere length

Two cylindrical, raw cores (1.27 cm) were removed from each muscle and used for determination of expressible moisture following the centrifugation method of Jauregui, Regenstein, and Baker (1981). Additionally, two samples were taken for measurement of sarcomere length according to the Cross, West, and Dutson (1981) procedures. Remaining portions of each muscle were frozen, pulverized, and used for subsequent determination of pH and total collagen content.

pH

Approximately 3 g of pulverized muscle tissue was blended with 30 mL distilled, deionized water until a smooth slurry was formed. This slurry was filtered through Whatman #1 filter paper (Whatman®, Maidstone, Kent, UK), and a glass-tipped, bench-top pH probe (Accumet Basic, Fisher Scientific, Pittsburgh, PA) was inserted for 30 to 60 sec to allow for equilibration before reading.

Total collagen content

Total collagen content was determined by isolating hydroxyproline from pulverized muscle samples as described by Hill (1966). Hydroxyproline concentration was determined with a colorimetric assay described by Bergman and Loxley (1963), and used to calculate collagen content according to the method set forth by Cross, Carpenter, and

Smith (1973). Collagen content was not determined on the *M. teres major* or *M. tensor fasciae latae* due to insufficient product for sampling.

Statistical analysis

Data were analyzed using the PROC GLM procedure of SAS (SAS Institute, Cary, NC). Muscle effects were tested for each factor analyzed. When significant ($P < 0.05$), least squares means were generated and separated using the PDIFF option.

Results & Discussion

Muscle dimension

Differences ($P < 0.05$) in physical measurements were observed among the eighteen individual muscles (Table 2). On average, the largest muscles identified were the *M. gluteobiceps*, *M. gluteus medius*, *M. longissimus lumborum*, *M. longissimus thoracis*, and *M. semimembranosus*. Muscles such as the *M. latissimus dorsi*, *M. pectoralis profundus*, and *M. serratus ventralis* were thin, but possessed large surface areas. The *M. adductor*, *M. infraspinatus*, *M. psoas major*, *M. rectus femoris*, *M. semitendinosus*, *M. supraspinatus*, *M. triceps brachii*, and *M. vastus lateralis* were moderate in terms of all physical measurements and the *M. teres major* and *M. tensor fasciae latae* were the smallest.

Table 2
Least squares means for physical measurements

Muscle	Weight (g)	Length (mm)	Width (mm)	Thickness (mm)	
				Minimum	Maximum
<i>M. adductor</i>	184.2de	131.4a	59.1def	10.6abc	35.7h
<i>M. gluteobiceps</i>	379.2k	297.8	66.5f	5.9ab	33.6gh
<i>M. gluteus medius</i>	300.3i	172.1cd	102.2j	6.9ab	31.2fg
<i>M. infraspinatus</i>	215.7fg	205.6e	55.8cde	18.4c	27.1e
<i>M. latissimus dorsi</i>	152.6c	248.6f	101.6i	2.6a	11.4a
<i>M. longissimus lumborum</i>	493.5n	275.1g	77.2g	13.5bc	29.5ef
<i>M. longissimus thoracis</i>	345.5j	312.6i	47.8bc	7.6ab	31.9fg
<i>M. psoas major</i>	233.0g	347.6j	42.7ab	5.3ab	23.2d
<i>M. pectoralis profundus</i>	263.6h	370.7k	93.2hi	3.2a	11.9ab
<i>M. rectus femoris</i>	203.3ef	158.6bc	54.5cd	11.2abc	39.45i
<i>M. semimembranosus</i>	403.9l	164.6bc	76.4g	10.4abc	46.9j
<i>M. semitendinosus</i>	170.3cd	183.4d	43.9b	4.9ab	29.4ef
<i>M. supraspinatus</i>	171.5cd	163.6bc	53.2cd	5.8ab	32.4fg
<i>M. serratus ventralis</i>	444.2	423.3l	117.9k	3.7a	16.0c
<i>M. triceps brachii</i>	335.2j	164.7bc	91.5h	8.6ab	42.4i
<i>M. tensor fasciae latae</i>	91.1b	155.6b	63.2ef	5.0ab	13.8abc
<i>M. teres major</i>	47.1a	141.0a	34.3a	5.0ab	14.9bc
<i>M. vastus lateralis</i>	193.7e	157.9b	78.8g	6.4ab	31.9fg
SEM ^a	7.29	4.92	3.10	3.35	1.15

Means within a column lacking a common letter differ ($P < 0.05$).

^aSEM is the standard error of the least squares means.

pH and expressible moisture

Least squares means for muscle pH and expressible moisture are reported in Table 3. Values for pH ranged from 5.9 for muscles such as the *M. longissimus thoracis*, *M. longissimus lumborum*, and the *M. semimembranosus*, to 6.5 for muscles such as the *M. serratus ventralis*. The *M. teres major* and *M. serratus ventralis* had the highest ($P < 0.05$) pH values of all the muscles evaluated. The pH values of the *M. longissimus thoracis* and *M. longissimus lumborum* (5.9) observed in this study were higher than the value of 5.74 reported for these muscles by Wheeler and Koochmaraie (1994).

The *M. triceps brachii*, *M. pectoralis profundus*, and *M. latissimus dorsi* were found to have among the lowest numerical expressible moistures, whereas the *M. adductor* and the *M. longissimus lumborum* had among the highest. As expected, higher expressible moisture values tended to correspond with lower muscle pH, and lower expressible moisture was associated with higher muscle pH.

Table 3
Least squares means for pH and expressible moisture

Muscle	pH	Expressible moisture, %
<i>M. adductor</i>	6.0bcde	39.4j
<i>M. gluteobiceps</i> - distal	6.0cde	37.1ghij
<i>M. gluteobiceps</i> - proximal	6.0bcde	38.1ij
<i>M. gluteus medius</i>	6.0abcd	37.7hij
<i>M. infraspinatus</i>	6.3gh	32.4bcd
<i>M. latissimus dorsi</i>	6.3h	29.4a
<i>M. longissimus lumborum</i>	5.9abc	39.7j
<i>M. longissimus thoracis</i>	5.9a	37.6hij
<i>M. psoas major</i>	6.0de	34.8defgh
<i>M. pectoralis profundus</i>	6.2f	29.3a
<i>M. rectus femoris</i>	6.2f	35.6efghi
<i>M. semimembranosus</i>	5.9ab	37.4ghij
<i>M. semitendinosus</i>	6.2fg	31.3ab
<i>M. supraspinatus</i>	6.2fgh	33.4bcdef
<i>M. serratus ventralis</i>	6.5i	31.7abc
<i>M. triceps brachii</i>	6.2f	29.2a
<i>M. tensor fasciae latae</i>	6.0cde	32.7bcde
<i>M. teres major</i>	6.4i	34.8defgh
<i>M. vastus lateralis</i>	6.1e	36.0fghi
SEM ^a	0.03	1.08

Means within a column lacking a common letter differ ($P < 0.05$).

^aSEM is the standard error of the least squares means.

Sarcomere length, collagen content, and Warner-Bratzler shear force

Least squares means for sarcomere length, collagen content, and Warner-Bratzler shear force are reported in Table 4. The *M. psoas major* had the longest ($P < 0.05$)

sarcomere length, which is in agreement with McKeith, DeVol, Miles, Bechtel, and Carr (1985). The *M. adductor*, *M. gluteobiceps*, *M. gluteus medius*, *M. longissimus lumborum*, *M. longissimus thoracis*, and *M. semimembranosus* had among the shortest sarcomere lengths. Values reported for sarcomere lengths are similar to those reported by Cross, Smith, and Carpenter (1972) for lamb *M. gluteobiceps*, *M. rectus femoris*, *M. semimembranosus*, *M. semitendinosus*, and *M. vastus lateralis*, and Wheeler and Koohmaraie (1994) for lamb *M. longissimus lumborum*.

The *M. infraspinatus* had the highest ($P < 0.05$) total collagen content when compared to all other muscles; however, the standard deviation for this muscle also was the highest (3.6; not reported in tabular form). This muscle contains a layer of heavy connective tissue running through its center, which is likely the cause of these observations. The *M. longissimus lumborum*, *M. longissimus thoracis*, *M. adductor*, *M. semimembranosus*, and *M. semitendinosus* had among the lowest collagen contents in the muscles studied.

The *M. serratus ventralis* had among the lowest numerical WBS values. One of the features contributing to the tenderness of this muscle is a high fat content

Table 4

Least squares means for sarcomere length, total collagen content, and Warner-Bratzler shear force (WBS) of individual lamb muscles

Muscle	Sarcomere length, μm	Collagen, mg/g	WBS, N
<i>M. adductor</i>	1.7a	3.2abc	31.6e
<i>M. gluteobiceps</i> - distal	1.7a	5.0efg	26.5bc
<i>M. gluteobiceps</i> - proximal	1.7a	5.6fg	28.1cde
<i>M. gluteus medius</i>	1.7a	6.1g	30.7de
<i>M. infraspinatus</i>	2.3e	9.0h	27.0bcd
<i>M. latissimus dorsi</i>	2.9i	5.0efg	28.1cde
<i>M. longissimus lumborum</i>	1.7a	2.6a	25.6abc
<i>M. longissimus thoracis</i>	1.8ab	2.9ab	23.4ab
<i>M. psoas major</i>	3.1j	4.5def	28.4cde
<i>M. pectoralis profundus</i>	2.8h	5.0efg	28.7cde
<i>M. rectus femoris</i>	2.0c	4.3cde	26.9bcd
<i>M. semimembranosus</i>	1.7a	3.5abcd	42.6f
<i>M. semitendinosus</i>	2.4f	3.7abcd	31.1e
<i>M. supraspinatus</i>	2.2d	5.5fg	30.6de
<i>M. serratus ventralis</i>	2.1d	4.1cde	21.8a
<i>M. triceps brachii</i>	2.6g	5.0efg	29.7cde
<i>M. tensor fasciae latae</i>	2.9i	--	30.9de
<i>M. teres major</i>	2.6g	--	26.4bc
<i>M. vastus lateralis</i>	1.9b	3.9bcde	29.4cde
SEM ^a	0.04	0.41	0.33

Means within a column lacking a common letter differ ($P < 0.05$).

^aSEM is the standard error of the least squares means.

(Brackebusch, McKeith, Carr, & McLaran, 1991). The *M. semimembranosus* had the highest WBS value of all of the muscles in the study (42.6 N). Belew, Brooks, McKenna, and Savell (2003) reported a similar value (4.53 kg or 44.4 N) for beef, and of the muscles represented in this study, the *M. semimembranosus* ranked last in terms of tenderness with only the *M. pectoralis profundus* being less tender. In agreement with our findings, Morgan et al. (1991) reported that steaks from the top round had the highest WBS values of the muscles evaluated. The WBS values for the *M. triceps brachii*, *M.*

supraspinatus, and *M. psoas major* from the current study (29.7, 30.6, and 28.4 N, respectively) are very similar to those reported for lamb by Shackelford, Wheeler, and Koohmaraie (1997).

Color

Least squares means for muscle color are shown in Table 5. Muscle L^* values indicated that the *M. latissimus dorsi* and *M. tensor fasciae latae* had the lightest ($P < 0.05$) colored lean (highest L^* values) and the *M. adductor* and *M. semimembranosus* had the darkest ($P < 0.05$) colored lean (lowest L^* values) when compared to all other muscles. The *M. supraspinatus* and *M. psoas major* had among the highest numerical redness (a^*) values.

Table 5
Least squares means for color measurements of individual lamb muscles

Muscle	L^*	a^*	b^*
<i>M. adductor</i>	41.0a	15.4ef	3.9cdef
<i>M. gluteobiceps</i> - distal	42.8b	16.0fgh	4.0cdef
<i>M. gluteobiceps</i> - proximal	43.8cde	16.3gh	4.2defg
<i>M. gluteus medius</i>	43.2bc	16.5hi	4.3efg
<i>M. infraspinatus</i>	46.3gh	16.9ij	3.9bcdef
<i>M. latissimus dorsi</i>	48.1i	14.0ab	3.3ab
<i>M. longissimus lumborum</i>	42.7b	14.7bcd	3.8bcde
<i>M. longissimus thoracis</i>	44.3de	15.6ef	4.2efg
<i>M. psoas major</i>	44.3de	17.4jk	4.4fg
<i>M. pectoralis profundus</i>	47.0h	13.9a	3.0a
<i>M. rectus femoris</i>	45.4fg	15.5ef	3.7bcd
<i>M. semimembranosus</i>	41.1a	15.3def	4.0cdef
<i>M. semitendinosus</i>	46.7h	15.2cde	4.2efg
<i>M. supraspinatus</i>	46.8h	17.7k	4.7g
<i>M. serratus ventralis</i>	46.5h	15.8efgh	4.1bcdef
<i>M. triceps brachii</i>	43.5bcd	15.7efg	3.5abc
<i>M. tensor fasciae latae</i>	48.2i	13.9a	3.9cdef
<i>M. teres major</i>	46.1gh	14.5abc	3.5abc
<i>M. vastus lateralis</i>	44.7ef	16.5hi	4.2defg
SEM ^a	0.34	0.26	0.20

Means within a column lacking a common letter differ ($P < 0.05$).

^aSEM is the standard error of the least squares means.

Conclusions

With a better understanding of individual muscle characteristics, the meat industry may be able to maximize potential from individual muscles to help increase quality and consistency in lamb products. This process should open many new opportunities in value-added and new-product development. Further research is needed to evaluate consumer

acceptance of individual lamb muscles with marketing strategies developed to positively alter consumer perception of lamb products.

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EFFECT OF STUNNING METHODS(ELECTRICAL VS CO₂) ON INCIDENCE OF PSE PORK

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Key Words: Pork, PSE, CO₂ stunning, Electrical stunning

Introduction

Postmortem metabolic rate has a significant effect of meat quality through its effect of protein denaturation and proteolysis, while pork has a faster postmortem metabolic rate than beef and sheep(March et al., 1972). Pearson(1987) reported that pork quality was largely affected by postmortem changes in muscle temperature and pH. Mitchell and Heffrom(1982) showed that pH decline was not significantly determined by stress gene, but pH decline itself has more than three times effect on PSE incidence.

Park et al. (2003) reported that approximately 45% of pork produced by Korea industry was PSE-like meat and that was largely related to pre-slaughter transit stress, feed restriction as well as stunning method. From the animal welfare viewpoint, it has been recommended that pig should be stunned prior to slaughter (Council Directive 93/119/CEE, 1993). Stunning method has a significant effect on pork quality despite the effect have varied depending on experimental design and purpose of studies(Gregory, 1994; Cannon et al., 2002; Casteels et al., 1995). Electronic and CO₂ have been largely used by pork industries across world, while the former method has been dominant in Korean pork industry, with only three slaughter plans have adopted the latter stunning method.

Objectives

The current study was conducted to investigate the effects of stunning methods (electrical vs CO₂ method) on PSE incidence and general pork quality.

Methodology

Animals, experimental design and treatment: date set comprised two separate experiments; 1) PSE incidence of five biggest slaughter house in Korea from 2003. 9. to 2004. 6. where electrical stunning method was applied and 2) comparison of electrical and CO₂ stunning methods from the same places conducted at 2003. 6. The number of animals are presented in Tables, 1, 2 and 3.

Objective meat quality and PSE incidence: After slaughter carcasses were placed at a 5 chiller longer than 18 hours and 4th/5th ribs were cut. Meat color, texture,

intramuscular fat, moisture score and separation score between muscles were determined by carcass graders from the Korean Animal Products Grading Service (APGS, 2001). For the determination of PSE incidence, carcass with color number 1 or 2, texture number 3 and moisture score 3 was determined as severe PSE, while carcass of color number 3, texture or moisture score number 2 was considered as light PSE. The rest carcass was considered to be normal. The data set was examined to determine the effect of stunning method on meat quality and PSE incidence using SAS package(SAS, 2001) by applying Duncan test and Chi-Square procedure.

Results & Discussion

Table 1 shows number of animals, carcass characteristics and PSE incidence. Pigs resulted in normal carcass traits were lighter weight with 74.6 kg than those for severe and light PSE of 76.9 and 77 kg, respectively. Average backfat thickness for normal, severe and light PSE were 16.2, 17.1 and 17.4 mm, respectively. The results indicated that PSE incidence was associated with heavier carcass weight with thicker backfat while intramuscular fat content was lower, suggesting that fast growth rate could result in PSE meat, as reported by an early study (Webb et al., 1982).

Table 2 presents the effects of various electrical stunning methods on PSE incidence. The results was rather expected, but demonstrated that a higher voltage increased the frequency of PSE, with 12.3, 17.41, 24.91, and 43.12% for 220, 240, 250, and 430 voltage, respectively. The data again indicated that high voltage stunning has a high risk of PSE incidence and that was likely related to a higher rate of postmortem metabolic rate when carcass temperature maintained near physical temperature which could result in protein denaturation. This result was similar to our early study(Park et al., 2002) where 230 voltage stunning resulted in a significantly lower PSE incidence with 42.3% than 500 voltage of 66.7%. Similarly Grandin(1994) recommended a 300 voltage system for big pigs and a lower voltage for more a lighter pig.

In order to identify the practical problem associated with stunning method in PSE incidence, 500 voltage system which has been largely used by Korean pig industry was compared with CO₂ method with putting an emphasize on PSE incidence. Table 3 presents the comparison between two methods on PSE incidence. The result indicated that CO₂ method reduced approximately 33% of PSE incidence by reducing 72.9% for 500 voltage system to 39.3% for CO₂ stunning system. As the current study was industrial based examination, biological aspects of the stunning methods were not examined in details, but the data might indicated that the latter method reduced stunning stress and consequently alleviated metabolic rate during rigor development.

Conclusions

Therefore, the result from this study suggested that the stunning methods had a significant effect on the incidence of PSE pork. Also, the low-voltage stunning and CO₂ stunning methods were highly recommended to control and maintain the pork quality.

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Table 1. Comparison of carcass and meat properties for normal, light and severe PSE pork

Item	Normal	Severe PSE	Light PSE
Number of animal	68,697	9,995	2,457
Carcass weight	74.63 ^b ± 0.03	76.87 ^a ± 0.08	76.96 ^a ± 0.16
Backfat thickness(mm)	16.17 ^c ± 0.02	17.13 ^b ± 0.05	17.43 ^a ± 0.11
Color score	3.51 ^a ± 0.00	2.44 ^c ± 0.01	3.19 ^b ± 0.01
Texture score	1.12 ^c ± 0.00	2.61 ^a ± 0.01	2.00 ^b ± 0.00
Marbling score	2.09 ^a ± 0.01	1.43 ^c ± 0.01	1.61 ^b ± 0.02
Moisture %	15.64 ^c ± 0.07	85.86 ^a ± 0.20	73.57 ^b ± 0.10
Moisture score	1.02 ^c ± 0.00	2.69 ^a ± 0.01	2.00 ^b ± 0.00
Separation score between muscles	1.03 ^c ± 0.00	1.20 ^a ± 0.00	1.11 ^b ± 0.01

^{a-c}Means having different letters in the same row are significantly different(p<.05).

Table 2. Incidence rate of PSE pork by using different electric voltage for stunning

Stunning Voltage	Normal	PSE			Total numbers of animal
		Severe	Light	Total	
220V	87.19	10.86	1.95	12.81	58,867
240V	84.64	9.08	6.28	15.36	8,771
250V	76.33	18.22	5.46	23.68	10,716
430V	63.26	30.55	6.19	36.74	2,795

Chi-Square:3505.99(p<.0001).

Table 3. Incidence rate of PSE pork by using high electrical voltage or CO₂ stunning methods

Stunning methods	Normal	PSE pork		
Stunning methods	Normal	Severe	Light	Total
High electrical stunning (500V)	27.14(19) ¹⁾	14.29(10)	58.57(41)	72.86(51)
CO ₂ stunning	60.71(34)	16.07(9)	23.21(13)	39.28(22)

¹⁾() : frequency

DISTRIBUTION OF PSE MEAT IN SKELETAL MUSCLES OF PIG CARCASS

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Keywords: pig half-carcasses, PSE meat, distribution in muscles

Introduction

Quality defects in pig meat generate considerable economic losses in meat production. Pospiech and Borzuta (1998) estimated that annual losses caused by occurrence of PSE and acid meat amounts to about 2,4% of raw material value. There are about 100 striped muscles in a pig half-carcass, but only five of them compose 1/3rd of carcass muscle weight and they are the main part of two most important cuts, i.e. loin and leg.

Quality defects in meat are developed in the most valuable muscle groups with a considerable percentage of pale fibers and sometimes only in a part of an examined muscle (Linke and Heinz 1972, Koćwin-Podsiadła *et al.* 1998, Kauffman *et al.* 1993, Borzuta *et al.* 2001). Present research concerns mainly the *longissimus dorsi* muscle and there is lack of thorough data concerning PSE meat distribution in other carcass muscles.

Objectives

The objective of the study is to define of the anatomic-spatial distribution of PSE meat in skeletal muscles of pig carcass depending on the rate of meat acidification after slaughter.

Methodology

Research material comprised of 80 pig carcasses chosen on the basis of pH₁ measurement in *longissimus lumborum* (LL) muscle, what took place on slaughter line in a slaughter house situated near Poznań. Carcasses were divided into following 4 groups, 20 half-carcasses each, depending on quality, differing as for pH₁ level in LL muscle, measured at first lumbar vertebrae about 45 min. after slaughter: group A – pH₁ ≤ 5,8, group B – pH₁ 5,81 – 6,00, group C – pH₁ 6,01 – 6,30, control group D – pH₁ > 6,30 and pH₂₄ < 6,2. Quality groups were chosen on the basis of results of research conducted by many authors who studied PSE meat (Briskey 1964, Kortz 2003, Krzywicki 1972, Kauffman *et al.* 1993, Pospiech and Borzuta 1998).

pH value of meat was measured using the Radiometer PHM 80 Portable pH-meter equipped in a combined electrode. pH measurements were conducted on hanged left carcasses in the following muscles: *longissimus dorsi* in 3 points (near neck part, back part and near leg part), *triceps brachii*, *semimebranosus*, *biceps femoris*, *quadriceps femoris*, and *gluteus medius*.

In the above mentioned muscles, electrical conductivity was also measured, 3h (EC₃) and 24h (EC₂₄) after slaughter using PQM Combi device, as well as lightness, 24 h after slaughter (Minolta Chroma Metters CR-300). Chilled half-carcasses were cut into joints and then the joints were de-boned.

Pale meat with PSE symptoms was cut out of the de-boned joint meat and weighed with accuracy to 5g. The pale meat suitable for cutting was examined by a team of 3 experts from Meat and Fat Research Institute, with the help of color quality standard included in annex 1 of Agriculture Canada Publication 5180/B. For the purpose of statistic calculation, variance analysis as well as Tukey test (Statistica PL 1997) was used.

Results & Discussion

The muscles most compliant to quality changes of PSE type are those from most valuable half-carcass parts, i.e. loin and leg and, in smaller degree, shoulder and neck (tab. 1). Pale meat was not found in fat joints of half-carcass, such as: belly, jawl and ventral part of belly.

Analysis of data included in tab. 1 raises reflection that the percentage of PSE meat in particular joints as well as in all half-carcass decreases considerably in particular quality groups A to D. However, in first two groups the percentage is the highest and exceeds 10% of carcass weight. In group C of half-carcasses, classified as partly watery meat group, the percentage of pale meat with PSE symptoms is 2 to 3 times lower than in groups A and B. Occasional occurrence of pale meat in some carcass joints classified as normal quality meat group can be explained by natural lightness change caused by factors other than glycolysis, e.g. lower level of myoglobin (Krzywicki 1972).

High percentage of PSE meat in groups A and B undermines the legitimacy of $pH_1 \leq 5,8$ limit as the indicator of watery meat and proves that more legitimate limit would be $pH_1 \leq 6,0$. Such a limit was proposed in earlier research conducted by Bendall and Lawrie (1964), Krzywicki (1972) and Kortz (2003).

Average value of investigated muscle pH is distinctly and statistically significantly diversified depending on quality group (tab. 2). The lower acidity is smaller the experimental group is closer to a control group. It means that pH_1 of all examined muscles increases from group A to D. Those results are connected with accepted concept of diversification of the research groups on the basis of pH_1 measurement in the middle part of *longissimus dorsi* muscle.

Within particular research groups the highest pH_1 values were observed in the muscles: *triceps brachii* and *quadriceps femoris*. Those differences are present also during the later period of after slaughter changes. Correlation between pH_1 of LD muscle and the results of other examined muscles is also an interesting issue. Correlation factors between pH_1 of LD muscle and pH_1 of other muscles were statistically significant ($P \leq 0,01$) and amounted to: *biceps femoris* muscle 0,9976, *gluteus medius* muscle 0,9945, *semimembranosus* muscle 0,9833, *triceps brachii* muscle 0,9822, *quadriceps femoris* muscle 0,9723, *psoas major* muscle 0,9524. The defined correlations as well as the results of examination of other LD muscle quality traits, such as electrical conductivity and lightness, enabled the researchers to provide a linear regression equation ($r^2 = 0,85$)

to estimate the percentage of pale meat with PSE traits in a carcass (Y), what is as follows:

$$Y = 234,4 - (5,21 \text{ pH}_1 + 9 \text{ EC}_3 - 6,96 \text{ EC}_{24} + 2,40 \text{ L})$$

where:

EC_3 – electrical conductivity 3h after slaughter, EC_{24} – electrical conductivity 24h after slaughter,

L – lightness 24h after slaughter, %).

Conclusions

- 1) The percentage of pale meat with PSE symptoms, estimated in the thesis, in carcass groups with pH_1 of LD muscle $\leq 5,8$ (A) and $5,81 - 6,00$ (B) is very high as it constitutes $1/3^{\text{rd}}$ of the meat obtained from carcass for group A and $1/5^{\text{th}}$ for group B. As for half-carcass weight, the percentage of PSE meat amounts adequately to 17,51 and 10,34%.
- 2) Muscles most prone to PSE meat occurrence are loin and leg muscles, and, in smaller degree, shoulder, neck and shank. PSE meat was not found in belly, jawl and ventral part of belly.
- 3) Among factors predicting the percentage of PSE meat in pig carcass, measured instrumentally, the best are: pH_1 , EC_3 , EC_{24} and photometric lightness (L^*) of *longissimus dorsi* muscle. Using those traits, a multiple regression equation was provided to estimate the percentage of PSE meat in carcass

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Tables and Figures

Table 1. Share of PSE meat in cuts and in the carcass (%)

Part of carcass	Quality groups			
	A	B	C	D
loin	48,37 ^a	28,96 ^b	23,54 ^c	0,12 ^d
leg with shank	37,49 ^a	23,39 ^b	8,71 ^c	0,81 ^d
shoulder with shank	9,28 ^a	5,12 ^b	0,46 ^c	0,00 ^d
neck	5,69 ^a	3,52 ^b	2,80 ^c	0,00 ^d
tenderloin	1,87 ^a	1,01 ^b	0,31 ^c	0,00 ^d
carcass	17,51 ^a	10,34 ^b	4,61 ^c	0,21 ^d

a,b,c,d – means with different indexes differ statistically significant ($P \leq 0,05$)

Table 2. Means of pH₁ of investigated muscle in different quality groups

Muscles	Trait	Groups			
		A	B	C	D
Longissimus dorsi (1)	\bar{x} s	5,62 ^a _A 0,07	5,92 ^b _A 0,05	6,18 ^c _B 0,05	6,72 ^d _C 0,10
Longissimus dorsi (2)	\bar{x} s	5,73 ^a _B 0,17	6,00 ^b _B 0,14	6,25 ^c _B 0,12	6,61 ^d _B 0,26
Longissimus dorsi (3)	\bar{x} s	5,74 ^a _B 0,15	5,92 ^b _A 0,15	6,26 ^c _B 0,14	6,68 ^d _B 0,14
Psoas major	\bar{x} s	5,85 ^a _B 0,13	6,02 ^b _B 0,17	6,02 ^b _A 0,17	6,48 ^c _A 0,13
Triceps brachii	\bar{x} s	5,99 ^a _C 0,16	6,23 ^b _C 0,12	6,32 ^b _C 0,14	6,64 ^c _B 0,13
Biceps femoris	\bar{x} s	5,88 ^a _B 0,16	6,09 ^b _B 0,07	6,29 ^c _B 0,09	6,70 ^d _B 0,14
Semimembranosus	\bar{x} s	5,76 ^a _B 0,10	6,06 ^b _B 0,10	6,25 ^c _B 0,11	6,71 ^d _B 0,19
Quadriceps femoris	\bar{x} s	5,97 ^a _C 0,12	6,29 ^b _C 0,10	6,34 ^b _C 0,12	6,69 ^c _B 0,17
Gluteus medius	\bar{x} s	5,85 ^a _B 0,10	6,07 ^b _B 0,09	6,30 ^c _B 0,14	6,65 ^d _B 0,17

1 –last rib

2 – near neck

3 – near ham

a,b,c,d,e - statistically significant differences calculated for the investigated groups.

A,B,C - statistically significant differences calculated for the investigated muscles.

EFFECT OF FEED INGREDIENT SOURCE AND MODIFIED ATMOSPHERE PACKAGING WITH CARBON MONOXIDE ON FRESH PORK QUALITY

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Key Words: Pork quality, modified atmosphere packaging, carbon monoxide, by-products

Introduction

Feeding pigs with diets containing animal by-products is common practice in New Zealand and is based on both the historical association of pig production with the dairy industry and the relatively high cost of imported plant-based ingredients (NZPIB, 1998). A typical New Zealand finisher pig diet may contain 10% meat and bone meal, representing 30% of total dietary protein (Hendriks et al., 2002). There is considerable worldwide variation in both regulation of the animal feed and production industries and approaches to the safe use of by-product ingredients (Machin, 2005). Considering that no evidence has emerged showing a susceptibility of pigs to transmissible spongiform encephalopathies through oral exposure (Brooks, 1991; Anil & Austin, 2005), continued use of meat and bone meal can be expected in countries where marketing opinion remains favourable towards this practice and where economic considerations make it the most feasible method of protein and fat incorporation. There is, however, a paucity of data comparing the effects of feed ingredients derived from different by-product sources on pork quality.

Preservative packaging is another key meat quality concern, particularly where products are shipped to distant markets. Modified atmosphere packaging (MAP) using a high carbon dioxide environment is an effective means of prolonging microbial shelf-life during extended storage (Sørheim et al., 1999). In the short term, oxygen, either residual or in the MAP gas mixture, is effective for maintaining an attractive “bloomed” colour in red meats, but prolonged exposure results in the irreversible formation of metmyoglobin and an overwhelming of the myoglobin reducing ability of meat, thus preventing the oxygenation required for an attractive meat colour at retail (Tewari et al., 2002). Addition of carbon monoxide, however, results in the formation of stable, bright red carboxymyoglobin, even at low levels of incorporation in the MAP environment (<1.0%; Luño et al., 2000).

Objectives

This research was undertaken to explore the effects of feeding ingredients from animal by-product and plant material sources on pork quality and to determine the impact of including carbon monoxide in a carbon dioxide MAP atmosphere on the storage of this fresh pork for 8 weeks.

Methodology

Eight pigs were fed from six weeks post-weaning to an average slaughter weight of 102.0 kg. During each growth phase (weaner, grower, finisher), pig diets were differentiated by their ingredient sources. Half of the pigs received a diet with protein and fat sourced from a combination of animal and plant ingredients (barley + blood meal, fish meal, meat and bone meal, skim milk powder, soybean meal, tallow). The remainder of the pigs received diets based solely on plant ingredients (barley + soybean meal, soy protein isolate, peas, soybean and linseed oils).

Carcass processing was conducted at a commercial plant. Following overnight chilling (~18 h), longissimus muscles were removed from each carcass. All cutting and packaging was completed in a well-sanitized commercial facility at the start of the work day. The lumbar portion (extending 30 cm from the last rib) of each left side muscle was vacuum packaged and returned to the meat lab for evaluation at 48 h postmortem. Fat thickness over the last rib was measured and a muscle tracing was taken and later measured (KP-90 N planimeter, Placom, Japan) to determine area. A pre-weighed 25 mm slice from each muscle was cooked for 60 min at 70°C in a waterbath. After chilling overnight, samples were reweighed to determine cooking loss and from each, six cores with 13 x 13 mm cross-sections were prepared for Warner-Bratzler shearing. Drip loss of a 40 mm cube from each muscle was determined after suspending pre-weighed samples inside pre-inflated plastic bags for 48 h at 0-1°C. Measurement of pH was made on 2.0 g meat samples homogenized with distilled water.

Each right side longissimus muscle was divided into 27 boneless pork chops. Each chop was placed on a polystyrene tray with soaker pad and overwrapped with a highly permeable polyethylene film to produce retail-ready samples. The overwrap film was scored with a knife to permit unrestricted gas exchange once samples were placed in master packs. Three chops from each muscle were designated for immediate evaluation (week 0) upon their return to the lab. The remaining 24 chops from each muscle were allocated to one of two MAP treatments consisting of the placement of six retail-ready samples in transparent, 7-layer, co-extruded barrier bags (O₂ transmission rate 0.13 cc/m²/24h/atm at 1°C and 100% RH, Vertex Pacific Ltd, New Zealand). The bags were evacuated and flushed twice (Securepack, Vertex) with an excess of either 100% carbon dioxide (CO-) or a mixture of 80% carbon dioxide, 19.6% nitrogen, and 0.4% carbon monoxide (CO+). Within each modified atmosphere packaging treatment, three chops from each muscle were allocated to refrigerated storage (3°C) for 2, 4, 6, or 8 weeks.

At the conclusion of each storage period, colour and bacteriology of each pork chop were evaluated. Immediately after opening each master pack, instrumental colour (L*, hue, chroma; Minolta ChromaMeter CR-200, Japan) was measured through the overwrap film. Pork chops were then aseptically sampled and prepared for enumeration of total aerobes and anaerobes (plate count agar; Merck, New Zealand) and presence/absence of *Listeria* spp. (pre-poured Difco Oxford agar, Fort Richards Laboratories Ltd, New Zealand; Single Path test, Merck, New Zealand). Colour measurements were repeated after microbiological sampling was completed. The remainder of each sample was freeze dried and prepared for thiobarbituric acid reactive substances assay (Inoue et al., 1998) to assess lipid oxidation status.

Statistical analyses of meat and storage quality data were conducted with the GLM procedure of SAS, using repeated measures for colour measurements. Means were deemed significantly different at $P < 0.05$.

Results & Discussion

Dietary treatment did not affect fat depth or longissimus muscle area (Table 1), indicating that similar carcass yields can be expected when pigs are fed to a common slaughter weight on diets containing either animal or plant ingredients. Similarly to Lettner et al. (2001) who reported a lack of significant difference on drip loss and taste panel scores for tenderness, juiciness, and flavour of pork from pigs fed diets containing 10-12% meat meal versus 25% soy, no other meat quality attributes were affected by dietary treatment (Table 1).

Table 1. Effect of ingredient source on carcass and meat quality attributes

Quality attribute	Ingredient source		SEM	P-value
	Animal by-products	Plant material		
Fat depth (mm)	11.9	12.0	0.53	0.86
Muscle area (cm ²)	40.3	40.8	1.50	0.77
Shear force (kg)	7.4	7.6	0.39	0.66
Cooking loss (%)	29.5	29.7	0.51	0.80
Drip loss (%)	3.9	3.9	0.35	0.89
pH	5.53	5.57	0.042	0.48

Dietary treatment of the live pigs did not affect the subsequent bacteriology of the pork chops, and differences in bacterial counts were neither practically significant nor consistent across MAP environments and storage time (Table 2). Mean aerobic counts moved from 3.2 to 3.6 log CFU·g⁻¹ between 0 and 8 weeks of storage ($P = 0.04$), while anaerobes averaged 2.0 log CFU·g⁻¹ at week 0, decreased to 1.5 log CFU·g⁻¹ by week 2 and gradually increased to 2.2 log CFU·g⁻¹ by the conclusion of the study ($P < 0.01$). Reflecting the general level of hygiene in the processing facility, bacterial counts did not reach levels anywhere near the 6.0 log CFU·g⁻¹ threshold for spoilage.

Table 2. Effect of ingredient source and presence of CO in the MAP environment on total plate counts of aerobic and anaerobic bacteria over 8 weeks of refrigerated storage

Plate count, log CFU·g ⁻¹	Ingredient source and CO presence				SEM	P-value		
	Animal byproducts		Plant material			IS	CO	IS x CO
	CO+	CO-	CO+	CO-				
Aerobes								
Week 0	3.3	3.3	3.0	3.0	0.09	0.48	-	-
Week 2	3.7	3.7	3.3	3.2	0.11	0.07	0.85	0.80
Week 4	2.9	3.3	3.0	3.6	0.20	0.75	0.03	0.75
Week 6	3.3	3.4	3.4	3.4	0.19	0.88	0.80	0.69
Week 8	3.8	3.4	4.1	3.0	0.34	0.76	0.04	0.41
Anaerobes								
Week 0	2.2	2.2	1.9	1.9	0.19	0.65	-	-
Week 2	1.7 ^a	1.3 ^b	1.2 ^b	1.6 ^{ab}	0.16	0.67	0.75	<0.01
Week 4	1.0	1.7	1.4	1.7	0.27	0.49	0.08	0.45
Week 6	1.7	1.9	1.7	2.1	0.25	0.94	0.28	0.70
Week 8	2.7	2.1	2.4	1.8	0.40	0.98	0.16	0.99

^{a,b}Means followed by common letters are not significantly different ($P > 0.05$)

The presence/absence of *Listeria* spp. was assessed as an indicator of the presence and distribution of psychrotrophic pathogens. Five of the eight muscles had at least one pork chop that tested positive for this organism and these were equally distributed across both dietary and MAP treatments. Once a sample tested positive, subsequent samples from that muscle tended to remain positive until the sixth week of storage, after which time no positive results were recorded. The relatively wide distribution of *Listeria* indicates that caution must be exercised and due attention paid to personal hygiene procedures where retail-ready products are produced. Although the source of contamination was not known, its introduction and spread across multiples samples within a muscle were suspected to have been by a member of the packaging team and not by cutting facility staff. While this did not affect the shelf-life of the product, it is clearly a point of concern.

Feed ingredient source had no effect on meat colour (Table 3), nor did Lettner et al. (2001) observe an effect on reflectance. The CO+ samples had a lighter (L*), more intense (chroma), red (hue) colour than the pork chops exposed to 100% CO₂. After exposure to atmospheric oxygen this MAP treatment effect was sustained. Upon visual assessment we concluded that the CO+ pork chops were a more attractive and appealing pink-red colour than the samples packaged without CO.

Table 3. Effect of ingredient source and presence of CO in the MAP environment on instrumental colour measurements of packaged meat and meat exposed to atmospheric oxygen

Colour	Ingredient source and CO presence				P-value			
	Animal by-products		Plant material		SEM	IS	CO	IS x CO
	CO+	CO-	CO+	CO-				
In package								
L*	58.1	57.1	57.7	56.9	0.28	0.78	<0.01	0.70
Chroma	11.4	8.6	11.1	8.4	0.20	0.86	<0.01	0.79
Hue	16.4	22.4	17.7	23.0	0.61	0.75	<0.01	0.62
Open package ^z								
L*	56.0	55.0	55.8	55.2	0.37	0.79	0.03	0.63
Chroma	14.14	13.0	13.8	12.4	0.39	0.39	<0.01	0.70
Hue	31.7	37.9	31.6	39.2	0.65	0.87	<0.01	0.31

^zEffect of exposure to atmospheric oxygen P <0.01 for all colour measurements

As expected, pork from animals fed a diet containing only plant-derived ingredients displayed a significantly higher level of lipid oxidation (animal = 0.63, plant = 1.42 ± 0.076 µg MDA·g⁻¹ fat, P <0.01) since propensity to oxidize increases with level of fat unsaturation (Wood et al., 2003). The MAP gas environment had no effect on lipid oxidation (CO+ = 1.02, CO- = 1.03 ± 0.067 µg MDA·g⁻¹ fat, P = 0.89), although Luño et al. (2000) reported increased inhibition of lipid oxidation with increased CO concentration in the MAP atmosphere.

Conclusions

Feeding pig diets containing either animal by-products or plant materials did not significantly affect quality or bacteriology of fresh pork, although lipid oxidation was greater in pork from pigs fed the plant ingredient diet, a source of polyunsaturated fatty acids. Either type of diet can be recommended for producing pork of acceptable quality, however, further investigation of the impact of plant ingredients on pork rancidity and palatability with extended storage is warranted. Where care is taken to control hygiene at the time of packaging, a carbon dioxide-MAP gas mixture with or without CO provides at least 8 weeks of spoilage-free refrigerated storage for retail-ready pork chops. Although it did not affect lipid oxidation, inclusion of CO provides a clear advantage for the maintenance of a bright, pink-red fresh pork colour under both MAP conditions and after exposure to atmospheric oxygen.

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MYOGLOBIN DENATURATION AS AN INDICATOR OF INTERNAL COOKED GROUND BEEF COLOR

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Key Words: Ground beef, Cooked color, Myoglobin, Endpoint temperature, Cooking rate

Introduction

Internal cooked color traditionally has been used by consumers to determine cooked meat product doneness. However, two phenomena (premature browning and persistent pinking) prohibit the use of internal cooked color to indicate doneness of ground beef. Premature browning has been defined as an internal well-done appearance when endpoint temperatures ensuring safety have not been reached, thereby creating a food safety issue. Persistent pinking is remaining redness/pinkness after the product has been cooked to a safe endpoint temperature. This often leads to overcooking, creating a food quality concern.

In ground beef, consumers often accept a patty with a brown internal color, which may not have been cooked to a safe temperature, while rejecting a patty with a pink internal color that was cooked to a safe temperature. For that reason, premature browning and persistent pinking present issues that food-service establishments must confront with regard to product safety and customer acceptance.

Marksberry (1990) first noted that some ground beef patties had a well-done appearance at much lower temperatures than those needed to ensure safety. Hague et al. (1994) termed this occurrence “premature browning” (PMB) and demonstrated that internal cooked color was not always a reliable indicator of patty doneness. Warren et al. (1996b) examined several chemical properties of ground beef that developed PMB and concluded that PMB was related to the oxidative state of myoglobin in raw product. In examining the importance of myoglobin oxidative state, Warren et al. (1996a) showed if oxidized myoglobin was present in the interior of the raw patty, the interior cooked color was brown. If reduced myoglobin was present, an expected reddish-pink cooked color was observed. Hunt et al. (1999) further found patties with DMb in the interior appeared red and undercooked at 55°C becoming browner with increasing temperature. Additionally, patties with OMb and MMb in the interior created a brown internal cooked color after being cooked to 55°C.

Just as PMB creates unreliability in determining doneness, so does persistent pinking. Trout (1989), and Mendenhall (1989), indicated that as pH increased, myoglobin became more heat stable, resulting in persistent pinking (Kropf and Hunt, 1998). *Nitroso pigments*, found in cured meat products, may also cause persistent pinking (Cornforth,

1994). Hunt et al. (1999) also demonstrated that the redox form of myoglobin affects persistent pinking.

The effects of rapid cooking on internal cooked color development of ground beef are unknown. Brewer et al. (1999) cooked ground beef patties at two rates, 0.7° or 3°C/min, to endpoint temperatures of 55, 60, 65, 70, 75 or 80°C. They found no differences in instrumental or visual color. However, no research data exist on the effects of very rapid cooking rates, such as those used widely in the American fast food industry, the subject of this research. Furthermore, there are no literature reports on the amount of myoglobin that must denature for a ground beef patty to have a well-done internal appearance.

Objectives

Our objective was to determine the role of myoglobin denaturation as an indicator of cooked ground beef patty doneness as influenced by cooking rate and endpoint temperature.

Methodology

Raw ground beef patties (113.5 g, 11.5cm × 12.5cm × 0.8cm) containing 19.7% fat and having a pH range of 5.9 to 6.1, were formulated, vacuumed packaged, and never frozen. Patties contained essentially 100% DMb on the interior, which should give the expected change in internal cooked color from red to pink to brown (Hunt et al., 1999). After 5d, ground beef patties were cooked to one of five endpoint temperatures 65.6, 71.1, 76.7, 82.2 or 87.8°C using either a double-sided grill (1.0°C/sec, very rapid rate) or flat single-surface grill (0.2°C/sec, slow rate). As patties reached their assigned endpoint temperature, they were removed from the cooking device and placed into a bag, which was then placed in an ice bath to limit post-cook temperature rise. Cooled patties were bisected parallel to the patty surface and CIE L* (lightness), a* (redness), and b* (yellowness) values for Illuminant A were obtained. A trained visual panel (n = 3) evaluated the internal cooked color of patties to the nearest 0.5 using the following 5-point cooked-color scale (Marksberry et al., 1993): 1 = reddish-pink center, pink border, tan edge; 2 = pinkish-red center, pink to light brown/tan to outer surface; 3 = slightly pink center, light brown to tan edge (medium); 4 = tan/brown center and edges, no evidence of pink; 5 = dry, brown throughout (well done).

Myoglobin concentration was quantified on raw and cooked patties by extracting myoglobin using a method described by Warriss (1979) and modified by Hunt et al. (1999). Total myoglobin content in raw patties was compared to total undenatured myoglobin in cooked patties to determine the total percent myoglobin denatured. The study was designed as a completely randomized 5 × 2 factorial consisting of 5 temperatures and 2 cooking rates with 5 replications. Data were analyzed using analysis of variance in the MIXED procedure of SAS (2001).

Results & Discussion

Rapid cooking had a profound impact on instrumental cooked color. At endpoint temperatures below 82.2°C, rapidly-cooked patties had greater ($P < 0.05$) a^* values than slow-cooked patties (Fig. 1-a) with the greatest difference in a^* value occurring at 65.6°C. While rapidly-cooked patties had greater a^* values than slow-cooked patties at 87.8°C, patties cooked using either rate were least red of all treatment combinations at this endpoint temperature. Patties cooked rapidly to 71.1°C were less red than 65.6°C and redder than 76.7°C ($P < 0.05$), while cooking to an endpoint temperature of 82.2°C was similar to 76.7 and 87.8°C ($P > 0.05$). Cooking rapidly to temperatures of 82.2 and 87.8°C resulted in a minimum a^* value indicating that little if any pink color remained. Slow-cooked patties had similar ($P > 0.05$) a^* values among endpoint temperatures below 87.8°C, with patties cooked to 87.8°C being the least red ($P < 0.05$). As all slow-cooked patties had low a^* values, it reasons that they all probably would have a similar well-done appearance. Regardless of cooking rate, a^* decreased as endpoint temperature increased.

Visual color data reflected those of instrumental color data. Rapidly-cooked patties were more red/pink ($P < 0.05$) than slow-cooked patties at all endpoint temperatures except 87.8°C (Fig 1-b). Only at 87.8°C were rapidly- and slow-cooked patties of equal appearance ($P > 0.05$). All slow-cooked patties appeared well done (visual score > 4.0) even at 65.6°C, indicating that premature browning had developed. Patties cooked rapidly to 71.1 and 76.7°C were still slightly pink on the interior (visual score < 4.0). Rapidly-cooked patties appeared well done at endpoint temperatures of 82.2 and 87.8°C.

Percent myoglobin denaturation provided an objective measure of pigment denaturation during cooking and accentuated visual and instrumental assessments of internal cooked color (Fig. 1-c). Slow-cooked patties had essentially 90% or greater denatured myoglobin at all endpoint temperatures corresponding with their well-done appearance. A greater range of values was seen in rapidly-cooked patties, similar to a^* and visual color data. Myoglobin denaturation was less ($P < 0.05$) for rapidly-cooked patties than slow-cooked patties at endpoint temperatures of 76.7°C and below, resulting in a redder, less well-done internal appearance. At endpoint temperatures of 82.2 and 87.8°C, both cooking rates were similar ($P > 0.05$) in myoglobin denaturation. Both temperatures resulted in greater than 90% myoglobin being denatured, which was reflected in the decreased a^* and greater visual scores.

Instrumental (Fig. 2-b) and visual (Fig. 2-a) color were highly correlated with myoglobin denaturation ($r = -0.91$ and 0.88 , respectively). Through regression analysis, it was determined that a patty with approximately 80% myoglobin denaturation would result in a well-done internal appearance (visual score > 4.0) with a corresponding a^* value of 12.4. Thus, approximately 80% myoglobin denaturation is needed to result in a well-done internal appearance regardless of cooking method. This will be achieved at lower temperatures with slower cooking rates and at higher endpoint temperatures with more rapid cooking rates.

Conclusions

Percent myoglobin denaturation appears to be an excellent objective indicator of internal cooked appearance, with approximately 80% myoglobin denaturation resulting in a well-done internal appearance. Cooking rate and endpoint temperature greatly influenced the amount of myoglobin denatured and resultant internal cooked color of ground beef patties. Very rapid cooking required greater endpoint temperature than necessary for safety to achieve a well-done appearance, while slow-cooking resulted in PMB. This study reaffirms that internal cooked color is not an adequate indicator of product doneness and/or safety.

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Figure 1: Internal (a) a^* (redness), (b) visual color, and (c) percentage myoglobin (Mb) denaturation of ground beef patties, slowly or rapidly cooked to assigned endpoint temperatures. Raw patties contained predominately deoxymyoglobin in the interior when cooked. Means within an endpoint temperature (a, b) or within a cooking rate (w, x, y, z) differ ($P < 0.05$). $n = 5$ patties/treatment combination.

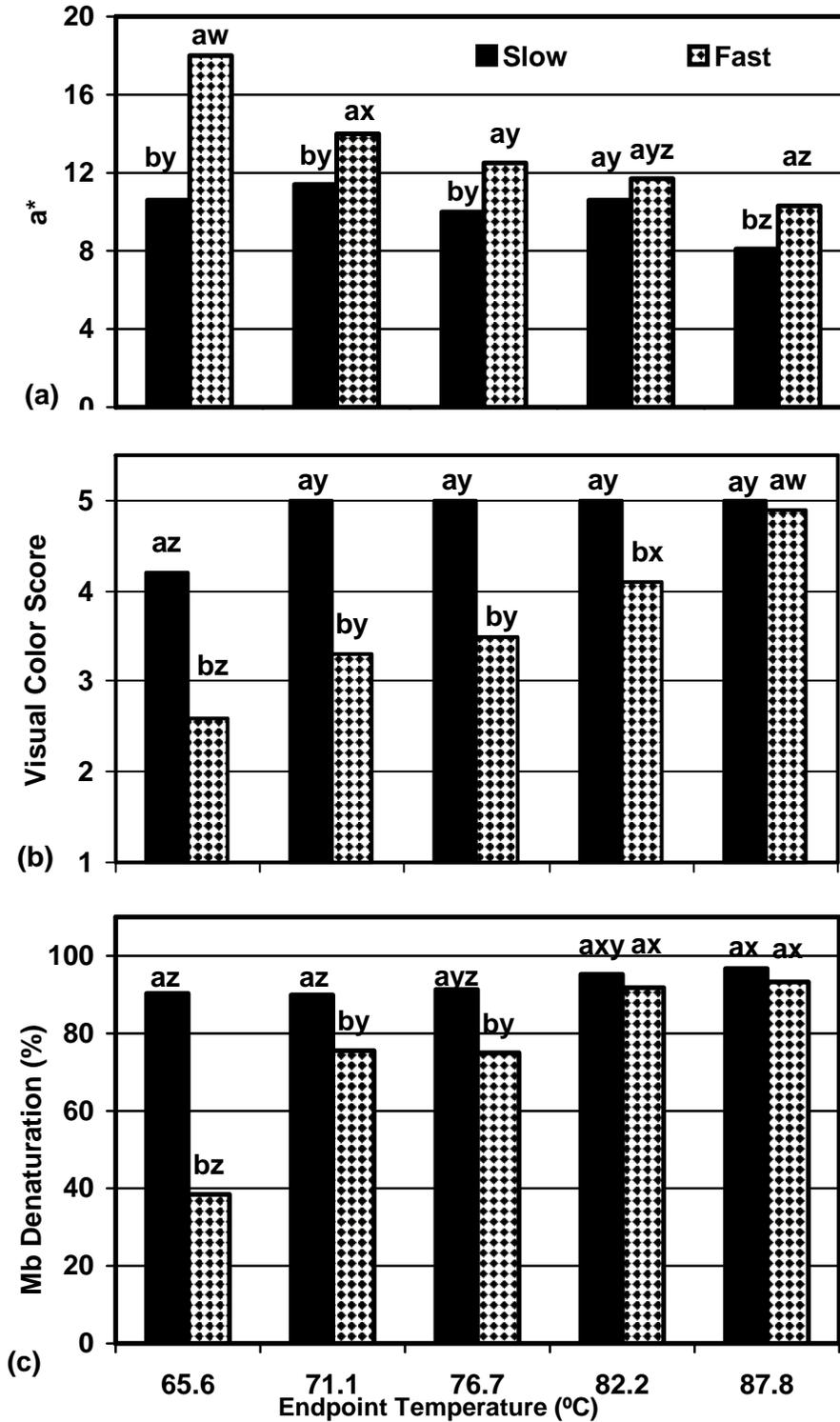
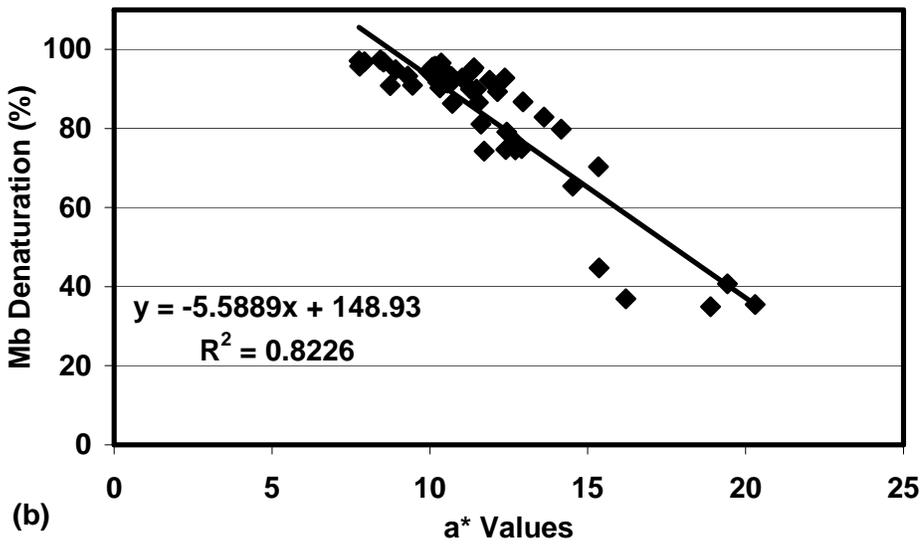
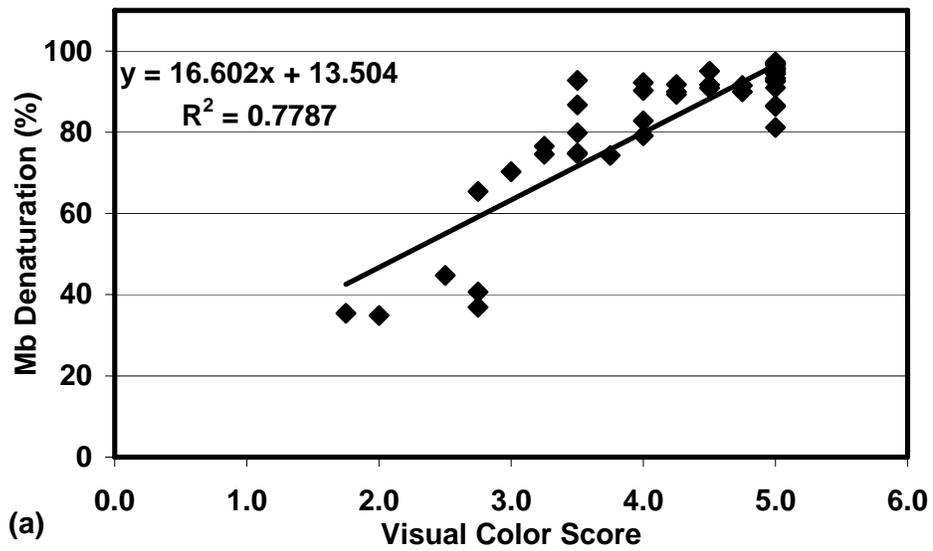


Figure 2: Regression analysis predicting myoglobin denaturation for (a) visual color and (b) a* (redness) values of ground beef patties, slowly or rapidly cooked to assigned endpoint temperatures.



CHEMICAL COMPOSITION, MEAT QUALITY AND CONSUMER ACCEPTABILITY IN MEXICAN (GUADALAJARA, CHIHUAHUA AND VERACRUZ) RETAIL BEEF

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Key Words: Beef quality; Chemical composition; Mexican Market

Introduction

Mexico beef cattle production is diverse and depends on the climate of the region, feeding systems and breeds used. In Mexico, the meat industry is governed by a variety of factors that range from raising the cattle under different conditions to the ways meat is displayed at the supermarket counter. Throughout this chain, many processes affect the final quality of the meat. Therefore, a great variation in the final beef quality is expected. Guadalajara, Chihuahua and Veracruz are three of the most important beef markets in Mexico. Variation in beef quality is generating much discussion in national forums in regards to whether the northern states are producing better meat than the southern regions. A previous research has reported few differences in the quality of beef because of regional differences (Delgado, Rubio, Méndez, Iturbe, Casís & Rosiles, 2005). However, more research is needed to further identify the differences in chemical composition and quality between Mexican meat sources.

Imported beef is toughening the competition for Mexican beef. However, there is only one previous study that has reported some of the differences between both types of meat (Delgado et al., 2005). This study is a continuation of research, which intends to present empirical data about chemical composition, quality traits and consumer acceptability of both Mexican and imported retail beef samples.

Objectives

The objective of this study is to evaluate chemical composition, beef quality and consumer acceptability of both Mexican (from Chihuahua, Guadalajara and Veracruz) and Imported beef samples (bought in the same cities).

Methodology

The study was conducted in three Mexican cities (Chihuahua, Guadalajara and Veracruz). These cities were selected because they represent some of the largest metropolitan areas (INEGI, 2003) and important distribution points for beef.

Samples of packaged (film-wrapped) refrigerated New York steaks of approximately 1 inch thick were purchased from different supermarkets. Meat samples of Mexican, USDA-Choice, and No Roll US beef were purchased, depending on the availability of the relevant sources of beef in the shops. Overall, 65 samples of Mexican beef (25 from Guadalajara, 20 from Chihuahua and 20 from Veracruz) and 65 imported beef (50 samples of USDA-Choice beef and 15 samples of No Roll US beef) were analyzed in the study. A sample unit consisted of three New York steaks from the same primal cut.

Samples were analyzed for moisture, fat and protein content following the procedures described by the AOAC (1990). Total and soluble collagen was estimated from the levels of hydroxyproline (Bergman and Loxley, 1963; Cross, Carpenter, & Smith, 1973). The pH was measured in refrigerated ground meat samples (2-4 °C) using a pH meter with automatic temperature compensation (HANNA pH meter, Model 8521) (AOAC, 1990). Warner Bratzler shear force (WBSF) and cooking loss were determined according to AMSA Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995). Objective color measurements were performed after the steaks were allowed to bloom for 15 min at room temperature (20-25 °C), on two different sites of each steak using a Minolta Chroma Meter CR-310 (Minolta, Osaka, Japan). The average lightness (L^*), redness (a^*), and yellowness (b^*) of each sample was recorded.

The sensory test was carried out in five different sessions more than 150 panelists Steaks of Mexican and both categories of imported beef (USDA-Choice and No Roll US beef) were cooked following AMSA guidelines, as previously described (AMSA, 1995). An affective evaluation test was accomplished using a 7-point hedonic scale from 1) I dislike it very much; to 7) I like it very much. Panelists were asked to assign scores for tenderness and overall desirability to each sample. Clenbuterol was measured using a competitive immuno-enzymatic assay called Quantitative ELISA (R-Biopharm, 1996).

The effect of the origin of beef (Mexican beef from northern, central and southern regions, and imported USDA-Choice and No Roll US beef) was tested for significance using one-way analysis of variance (Lentner & Bishop, 1986). Means were discriminated using the Tukey's range procedure (Statgraphics Plus 2.1).

Results & Discussion

Table 1 shows the chemical composition and meat quality of national vs imported (Choice and No Roll) beef. All sources of Mexican beef and No Roll US beef were comparable in terms of moisture, fat, total collagen, and soluble collagen content ($P > 0.05$). These results agree with those found previously by Delgado et al (2005), who reported these similarities between both types of meat.

The USDA-Choice beef had lower moisture content ($P < 0.05$) and higher fat content ($P < 0.05$) than the other beef types. USDA-Choice meat was expected to contain a high fat content, which agrees with previous studies (Delgado et al 2005 and Luchak et al., 1998). No significant differences were found in the collagen content among beef sources. Delgado et al (2005) found USDA-Choice to have lower collagen content than the other imported and Mexican sources.

The pH of the No Roll meat was higher than the other beef sources ($P < 0.05$). USDA-Choice beef had the lowest WBSF value and the highest cooking loss compared to the

other sources of beef, this matches the previous research on Mexican meat by Delgado et al. (2005). Mexican beef showed a more intense red color compared to the imported samples, this could be an indication of older cattle being slaughtered or the slaughtering conditions in Mexican abattoirs.

Table 2 shows the chemical composition and meat quality of Mexican beef by city. Beef from Guadalajara had the highest fat content and the lowest soluble collagen percentage. Veracruz beef had the lowest fat and protein content and the highest percentage of moisture. Meat quality characteristics show beef from Veracruz to be the most tender compared to the other Mexican beef sources.

The sensory evaluation showed that consumers found similar tenderness for Mexican and No Roll beef; however they found USDA-Choice to be the most tender of all. Results showed no samples with clenbuterol were found.

Conclusions

All sources of Mexican beef and No Roll US beef had comparable chemical compositions. Consumer acceptability was similar for all sources of Mexican beef and for No Roll beef. The present study identified that retail beef in the Mexican market was variable in fat content, Warner-Bratzler shear force, cooking loss, redness (a^* -values), and consumer acceptability, depending on the origin of the meat.

Mexico does not implement a carcass evaluation system that segregates meat by quality. This condition makes all sources of Mexican meat to be accounted as one in this study, which decreases the overall quality. However, imported meat found in Mexican markets was graded by quality (Choice and No Roll) which gives an advantage to the USDA-Choice, which has been specifically selected to have a greater meat quality compared to the lower grades like No Roll meats. Therefore, we strongly recommended Mexico to implement a carcass evaluation in order to be competitive with imports.

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Tables and Figures

Table 1. Chemical composition and meat quality of Mexican and Imported beef (USDA-Choice and No Roll) in Veracruz, Chihuahua and Guadalajara.

	Mexican Beef	No Roll Beef ^{1}	USDA- Choice ^{2} }	SE
n	65	15	50	
Moisture %	72.55 ^{a}	72.58 ^{a}	67. ^{b}	0.14
Fat %	2.45 ^{a}	1.95 ^{a}	6.52 ^{b}	0.07
Protein (Nx6.25) %	20.17 ^{a}	19.62 ^{b}	19. ^{b}	0.07
Total collagen mg/g	9.28	9.28	9.17	0.15
Soluble collagen %	14.01 ^{a}	13.83 ^{a,b}	12.88 ^{b}	0.21
pH	5.73 ^{a}	5.88 ^{b}	5.73 ^{a}	0.01
Shear force, Newtons	44.43 ^{a}	45.11 ^{a}	25.40 ^{b}	0.50
Cooking loss %	24.72 ^{a}	18. 85 ^{b}	26.38 ^{c}	0.32
L (lightness)	42.13 ^{a}	38.67 ^{b}	40.93 ^{c}	0.19
a (red)	18.14 ^{a}	17.05 ^{b}	13.87 ^{c}	0.15
b (yellow)	6.94 ^{a}	5.30 ^{b}	7.32 ^{a}	0.09
Price dollars/kg	7.03 ^{a}	5.88 ^{b}	16.17 ^{c}	0.01

^{1}US beef with no quality grade specified on the label at the point of sale

^{2}Beef labeled as USDA-Choice at the point of sale

^{a,b}Means with different letters in the same row are significantly different (P<0.05)

Table 2. Chemical composition and meat quality for Mexican beef bought in Guadalajara, Chihuahua and Veracruz supermarkets.

	Chihuahua	Guadalajara	Veracruz	SE
n	20	25	20	
Moisture %	72.41 ^{a}	71.92 ^{a}	73.49 ^{b}	0.36
Fat %	2.28 ^{a}	3.07 ^{b}	1.85 ^{a}	0.13
Protein (Nx6.25) %	20.16 ^{a}	20.79 ^{b}	19.39 ^{c}	0.17
Total collagen mg/g	9.15	9.50	9.15 ^a	0.37
Soluble collagen %	14.02 ^{a,b}	13.10 ^{a}	15.15 ^{b}	0.41
PH	5.63 ^a	5.87 ^{b}	5.66 ^{a,b}	0.01
Shear force, Newtons	46.19 ^a	46.28 ^{a}	40.60 ^{b}	1.47
Cooking loss %	18.76 ^{a}	34.20 ^{b}	21.33 ^{c}	0.52
L (lightness)	38.63 ^{a}	43.05 ^{b}	43.78 ^{b}	0.40
a (red)	18.89 ^{a}	16.32 ^{b}	19.15 ^{a}	0.33
b (yellow)	6.05 ^{a}	7.70 ^{b}	6.92 ^{c}	0.20
Price dollars/kg	6.89 ^{a}	6.67 ^{a}	7.80 ^{b}	0.12

^{a,b}Means with different letters in the same row are significantly different (P<0.05)

IN-HOME CONSUMER EVALUATIONS OF INDIVIDUAL MUSCLES FROM BEEF ROUNDS SUBJECTED TO TENDERIZATION TREATMENTS

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Key Words: Beef rounds; In-home consumer survey; Tenderization

Introduction

The U. S. beef industry is moving towards merchandising individual muscles, and has been focused on identifying and improving underutilized muscles from the round and chuck as a way to increase the value of the entire carcass. Kollé et al. (2004) investigated the effects of blade tenderization, enzymatic tenderization, and injection with salt and phosphate solutions on individual muscles from beef rounds. Kollé et al. (2004) found that responses to tenderization systems were largely muscle dependent, with the *M. semimembranosus*, *M. adductor*, *M. rectus femoris*, and *M. vastus lateralis* showing promising improvements in tenderness for all tenderization treatments. Although most research has suggested that tenderness is the most important determinant of beef palatability, a steak that is lacking in any palatability criteria is likely to disappoint consumers. In addition, consumer controlled factors such as degree of doneness and cooking method can have a great impact on consumer satisfaction (Lorenzen et al., 1999; Neely et al., 1999; Savell et al., 1999).

One of the limitations of objective measures of meat tenderness is that many factors that can influence tenderness and palatability, such as cooking method and degree of doneness, are controlled (Kollé et al., 2004). In-home consumer evaluation studies have given excellent insights into how consumers prepare different types of steaks, what degree of doneness they prefer, and how these factors impact their satisfaction with beef products. An in-home study by Neely et al. (1998) found low customer satisfaction ratings for top round steaks were observed primarily because consumers cooked with dry heat methods rather than moist heat.

Of the muscles that responded positively to the various tenderization methods in Kollé et al. (2004), it is unclear which factors augment the existing palatability characteristics of the steaks from individual round muscles in the most beneficial way. An in-home study was conducted to give insight into consumer's ability to pick up both positive and negative effects of tenderization treatments, and subsequent tenderization recommendations can be made to retailers and processors so that muscles from beef rounds are tenderized in a manner that maximizes palatability.

Objectives

To determine in-home consumer palatability responses for steaks from individual muscles from beef rounds that have been tenderized using blade tenderization or injection with a salt and phosphate solution, and determine which treatment works best with each muscle to optimize palatability.

Methodology

USDA Select, beef inside rounds (IMPS#169A) (n = 67) and knuckles (IMPS# 167A) (n = 66) were purchased from a local processing facility and shipped to the Rosenthal Meat Science and Technology Center at Texas A&M University. Subprimals were assigned randomly to one of three treatments (control, blade tenderization, or injection with salt and phosphate solution). Inside rounds were separated into *M. semimembranosus* and *M. adductor*, and knuckles were separated into *M. rectus femoris* and *M. vastus lateralis*. Muscles were trimmed, defatted, and treated according to the treatment group they were assigned. Final pH for post-processing control muscles were 5.75 ± 0.12 , 5.75 ± 0.11 , 5.74 ± 0.08 , and 5.75 ± 0.08 for the *M. semimembranosus*, *M. adductor*, *M. rectus femoris*, and *M. vastus lateralis*, respectively. The blade tenderization treatment consisted of a double pass through a 286 blade (13 rows of 22 blades) TEND-R-RITE Blade tenderizer (TR-2, Bettcher Industries, Inc., Birmingham, OH). Final pH for post-processing blade tenderized muscles were 5.70 ± 0.13 , 5.69 ± 0.09 , 5.48 ± 0.69 , and 5.51 ± 0.05 for the *M. semimembranosus*, *M. adductor*, *M. rectus femoris*, and *M. vastus lateralis*, respectively. The injection/enhancement treatment consisted of a water solution containing 5.0% sodium chloride and 2.95% sodium tripolyphosphate (Brifisol ® 512, BK Giulini Corp., Simi Valley, CA). The solution (pH 7.61 ± 0.23 at $16.1^\circ \pm 1.07^\circ\text{C}$) was injected into the muscles at a 15% level using a single pass through a commercial injection machine (Inject Star BI 72, Inject Star, Inc., Brookfield, CT). Final concentrations of sodium chloride and sodium tripolyphosphate in the muscles were estimated to be 0.71% and 0.42%, respectively. Final post processing pH for injected muscles were 5.96 ± 0.13 , 5.92 ± 0.21 , 5.78 ± 0.14 , and 5.89 ± 0.17 for the *M. semimembranosus*, *M. adductor*, *M. rectus femoris*, and *M. vastus lateralis*, respectively. After processing, muscles were cut into 2.54 cm steaks, individually vacuum-packaged, and frozen. Three steaks were cut from each muscle perpendicular to the muscle fiber orientation starting from the most cranial aspect of each muscle and moving towards the most caudal aspect (or proximal and distal when appropriate).

Steaks from each treatment group (control, blade tenderized, and injected) within a muscle from both muscles within a subprimal were assigned randomly to a box so that each box had six steaks (two muscles * three treatments). Each box also contained a survey with directions, a cooked beef color guide, a food safety guide, and a stamped, addressed envelope. Boxes were stored at -10°C until delivery to consumers.

Beef consumers (n=395) were solicited through direct contact by Texas A&M University personnel (261 consumers completed the study). Participants were given a box of steaks and asked to prepare those steaks as they normally would if they had purchased them from the supermarket. Consumers were directed to cook only one steak per meal, per consumer, and it was suggested that they cook two steaks per week for a total of three

weeks for timeliness purposes. Preparers were asked to identify the cooking method (outdoor or indoor grill, pan-broil, pan-fry, stir-fry, broil, oven roasted, uncovered, braise and simmer, or stew) used by referring to the definitions provided in the included directions. The approximate degree of doneness was determined by consumers using the National Cattlemen's Beef Association beef steak color guide provided for them in the box. The consumers were asked to evaluate steaks for overall-like, tenderness, juiciness, flavor intensity, and flavor desirability using a 10-point scale (10 = extreme like, extremely tender, extremely juicy, extremely intense, and extremely desirable; 1=extreme dislike, extremely tough, extremely dry, extremely bland, and extremely undesirable). This study was approved by the Institutional Review Board at Texas A&M University, and informed consent was obtained from all participants.

Data were analyzed for each muscle individually using the PROC GLM procedures of SAS (SAS Institute, Cary, NC). Initial models tested the main effects of tenderization treatment and cooking method and their interaction. Cooking methods were pooled into four categories including: grill (outdoor and indoor grilling), oven (broil and oven roasted, uncovered), skillet (pan-broil, pan-fry, and stir-fry), and moist cookery (braise and simmer and stew). Within cooking methods displaying sufficient numbers of steaks cooked to various degrees of doneness (grilling and skillet methods), the effects of degree of doneness and tenderization treatment were tested. If there was no interaction, only the degree of doneness means were presented as other means were presented in the previous analysis. A predetermined α of 0.05 was used for all determinations of statistical significance.

Results & Discussion

Tenderization Treatments

Least-squares means for consumer evaluations of steaks are reported in Table 1. *M. semimembranosus* steaks from the salt/phosphate treatment received the highest ($P < 0.05$) ratings for all traits compared to those from the control and blade tenderized treatments. For the *M. rectus femoris*, consumers gave steaks from the salt/phosphate treatment higher ($P < 0.05$) palatability ratings compared to the control steaks, and higher ($P < 0.05$) tenderness and juiciness ratings than steaks from the blade tenderized treatment. For the *M. vastus lateralis* steaks, those that were from the salt/phosphate treatment received higher ($P < 0.05$) palatability ratings for all traits compared to the steaks from the blade tenderization treatment and the controls. In general, the salt/phosphate treatment resulted in improved palatability compared to the blade tenderized treatment and to the controls. In most cases, blade tenderizing did not improve palatability compared to controls.

Table 1
Least-squares means (SEM) for consumer evaluations of beef steaks treated with blade tenderization or salt/phosphate injection

Muscle	Attribute	Treatment			P > F
		Control	Blade	Inject	
<i>M. adductor</i>	Tenderness	5.7 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	0.06
	Juiciness	5.4 ± 0.2	5.7 ± 0.2	6.0 ± 0.2	0.13
	Flavor Intensity	5.9 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	0.15
	Flavor Desirability	6.0 ± 0.2	6.3 ± 0.2	6.3 ± 0.2	0.35
	Overall Like	6.3 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	0.10
<i>M. semimembranosus</i>	Tenderness	6.0 ± 0.2b	6.1 ± 0.2b	6.6 ± 0.2a	0.04
	Juiciness	5.5 ± 0.2b	5.3 ± 0.2b	6.2 ± 0.2a	<0.01
	Flavor Intensity	5.9 ± 0.2b	6.0 ± 0.2b	6.5 ± 0.2a	0.05
	Flavor Desirability	6.0 ± 0.2b	6.0 ± 0.2b	6.7 ± 0.2a	0.01
	Overall Like	6.4 ± 0.2b	6.5 ± 0.2b	7.1 ± 0.2a	0.02
<i>M. rectus femoris</i>	Tenderness	6.3 ± 0.2b	6.7 ± 0.2b	7.2 ± 0.2a	<0.01
	Juiciness	5.9 ± 0.2b	6.1 ± 0.2b	6.9 ± 0.2a	<0.01
	Flavor Intensity	6.3 ± 0.2b	6.5 ± 0.2ab	7.0 ± 0.2a	0.02
	Flavor Desirability	6.2 ± 0.2b	6.5 ± 0.2ab	6.9 ± 0.2a	0.02
	Overall Like	6.5 ± 0.2b	6.9 ± 0.2ab	7.3 ± 0.2a	<0.01
<i>M. vastus lateralis</i>	Tenderness	5.4 ± 0.2b	5.7 ± 0.2b	6.5 ± 0.2a	<0.01
	Juiciness	5.3 ± 0.2b	5.6 ± 0.2b	6.2 ± 0.2a	0.01
	Flavor Intensity	5.5 ± 0.2b	5.8 ± 0.2b	6.6 ± 0.2a	<0.01
	Flavor Desirability	5.6 ± 0.2b	6.0 ± 0.2b	6.7 ± 0.2a	<0.01
	Overall Like	5.8 ± 0.2b	6.1 ± 0.2b	6.9 ± 0.2a	<0.01

Means within the same row lacking common letters (a,b) differ ($P < 0.05$).

Cooking methods

When looking at the various cooking methods (Table 2), *M. adductor* steaks cooked in the skillet and using a grilling method received higher ($P < 0.05$) consumer ratings for juiciness than steaks cooked in an oven or using moist cookery. For flavor intensity and desirability, steaks cooked in a skillet, on the grill, and in the oven were ranked higher ($P < 0.05$) than those cooked using moist cookery. This might suggest that cooking with moist heat cookery may reduce the flavor attributes of the *M. adductor* steak to an unacceptable level for consumers. For *M. rectus femoris* steaks, tenderness ratings were higher ($P < 0.05$) for those steaks cooked using moist cookery and in a skillet than those cooked in an oven or on a grill. For *M. vastus lateralis*, the steaks cooked with moist cookery were given higher ratings ($P < 0.05$) for tenderness, whereas, those cooked on a skillet, in the oven, or on a grill, were given similar and lower ratings. The increase in tenderness of the *M. vastus lateralis* steaks cooked using moist heat also created higher ($P < 0.05$) ratings for overall like suggesting that the role that moist heat cookery played in increasing tenderness, increased the overall palatability of the steak for consumers.

In general, cooking methods did not provide substantial increases in consumer palatability attributes. For the knuckle steaks (*M. rectus femoris* and *M. vastus lateralis*),

moist-heat cookery did create an increase in tenderness ratings from consumers over the dry-heat methods (grill and oven).

Table 2
Least-squares means (SEM) for consumer evaluations of beef steaks cooked with grilling, oven moist, cookery methods, or in a skillet

Muscle	Attribute	Cooking Method				P > F
		Grill	Oven	Moist	Skillet	
<i>M. adductor</i>	Tenderness	6.1 ± 0.2	6.1 ± 0.3	5.7 ± 0.4	6.5 ± 0.2	0.17
	Juiciness	6.0 ± 0.2ab	5.4 ± 0.3b	5.3 ± 0.4b	6.2 ± 0.2a	0.05
	Flavor Intensity	6.4 ± 0.1a	6.2 ± 0.3ab	5.4 ± 0.4b	6.6 ± 0.2a	0.02
	Flavor Desirability	6.3 ± 0.1a	6.4 ± 0.3a	5.4 ± 0.4b	6.7 ± 0.2a	0.02
	Overall Like	6.4 ± 0.1	6.8 ± 0.3	6.4 ± 0.4	6.9 ± 0.2	0.21
<i>M. semimembranosus</i>	Tenderness	5.9 ± 0.1	6.3 ± 0.4	6.6 ± 0.4	6.0 ± 0.2	0.33
	Juiciness	5.8 ± 0.2	5.4 ± 0.4	5.8 ± 0.4	5.6 ± 0.2	0.72
	Flavor Intensity	6.1 ± 0.1	6.1 ± 0.3	6.4 ± 0.4	6.1 ± 0.2	0.90
	Flavor Desirability	6.1 ± 0.1	6.2 ± 0.4	6.4 ± 0.4	6.2 ± 0.2	0.91
	Overall Like	6.5 ± 0.2	6.8 ± 0.3	7.0 ± 0.3	6.4 ± 0.2	0.38
<i>M. rectus femoris</i>	Tenderness	6.4 ± 0.2b	6.3 ± 0.4b	7.2 ± 0.3a	6.9 ± 0.2a	0.03
	Juiciness	6.3 ± 0.2	5.9 ± 0.4	6.6 ± 0.3	6.6 ± 0.2	0.26
	Flavor Intensity	6.4 ± 0.2	6.3 ± 0.4	6.8 ± 0.3	6.9 ± 0.2	0.29
	Flavor Desirability	6.4 ± 0.2	6.2 ± 0.4	6.7 ± 0.3	6.8 ± 0.2	0.21
	Overall Like	6.7 ± 0.1	6.6 ± 0.3	7.2 ± 0.3	7.1 ± 0.2	0.21
<i>M. vastus lateralis</i>	Tenderness	5.6 ± 0.2b	5.1 ± 0.3b	7.1 ± 0.3a	5.6 ± 0.2b	<0.01
	Juiciness	5.7 ± 0.2	5.3 ± 0.4	6.1 ± 0.3	5.7 ± 0.2	0.40
	Flavor Intensity	6.1 ± 0.2	5.4 ± 0.3	6.2 ± 0.3	6.1 ± 0.2	0.26
	Flavor Desirability	5.9 ± 0.2	5.7 ± 0.3	6.6 ± 0.3	6.2 ± 0.2	0.21
	Overall Like	6.1 ± 0.2b	5.7 ± 0.3b	7.1 ± 0.3a	6.1 ± 0.2b	0.01

Means within the same row lacking common letters (a,b) differ ($P < 0.05$).

Degree of Doneness

M. semimembranosus steaks cooked on a grill (Table 3) to medium rare and below received the highest ($P < 0.05$) ratings for juiciness, flavor intensity, and desirability. The method of cookery clearly influenced consumer palatability ratings for *M. rectus femoris* steaks cooked on a grill. Lower degrees of doneness (medium rare and below and medium) received higher ($P < 0.05$) tenderness ratings than steaks cooked to well done. Juiciness ratings increased as the degree of doneness decreased, with steaks cooked to medium rare or below receiving higher ($P < 0.05$) ratings than those cooked to medium well. In addition, those steaks cooked to medium well produced higher ($P < 0.05$) juiciness ratings than those cooked to well done. Steaks cooked at medium rare and below received the highest ($P < 0.05$) ratings for flavor intensity. Flavor desirability and overall like ratings received similar ratings as those steaks cooked to medium rare and below. In general, lower degrees of doneness produced higher palatability ratings. This, combined with other findings, suggest that a dry heat cookery method like grilling may be acceptable for the *M. rectus femoris* if cooked to lower degrees of doneness.

Table 3
Least-squares means (SEM) for consumer evaluations of beef steaks cooked on a grill.

Muscle	Attribute	Degree of Doneness				P > F
		Med Rare and Rare	Medium	Medium Well	Well Done	
M. adductor	Tenderness	5.8 ± 0.4	5.7 ± 0.3	6.4 ± 0.3	6.4 ± 0.3	0.22
	Juiciness	5.9 ± 0.4	5.7 ± 0.3	6.2 ± 0.3	6.1 ± 0.4	0.67
	Flavor Intensity	6.5 ± 0.4	6.2 ± 0.2	6.7 ± 0.2	6.3 ± 0.3	0.36
	Flavor	6.4 ± 0.4	6.0 ± 0.3	6.6 ± 0.3	6.1 ± 0.3	0.36
	Desirability					
	Overall Like	6.1 ± 0.4	6.1 ± 0.2	6.7 ± 0.2	6.8 ± 0.3	0.22
M. semimembranosus	Tenderness	6.8 ± 0.4	5.8 ± 0.2	5.6 ± 0.3	6.0 ± 0.3	0.09
	Juiciness	7.0 ± 0.4a	5.9 ± 0.3	5.7 ± 0.3B	5.1 ± 0.4b	<0.01
	Flavor Intensity	7.3 ± 0.4a	6.0 ± 0.2b	5.7 ± 0.2b	5.8 ± 0.3b	<0.01
	Flavor	7.4 ± 0.4a	6.0 ± 0.2b	5.8 ± 0.3b	5.9 ± 0.3b	0.01
	Desirability					
	Overall Like	7.1 ± 0.4	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.3	0.38
M. rectus femoris	Tenderness	6.9 ± 0.3a	6.6 ± 0.3a	6.2 ± 0.3ab	5.5 ± 0.4b	0.04
	Juiciness	7.2 ± 0.3a	6.5 ± 0.3ab	6.0 ± 0.3b	4.3 ± 0.4c	<0.01
	Flavor Intensity	7.4 ± 0.3a	6.4 ± 0.3b	6.0 ± 0.3b	5.7 ± 0.4b	<0.01
	Flavor	7.2 ± 0.3a	6.5 ± 0.3ab	5.9 ± 0.3b	5.4 ± 0.4b	0.01
	Desirability					
	Overall Like	7.2 ± 0.3a	6.9 ± 0.2ab	6.3 ± 0.3b	6.1 ± 0.4b	0.05
M. vastus lateralis	Tenderness	5.5 ± 0.3	5.8 ± 0.3	5.1 ± 0.3	6.7 ± 0.5	0.07
	Juiciness	6.1 ± 0.4	5.9 ± 0.3	5.2 ± 0.3	5.2 ± 0.5	0.18
	Flavor Intensity	6.1 ± 0.3	6.1 ± 0.3	5.7 ± 0.3	7.0 ± 0.5	0.22
	Flavor	5.9 ± 0.4	6.1 ± 0.3	5.4 ± 0.3	7.0 ± 0.5	0.10
	Desirability					
	Overall Like	6.1 ± 0.3	6.2 ± 0.3	5.6 ± 0.3	6.9 ± 0.5	0.20

Means within the same row lacking common letters (a-c) differ ($P < 0.05$).

In Table 4, least squares means for consumer evaluations of steaks cooked using moist cookery are presented. For the attribute of overall like, consumers rated those *M. semimembranosus* steaks cooked medium rare and below the lowest ($P < 0.05$).

Table 4
Least-squares means (SEM) for consumer evaluations of beef steaks cooked using moist cookery.

Muscle	Attribute	Degree of Doneness				P > F
		Med Rare and Rare	Medium	Medium Well	Well Done	
M. adductor	Tenderness	-	4.7 ± 1.2	4.9 ± 0.8	6.1 ± 0.5	0.32
	Juiciness	-	4.7 ± 1.1	4.9 ± 0.7	5.4 ± 0.5	0.76
	Flavor Intensity	-	5.0 ± 1.0	5.4 ± 0.7	5.3 ± 0.4	0.95
	Flavor	-	5.0 ± 1.1	4.3 ± 0.8	5.8 ± 0.5	0.24
	Desirability	-	5.3 ± 1.0	6.7 ± 0.6	6.4 ± 0.4	0.52
M. semimembranosus	Tenderness	1.1 ± 2.0	7.8 ± 1.2	6.3 ± 0.9	6.8 ± 0.4	0.06
	Juiciness	2.5 ± 2.4	7.3 ± 1.4	5.6 ± 1.1	5.8 ± 0.5	0.41
	Flavor Intensity	3.1 ± 2.1	6.5 ± 1.2	5.7 ± 1.0	6.6 ± 0.4	0.34
	Flavor	2.0 ± 2.0	6.7 ± 1.2	6.0 ± 1.0	6.7 ± 0.4	0.17
	Desirability	2.0 ± 2.0b	8.7 ± 1.2a	6.1 ± 0.9a	7.1 ± 0.4a	0.05
M. rectus femoris	Tenderness	7.0 ± 2.1	6.9 ± 1.0	7.3 ± 0.5	7.4 ± 0.4	0.98
	Juiciness	5.1 ± 2.4	5.4 ± 1.1	6.8 ± 0.6	6.9 ± 0.5	0.54
	Flavor Intensity	4.5 ± 2.4	5.5 ± 1.1	7.6 ± 0.6	6.8 ± 0.4	0.29
	Flavor	3.7 ± 2.3	5.2 ± 1.1	7.5 ± 0.6	6.9 ± 0.4	0.17
	Desirability	5.9 ± 2.0	6.1 ± 0.9	7.1 ± 0.5	7.7 ± 0.4	0.33
M. vastus lateralis	Tenderness	7.8 ± 1.6	6.2 ± 0.8	6.9 ± 0.6	7.4 ± 0.5	0.63
	Juiciness	8.4 ± 1.7	5.2 ± 0.9	6.3 ± 0.6	5.8 ± 0.5	0.39
	Flavor Intensity	8.4 ± 1.3	4.7 ± 0.7	6.6 ± 0.5	6.1 ± 0.4	0.06
	Flavor	8.3 ± 1.5	4.9 ± 0.8	6.8 ± 0.5	6.7 ± 0.5	0.15
	Desirability	8.3 ± 1.4	6.4 ± 0.7	7.2 ± 0.5	7.1 ± 0.4	0.57

Means within the same row lacking common letters (a,b) differ ($P < 0.05$).

Consumers who prepared *M. semimembranosus* steaks in an oven (Table 5) ranked steaks cooked to medium rare and below higher ($P < 0.05$) than those cooked to medium well and well done for overall like. For *M. rectus femoris* steaks cooked in an oven to medium rare and below, higher ratings ($P < 0.05$) were given for juiciness than for all other attributes. For *M. vastus lateralis* steaks cooked in an oven, ratings for juiciness and overall like were higher ($P < 0.05$) for steaks cooked to medium rare and below than those cooked to medium well and lowest ($P < 0.05$) for those cooked well done. For flavor intensity, those cooked to medium rare and below and medium were rated higher ($P < 0.05$) than those cooked well done. Ratings for overall like patterned those for juiciness signifying that juiciness plays an important role in the overall palatability of a round steak when cooked using a dry heat method.

Table 5
Least-squares means (SEM) for consumer evaluations of beef steaks cooked in an oven.

Muscle	Attribute	Degree of Doneness				P > F
		Med Rare and Rare	Medium	Medium Well	Well Done	
M. adductor	Tenderness	5.3 ± 1.1	6.4 ± 0.7	5.9 ± 0.7	6.3 ± 0.6	0.79
	Juiciness	6.0 ± 1.3	6.1 ± 0.8	5.7 ± 0.8	4.4 ± 0.7	0.36
	Flavor Intensity	6.0 ± 1.2	6.5 ± 0.7	6.8 ± 0.8	5.5 ± 0.6	0.57
	Flavor	6.6 ± 1.2	6.8 ± 0.7	6.7 ± 0.8	6.0 ± 0.6	0.83
	Desirability Overall Like	6.5 ± 1.0	7.0 ± 0.6	6.6 ± 0.7	7.1 ± 0.5	0.92
M. semimembranosus	Tenderness	5.2 ± 0.9	7.0 ± 0.8	5.4 ± 1.0	6.5 ± 0.6	0.37
	Juiciness	5.2 ± 1.0	6.8 ± 0.8	4.4 ± 1.1	5.1 ± 0.7	0.31
	Flavor Intensity	5.6 ± 1.0	7.2 ± 0.8	5.0 ± 1.0	6.1 ± 0.6	0.35
	Flavor	5.1 ± 0.9	7.0 ± 0.8	5.2 ± 1.0	6.5 ± 0.6	0.31
	Desirability Overall Like	5.8 ± 0.6a	6.9 ± 0.6ab	5.6 ± 0.7b	7.7 ± 0.4b	0.04
M. rectus femoris	Tenderness	7.4 ± 0.8	6.7 ± 0.5	6.3 ± 0.5	5.4 ± 0.6	0.21
	Juiciness	7.9 ± 0.7a	6.1 ± 0.4b	5.3 ± 0.5b	5.2 ± 0.5b	0.03
	Flavor Intensity	7.4 ± 0.8	6.7 ± 0.5	5.9 ± 0.5	5.9 ± 0.6	0.32
	Flavor	7.6 ± 0.8	6.4 ± 0.5	6.0 ± 0.5	5.3 ± 0.6	0.13
	Desirability Overall Like	8.2 ± 0.8	6.6 ± 0.4	6.4 ± 0.5	6.0 ± 0.6	0.17
M. vastus lateralis	Tenderness	6.1 ± 0.6	5.6 ± 0.5	4.6 ± 0.5	3.8 ± 0.7	0.06
	Juiciness	6.7 ± 0.5a	5.6 ± 0.5ab	5.1 ± 0.5b	3.3 ± 0.6c	<0.01
	Flavor Intensity	6.3 ± 0.6a	6.1 ± 0.5a	5.0 ± 0.5ab	4.0 ± 0.6b	0.02
	Flavor	7.2 ± 0.6a	6.2 ± 0.5ab	5.0 ± 0.5b	4.4 ± 0.6b	0.01
	Desirability Overall Like	6.7 ± 0.6a	6.1 ± 0.5ab	5.1 ± 0.5b	4.1 ± 0.6c	0.02

Means within the same row lacking common letters (a-c) differ ($P < 0.05$).

M. adductor steaks cooked in a skillet (Table 6) to a well done degree of doneness received lower ($P < 0.05$) ratings for juiciness than those cooked to medium rare and below and those cooked to medium well. For *M. vastus lateralis* steaks cooked in a skillet, juiciness ratings were higher ($P < 0.05$) for those steaks cooked to medium rare and below than for those cooked to medium well and well done.

Table 6
Least-squares means (SEM) for consumer evaluations of beef steaks cooked in a skillet.

Muscle	Attribute	Degree of Doneness				P > F
		Med Rare and Rare	Medium	Medium Well	Well Done	
M. adductor	Tenderness	6.8 ± 0.6	6.7 ± 0.3	6.8 ± 0.3	6.1 ± 0.3	0.47
	Juiciness	7.3 ± 0.6a	6.3 ± 0.3ab	6.6 ± 0.3a	5.6 ± 0.3b	0.03
	Flavor Intensity	7.3 ± 0.6	6.6 ± 0.4	7.0 ± 0.4	6.1 ± 0.3	0.19
	Flavor	7.3 ± 0.6	6.5 ± 0.3	7.1 ± 0.3	6.4 ± 0.3	0.43
	Desirability					
	Overall Like	7.5 ± 0.6	6.9 ± 0.3	7.3 ± 0.3	6.5 ± 0.3	0.29
M. semimembranosus	Tenderness	5.2 ± 0.5	6.0 ± 0.4	6.5 ± 0.4	5.9 ± 0.4	0.32
	Juiciness	5.6 ± 0.5	5.9 ± 0.4	5.7 ± 0.4	5.4 ± 0.4	0.74
	Flavor Intensity	6.3 ± 0.5	6.2 ± 0.3	6.2 ± 0.4	5.9 ± 0.4	0.94
	Flavor	5.9 ± 0.5	6.2 ± 0.4	6.1 ± 0.4	6.3 ± 0.4	0.94
	Desirability					
	Overall Like	5.4 ± 0.5	6.3 ± 0.4	6.8 ± 0.4	6.5 ± 0.4	0.24
M. rectus femoris	Tenderness	6.7 ± 0.5	7.3 ± 0.3	6.7 ± 0.3	6.9 ± 0.4	0.57
	Juiciness	7.5 ± 0.5	6.6 ± 0.3	6.4 ± 0.3	6.0 ± 0.4	0.11
	Flavor Intensity	7.5 ± 0.5	6.8 ± 0.3	6.9 ± 0.3	6.5 ± 0.4	0.53
	Flavor	7.1 ± 0.5	6.9 ± 0.4	6.7 ± 0.3	6.7 ± 0.4	0.91
	Desirability					
	Overall Like	7.6 ± 0.5	7.1 ± 0.3	6.8 ± 0.3	7.0 ± 0.4	0.52
M. vastus lateralis	Tenderness	5.9 ± 0.5	5.7 ± 0.4	5.7 ± 0.3	4.8 ± 0.4	0.24
	Juiciness	6.7 ± 0.5a	5.9 ± 0.4ab	5.4 ± 0.4b	4.9 ± 0.4b	0.04
	Flavor Intensity	6.8 ± 0.4	6.1 ± 0.4	6.3 ± 0.3	5.4 ± 0.4	0.10
	Flavor	6.7 ± 0.5	6.1 ± 0.4	6.3 ± 0.4	5.7 ± 0.4	0.46
	Desirability					
	Overall Like	6.5 ± 0.5	6.1 ± 0.4	6.4 ± 0.4	5.4 ± 0.4	0.28

Means within the same row lacking common letters (a,b) differ (P < 0.05).

Conclusions

In conclusion, injecting round muscles with a salt and phosphate solution improved most palatability traits compared to those that were blade tenderized or were not treated. For the most part, cooking method and degree of doneness had little influence on consumer palatability ratings. Where differences occurred, they were muscle specific, which may allow limited recommendations for certain muscles with respect to the most appropriate cooking method and degree of doneness.

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AROMA COMPONENTS OF WAGYU BEEF AND IMPORTED BEEF

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Introduction

We have clarified the existence of a preferable odor of Wagyu (Japanese Black cattle) beef, i.e., Wagyu beef aroma (Matsuishi et al., 2001). This aroma is generated remarkably in highly-fat-marbled Wagyu beef which is sliced, stored in the presence of oxygen for several days and then cooked at 80°C. The odor quality is sweet and fatty. This aroma is specific to Wagyu beef, while imported beef with low level of fat-marbling has little amount of the aroma. Japanese people are considered to prefer Wagyu beef because of the existence of this aroma (Japan Meat Information Service Center, 1998).

Thus far, there are a few reports on aroma components of Wagyu beef. Sato et al. (1994) analyzed aroma components in Wagyu lean meat patties containing fat from Wagyu beef, Holstein beef or imported beef by gas chromatography-mass spectrometry (GC-MS) and showed that the contents of lactones and ketones were higher in the patties containing Wagyu beef fat than other fat. Boylston et al. (1996) analyzed volatile compounds of Japanese Wagyu beef, American Wagyu beef and American Angus beef and found that the contents of aldehydes were higher in the Japanese and American Wagyu beef than in the American Angus beef. However, what components contributed to Wagyu beef aroma was not demonstrated in those reports.

Objectives

In this study, the aroma components of Wagyu beef were compared with those of imported beef to reveal the components contributing to Wagyu beef aroma. Further, the contribution rate of each aroma component of Wagyu beef to Wagyu beef aroma was presumed by the odor quality determined by gas chromatography (GC)-sniffing and the flavor dilution factors (FD factors) determined by aroma extract dilution analysis (AEDA).

Methodology

The chuck rolls of the commercial domestic Wagyu beef and imported Australian beef (Angus breed beef) were used. The aging times and packaging/storage conditions for both beef were unknown. Twenty gram of minced beef was homogenized with 100 ml of 1% NaCl solution and 0.1 ml of 0.05 µg/ml n-hexadecane/diethyl ether solution (as a internal standard). The obtained homogenate was subjected to the simultaneous distillation/extraction for 30 min according to the method of Nickerson and Likens (1996), so that aroma components were collected in a diethyl ether. This procedure was repeated thirty times and the aroma fraction was obtained from 600 g of meat. The aroma fraction was dehydrated and concentrated for the analysis by GC, GC-MS and GC-sniffing.

Conditions for the GC analysis were as follows: The carrier gas was N₂. The column was TC-WAX (0.25 mm i.d. x 60 m) (GL Sciences Inc., Tokyo). The column temperature was programmed 50°C for 4 min at the initial stage, then to increase to 190°C at 4 °C/min. The split ratio was 20:1. The detector was FID.

Conditions for the GC-MS analysis was as follows: The carrier gas was He. The split ratio was 50:1. The ion source was EI at 70 eV. Compounds were identified by comparing their mass spectra with those contained in the NIST. Other conditions were as for the GC analysis.

In the GC-sniffing analysis, at the end of the capillary column, the effluent was split 1:1 into a FID and a sniffing port (GL Sciences Inc., Tokyo). Other conditions were as follows: The carrier gas was He. The column was TC-Wax (0.53 mm i.d. x 30 m). The split ratio was 15:1. The temperature program was as for the GC analysis.

In AEDA (Gasser and Grosch, 1988), the aroma fractions which were stepwise diluted with diethyl ether (4⁰ to 4⁷ times) were subjected to GC and at the end of the capillary column panelists sniffed the odor. The FD factor is the highest dilution rate at which a substance is still smelled.

Results & Discussion

The analysis of GC-MS resulted in the identification of forty-eight components, which were five lactones, five ketones, eight alcohols, three unsaturated alcohols, two esters, nine aliphatic aldehydes, eight aliphatic unsaturated aldehydes, two acids and six other components. These components were classified into five groups on the basis of odor quality and the relative amounts, the detailed odor quality and the FD factors in Wagyu beef of the five groups were shown in Table 1. The total amount of identified components of Wagyu beef was about 2.5 times as much as that of imported beef. In comparison of relative amount of each component between Wagyu beef and imported beef, 1-nonenal, hexadecanal and benzaldehyde were in larger amount in imported beef, while most of lactones, ketones, aldehydes and alcohols were in larger amount in Wagyu beef. Especially, the Wagyu beef/imported beef ratios of lactones were remarkably large. Sato et al. (1994) and Boylston et al. (1996) also reported that lactones, ketones, aldehydes, alcohols were in larger amount in Wagyu beef than in imported beef or Angus breeds beef. The fat oxidation would occur more markedly in Wagyu beef with more

marbled fat than other beef, so that oxidation products such as those compounds would increase more remarkably in Wagyu beef than in other beef.

Wagyu beef contained remarkably large amount of lactones with the coconuts-like and peach-like odor, and the FD factor of γ -nonalactone was high value of 1024. Lactones of which odor quality was accompanied with sweetness were considered to contribute to the part of the sweet sensation of Wagyu beef aroma. The compounds of which odors were fruity except coconuts-like and peach-like or green were mainly composed of ketones, alcohols and aldehydes, which were detected in larger amount in Wagyu beef than in imported beef. However, the Wagyu beef/imported beef ratios of those compounds were less than that of lactones. Although among those compounds, hexylpropionate and hexanal showed high FD factor of 256 and 4096, respectively, those would not be major contributor to Wagyu beef aroma judging from their odor quality. Among the compounds with butter-like or fatty odor, diacetyl, acetoin and *E*-2-nonenal showed high FD factor of 256. These compounds would contribute to the part of the fatty sensation of Wagyu beef aroma judging from their odor quality. The compounds with other various odors such as sour, rose-like and mushroom-like odors would not be major contributor to Wagyu beef aroma judging from their odor quality.

Conclusions

Most of forty-eight compounds identified were detected in larger amount in Wagyu beef than in imported beef. Among these compounds, lactones with coconuts-like and peach-like aroma would contribute to the part of the sweet sensation of Wagyu beef aroma, while compounds such as diacetyl, acetoin and *E*-2-nonenal with butter-like or fatty aroma would contribute to the part of the fatty sensation of Wagyu beef aroma.

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Tables and Figures

Table 1. The relative amount, odor quality and FD factor of identified compounds from Wagyu beef and imported beef

K.I.†	Identified compound	Relative amount¶		Odor quality	FD factor of Wagyu beef
		Wagyu beef	imported beef		
<u>coconuts-like, peach-like</u>					
2026	γ -nonalactone	1.45±1.44	tr	coconuts-like, sweet	1024
2124	γ -decalactone	0.29±0.12	-	peach, coconuts-like	16
2200	γ -decalactone	0.73±0.24	tr	peach-like, milky	16
2327	γ -undecalactone	0.37±0.05	tr	peach-like, milky	4
2431	γ -dodecalactone	tr	-	peach-like, milky	1
<u>fruity except coconuts-like and peach-like</u>					
1381	hexylpropionate	0.10*	tr	pear-like	256
1459	decanal	0.99*	0.63±0.10	orange, lemongrass-like	256
1822	2-tridecanone	0.20±0.08	0.09±0.07	fruity, waxy	64
1206	2-heptanone	2.72±0.61	2.05±0.53	fruity	16
1372	1-hexanol	3.83±0.87	1.03±0.35	fruity, floral	16
1564	1-octanol	5.56±3.30	3.18±0.56	fruity, milky	16
1630	1-nonanol	0.40±0.47	0.44±0.26	fruity, floral	16
1678	dodecanal	2.11±2.05	1.84±0.87	lemongrass-like, rose	16
1915	tetradecanal	4.49*	0.23±0.10	lemongrass-like, cardboard	16
1214	<i>d</i> -limonene	4.13±1.98	1.10±0.16	citrus, fatty	16
1290	2-octanone	0.24±0.16	0.08*	fruity, passion fruits	1
1475	1-heptanol	2.82±0.50	0.83±0.35	fruity, citrus	1
1950	1-dodecanol	1.09*	0.61±0.09	fruity, fatty	1
1552	benzaldehyde	4.72±0.84	4.75±4.05	fruity, orange, fatty	1
1641	2-decenal	3.36±2.43	1.08±0.58	citrus, rose-like	1
<u>green</u>					
1078	hexanal	50.55±29.49	6.01±3.04	green apple-like, floral	4096
1794	(<i>E,E</i>)-2,4-nonadienal	2.41±1.27	0.82±0.36	green, metallic	256
1396	nonanal	4.70±5.10	0.29±0.09	pine-like	64
1292	octanal	0.45±0.17	0.17±0.07	green, citrus	16
1844	(<i>E,E</i>)-2,4-decadienal	0.19±0.14	0.05±0.01	green, metallic,	16
1871	hexanoic acid	4.50±1.00	3.54±5.13	green, fatty	16
2151	hexadecanal	9.98±5.60	11.8±3.39	green, strawberry, apricot	4
1252	<i>E</i> -2-hexenal	2.58±1.34	0.60±0.40	green, aldehydic, herbal	4
<u>butter-like, fatty</u>					
979	diacetyl	8.64±6.70	2.36±0.52	butter-like, fermented	256
1311	acetoin	4.95±4.51	3.16±1.10	butter-like, fatty	256

1541	<i>E</i> -2-nonenal	1.30±0.52	0.55±0.09	green, citrus, fatty	256
1428	<i>E</i> -2-octenal	2.78±2.20	0.78±0.09	green, citrus, fatty	16
2046	pentadecanal	1.71±0.75	0.83±0.54	sweet fatty	4
1133	ethylbenzene	0.14±0.16	0.13±0.05	sweet fatty corn cream	1
1469	7-octen-4-ol	13.22±3.41	2.96±0.94	corn creamy, milky, oily	—
	<i>others</i>				
1739	2-undecenal	0.83±0.13	0.52±0.13	withered rose-like	256
1482	acetic acid	0.26±0.22	0.38±0.30	sour	256
1268	1-pentanol	17.37±8.52	4.69±0.25	coumarin-like	16
1641	1-nonen-4-ol	0.48±0.18	0.58±0.37	cardboard	16
1685	phenylacetaldehyde	0.65±0.73	1.57*	rose-like	16
1218	2-pentylfuran	0.61±0.18	0.13*	petroleum-like, oxidized fat	16
1994	1,3,5-undecatriene	0.08*	0.52±0.53	violet, seaweed-like	16
1108	1-penten-3-ol	1.11±0.22	0.62±0.28	mushroom-like	4
1150	1-butanol	2.72±1.60	0.96±0.28	alcoholic	4
1703	(<i>E,E</i>)-2,4-octadienal	1.13±1.31	2.27±1.00	rubbery	4
878	ethyl acetate	9.12±3.09	7.26±4.48	rum	1
1782	tridecanal	1.45±0.12	0.75±0.49	solvent-like	1
1496	2-propyl-1-pentanol	0.83±0.24	0.74±0.57	alcoholic	—
	total	184.34	73.34		

†Kovats Index

relative peak area of each peak when the value of the internal standard (n-hexadecane) is regarded as 100.

Mean±standard deviation(n=3)

*Mean (n=2)

tr; trace amount

— : not determined

REGULATION OF TASTE-ACTIVE COMPONENTS OF MEAT BY DIETARY LYSINE LEVELS

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Background

Meat quality is an important quality attribute for consumers. For example, breeding technique and dietary antioxidants were mainly used for improving the meat quality in the world. However, there are few reports about improving the taste active components of meat by diet. Previously, main taste component, free glutamate (Glu), content of meat was regulated by dietary crude protein (CP) and some amino acids (Fujimura et al., 2004, Imanari et al., 2004). In this study, we investigated the effect of dietary amino acids on the Glu contents and sensory score of meat.

Objectives

Effect of dietary CP and amino acids on the taste active components, Glu and ATP metabolites, and sensory score of meat were studied. 3 experiments were conducted. Essential amino acids (EAAs) were divided into 5 groups, and their dietary levels were changed. As a result, basic amino acids were available to increase the Glu of meat, we focused to dietary Lys levels. Then, the Glu-related enzyme activities in muscle were measured to elucidate the regulatory mechanism of Glu in the muscle.

Materials and methods

Experiment 1: The 28-day-old female Cobb strain broiler was used for 5 experiments. Essential amino acids (EAA) were divided into 5 groups, branched chain amino acids (Ile+Val), basic amino acids (His+Lys+Arg), sulfur amino acid (Met), aromatic amino acids (Trp+Phe) and Thr. The contents of objective EAA(s) were 100, 150, or 200% of the NRC requirements (1994). The chickens were fed diets in ad libitum for 10 days. On 11th day, all chickens were slaughtered, and breast muscles were taken for analyses. Free amino acids and ATP metabolites in meat extract were measured by HPLC. Experiment 2: The 35-day-old female Cobb strain broiler divided into 4 groups. Lys contents of diet were 100 (control) and 150% of the NRC requirements. Sensory evaluation was carried out with 16 trained panelists using paired difference test and Scheffe's paired comparison test. Experiment 3: for the investigation of the mechanism of Glu regulation, the muscle glutamate dehydrogenase (GDH), glutaminase (GA), glutamine synthetase (GS) and Alanine transaminase (ALT) activities were measured by enzymatic methods (Bergmeyer, 1974).

Result and discussion

In Experiment 1, free Glu contents in breast muscle were increased with Ile+Val and His+Lys+Arg levels. Especially, Glu content of latter was 45.0% higher than that of control. No differences were found in Glu contents between Met and control group. As a result, we focused in dietary Lys for improving the meat taste. In Experiment 2, free Glu content of meat in the Lys 150% was significantly 53.4% higher than that of control ($P<0.01$). In sensory evaluation, all panelists were answered a difference in meat taste between Lys 150% and control in the paired difference test ($P<0.01$). Furthermore, meat of Lys 150% had a significantly higher score in overall preference, umami taste and taste intensity by Scheffe's paired comparison test ($P<0.05$). These results suggested that the meat taste of Lys 150% group was superior to that in the Lys 100% group. In experiment 3, GDH activity in muscle was significantly increased with increasing dietary Lys levels ($P<0.05$). Therefore, we considered that GDH activity related to the change of Glu contents in the muscle.

Conclusions

In order to clarify the relationship of meat taste and dietary components, effects of dietary CP and EAAs on taste active components of meat were studied. As a result, some EAA increased the free Glu contents of muscle, but the increment ratio were difference in EAAs. Especially, most of higher Glu contents of meat were found in the dietary Lys group. Meat of dietary Lys group obtained high score in sensory evaluation. From the enzymatic analysis, the dietary Lys levels affected GDH activity in muscle. We conclude that dietary Lys regulates the free Glu contents in muscle, and improve the meat taste.

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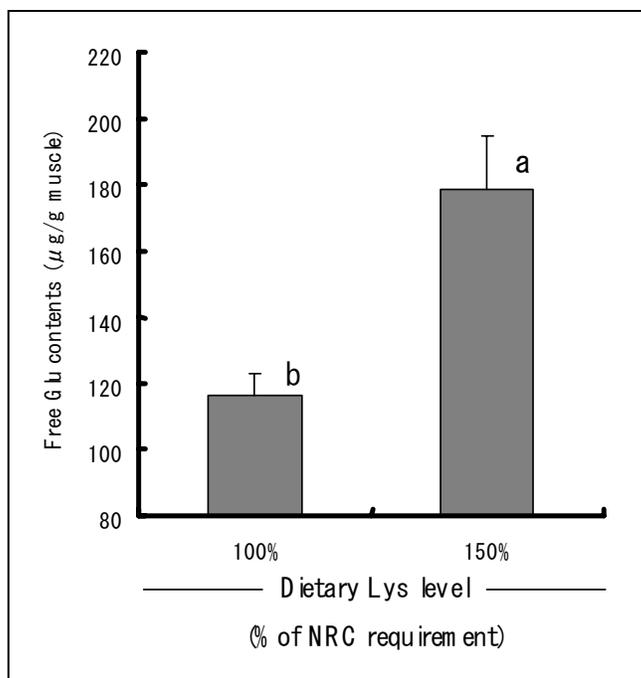


Figure 1 Effect of dietary Lys levels on free Glu contents in chicken breast muscle. Values are means±SE, n=6 chicks per each group. a,b: p<0.01

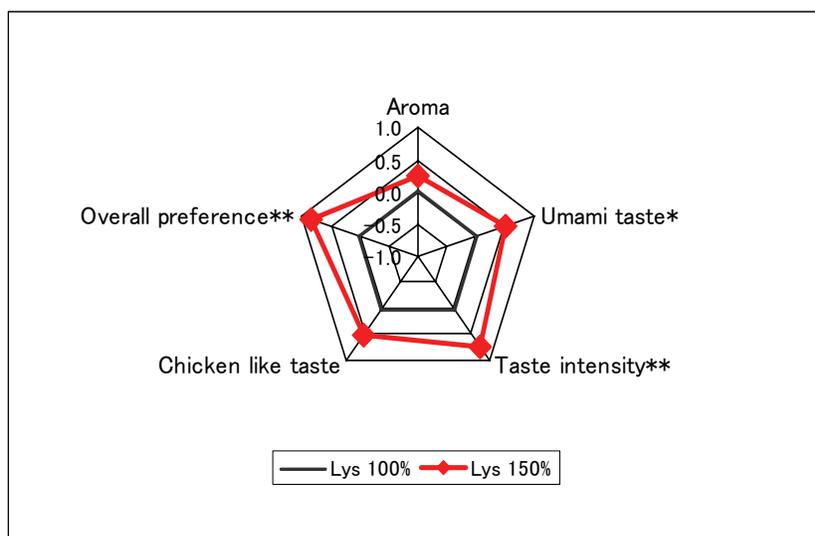


Figure 2 Sensory evaluation of meat by Scheffe's paired comparison test. *<0.05, **<0.01

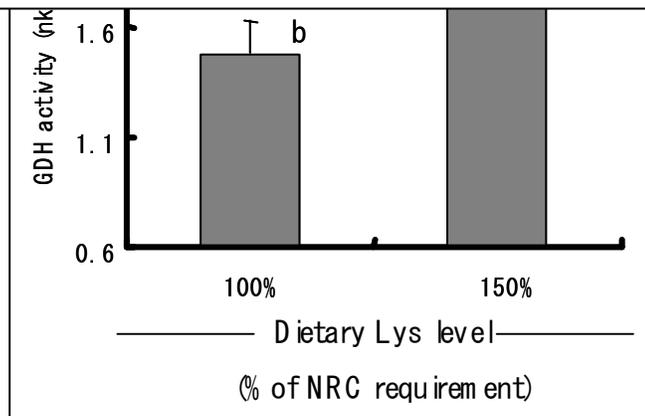
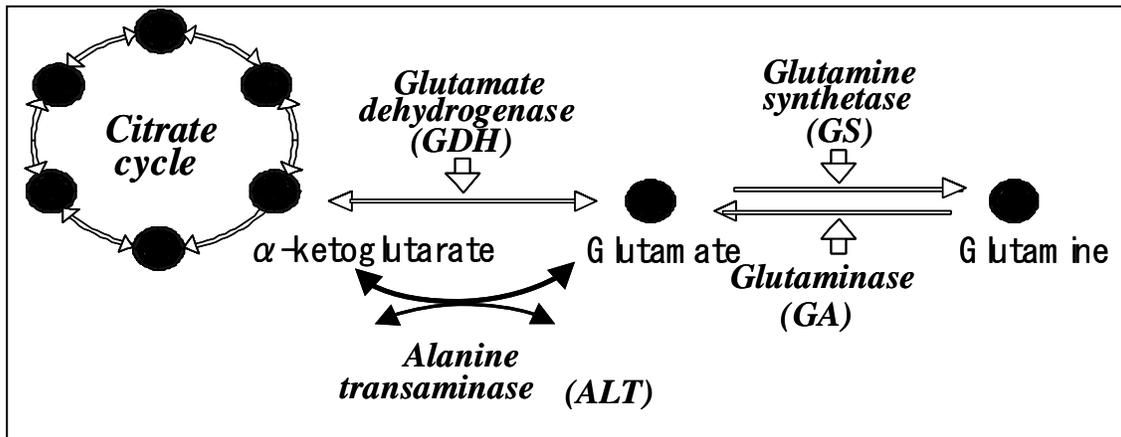


Figure 4 Effect of dietary Lys levels on GDH activity in breast muscle. Values are means \pm SE, n=6 chicks per each group. a,b: p<0.05

**REGULATION OF TASTE-ACTIVE COMPONENTS OF MEAT BY DIETARY
PROTEIN LEVELS: EFFECT OF SHORT-TIME FEEDING OF HIGH PROTEIN
DIET**

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Key Words: meat taste, diet, protein, sensory evaluation, chicken, amino acid metabolism

Introduction

Dietary nutrients play a significant part in determining growth rate and meat yield. It is known that the compositions of protein and total amino acids of meat are invariable by feeding treatments, hence the meat taste is considered to be invariable. However, the relationship of taste components of meat with nutrients is not fully elucidated, and there have been few reports on the effect of feeding treatments on taste-active components of chicken meat. Previously, restricted feeding and dietary low metabolizable energy levels decreased the free Glu contents of meat, and the meat taste was deteriorated (Fujimura et al., 1997, 2001). While, dietary protein and leucine levels increased the Glu content of meat, and improved the meat taste (Fujimura et al., 2004, Imanari et al., 2004). Therefore, meat taste can be affected by dietary components. In this study, the increase in free Glu and sensory score of meat by the diet was studied.

Objectives

In this study, the relationship of dietary crude protein (CP) levels with taste components in chicken meat extract was studied, focusing in particular on the taste-active components. As a result, meat taste was improved by the dietary protein levels, and then Glu regulation mechanism was studied by Glu-related enzymes in muscle. Three experiments were conducted, Experiment 1: meat type chickens were fed graded CP level diets for 10 days, and the meat composition, free amino acids and ATP metabolites were measured at 0, 3, 5 and 10 days. Experiment 2: meat taste was evaluated by sensory evaluation. Experiment 3: Glu-related enzymes of meat were measured at 0, 3, 5 and 10 days.

Methodology

Experiment 1: The 14-day-old female Cobb strain broiler chickens were divided into four groups with twelve chicks in each group. The chicks were fed CP 17.6, 26.3, 30.7 and 35.1% diet. All the chickens were kept in individual wire cages. Free amino acids and ATP metabolites of the pectoral meat extract were measured at 0, 3, 5 and 10 days. Experiment 2: Two sensory evaluations, paired comparison test and Scheffe's paired difference test, were conducted between meats of CP17.5% and 30.7% groups.

Experiment 3: The 14-day-old broiler chicks were fed CP 30.7% diet for 10 days. At 0, 3, 5 and 10 days, Glu-related enzymes in muscle were measured. For the investigation of Glu regulation mechanism, glutamate dehydrogenase (GDH), kidney type glutaminase (KGA), glutamine synthetase (GS) and alanine transaminase (ALT) activities were measured by enzymatic methods (Bergmeyer, 1974).

Results & Discussion

In Experiment 1, free Glu of muscle after 3 and 5 days feeding of high CP diet was higher than that of 0 day ($P < 0.01$), and tended to decrease after 5 day, while 5'-inosinic acid (IMP) was invariable. Because free Glu contents of meat were above the taste threshold value of Glu, these variations were considered to have affected the meat taste. In the sensory evaluation (Experiment 2), a significant difference showed that in the paired comparison test ($P < 0.01$). The result of the paired comparison test, the taste of the meat in the high CP diet group was superior to that in the control group, especially overall preference, thickness and umami taste. These results suggested that dietary CP levels could affect taste-active components, especially free Glu. In experiment 3, at 3 day, KGA activity in muscle of CP 30.7% diet was lower than that of control ($P < 0.05$). GDH activity in CP 30.7% diet was tended to increase after 5 days. There were no differences in GS and ALT activities. KGA was considered to contribute to the free Glu increase. And feedback inhibition of KGA activity by high Glu concentration at 5 day may have affected the free Glu concentration of muscle at 10 day. As a result of this study, we concluded that short-time feeding of high CP diet was appropriate to increase the free Glu and sensory score of meat.

Conclusions

In order to elucidate the factors affecting the taste of chicken meat, the effect of dietary CP levels before marketing on broiler meat composition, i.e., free amino acids, and ATP metabolites, and regulation mechanism were studied using Cobb strain female broilers. As a result of this study, free Glu and sensory score in meat were increased by dietary CP levels for 3 to 10 days. Because the variation of the taste component improves the meat taste, the feeding condition is one of the important factors affecting the meat taste of chickens. Muscular KGA and GDH activities were changed by the dietary proteins. Functional feedback regulation affected the Glu in muscle after 5 day, therefore, the Short-time feeding of high CP diet, especially for 3 to 5 days, is more appropriate to improve the meat taste.

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Tables and Figures

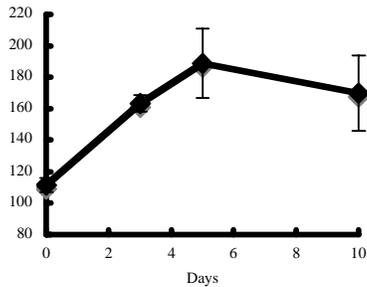


FIGURE 1 Effect of CP 30.7% diet on free Glu contents of pectoral meat extract. Values are means+SE, n=6 chicks per each group. (P<0.01)

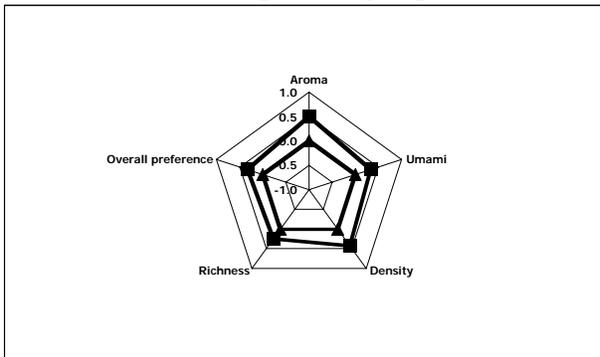


FIGURE 2 Scheffe's paired difference test between pectoral meat extracts of CP 17.6 and 30.7% diets.

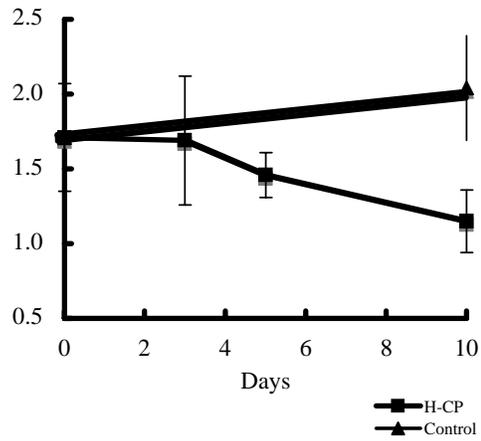


FIGURE 3 Effect of dietary CP levels on glutaminase activity of pectoral muscle. Values are means+SE, n=6 chicks per each group. (P<0.01)

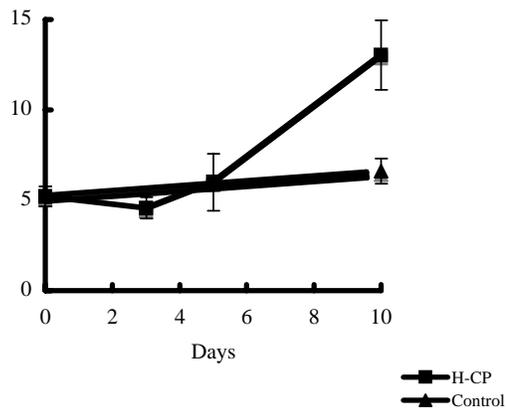


FIGURE 4 Effect of dietary CP levels on GDH activity of pectoral muscle. Values are means+SE, n=6 chicks per each group. (P<0.01)

EFFECT OF CONFORMATION AND FATNESS SCORE ON VOLATILE COMPOUNDS PROFILE

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Key Words: Beef, Carcass Grading, Volatile Compounds

Introduction

Labels and quality marks have arisen around breeding of local breeds. They have to, among other objectives, characterise marked products to fix prices according to a described quality. The effect of volatile compounds on the sensory acceptability of meat is very important. Raw beef contains a lot of precursors that during cooking will contribute to meat aroma and flavour (Mottram and Edwards, 1993). In Spain, beef carcass prices are fixed according the conformation and fatness score of the European beef grading system (OJEU, 1991). So, to characterise the products of a local breed and to link these characteristics to an economic value, it would be necessary to describe the differences among the conformation and fatness scores.

Objectives

The objectives of this work were to describe the volatile compound profile of cooked beef from *Pirenaica*, a Spanish cattle breed, bulls and to study the differences on volatile compounds among conformation and fatness scores.

Methodology

Forty carcasses from yearling bulls of *Pirenaica* breed were graded for fatness and conformation according to the European beef grading system (OJEU, 1991). After ageing *l. dorsi* (12th rib) samples for 24 hours, they were wrapped in aluminium foil and broiled at 180°C until achieve 70°C of internal temperature. Volatile compounds were extracted by purge and trap and tentatively identified by gas chromatography with mass spectrometry. The relative percentage of area of each volatile compound was calculated to describe volatile compounds profile.

The discriminant analysis was accomplished with volatile compounds detected in all of the studied samples, to find out which of them contributed to a greater extent to separate conformation or fatness scores. In view of the assignment of the carcasses across grades (67% of carcasses were graded as U⁰ in the conformation scale and 65% as 2⁰ in the fatness scale) it was decided to group the carcasses to create groups which provide enough number of carcasses for meaningful statistical analysis. The carcasses with higher and lower conformation scores than U⁰ were grouped to create groups of high and low

conformation, and the carcasses assigned exactly to conformation U^0 was the group of average conformation. Similarly, carcasses that scored below 2^0 for fatness scores were grouped as low fatness, carcasses score above 2^0 as high fatness, and carcasses that scored exactly 2^0 as average fatness score.

Results & Discussion

Thirty volatile compounds were tentatively identified from the headspace of the beef samples. The most abundant detected compounds by their relative percentage of area were aliphatic ketones (50.8%), followed by aliphatic aldehydes (26.96%), aliphatic alcohols (12.8%), aliphatic hydrocarbons (6.3%), benzenoid compounds (1.9%), sulphur compounds (0.9%), and alicyclic hydrocarbons (0.3%) (Figure 1). Hydrocarbons, aldehydes, alcohols, ketones and benzenoid compounds are originated from lipid thermal oxidation (Mottram & Edwards, 1983; Fogerty et al., 1990; Bolyston et al., 1996). The extensive presence of lipid oxidation-derived products and the sparse presence of Maillard reaction-derived products could be due to the mild cooking conditions that led to a medium cooked meat. (Mottram, 1998). Among detected volatile compounds the aliphatic ketone 2-propanone showed the highest RPA (RPA=43.7%), followed by the aliphatic aldehyde hexanal (17.8%). Only 8 volatile compounds were detected in all the analysed samples: the aliphatic hydrocarbons 3-octene and 2,2,4,6,6-pentamethylheptane; the aliphatic aldehydes ethanal and hexanal; the aliphatic ketones 2-propanone and 2-butanone; the sulphur compound methanethiol and the aliphatic alcohol thioethanol.

When defining groups by conformation score there were obtained two canonical discriminant functions that classified correctly 67.5% of the cases (Table 1; Figures 1A). Ethanal, hexanal, 2-propanone and thioethanol contributed to separate average conformation score against low and high conformation score. Function 2 separated extreme conformation scores on the basis of 3-octene, 2,2,4,6,6-pentamethylheptane, 2-butanone and methanethiol. When defining groups by fatness scores, there were obtained two canonical discriminant functions that classified correctly 67.5% of the cases (Table 1; Figures 2B). Function 1 separated average fatness score against low and high fatness score on the basis of 2,2,4,6,6-pentamethylheptane, ethanal, 2-propanone and thioethanol. Function 2 separated low against high fatness score on the basis of 3-octene, hexanal, 2-butanone and methanethiol.

There were three volatile compounds (3-octene, 2-butanone and methanethiol) that contributed to separate extreme conformation and fatness scores. Those carcasses scored as U^{2^0} , which were the most abundant carcasses in the current research, would be characterised by their higher content of 2-propanone and thioethanol.

Conclusions

Thirty volatile compounds were tentatively identified in broiled beef from yearling bulls of *Pirenaica* breed, with an extensive presence of lipid oxidation-derived products. Beef carcasses of *Pirenaica* breed currently traded would be relatively homogeneous: Most of the carcasses were graded as U^0 in the conformation scale and as 2^0 in the fatness scale. The animals U^{2^0} would be characterised by their high content of 2-propanone and thioethanol.

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Tables and Figures

Table 1. Correlation coefficients between canonical discriminant functions and volatile compounds on groups defined by conformation and fatness score

Percentage of explained variability	Conformation score		Fatness score	
	67.2%	32.8%	87.9%	12.1%
	0.45	0.54*	0.10	0.59
	0.36	0.37*	0.28*	0.13
	0.25*	0.04	0.67*	0.39
	0.25*	0.13	-0.07	0.49*
	0.51*	-0.03	-0.24*	-0.21
	0.02	0.53*	0.13	-0.23*
	0.05	0.15*	-0.06	0.09*
	0.17*	0.08	-0.12	-0.03

*: denotes largest absolute correlation between each variable and any discriminant function

Figure 1. Percentage of area counts of volatile compounds according to their chemical nature

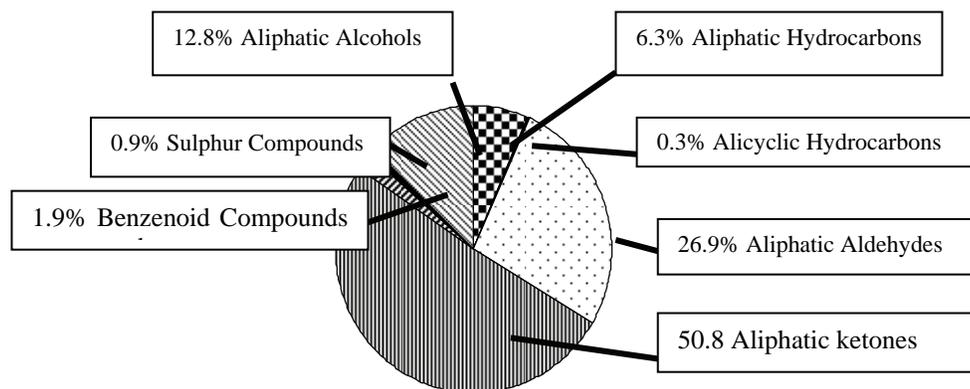
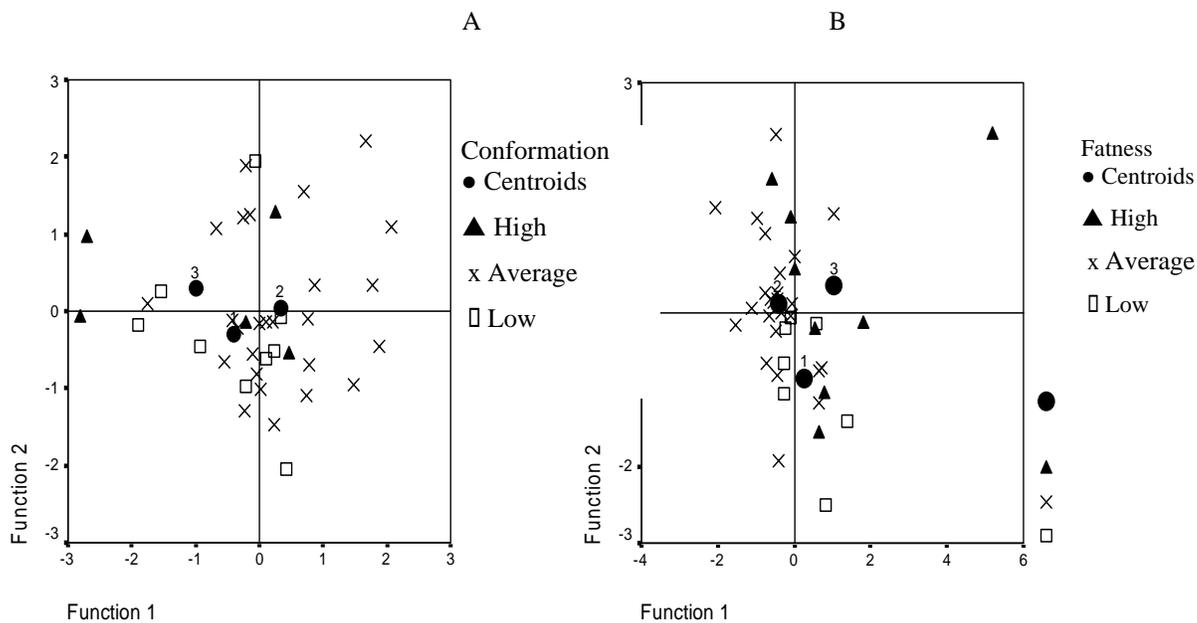


Figure 2. Separation of conformation (A) and fatness (B) groups applying the discriminant analysis to volatile compounds of the *l. dorsi* of beef of Pirenaica young bulls



MEAT VOLATILES OF FIVE DIFERENT GOAT GENOTYPES

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Key Words: Flavor compounds, goat meat, genotypes

Introduction

The high nutritional and climatic adaptation of goats, as well as the fact that they provide low fat meat, makes it an important protein source for hot dry climates as the Brazilian northeast region where most of Brazil's goats are located. They are there extensively reared under native pasture without feed supplementation constituting an important factor for the subsistence economy.

Brazilian herd is constituted of SRD animals – without a definite breed – originated from random crossbreeding between native (Canindé, Moxotó, Marota and Repartida) and foreign breeds from Asia, Africa and Europe. Though characterized as highly rustic they were not selected for meat production.

However, from 1992 to 2002 Brazilian demand for goat meat augmented 24.5% (COUTO, 2003). At the same time consumer demands for better quality meat also increased demanding better slaughter conditions, new breed introduction and younger animals. Yet, Brazilian goat meat is still considered as presenting strong flavor and tenderness problems limiting a stronger expansion of its consumption consolidating this activity as an important one for the Brazilian agribusiness.

Breed, age, sex and feeding regimen have been pointed as important factors affecting the characteristic flavor of goat meat (SOBRINHO e NETO, 2001; MADRUGA et al., 2003). Breed has been especially implicated as a factor of meat quality (SAÑUDO et al. 1998). However, few studies based on genetic group comparison have been conducted in Brazil. Evaluation of pure breeds and crossbreeds or both may provide information leading to opportunities for genetic improvement of goat meat quality.

Objectives

To obtain information allowing development of Brazilian goat herds with better meat flavor.

Methodology

F1 intact male goats were obtained from the EMEPA's Experimental Station of "Pendência", located in the Cariri region of Pernambuco State, Brazil, by crossbreeding Brazilian native (Moxotó and SRD) and foreign animals (Boer, Savanna, Kalahari and Anglo Nubian) as to constitute five genetic groups: ½ Moxotó + ½ Boer; ½ Boer + ½ SRD; ½ Savanna + ½ SRD; ½ Anglo Nubian + ½ SRD; ½ Kalahari + ½ SRD. Until four months of age they were raised under field conditions with their mothers feeding on brachiaria (*Brachiaria decumbens*), mombaça (*Panicum maximum*), pangola (*Digitaria eriantha*), and tifton (*Cynodon* sp.) grass and supplemented with a concentrated mixture to provide their nutritional needs. They were then separated from their mothers and confined in collective pens for 60-65 days, receiving a concentrated mixture (89.9% of dry matter, 93% of organic matter, 18.6% of crude protein, 4.2% of fat, 32% of neutral detergent fiber, 14% of acid detergent fiber and 3,26 kcal/kg DM of digestible energy) of broken maize (50%), maniçoba hay (20%), soy bean meal (23%), vegetal oil (1%) and mineral salt (2%). By this time the animals were 6-6.5 month old and weighed between 18 and 28 kg.

After fasting for 24 hours with free access to water six animals of each genetic group were slaughtered. Hot carcasses were held at room temperature (25-30 °C) for six to eight hours and chilled (-4 °C ; 24 h). Chilled carcasses were dissected into bone and edible meat. Muscles from the left legs were frozen (-18 °C) until conduction of chemical analysis.

Volatile compound determination were conducted in the quadriceps muscle after volatile extraction by the simultaneous extraction and distillation technique (LIKENS & NICKERSON, 1964) followed by separation and identification in a GC-MS chromatographer. Goat quadriceps muscle was defrosted overnight, and 150 g portions were cut into 1 cm³ cubes which were cooked (~ 55 min) in a pre-heated (200 °C) oven to an internal temperature of 75 °C. Extraction of flavor volatile compounds (MADRUGA et al., 2000) was performed. Distilled volatiles were analyzed in a GC chromatographer (Shimadzu GC-17) connected to a QP 5050A mass spectrophotometer. A split/splitless (1:10) injector set at 230 °C was used to introduce 0.5 µL aliquots of each of the distillation extract onto a fused silica capillary column. The capillary column used was a DB-5; 30 m x 0,257 mm (i.d.) x 0,25 µm film thickness (US 1372916H - J&W Scientific Inc, UK). Helium at 1 ml/min was used as a carrier gas. The initial column temperature was set at 30 °C. Five minutes after injection the temperature was increased (20 °C/min) to 60 °C and maintained for 5 min. Then it was programmed to increase (4 °C/min), to a final temperature of 250 °C. The temperature was held at 250 °C for 10 min. Column end was connected to an ion source (250 °C, ionization energy of 70 eV). The MS operated through an electron impact system with a 40 to 350 m/z mass variation amplitude and a scan rate of 1 scan/sec. Volatiles were identified by comparing their mass spectra with those of a NIST (National Institute of Standards & Technology, E.U.A.) computer data base library (150,000 reference spectra).

To calculate the linear retention indices (LRI) volatile compound, a solution containing C₆-C₂₅ n-alkanes was run through the chromatographer before each run of the GC-MS (MADRUGA et al., 2000). LRI values were compared with those of literature

(JENNINGS & SHIBAMOYO, 1980; KONDJAYAN & BERDAGUÉ, 1996) authentic compounds.

Data from volatile compound composition were only descriptively analyzed since it was necessary to group the quadriceps muscles from the six animals of each genetic group to provide sufficient material.

Results & Discussion

Fifty one flavor volatiles peaks were identified (Table 1) as aldehydes (14), alcohols (10), hydrocarbons (9), ketones (7), esters (5), furans (2), aromatics (2) and terpenoids (2). Thirty eight were identified both by mass spectroscopy (MS) and comparison of their linear retention indexes (LRI) to those presented in the literature. The remaining 13 were identified only by MS. These compound classes have been pointed as the major ones in cooked goat meat volatiles (MADRUGA et al., 2000). The number of volatile compounds is lower than those (108) reported by MADRUGA et al. (2000) in mixed race goats of different age and gender. However, these authors only found 53 volatiles in similar animals (entire males slaughtered after 175 days).

Table 1 shows that, based on both relative area and number of volatiles, four classes stick out: aldehydes, alcohols, hydrocarbons and ketones, which are the major compounds in the aromatic profile of cooked meats (MADRUGA et al., 2003) resulting from thermal degradation of long chain fatty acids (MADRUGA et al., 2000).

Aldehydes are volatiles found in greater concentration and number. Their relative amount varied from 17.59% in the ½ Boer + ½ SRD to 63,65% in the ½ Anglo Nubiana + ½ SRD genetic group.

Aliphatic aldehydes have been associated with the characteristic goat meat odors (SUTHERLAND & AMES, 1995), but not in lamb (NIXON et al., 1979).

Furans, produced from Maillard thermal degradation of carbohydrates are important to beef and pork flavor but not widely identified in lamb and goat meat. However, alkyl furans, especially 2-pentil-furan, have been verified in almost every meat and may result from the cyclization of the 4-keto nonanal derived from linoleic acid (MOTTRAM, 1991).

Though expected as one of the major class of compounds, originating from the thermal degradation of sulfur-containing amino acids and from thiamine or Maillard reactions (MOTTRAM, 1991), especially in lean meats source such as goat, no sulfur volatiles were identified. On the contrary, most of the volatiles identified originate from fats (MOTTRAM, 1991; ELMORE et al., 1999). A possible reason for this absence of sulfur volatiles is the low cooking temperature (70 °C) since they are generated under higher (> 80 °C) cooking temperatures (MACLEOD & COPPOCK, 1977; LAWRIE, 1979; CAMBERO et al., 1992). However, MADRUGA et al. (2000) reported 2 sulfur volatiles (0.7% of the total volatiles) in similar animals (entire males slaughtered at 175 days of age) cooked to a similar internal temperature.

Similar to MADRUGA et al. (2000) no carboxylic acids were identified which is probably due to the fact that this class of compounds is not very volatile (MOTTRAM, 1991).

Among the identified volatiles five presented greater chromatographic areas in every genetic breed: hexadecanal, butylated hydroxytoluene (BHT), hexanal, nonanal and pentanol-2.

Hexadecanal was first identified in lamb (SUTHERLAND & AMES, 1995).

BHT detection in every sample analyzed is probably associated to the use of this antioxidant in animal feed.

Table 1 also shows that genetic group influenced the major groups of volatile compounds. Animals of the ½ Anglo Nubiana + ½ SRD genotype generated a greater proportion of aldehydes even though only 2 (hexadecanal and octadecanal) have been identified. Animals of this genetic group also presented significant amounts of BHT and insignificant amounts of the remaining volatile groups. Animals of the ½ Kalahari + ½ SRD genotype generated similar amounts of aldehydes (hexanal, benzaldehyde, nonanal and hexadecanal) and aromatic compounds (BHT) which, together, accounted for 72.48% of all volatile identified. Except for terpenoids, though in smaller amounts, the remaining volatile groups are also present in the meat volatile profile of this genetic group. Animals of the ½ Moxotó + ½ Bôer genotype generated greater amounts of aldehydes (all of the 14 identified) which, together with 9 different alcohols (20,25%) and 2 aromatics (25,04%) make up 94.58% of all volatiles. Animals of the ½ Savanna + ½ SRD genotype were characterized by greater generation of aromatic compounds (BHT and toluene – 56.1%) and 5 different aldehydes (2-methyl butanal, hexanal, octanal, nonanal e hexadecanal) which together account for 89.73% of all volatiles. Finally, ½ Bôer + ½ SRD animals were characterized by the greater generation of aromatic volatiles (BHT and toluene – 38.9%) and 7 different alcohols (35.81%) which together with 9 different aldehydes account for 92.73% of all volatiles.

Though there were no literatures evaluating goat meat volatile profile related to genetic groups, except for hydrocarbons, breed differences on all classes of volatile compounds have been reported in lambs (ELMORE et al., 2000). However, for beef there were differences only in some volatiles (ELMORE et al., 2004).

The effect of genetic group on goat meat volatile profile can also be observed according to the number of volatiles identified. In this respect, the volatile profile from animals of ½ Moxotó + ½ Bôer, followed by ½ Boer + ½ SRD, genetic groups presented greater number of different volatile compounds. In these genetic groups, respectively, 37 and 32 different types of volatile compounds were identified. These numbers are lower than those (53-54) reported by MADRUGA et al. (2002) in the volatile of mixed breed (Creole X Anglo Nubian, Saanen or English Alpine) intact male goats slaughtered between 175 and 220 days of age and also indicate flavor differences due to genetic groups. These differences in volatile profiles are probably related to the observed (data not shown) genotype differences in fatty acid composition.

Conclusions

Genetic group affect goat meat flavor and may be related to differences in fatty acid profiles. Under the experimental conditions genetic group having Bôer blood presented a greater number of volatiles, especially aldehydes, alcohols, hydrocarbons and ketones.

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Tables and Figures

Table 1 – Meat volatile compounds from intact male goat genotypes and their relative proportion.

Volatile Compounds and class	Identification form *	LRI	½ Boer	+	½Savanna	½Moxotó	½Kalahari +	½ Anglo
			½SRD		+ ½SRD	+ ½Boer	½SRD	Nubian + ½SRD
<i>Aldehydes (14)</i>								
3-methyl butanal	A,C,E,F	643	0,05		Nd	1,70	Nd	Nd
2- methyl butanal	A,C,E,F	655	Nd		1,40	0,60	Nd	Nd
Hexanal	A,C,D,E,F	800	3,76		17,79	6,09	9,19	Nd
Heptanal	A,B,C,D,E,F	901	0,82		Nd	3,28	Nd	Nd
Benzaldehyde	A,C,E	959	0,85		Nd	1,82	1,06	Nd
Octanal	A,B,C,D,E,F	1002	0,52		0,75	2,44	Nd	Nd
Nonanal	A,B,C,D,E,F	1103	1,49		4,43	4,98	5,76	Nd
2-nonenal	A,D,E,F	1160	Nd		Nd	0,43	Nd	Nd
2-decenal	A,C,D,F	1262	Nd		Nd	0,50	Nd	Nd
Trans-2-undecanal	A,D,E	1364	Nd		Nd	0,38	Nd	Nd
Tetradecanal	A,D,E,F	1612	0,21		Nd	0,19	Nd	Nd
Hexadecanal	A,B,C,D,E,F	1817	9,25		9,27	22,48	18,69	61,00
9-octadecenal	A,D	1997	Nd		Nd	0,31	Nd	Nd
Octadecanal	A,D	2021	0,64		Nd	3,40	Nd	2,65
Relative area (%)			17,59		33,64	49,29	34,70	63,65
<i>Alcohols (10)</i>								
Trimethyl nonanol	A	667	Nd		1,49	Nd	Nd	Nd
Pentanol-2	A,B	701	19,89		1,00	4,62	2,22	2,65
3-methyl 2-heptanol	A	704	4,77		Nd	1,56	Nd	Nd
3- methyl 2-butanol	A	711	0,67		Nd	0,69	Nd	Nd
1-hexenol-3	A,B	852	2,52		Nd	2,68	Nd	Nd
1-hexanol	A,B,F	854	7,21		Nd	8,91	Nd	Nd
1-octenol-3	A,D,E,F	980	0,49		Nd	0,84	Nd	Nd
1-octanol	A,D,E,F	1073	Nd		Nd	0,31	Nd	Nd

Pentadecanol	A,D	1714	0,26	Nd	0,50	Nd	Nd
Hexadecanol	A,D	1882	Nd	Nd	0,12	Nd	Nd
Relative area (%)			35,81	2,49	20,25	2,23	2,65
<i>Hydrocarbons (9)</i>							
Cycloheptane	A,D	629	Nd	Nd	Nd	2,02	Nd
Tridecane	A,F	1300	0,10	Nd	Nd	Nd	Nd
Tetradecane	A,B,C,D,E,F	1400	0,26	Nd	Nd	Nd	Nd
Pentadecane	A,D,E,F	1485	0,26	Nd	Nd	Nd	Nd
2- methyl Pentadecane	A	1500	Nd	Nd	0,31	Nd	Nd
Hexadecane	A,D,F	1600	0,23	Nd	0,12	Nd	Nd
Heptadecane	A,B,C,D,E,F	1699	Nd	Nd	0,43	Nd	Nd
Octadecane	A,D,F	1800	0,28	Nd	Nd	Nd	Nd
Nonadecane	A,B,D,E,F	1890	0,15	Nd	0,41	Nd	Nd
Relative area (%)			1,29	0	1,27	2,02	0
<i>Ketones (7)</i>							
2,3-pentanedione	A,E,F	681	Nd	Nd	0,10	Nd	Nd
2-pentanone	A,B,E,F	690	2,06	Nd	2,52	1,97	Nd
3-methyl-2-pentanone	A	697	1,93	Nd	Nd	9,34	Nd
2,3-hexanedione	A	778	0,10	1,25	Nd	Nd	Nd
2-undecanone	A,D,F	1294	Nd	Nd	0,17	Nd	Nd
6,10-dimethyl-5,9-undecadien-2-one	A,C	1452	0,26	Nd	Nd	Nd	Nd
2-tridecanone	A,D,E	1496	Nd	Nd	0,24	Nd	Nd
Relative area (%)			4,35	1,25	3,02	11,31	0
<i>Esters(5)</i>							
1,1-dodecanodiol diacetate	A	658	Nd	2,84	0,10	Nd	Nd
Butyl acetate	A	825	Nd	Nd	Nd	1,87	Nd
3-methyl butanoate butylate	A	1092	0,46	Nd	0,07	Nd	Nd
Isopropyl Pentanate	A	1126	Nd	Nd	Nd	2,73	Nd
Di methyl Carbamoditianate	A	1379	1,29	Nd	Nd	Nd	Nd
Relative area (%)			1,75	2,84	0,17	4,60	0

Furanes (2)

Furfural	A,E,F	829	Nd	Nd	Nd	5,91	Nd
2-pentyl-furane	A,C,D,F	991	0,13	3,69	0,84	Nd	Nd
Relative area (%)			0,13	3,69	0,84	5,91	0

Aromatics (2)

Toluene	A,C,D	769	0,62	2,79	0,50	Nd	Nd
Butylated hydroxytoluene (BHT)	A,C	1514	38,36	53,31	24,54	37,78	33,71
Relative area (%)			38,97	56,10	25,04	37,78	33,71

Terpenoids (2)

α -terpineol	A	1194	0,10	Nd	Nd	1,46	Nd
α -copaene	A	1919	Nd	Nd	0,12	Nd	Nd
Relative area (%)			0,10	0	0,12	1,46	0

LRI – Linear Retention Index

¹ID: identification comparing mass spectra (A – MS);

Literature LRI

B – Jennings e Shibamoyo (1980);

C – Kondjoyan e Berdagué (1996);

D – Madruga (2000);

E – Madruga *et al.* (1997);

F – Sutherland & Ames (1995).

FLAVOR RELATIONSHIPS AMONG MUSCLES OF THE BEEF CHUCK AND ROUND

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Introduction

New cuts from the beef round and chuck have gained popularity. There have been anecdotal reports of off-flavors, especially a liver-like flavor, in some of these beef value cuts. The incidence and intensity of liver-like flavor in various muscles is unknown. Flavor is highly correlated with overall-like ratings in beef (Neely et al., 1998; Goodson et al., 2002). Goodson et al. (2002) concluded that flavor was the overwhelming trait of importance in clod steaks. With the importance of flavor to the consumer, it is likely that they will not try the same cut again if they have a bad experience. The objective of this research was to compare different beef muscles for off-flavor notes and to determine the relationship of pH and heme-iron content to off-flavor.

Methods and Materials

After grading, knuckles and shoulder clods were removed from 16 Choice and 14 Select-grade beef carcasses. Hot carcass weight, fat thickness, marbling, rib-eye area, and percentage kidney, pelvic, and heart (KPH) fat were recorded and yield grade was calculated. The knuckles and shoulder clods were stored in a 1°C dark cooler until 7 d postmortem. The *m. rectus femoris* (REC), *m. vastus lateralis* (VAL), *m. vastus medialis* (VAM), *m. infraspinatus* (INF), *m. teres major* (TER), and *m. triceps brachii* caput longum (TRI) were fabricated from each carcass. The INF was filleted, and the connective tissue running laterally through the middle of the muscle was removed. Each half of the INF was then cut into three steaks. A small sample was cut from the dorsal, anterior end of each muscle, minced, and placed in a plastic bag for chemical analysis. All muscles except for the INF were cut into 2.54 cm steaks, wrapped, and frozen at -20°C.

Samples were prepared by cubing, freezing in liquid nitrogen, powdering the frozen sample with a blender, and storing at -80°C. The powdered samples were used for moisture analysis, pH, and heme-iron concentration.

Powdered sample was used to measure moisture content using a LECO Thermogravimetric Analyzer-601 (Model 604-100-400, LECO Corp., St. Joseph, MI). The pH of the samples was determined by combining 10 g of meat with 90 mL of distilled water. Total heme-iron concentration was determined using the method described by Hornsey (1956) as modified by Lee, B.J., Hendricks, D.G., and Cornforth, D.P. (1998). Two grams of powdered sample were weighed into tubes and concentration was determined in triplicate. Samples were homogenized with 8.1 mL of acetone and 0.2 mL of hydrochloric acid. This mixture was filtered and tubes were stored for approximately

15 minutes in a dark cabinet to limit light exposure. The filtrate was then read with a spectrophotometer at 640 nm. The absorbance value was then multiplied by 680 to give the amount total pigment. Total pigment was converted to heme-iron content using the following formula: (total pigment (ppm) x 8.82/100).

Frozen steaks were tempered for 1 day in a 1°C cooler before cooking. The steaks were weighed and trimmed of external fat before cooking. Each steak was grilled to an internal temperature of 65°C. Thermocouples were inserted in the approximate center of each steak. An Omega handheld digital thermometer model 450-ATT (Omega Engineering Inc., Stamford, CT) was also used to confirm the internal temperature. Steaks were turned for the first time after two minutes and then flipped as needed to minimize charring.

When the steak reached the desired internal temperature, the steak was removed from the grill and weighed. The steak was then covered in foil for no more than 10 minutes. The steaks were cut into 1.27 cm x 1.27 cm x 2.54 cm steak cubes and placed in double broilers until served (<15 min).

Taste panelists were trained using the guidelines and procedures of Meilgaard, M., Civille, G.V., and Carr, B.T. (1991). Taste panels were held mid-morning or mid-afternoon and panelists were asked to avoid soft drinks, coffee, and food one hour prior to the sampling session. The panelists received between six and eight samples per session. All eight samples were either from the same muscle type or they were in groups of four from two different muscles. On days that samples from two muscles types (such as steaks from both the INF and TER) were served, a five minute break was given to separate the two muscles. All steaks were from a uniform location on the muscle. The steaks were from the second to fourth steaks counted from the anterior end of the muscle for the REC, VAL, INF, and TRI. Because of the small size of the TER and VAM, they were cooked as whole muscles. The order of the day that each muscle was served was random and steaks for each muscle were served in random order. Panelists were not aware of which type of steak they were eating.

Panelists were isolated in individual booths to reduce collaboration and samples were served under red incandescent light to eliminate visual differences. Distilled water and unsalted crackers were provided for panelists between samples to cleanse their palate. The steak cubes were served on ceramic plates to the panelists. Charred edges were removed to allow for consistent sampling.

Panelists used an 8-point hedonic rating scale with 8=extremely juicy, extremely tender, no connective tissue and no off-flavor, and 1=extremely dry, extremely tough, abundant amount of connective tissue, and extreme off-flavor. They also identified off-flavor notes including charred, liver-like, metallic, musty/oxidized, acidic, rancid, and sour flavors. Oxidized was described as a “warmed-over” flavor while rancid was the flavor associated with lipid oxidation.

Statistics

Muscle carcass traits were analyzed by analysis of variance using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Muscle and grade were included in the model. Least square means were separated at a predetermined significance level of $P < 0.05$ using the PDIFF function of SAS. Muscle off-flavor notes were analyzed by analysis of variance

using the MIXED procedure of SAS. Fixed effects included muscle and group. Animal within group was a blocking factor and considered a random effect. Least square means were obtained and separated using the PDIFF function. Muscle off-flavor notes were analyzed by analysis of variance using the GLM procedure of SAS. The model included muscle and grade. The linear and quadratic functions, as well as the interaction of, heme-iron and pH were analyzed to obtain the coefficients of determination.

Results

Only percent KPH fat and marbling differed between Choice and Select cattle, with Choice-grade cattle having a greater amount of both. This result is expected because carcasses are sorted into quality grades based primarily on marbling.

Off-flavor intensity differed among muscles. The INF had the lowest off-flavor intensity rating and juiciness rating and was among the most tender and juicy of the muscles tested while the VAL had the most intense off-flavor ratings and was the least tender, had the most connective tissue, and had the lowest amount of juiciness ($P<0.05$) (Table 1). This could be due to a “halo effect” where a sample that has a good flavor is rated more tender or juicy than one with bad flavor. The INF, TER, and VAM had the highest pH values of the muscles tested. There were no differences ($P<0.05$) among muscles for heme-iron concentration.

Liver-like, bloody, and rancid flavors were not affected by muscle type (Table 2). The INF, which had the lowest amount of off-flavor, was among the lowest in percentage of panelists detecting sour, metallic, and oxidized flavors, although it received a higher rating of fatty flavor than the other muscles ($P<0.05$). The VAL, which had the most intense off-flavor, was among the highest in percentage of panelists detecting sour, charred, and oxidized flavors ($P<0.05$). Most of the other muscles were rated as being intermediate in the percentage of panelists detecting specific off-flavor notes.

Table 1. The effect of muscle on sensory characteristics, heme-iron concentration, and pH

Muscle	Tenderness	Connective tissue	Juiciness	Off-flavor intensity	Heme-Iron Concentration	pH
Infraspinatus	6.50 ^{ab}	5.77 ^{ab}	6.22 ^a	6.03 ^a	44.42	5.70 ^a
Rectus femoris	6.11 ^b	5.44 ^b	5.69 ^b	5.68 ^b	46.25	5.59 ^b
Teres major	6.58 ^a	5.85 ^a	6.15 ^a	5.41 ^{bc}	42.99	5.71 ^a
Triceps brachii	5.45 ^c	4.32 ^c	5.68 ^b	5.54 ^b	45.43	5.47 ^c
Vastus lateralis	4.66 ^d	3.63 ^d	5.07 ^c	5.10 ^c	45.60	5.54 ^{bc}
Vastus medialis	5.45 ^c	4.18 ^c	6.04 ^a	5.58 ^b	47.47	5.66 ^a

^{a,b,c,d} Means within a column (for sensory traits) with different superscripts are significantly (P<0.05) different

Taste panel scale: 8=extremely juicy, extremely tender, no connective tissue and no off-flavor, and 1=extremely dry, extremely tough, abundant amount of connective tissue, and extreme off-flavor

Table 2. The effect of muscle on percentage of panelists detecting each off-flavor note

Muscle	Liver-like	Sour	Metallic	Charred	Bloody	Oxidized	Fatty	Rancid
Infraspinatus	0.09	0.23 ^a	0.09 ^a	0.30 ^b	0.02	0.09 ^{ab}	0.14 ^b	0.09
Rectus femoris	0.10	0.44 ^b	0.13 ^a	0.20 ^{ab}	0.03	0.07 ^a	0.03 ^a	0.05
Teres major	0.09	0.49 ^b	0.15 ^{ab}	0.22 ^{ab}	0.02	0.08 ^{ab}	0.03 ^a	0.06
Triceps brachii	0.08	0.49 ^b	0.20 ^b	0.22 ^{ab}	0.01	0.13 ^{abc}	0.02 ^a	0.06
Vastus lateralis	0.09	0.48 ^b	0.15 ^{ab}	0.31 ^b	0.01	0.17 ^c	0.01 ^a	0.07
Vastus medialis	0.11	0.49 ^b	0.17 ^{ab}	0.15 ^a	0.03	0.15 ^{bc}	0.02 ^a	0.07

^{a,b,c} Means within a column (for off-flavor notes) with different superscripts are significantly (P<0.05) different

When the off-flavor intensity scores were assessed, it became obvious that when one muscle of a given carcass was off-flavored, all muscles were off-flavor (Table 3). Sixteen of the 18 muscles from animals six, seven, and nine had off-flavor intensity scores below five.

Table 3. Off-flavor intensity scores among muscles

Animal	Grade	INF	TER	TRI	REC	VAL	VAM
1	Choice	6.36	4.20	6.06	6.44	5.58	5.25
2	Choice	6.25	6.17	6.00	5.75	5.14	5.65
3	Choice	6.75	6.45	6.31	6.78	5.44	6.05
4	Choice	7.19	5.44	6.11	6.75	5.86	6.33
5	Choice	6.61	5.00	5.56	6.75	5.72	5.65
6	Select	4.17	2.55	3.56	3.83	3.36	3.10
7	Select	4.38	3.39	4.39	3.31	4.14	4.90
8	Select	6.07	6.05	4.89	6.38	4.86	5.50
9	Select	4.56	5.35	5.06	4.94	4.60	4.00
10	Select	6.55	5.33	4.88	6.31	4.56	6.22

Taste panel scale: 8= no off-flavor, and 1= extreme off-flavor

In an attempt to explore the off-flavor intensity ratings among these muscles, the muscles were grouped. All muscles that were rated a five or below in off-flavor intensity, where at least 30% of the panelists recognized the off-flavor as liver-like, were placed together in an “off-flavor” grouping while the other muscles were left in a “normal” group. There were no group by muscle interactions for sour, metallic, fatty, bloody, or oxidized off-flavor notes (Table 4). When grouped this way, the percentage of panelists detecting liver-like scores was very high, which is to be expected as this is how they were grouped ($P < 0.05$). Charred flavors were lower for the off-flavor group than for the normal group ($P < 0.05$). This could be because the intense liver-like flavor overwhelms the charred flavor. There was also an interaction among rancid samples which was only significant for the VAM, where off-flavor samples were less rancid than normal samples ($P < 0.05$). This suggests that liver-like flavor does not contain a significant amount of other flavor notes.

Table 4. The effect of normal vs. off-flavor group^a and muscle on percentage of panelists detecting each off-flavor note

Muscle	Liver-like			Charred			Rancid			
	Normal	Off-flavor	Sour	Metallic	Normal	Off-flavor	Bloody	Oxidized	Fatty	Normal
	Infraspinatus	0.83 ^y	0.04 ^x	0.14 ^b	0.05 ^b	0.06	0.32	0.01	0.05 ^b	0.10
Rectus femoris	0.48 ^y	0.05 ^x	0.28 ^b	0.18 ^{cd}	0.23	0.21	0.02	0.04 ^b	0.02	0.08
Teres major	0.49 ^y	0.04 ^x	0.37 ^c	0.13 ^{bc}	0.69 ^y	0.17 ^x	0.01	0.11 ^{bc}	0.03	0.07
Triceps brachii	0.41 ^y	0.05 ^x	0.34 ^c	0.24 ^d	0.52 ^y	0.20 ^x	0.00	0.18 ^c	0.01	0.05
Vastus lateralis	0.48 ^y	0.04 ^x	0.38 ^c	0.14 ^{bcd}	0.65 ^y	0.27 ^x	0.01	0.19 ^c	0.01	0.13
Vastus medialis	0.60 ^y	0.05 ^x	0.48 ^c	0.14 ^{bcd}	0.20	0.15	0.02	0.17 ^c	0.03	0.23 ^x

^aOff-flavor group includes all muscles with a off-flavor intensity score of five or less, when the flavor note indicated was livery. Normal is all other muscles

^{b,c,d} Means within a column (for sour, metallic, bloody, oxidized, and fatty) with different superscripts are significantly (P<0.05) different

^{x,y} Means within a row (for liver-like, charred, and rancid) with different superscripts are significantly (P<0.05) different

Regression equations containing the linear and quadratic functions of heme-iron concentration, muscle pH, and their interaction were established for the frequency of off-flavor notes within each muscle for each quality grade. Within Choice, only the VAL and INF showed a relationship between pH, heme, and bloody flavor ($P < 0.05$). There were no significant relationships between pH, heme-iron concentration, and metallic flavors or oxidized flavors for either Choice or Select-grade muscles. Select-grade stronger relationships between off-flavor notes and pH and heme-iron, possibly because the three carcasses with strong, liver-like off-flavor were Select. Heme-iron and pH explained some of the off-flavor intensity of the TER, VAL, and VAM ($P < 0.05$).

Bloody flavor notes in the TRI showed a relationship ($P = 0.003$) for heme-iron concentration and pH. Heme-iron concentration and pH influenced liver flavor ($P = 0.0003$) and sour flavor ($P = 0.042$) in the REC. Liver-like flavor in the VAM was also influenced ($P = 0.042$). Heme-iron concentration and pH influenced charred flavor ($P = 0.032$) and rancid flavor ($P = 0.042$) in the TER.

Conclusion

These data suggest that when one muscle from a carcass contains liver-like off-flavor, all muscles contain that flavor. Muscles from the chuck and round have different off-flavor amounts as well as different sensory characteristics. Heme-iron concentration and pH influenced off-flavor in some select grade muscles, although there appears to be only a slight relationship. More research is needed to explain this relationship.

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**TENDERNESS AND COLLAGEN CHARACTERISTICS OF STEERS FINISHED
AS
CALVES OR YEARLINGS**

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Key Words: Beef, Finishing, Management Systems, Tenderness, Collagen

Introduction

Lack of consistency is one of the biggest concerns for today's beef industry. Inadequate tenderness and lack of uniformity in cattle ranked first and second, respectively, as the greatest challenges for all sectors of beef production (Smith, Savell, Morgan, & Montgomery, 2000). The National Beef Quality Audit stated that lack of uniformity in cattle is the concern for purveyors, restaurateurs, and retailers, which has shown the least improvement of the tracked concerns since 1991. Animal management plays a key role in the quality of the final end product. Calf finishing systems in the U. S. use large amounts of grain to feed high concentrate diets to weanlings for extended periods of time. In contrast, yearling systems are more extensive, growing calves for a longer period of time on forage before being fed a high concentrate diet for a short period prior to harvest. This reduction of days on a high concentrate diet may reduce costs; however, decreased days on feed have been associated with lower quality grades and less tender beef (Miller, Cross, Crouse, & Tatum, 1987; May, Dolezal, Gill, Ray, & Buchanan, 1992). Regardless of the management practice used to increase profitability, cattle produced must be of the type that are acceptable for the feedlot and yield a final product desirable to the consumer.

Literature provides varied results when comparing meat quality from finished calves and yearlings. Calves have been reported to produce carcasses with increased fat thickness, higher numerical yield grades and quality grades (Lunt and Orme, 1987), and more tender meat (Dikeman, et al., 1985; Johnson, Huffman, Williams, & Hargrove, 1990). However, others have shown minimal effects on carcass quality grade or meat palatability (Dikeman, Dayton, Hunt, Kastner, Axe, & Ilg, 1985; Huffman, Williams, Hargrove, Johnson, & Marshall, 1990).

Collagen solubility decreases (Hill, 1966; Cross, Carpenter, & Smith, 1973) and collagen cross-linkages increase (Goll, Bray, & Hoekstra, 1964; Goll, Hoekstra, & Bray, 1964; Cross, et al., 1973) in muscle as physiological age increases. Miller, Cross, Crouse, & Jenkins (1987) found decreased insoluble collagen and a higher percentage of newly synthesized soluble collagen in mature cows that had received a high energy diet prior to slaughter. This led to lower Warner-Bratzler shear force values and higher sensory tenderness scores compared to mature cows on a low-energy diet.

Objectives

Therefore, this study was conducted over a two year span to determine if collagen characteristics were responsible for tenderness differences of steers produced in the calf-fed and yearling systems when fed to a constant fat thickness.

Methodology

Steers (3/4 British, 1/4 Continental) were randomly assigned to be finished as calves (CF) or yearlings (YF) at weaning. Thirty-five and 41 calves and 42 and 41 yearlings were designated in year 1 and year 2, respectively.

Each year at weaning, CF steers were implanted (Synovex-S[®]) and were adapted from a 50% concentrate diet to a 92.5% concentrate finishing diet (TDN 84%, CP 12%) fed until harvest. Reimplantation (Revalor-S[®]) occurred after 90 d on feed. All steers were fed to an estimated 12th-rib fat thickness endpoint of 1 cm. To achieve this, Year 1 steers were on feed for 203 d and Year 2 steers were fed for 180 d. The CF steers were approximately 13 to 14 months old at the time of harvest.

The YF steers were drylot for 60 d, until corn stalks became available for grazing. While in drylot, these steers were fed ammoniated wheat straw ad libitum and supplemented with mineral and 2.27 kg/head/d (DM basis) of wet corn gluten feed. Steers then grazed corn stalks for 78 d in Year 1 and 91 d in Year 2. Hay was supplemented during heavy snow cover. After grazing corn stalks, steers were again drylot for the remainder of the wintering period until pasture was available for spring and summer grazing. Spring drylot was 64 d in Year 1 and 50 d in Year 2. Following the spring drylotting period, steers grazed pastures for 96 d in Year 1 and 103 days in Year 2. Steers were implanted (Revalor-G[®]) prior to summer grazing. Spring grazing pastures consisted of smooth brome grass. Summer grazing pastures consisted of big bluestem, indiagrass, and switchgrass. Following the summer grazing period, steers entered the feedlot, were reimplanted (Revalor-S[®]), blocked by weight, and assigned randomly to one of two pens. Steers were then fed similarly to the CF for receiving and finishing periods. This final finishing period consisted of 93 d in Year 1 and 90 d in Year 2. The YF steers were approximately 19 to 20 months old at the time of harvest.

Steers were harvested in a commercial slaughter facility. Shortly after being bled, carcasses were electrically stimulated with 8 to 10 low voltage (40 V) pulses. Hot carcass weights were obtained from all steers at the time of slaughter. In Year 1, carcasses were chilled for an extended 48 hour weekend chill period. Carcasses in Year 2 were chilled for approximately 42 hours. A marbling score was assigned to the carcass by the USDA grader. Other carcass data were measured and evaluated by experienced University of Nebraska personnel. Carcass measurements were used to calculate yield and quality grades. A boneless beef strip loin (IMPS #180) was collected from the left side of each carcass. Two strip loins from CF cattle were lost during the fabrication process, so additional data analysis continued on 34 and 40 strip loins in Year 1 and Year 2, respectively.

At 7 d postmortem, strip loins were cut into 2.54 cm *M. longissimus dorsi et lumborum* steaks for proximate analysis, Warner-Bratzler shear force (WBS) and sensory

panel evaluation. Individual steaks were designated for aging time (7, 14, and 21 d), shear force, and sensory evaluations, wrapped, and frozen at -22 °C until further analyses.

Frozen steaks were thawed at 4 °C for 24 h prior to cooking. Steaks were cooked to an internal temperature of 70 °C (AMSA, 1995) on a Farberware Open-Hearth broiler (Model 455N, Walter Kidde and Co., Bronx, NY). After cooling, 8 to 10 cores (1.27 cm in diameter) were removed and sheared using a Warner-Bratzler shear attachment to an Instron Universal testing machine (Model 55R1123, Canton, MA). The mean peak shear force (kg) of at least 6 cores was calculated for each steak.

Samples from the CF and YF steers were analyzed for soluble, insoluble, and total collagen according to Hill (1966) and Cross, et al., (1973). Soluble collagen was calculated with a 7.52 constant, and insoluble was calculated with a 7.25 constant (Goll, Bray, & Huekstra, 1963).

Steaks for sensory evaluation were cooked by the same procedure as described for WBS. After cooking the steaks were cut into 1 x 2 x 1 cm pieces for evaluation. Samples were served to a consumer sensory panel ($n \geq 30$) in individual booths under red lighting to mask differences in meat color. An 8-point Hedonic scale (8 = extremely desirable; 1 = extremely undesirable) was used to evaluate tenderness, juiciness, flavor, and overall acceptability.

Carcass traits, chemical characteristics, WBS, and consumer sensory panel data were analyzed using GLM procedure of SAS (1999). Both years were pooled and each steer was an experimental unit for carcass traits and chemical characteristics. Steaks at each aging time were experimental units for shear force and sensory evaluations.

Results & Discussion

Carcass characteristics for CF and YF steers are summarized in Table 1. The YF steers yielded heavier ($P < 0.01$) carcass weights with larger ($P < 0.01$) longissimus muscle areas, and less ($P < 0.01$) internal fat. They also had lower ($P < 0.01$) marbling scores, USDA quality grades, and percentage of carcasses grading USDA Choice or higher when compared to carcasses of CF steers. The differences in marbling scores were confirmed with chemical analysis (8.5 versus 5.5% fat).

As expected, increased aging time from 7 to 14 to 21 d produced steaks with lower ($P < 0.05$) shear force values, regardless of production system (Table 2). Steaks from CF had lower ($P < 0.01$) shear force values at 7, 14, and 21 d of age (Table 2) than steaks from yearlings. The YF steers had steaks that, after aging 21 d, had similar shear force values to CF steaks aged 7 d. They were also rated higher ($P < 0.05$) for tenderness, as well as juiciness, flavor, and overall acceptability (Table 3) after 7 and 14 days of aging. This was also true when comparing all Choice steaks or all Select steaks ($P < 0.05$).

The lower WBS values and higher sensory tenderness scores may be attributed to the collagen differences in the two groups. The CF had a more insoluble ($P < 0.01$), soluble ($P < 0.01$), and total collagen ($P < 0.01$) than the yearlings (Table 4). The higher total and insoluble values are probably due to dilution of collagen by muscle fiber growth. However, the collagen from the CF steers was much more soluble ($P < 0.01$) than from the YF steers (39.7 versus 24.4%, respectively). Hill (1966) demonstrated that collagen solubility decreases in muscle as physiological age increases. Solubility of collagen has been shown to be positively correlated to textural characteristics (Goll, et al., 1963).

Miller, Tatum, Cross, Bowling, and Clayton (1983) did not find these results. However, Aberle, Reeves, Judge, Hunsley, and Perry (1981) hypothesized that beef cattle feed high concentrate diets would have higher protein turnover and thus greater collagen solubility that would allow for less cross-linkages and more tender beef. The CF steers were on a high concentrate diet for a longer period of time which supports that rationale.

Conclusions

Growing steers for a longer period of time on forage with a short finishing period resulted in heavier carcasses with lower quality grades and beef that was less tender than CF steers. Steers finished as calves spent more days in the feedlot and in this study produced beef that was more tender and possessed more acceptable eating characteristics than yearlings. Collagen differences, especially soluble collagen amounts, may help explain the lower shear force values and higher tenderness ratings for the CF steers.

Table 1. Comparison of means for carcass characteristics from calf- and yearling-finished steers.

Trait	Calves		Yearlings		P-value
	Mean	SE	Mean	SE	
Hot carcass weight, kg	315.4 ^b	3.37	375.9 ^c	3.18	<0.0001
Fat thickness, cm	1.39	0.045	1.30	0.042	0.1880
Adjusted fat thickness, cm	1.50	0.039	1.42	0.037	0.1479
Longissimus muscle area, cm ²	72.76 ^b	0.67	81.06 ^c	0.63	<0.0001
Kidney, pelvic, heart fat, %	2.33 ^c	0.053	2.07 ^b	0.050	0.0004
Yield grade	3.49	0.054	3.46	0.050	0.6423
Marbling score ^a	454.1 ^c	8.80	346.1 ^b	8.28	<0.0001

^aMarbling score: modest = 500-599; small = 400-499; slight = 300-399.

^{bc}Means in the same row without a common superscript are different ($P<0.01$).

Table 2. Mean shear force values for steaks aged 7, 14, and 21 days from calf- and yearling-finished steers.

Age, d	Calves		Yearlings		P-value
	Mean	SE	Mean	SE	
7	3.30 ^{b,c}	0.089	4.09 ^e	0.109	<0.0001
14	3.07 ^b	0.089	3.74 ^d	0.109	<0.0001
21	2.79 ^a	0.089	3.40 ^c	0.109	<0.0001

^{abcde}Means without common superscripts are different ($P<0.05$).

Table 3. Mean sensory panel ratings for steaks aged 7 or 14 days from calf- or yearling-finished steers.

Age, d	Trait ^z	Calves		Yearlings	
		Mean	SEM	Mean	SEM
7	Juiciness	5.08 ^d	0.032	4.88 ^e	0.039
	Tenderness	5.46 ^a	0.034	4.56 ^c	0.043
	Flavor	4.96 ^d	0.033	4.64 ^e	0.041

	Overall Acceptability	5.07 ^d	0.032	4.47 ^e	0.039
14	Juiciness	4.86 ^e	0.032	4.61 ^f	0.039
	Tenderness	5.59 ^b	0.034	4.63 ^c	0.043
	Flavor	4.99 ^d	0.033	4.70 ^e	0.041
	Overall Acceptability	5.03 ^d	0.032	4.49 ^e	0.039

^{abc}Means for a given trait without a common superscript are different ($P < 0.05$).

^{def}Means for a given trait without a common superscript are different ($P < 0.01$).

^zConsumer panel rating for each trait: 8=extremely desirable; 1=extremely undesirable.

Table 4. Collagen (mg/g) in the longissimus muscle of calf- or yearling-finished steers.

Collagen	Calves		Yearlings		P-value
	Mean	SE	Mean	SE	
Insoluble	8.11 ^a	0.391	6.19 ^b	0.357	0.0004
Soluble	6.23 ^a	0.408	2.21 ^b	0.372	<0.0001
Total	14.52 ^a	0.742	8.46 ^b	0.678	<0.0001
% Soluble	39.72 ^a	2.528	24.38 ^b	2.308	<0.0001

^{ab}Means in the same row without common superscript are different ($P < 0.01$).

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COMPARISON OF BEEF FLAVOUR AND RELATED COMPOUNDS BETWEEN COWS AND YOUNG BULLS AS INFLUENCED BY AGEING

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Key Words: beef, ageing, flavour compounds, peptides, fatty acids

Introduction

The positive influence of ageing on beef tenderness has been convincingly demonstrated (e.g. Davey et al., 1976). Other sensory properties such as taste or flavour have been shown to change with ageing time as well, although the effects are not consistent (Spanier et al., 1997). Nevertheless, in the Belgian meat industry, it is generally accepted that beef ageing improves meat flavour. It is also believed that female cattle, having bred at least once, yield meat with more intense flavour than young male slaughter cattle.

Objectives

To investigate the effect of sex (cows vs. bulls) and ripening time on flavour properties and related parameters of bovine *Longissimus thoracis* (LT) muscle in the Belgian Blue beef strain breed.

Methodology

Three young bulls and 3 cows (aged between 12.6 and 14.6 and between 31.2 and 48 months respectively) of the Belgian Blue beef strain breed were slaughtered in the experimental slaughterhouse of our department after captive bolt stunning. The bulls and one cow had been fattened on a high concentrate diet, whereas two cows had been fattened at pasture supplemented with dried sugarbeet pulp. pH was measured between the 7th and 8th thoracic rib in the LT muscle after 1, 3, 5 and 24 hours *post mortem* (pm). At 1 day pm following conventional cooling, the LT between the 6th and 8th rib of the left carcass half and between the 6th and 9th rib of the right carcass half were excised. Sub-samples were taken in the same order of anatomical location, vacuum packed and frozen at -18°C until analysis either immediately (1 day ageing), or after 6 or 13 days additional storage at 3°C (7 and 14 days ageing). Shear force (SF) and aroma profiling by GC-MS after Likens-Nickerson extraction of volatile aroma compounds were assessed according

to the methods described in Raes et al. (2003). The concentration of 3-10 kDa peptides was determined by SDS-PAGE and expressed as cytochrome c equivalents (Claeys et al., 2004). Taste intensity was scored by a semi-trained 6 member panel, to which in each session 2 plates with each 3 grilled samples (of the three ageing times) of one female and one male animal were presented. Panellists were asked to rank the 3 samples of each plate according to taste intensity. In addition, they were asked to indicate an overall preference for one of the two plates.

Fatty acid composition was analysed on a sample of 1 day ageing (Raes et al., 2003). Determination of the L*, a*, b* colour co-ordinates and sarcomere length was done on the same sample of 7 days ageing used for shear force (Raes et al., 2003).

Due to the low number of observations, non-parametric tests (Exact Wilcoxon rank-sum test, Kruskal-Wallis rank sum test) were used to compare sex and ageing groups.

Results & Discussion

Live weight at slaughter, pH values of the LT at various times pm and LT sarcomere length were not significantly different between bulls and cows (Table 1). There was a tendency ($P=0.1$) for lower CIE L* and b* values and higher CIE a* values for the cow samples, indicating a darker colour measured at 7 days pm. This confirms the finding that meat of older animals is darker (Fiems et al., 2003), a consequence of its higher myoglobin content (Monin and Ouali, 1991). Across sexes, the effect of ageing on shear force and 3-10 kDa peptide concentration was significant with the values for 1 day ageing being significantly different from those of 7 and 14 days ageing ($P<0.01$). Average shear force values declined similarly up to 7 days pm for cows and bulls, but continued to decline in the cow samples with further ageing, whereas this was not seen for the bull samples (Fig. 1). This corresponds with the changes in peptide concentration that were observed (Fig. 2), indicating a higher degree of proteolysis in the cow compared to the bull samples at 14 days pm. However, it should be mentioned that the differences in peptide concentration and shear force between bull and cow samples at 14 days pm were not significant.

As could be expected from the sex and age difference (Fiems et al., 2003), a tendency ($P=0.1$) for a higher intramuscular fat content (reflected in total fatty acid content) was found for the cow compared to the bull LT samples (Table 2). This was accompanied by a higher proportion of saturated and n-3 poly-unsaturated fatty acids ($P=0.1$), whereas there was no significant difference for the mono-unsaturated and n-6 poly-unsaturated fatty acid proportions. Dietary factors as well as differences in fat content may be responsible for these differences.

Since taste intensity was evaluated by ranking of the samples from the three ageing times within each animal, this does not allow to test for the effect of sex. Samples of 7 and 14 days ageing were ranked lowest and highest respectively for taste intensity with 1 day ageing samples being intermediate (mean values 2.07, 1.72 and 2.24 for 1, 7 and 14 days ageing respectively), at the borderline of significance ($P=0.07$). Campo et al. (1999) found, depending on the breed type, little differences in overall flavour intensity scores with time pm. Spanier et al. (1997) found that the ageing process is characterised by an enhancement of beef sensory quality due to tenderization, whereas this is not the case for the overall flavour because desirable flavours decline with ageing, while off-flavours

such as bitter and sour increase. Panellists, however, preferred cow samples more than bull samples in our study (14 vs. 4 times first choice respectively).

Aroma profiling (GC-MS) did not reveal a significant effect of sex for any of the groups of volatiles (Table 3). Only short-chain aldehydes, esters and S-compounds were slightly higher at each ageing time for the cow compared to the bull samples. With respect to ageing, the concentration of short-chain aldehydes was significantly higher at 7 and 14 days ageing compared to 1 day ageing ($P < 0.01$) For the nitrogen containing compounds (N-compounds, e.g. pyrrol derivatives, pyrazines, thiazole derivatives and pyridine derivatives) and the lactones, there was a significant increase between 1 and 7 days ageing ($P < 0.05$), and a non-significant decrease thereafter. The concentration of ketones was significantly lower for the 14 days ageing samples compared to the 1 and 7 days samples. Overall, volatile concentrations tended to be higher in 7 days ageing samples, which does not correspond with the lowest taste intensity scores for these samples. The absence of a sex affect is also remarkable in view of the higher intramuscular fat content in the cow meat. Similar values for the long-chain aldehydes and the alcohols for the bull and cow samples probably indicates that there were no differences in the degree of auto-oxidation of unsaturated fatty acids for these two types of samples (Elmore et al., 1999).

Conclusions

Comparison between cow and young bull Belgian Blue LT muscle samples showed a darker colour for the cow samples. LT samples from cows had a higher total fatty acid content, which was accompanied by a higher saturated and n-3 poly-unsaturated fatty acid proportion. At 14 days pm, there was a tendency of the cow samples to be somewhat more tender compared to the bull samples, probably resulting from a higher degree of proteolysis, indicated by higher concentrations of 3-10 kDa peptides. The effect of ageing on taste intensity was unclear. There were significant effects of ageing for several of the volatile aroma compounds as determined by GC-MS, but there was no effect of sex. Meat from cows, however, was preferred to that of young bulls. Ageing brought about only clear changes in shear force values and peptide concentrations.

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Tables and Figures

Table 1. Mean live weight and pH, sarcomere length (SL) and colour coordinates of the LT muscle according to sex

	Bulls	Cows	P-value
Live weight (kg)	665	623	0.7
pH 1 h pm	6.87	6.82	0.7
pH 3 h pm	6.32	6.31	1.0
pH 5 h pm	5.83	5.75	1.0
pH 24 h pm	5.63	5.63	1.0
CIE L*	38.1	27.3	0.1
CIE a*	22.1	25.5	0.1
CIE b*	24.4	22.3	0.1
SL (μm)	1.72	1.68	0.7

Table 2. Mean total fatty acid content (mg/100g muscle) and proportions of the major classes (%) of the LT muscle according to sex

	Bulls	Cows	P-value
Sum FA	845	1225	0.1
SFA	37.0	41.2	0.1
MUFA	33.5	34.4	0.7
n-6 PUFA	16.9	11.7	0.2
n-3 PUFA	1.02	2.41	0.1

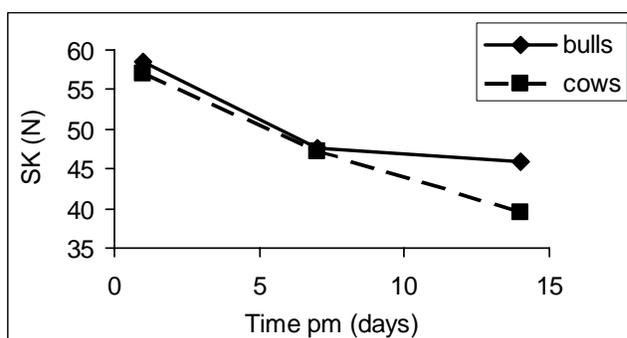


Fig. 1. Mean shear force values (N) of cow and bull LT samples with time of ageing (n=3). Differences between sexes are not significant.

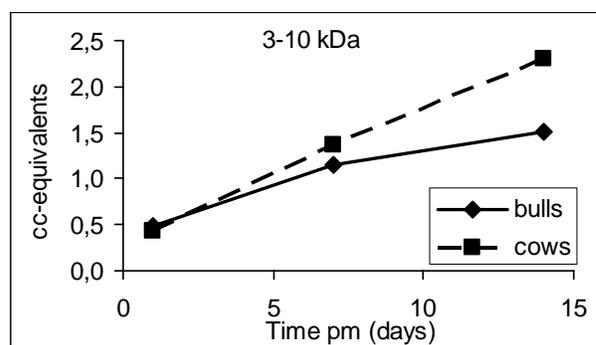


Fig. 2. Mean 3-10 kDa peptide concentration (mg cytochrome c equivalents/g muscle) of cow and bull LT samples with time of ageing (n=3). Differences between sexes are not significant.

Table 3. Mean values for the GC-MS aroma profiling of cow and bull LT samples at 1, 7 and 14 days of ageing (n=3). Concentrations as $\mu\text{g nonane/ kg muscle}$ (nonane = internal standard). The effect of sex was not significant for any of the groups of volatiles. For significant differences between ageing times, see text.

	Day 1 pm		Day 7 pm		Day 14 pm	
	Bulls	Cows	Bulls	Cows	Bulls	Cows
Short-chain aldehydes	240	277	440	507	453	559
Long-chain aldehydes	28665	29580	34322	32923	29307	34336
Alcohols	8.1	9.3	13.6	10.5	14.9	10.6
Ketones	2713	2708	2738	2521	2106	2051
N-compounds	331	325	729	469	411	469
S-compounds	59.2	67.7	75.1	80.6	76.7	89.1
Furan derivates	89.6	88.7	112	126	106	151
Esters	49.5	60.3	57.3	58.6	44.5	56.8
Lactones	87.0	76.1	144	106	103	116

**THE DEVELOPMENT IN INOSINE MONOPHOSPHATE AND ITS
DEGRADATION PRODUCTS DURING AGING OF PORK OF DIFFERENT
QUALITIES IN RELATION TO BASIC TASTE - AND RETRO NASAL FLAVOR
PERCEPTION OF THE MEAT**

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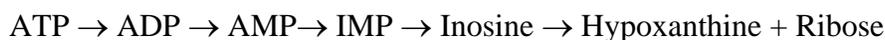
Key Words: pork, meat quality, flavor, IMP, hypoxanthine

Introduction

The characteristic flavor of meat mostly develops through the heating of the meat, however, raw meat also inherits several non-volatile constituents that can contribute to the overall flavor of meat (MacLeod, 1986). The 5'-ribonucleotide, inosine monophosphate (IMP) originating from dephosphorylation of the triphosphate 5'-ribonucleotide, adenosine triphosphate (ATP) and its degradation products ribose and hypoxanthine are all considered to be important constituents in meat flavor formation and development (see Scheme 1). IMP contributes with umami taste (Durnford, Shahidi, 1998; Spurvey et al, 1998) and has flavor-enhancing properties, and are reported to enhance meaty, brothy, mouth filling, dry and astringent qualities and suppress sulphurous notes (Kuninaka, 1981) while hypoxanthine displays bitter characteristics, and ribose is considered the most important reducing sugar participating in flavor-producing Maillard reactions in meats upon heating (Mottram, 1998).

Considering that IMP, ribose and hypoxanthine are all considered important constituents in meat flavor formation and development, an understanding of the post mortem metabolism in muscle and the subsequent degradation of the adenosine triphosphate (ATP) metabolite, IMP, during aging and cooking, as schematically outlined below (Scheme 1), becomes crucial in the further exploitation of flavor development in meat.

Scheme 1



ATP ≡ adenosine 5'-triphosphate
ADP ≡ adenosine 5'-diphosphate
AMP ≡ adenosine 5'-monophosphate
IMP ≡ inosine 5'-monophosphate

The present study aims to exploit the value of inherent inosine monophosphate during aging of pork of two qualities, normal pH versus high pH, in relation to basic taste perception and retro nasal flavor perception of the cooked meat.

Objectives

The objective of the study is to identify potential connections between the concentration of flavor precursors and sensory attributes in whole meat, meat juice, and the remaining meat residue in two pork qualities during aging considering both basic taste perception and retro-nasal flavor perception.

Methodology

Pork carcasses were randomly selected at the slaughter-line at Danish Crown, Ringsted, Denmark according to hot carcass weight (75-79 kg), meat percent (58.5-63.0%), and twenty-eight carcasses were chosen and grouped according to ultimate pH (16 carcasses with $5.5 < \text{pH} < 5.6$, normal pH, and 12 carcasses with $\text{pH} > 5.7$, high pH). The pH was measured with a Knick Portamess pH-meter no. 751 (Berlin, Germany) equipped with an Ingold LOT glass electrode type 3120 (Mettler Toledo, Urdorf, Switzerland). Both loins from the carcasses were excised the day after slaughter, vacuum-packed and aged at 2°C for either 2 days (16 loins with normal pH and 8 loins with high pH), 15 days (8 loins from each pH group) or 21 days (8 loins from each pH group), before they were frozen and stored at -20°C until further analysis. The experimental design is schematically outlined in Figure 1.

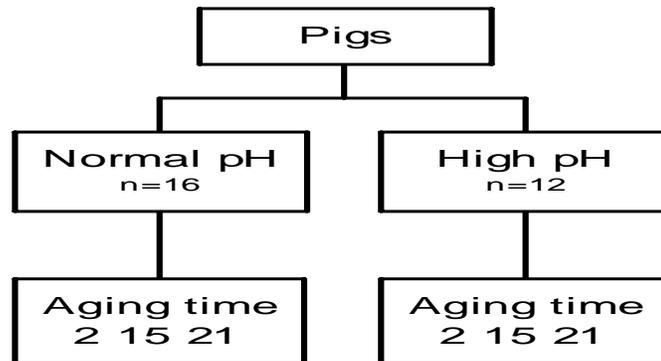


Figure 1. Experimental design (n – number of animals)

For sensory analysis the loins were thawed at 5°C over a period of 20 hours, slice for chemical analysis separated, and subsequently roasted in an oven at 100°C to a core temperature of 75°C. The roasted loins were allowed to rest for 30 minutes at room temperature before they were cut into five 1.5 cm-thick slices. The meat was served both as whole meat, meat juice and residue. Meat juice and residue were obtained by squeezing 2/3 of the remaining part of the roast in a pneumatic press. The meat juice was centrifuged (1000 rpm, 14°C, 5 min) to remove dissolved fat globules and subsequently brought to 30°C using a water bath before serving while whole meat and residue were served at room temperature.

The sensory attributes were as follows: *salty*, *sour*, *sweet*, *bitter*, *umami*, *meaty*, *brothy*, *piggy*, *fatty*, and *cooked root vegetables*. The intensity was evaluated using a 15 cm non-structured line scale. The assessors were either served 2 identical meat samples or 2 identical residue samples (1x2 cm). The meat juice (15-20 ml) was served in small plastic cups. Panelists used nose clips when evaluating the first sample (basic taste perception) and without the nose clip when evaluating the second sample (retro nasal

flavor perception). All three fractions from the same animal were served in the same session in a randomized design.

Pork samples (50 mg) for chemical analysis were homogenized (Polytron PT-MR 2100) for 10 seconds in 3 ml of ice-cold 0.6 M perchloric acid (PCA) containing a pH indicator (bromthymolblue and phenolphthalein 0.004% of each) using Sarstedt 15 ml conical vials. The samples were left on ice-bath for 15 minutes before neutralization with 2.7 ml of ice-cold 0.8 M KOH and addition of 0.125 ml ice-cold KH_2PO_4 buffer. Subsequently the mixtures were mixed for 10 seconds using an IKA MS 2 Minishaker, and the pH was adjusted to 7-8 using either KOH or PCA. Finally, the mixtures were centrifuged using an Eppendorf Centrifuge 5417R (4000 rpm for 10 min at 4°C), and 1 ml supernatant (in duplicate) was transferred to an Eppendorf vial and frozen at -80°C until further analysis.

Analysis of inosine 5'-monophosphate, inosine and hypoxanthine was carried out by high-performance liquid chromatography (HPLC) on a Hewlett-Packard HPLC system series 1100 using UV detection (210 nm). The samples were thawed and centrifuged, and the supernatants were transferred to cold HPLC vials and placed in a thermostatted auto sampler (1-2°C). A 10 μl sample was injected on a Lichrospher 250 x 4 mm RP18 column from which the three compounds were separated by isocratic elution using a solvent based on a buffer containing 10 mM tetrabutylammonium hydrogensulfate and 215 mM KH_2PO_4 to which 7.5 ml methanol/l was added. The following flow gradient was used to obtain optimal separation: 0.5 ml/min for 5 min, increasing to 1.5 ml/min in 1 min and keeping this flow for 9 min before a final decrease to 0.5 ml/min in 0.5 min. Quantification was based on standard curves using external standards and calculations carried out in the included software (HP Chemstation).

Data analysis was performed using Unscrambler v. 9.1 (CAMO PROCESS AS, Oslo, Norway) for Principal Component Analysis (PCA) and SAS v. 8.02 (SAS Institute Inc., Cary, NC, USA) for analysis of variance with the MIXED procedure.

Results & Discussion

Figure 2 shows that IMP independent of pH in the fresh meat decreased significantly ($p < 0.0001$) both in the samples aged from 2 to 15 and from 2 to 21 days. The difference in IMP concentration between pork samples from the normal and high pH group with the concentration being highest in the high pH group was only statistical significant after 2 days of aging. This difference in concentration between the normal and high pH group is in agreement with the fact that the stability of IMP is both temperature- and pH-dependent due to the presence of weak chemical bonds, e.g. glucoside and ester bonds (Matoba et al. 1988), with low pH accelerating the dephosphorylation of IMP. The decrease in IMP preceded a simultaneous increase in both inosine and hypoxanthine concentrations in meat samples of both qualities aged from 2 to 15 days and from 2 to 21 days, respectively. The rate by which IMP was degraded to inosine and the rate by which inosine was degraded to hypoxanthine during aging was found to be in agreement with the difference in the rate constants of the dephosphorylation of IMP and the hydrolysis of inosine described by Dunford and Shahidi (1998).

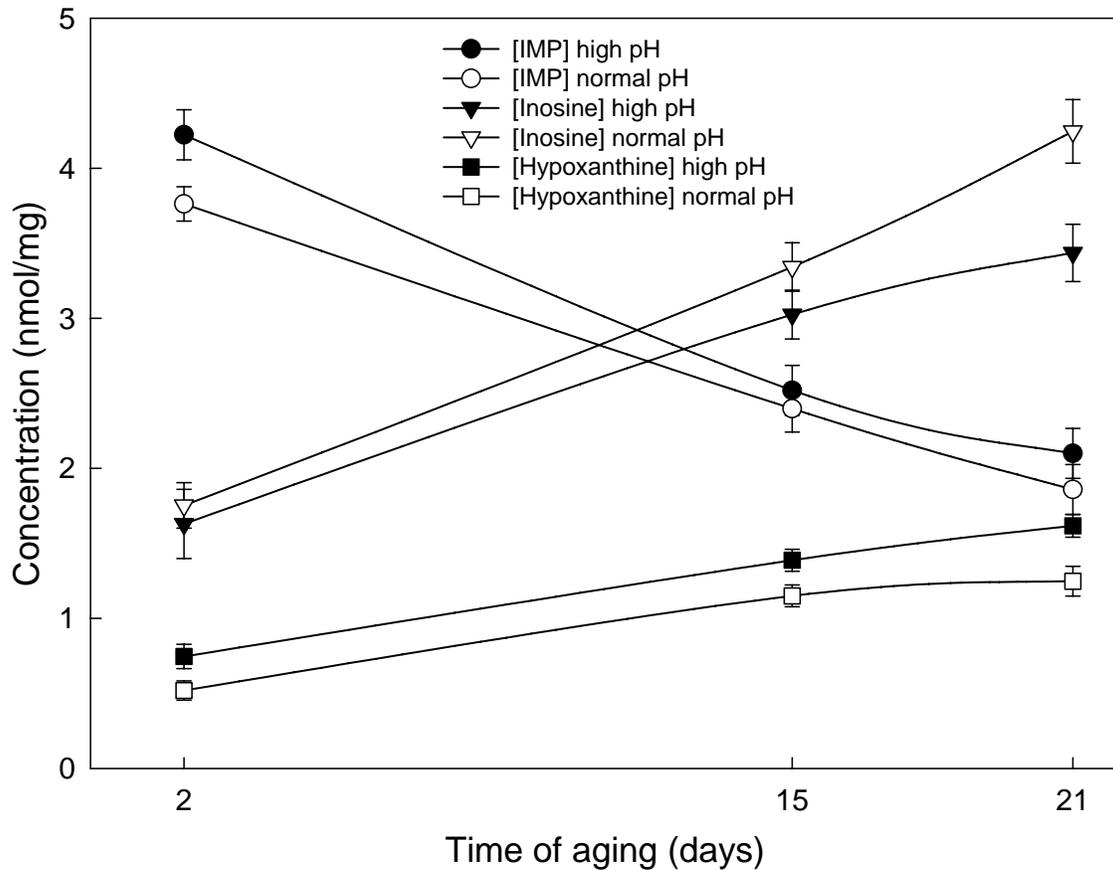


Figure 2. The change in the concentration of IMP, inosine and hypoxanthine of meat with high and normal pH as a function of aging expressed as *lsmeans* with standard errors

Performing sensory analysis with and without nose clip clearly showed that the basic tastes; *salty*, *sour*, *bitter*, and *umami*, displayed almost the same intensity in the individual samples independent of use of nose clip, while the intensity of the other sensory attributes, e.g. *meaty*, *brothy*, and *fatty*, decreased drastically when the sensory analysis was performed with nose clip. Consequently, the aim of performing the sensory analysis both with and without nose clip to differ between basic taste perception and retro nasal flavor perception of the samples seems to be fulfilled using this approach.

Independent of the two sensory analyses approaches, the sensory attribute *sour* for retro nasal perception ($p=0.0014$) and for basic taste perception ($p<0.0001$) was as expected found to be significantly more pronounced in pork of the normal pH. Moreover, the sensory attribute *salty* identified only by basic taste perception was significantly influenced by pH in meat (0.006) and meat juice (0.0134) with pork with normal pH appearing more salty while the sensory attribute *bitter* tended to be more pronounced in the pork juice fraction of the high pH quality ($p=0.0793$).

The sensory attribute *bitter* determined by retro nasal flavor perception in the pork residue was affected by aging of the meat ($p=0.0359$), as the residue became more bitter upon prolonged aging. Moreover, the sensory attribute *piggy* seemed more pronounced in whole meat ($p=0.0791$) and meat juice ($p=0.0363$) determined by basic taste perception, and in whole meat ($p=0.0513$) by retro nasal taste perception. Finally, aging of the meat had a positive effect on the sensory attribute *salty* in whole meat determined by both retro

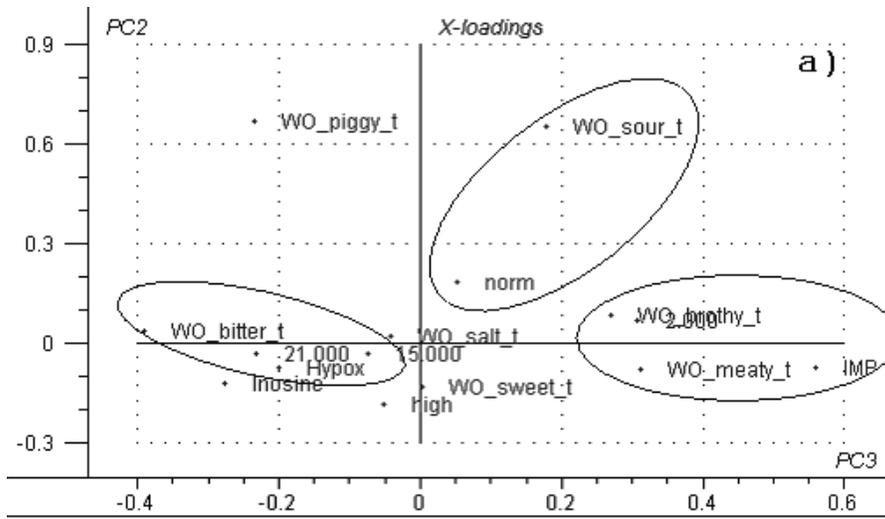
nasal flavor perception ($p=0.039$) and basic taste perception ($p=0.0297$) and in pork residue ($p=0.0722$) determined by basic taste perception.

To compare data obtained by sensory analysis with the chemical data on the meat as a function of aging, principal component analyses (PCA) were performed on the data. PCA analysis was carried out on data from whole meat, meat juice and the residue, respectively, and both on sensory data obtained with and without nose clip. Figure 3 shows loading plots of whole pork (a) and the corresponding meat juice (b) and residue (c) on retro nasal flavor perception and chemical data. In Figure 3a **PC3** expands the aging time and **PC2** the meat quality (*norm* - $5.5 < \text{pH} < 5.6$ & *high* - $\text{pH} > 5.7$) while **PC1** expands aging time and **PC2** the meat quality in both Figure 3b and 3c. Figure 3a and 3c shows that the sensory attribute *brothy* is associated with meat aged for only 2 days (fresh meat) and presence of the flavor enhancer, IMP. Subsequent correlation analysis showed that *brothy* determined by retro nasal flavor perception was significantly correlated with IMP in the pork residue ($R=0.36$, $p=0.0265$). These data confirm previous data, showing that IMP is a desirable flavor enhancer in meat and fish (Maga, 1987; Madruga, 1997; Murata and Sakaguchi, 1989). Moreover, Figure 3 clearly shows that the sensory attribute *bitter* is associated with meat aged for 21 days and presence of high concentrations of hypoxanthine. Subsequent correlation analysis showed that hypoxanthine and bitterness determined by retro nasal flavor perception tended to correlate positively ($R=0.31$, $p=0.0621$). Consequently, present data indicate that formation of bitter taste through degradation of IMP to hypoxanthine during aging might be an element in flavor deterioration, as previously suggested to be the case during prolonged storage of fish (Bremner et al., 1988).

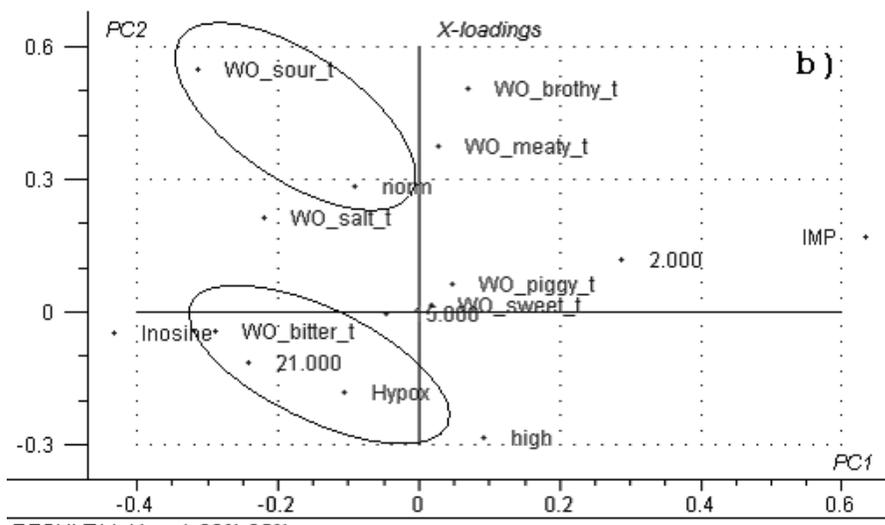
Finally, Figure 3 confirms that the normal meat quality is associated with the sensory attribute *sour*, as also found by analysis of variance.

Conclusion

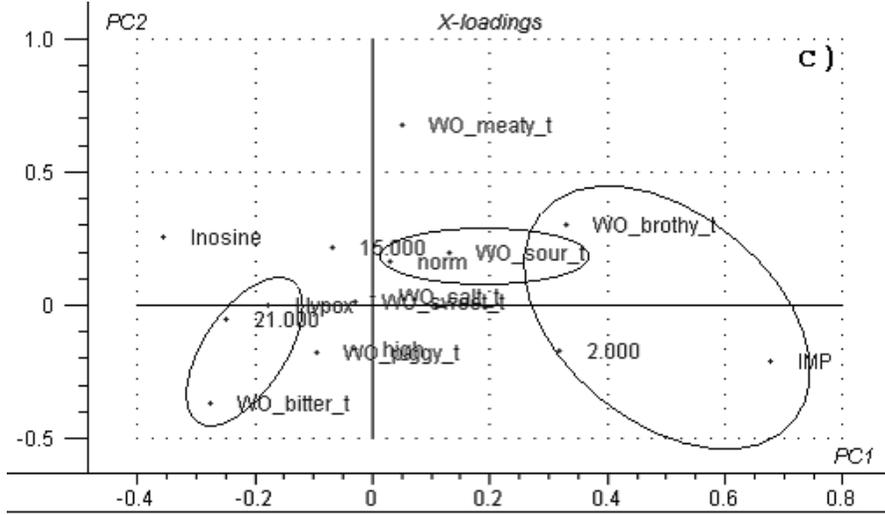
The present study shows that the flavor enhancer inosine monophosphate and its degradation product hypoxanthine contribute to the sensory attributes *brothy* and *bitter* in pork and that the change in sensory attributes from *brothy* to *bitter* taking place upon prolonged storage of pork resembles continuous degradation of inosine monophosphate to hypoxanthine.



RESULT8, X-expl: 16%,19%



RESULT11, X-expl: 30%,25%



RESULT112, X-expl: 29%,19%

Figure 3. The loading plots of principal component analysis on meat (a), meat juice (b) and residue (c) by retro nasal flavor perception

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**SPME-GC/MS ANALYSIS OF KEY VOLATILE COMPOUNDS GENERATED
FROM UNHEATED AMINO ACID AND REDUCING SUGAR MIXTURES**

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Key Words: Solid phase microextraction, Maillard reaction products, volatile compounds

Introduction

Flavor is essential for consumer satisfaction and the commercial success of meat products. Dry-cured meat products of excellent flavor are popular to consumers. Only through a long ripening period (12–22 °C for about six months or more), the typical flavor of dry-cured meat products can be developed (Toldrá, Flores and Sanz, 1997; Martín, Córdoba, Antequera, Timón and Ventanas, 1998). Ventanas, *et al.* (1992) reported that there were Maillard reactions during dry-cured ham processing. The Strecker degradation of amino acids, a minor pathway of the Maillard reaction, is very important in relation to the formation of a series of volatile organic flavor compounds: the Strecker aldehydes. This seems to be the important pathway for the development of dry-cured ham flavor.

To understand the contribution of Maillard reaction to meat flavor, the reactions between heated amino acids and sugar have frequently been studied in order to identify volatile compounds formed (Meynier and Mottram, 1995; Venskutonis, Vasiliauskaite, Galdikas and Šetkus, 2002; Hofmann and Schieberle, 1998; Adamiec, Rössner, Velíšek, Karel and Jan, 2001; Cremer, Vollenbroeker and Karl, 2000), but the volatile compounds obtained from unheated amino acids and reducing sugar mixtures have not been extensively studied, which may be a significant contributor to the flavor formation of dry-cured meat products during long-term ripening.

Objectives

The purpose of this study was to use HS (headspace) SPME (solid phase microextraction) coupled to GC/MS to investigate the generation of volatile compounds under a controlled temperature of 20 ± 2 °C which mimics the dry-cured meat ripening condition.

Methodology

Materials

L- Alanine, L- Arginine, L- Asparagines, L- Aspartic acid, L- Cysteine, L- Glutamine, L- Glutamic acid, L- Glycine, L- Histidine, L- Isoleucine, L- Leucine, L- Lysine, L- Methionine, L- Phenylalanine, L- Proline, L- Serine, L- Threonine, L- Tyrosine, L- Valine, D- Ribose were purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China); D- Glucose, NaH₂PO₄, Na₂HPO₄ were purchased from Beijing Chemical Reagents Company (Beijing, China).

Preparation of Maillard reaction products (MRPs) in model system

Equimolar mixture of each of two sugars and each of 19 amino acids were dissolved in a 0.2 M sterilized phosphate buffer (pH 6.5). At the same time, solutions containing only D- Glucose or D- Ribose or L- Lysine were made as the control, and the final concentration of each substance was 0.02 M. Test tubes (15 × 200 mm) containing 15 ml of each mixed solution were sealed and placed in an incubator with the temperature of 20 ± 2 °C for 10 d and 30 d. Equimolar mixtures of the selected sugar and amino acids were prepared as the above method.

Sampling

In order to select model systems, volatile compounds of MRPs generated from equimolar mixture of each of two sugars and each of 19 amino acids were detected by HS-SPME coupled to gas chromatography respectively on 10 d and 30 d.

For the selected model systems, volatile compounds were detected on 15 d by coupling HS-SPME to gas chromatography-mass spectroscopy and the color intensities were determined on 0 d, 10 d, 15 d, 25 d and 30 d.

Volatile compounds analysis

SPME method

The SPME device and fiber [Carboxen/ Polydimethylsiloxane (CAR/PDMS), 75 μm film thickness] used were purchased from Supelco (Bellefonte, PA, USA). The fiber was exposed to the splitless/split injection port of an Agilent 6820 gas chromatograph (Agilent Technologies, USA) under nitrogen flow and conditioned before use as recommended by the manufacturer. Fifteen-ml screw-top clear vials, hole cap with PTFE/Silicone septa (Supelco, Bellefonte, PA, USA) containing 5 ml of liquid MRPs were introduced onto the sample table maintained at room temperature for 20 min to form the headspace and a 15 min adsorption of the volatile compounds onto the fiber were chosen.

Gas Chromatography

After extraction, analyses were performed using an Agilent 6820 gas chromatograph (Agilent Technologies, USA). SPME fiber was thermally desorbed at 220 °C in the injector port for 10 min, the split valve being opened after 1 min (split ratio 15). A DB-1701 column (30 m × 0.32 mm i.d.) coated with cyanopropyl-phenyl (1.0 μm film

thickness) (Agilent Technologies, USA) was used to separate the volatile compounds of the model systems, the solutions containing only D- Glucose or D- Ribose or L- Lysine were made as the controls. The oven was held at 38 °C for 2 min, heated to 90 °C at 3 °C /min, then raised to 220 °C at 10 °C /min and held at 220 °C for 3 min, the program used 34.33 min. Nitrogen was used as carrier gas with a constant flow of 1.0 ml/min. The temperature of FID was 250 °C.

Gas Chromatography / Mass Spectrometry

After extraction, analyses were performed using an Agilent 5973 ion-trap mass spectrometer (mass selective detector) (Agilent Technologies, USA) fitted with an Agilent 6890 gas chromatograph (Agilent Technologies, USA). SPME fiber was thermally desorbed at 220 °C in the injector port for 10 min, the split valve being opened after 1 min. The above column was used to separate the volatile components of the model systems. The oven was held at 35 °C for 2 min, heated to 90 °C at 3 °C/min, then raised to 220 °C at 10 °C/min and held for 3min and finally by 20 °C to 250 °C and held for 2min at this temperature, the program used 39.83 min. Helium was used as carrier gas with a constant flow of 1.4 ml/min. Transition – line temperature was 250 °C. The mass spectrometer scanned from 12 450 amu. Ionization energy was set at 70 eV. Identification of peaks was based on the comparison with the MS computer library NIST98. L – Software package and the volatile standards when they are available.

Determination of color intensity

The color intensities of each of the selected model MRPs were determined with a 752 UV spectrophotometer (Shanghai analytical equipment factory, China) as the absorbance at 420 nm against phosphate buffer (c.f. Kwak and Lim, 2004).

Statistical analysis

Data were analyzed using ANOVA (The SAS System for Windows V8).

Results & Discussion

The results of color change and SPME-GC determination showed that D- Glucose did not yield detectable browning with any of the amino acids under conditions used in this study (data were not given). D- Ribose could react with 19 amino acids resulting in a color change, yet the SPME-GC determination showed that only the reactions of D- Ribose with L- Leucine, L- Isoleucine, L- Valine and L- Methionine generated detectable volatile compounds. Therefore, these four model systems were chosen to study the color change at different reacting periods and to identify volatile compounds produced.

The identified key volatile compound obtained from the Leu+R model system was 3-methylbutanal and that from the Ile+R model system was 2-methylbutanal (Table 1). Volatiles from the Val+R were 2-methyl-2-propenal and 2-methylpropanal, and from the Met+R system was dimethyl disulfide. These volatile compounds were found in Parma ham (Hinrichsen and Pedersen, 1995) and Serrano ham (Dirinck, Van Opstaele and Vandendriessche, 1997) during processing.

The volatile compounds generated from unheated amino acids (L- Leucine, L- Isoleucine, L- Valine and L- Methionine) and reducing sugar mixtures under acidic

conditions in this study probably were *via* the generally accepted Strecker degradation pathway in thermal treated alkali conditions (Mottram and Donald, 1998; Cremer, Vollenbroeker and Karl, 2000). Considering the difference of the pH, the mechanisms for the formation of these volatile compounds should be studied in details in the future.

Figure 1 shows changes of color intensity of the four model MRPs at 0 d, 10 d, 15 d, 25 d and 30 d. The browning of the four model MRPs increased significantly ($P < 0.05$) along with the reacting time. At 10 d and 25 d, the color intensities of the Val+R and Met+R model systems were significantly deeper ($P < 0.05$) than that of Ile+R and Leu+R mixture.

Conclusions

Key volatile compounds were generated from the reaction of D- Ribose with four amino acids L- Leucine, L- Isoleucine, L- Valine and L- Methionine at 20 ± 2 °C. These compounds might be important in the flavor development of dry-cured meat products.

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Tables and Figures

Table 1. Key volatile compounds identified from the four model systems by SPME-GC/MS

Model system*	Key volatile compounds
Leu+R	3-methylbutanal
Ile+R	2-methylbutanal
Val+R	2-methylpropanal, 2-methyl-2-propenal
Met+R	dimethyl disulfide

* Model system D- Ribose and L- Leucine mixture (Leu+R), D- Ribose and L- Isoleucine mixture (Ile+R), D- Ribose and L- Valine mixture (Val+R), D- Ribose and L- Methionine mixture (Met+R).

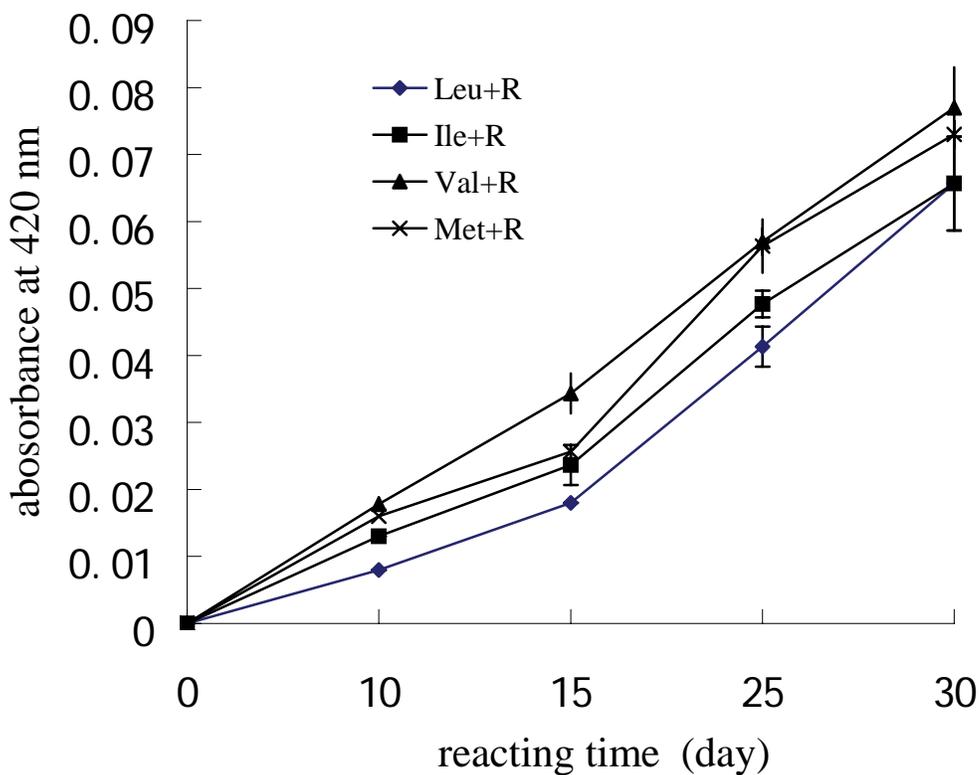


Fig.1 Changes of color intensity of four model MRPs prepared by D-Ribose with four amino acids at 20 ± 2 °C during reacting (n = 3).

THE EFFECT OF FRYING FAT ON FLAVOUR OF PORK CHOPS

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Key Words: pork loin, frying fat, flavour, consumers behaviour

Introduction

Frying chops on a pan is a commonly used cooking method for pork in Denmark. Using this method, only a small amount of fat is used compared to deep-frying. The fat ensures that the meat does not stick to the pan and will furthermore add flavour to the meat. A small consumer survey with 29 Danish consumers showed a large variety of fat types chosen by the consumers. Ten consumers used margarine, six used oil especially olive oil but rapeseed oil and grape seed oil were used as well, eight used a combination of butter and oil, one used butter, one used the fat from a marinade, one used the fat from fried bacon, and the last two fried without fat. On this background we wanted to investigate the effect of frying fat on the flavour of pork when using a traditional Danish cooking method.

Methodology

Meat

Left loins of 20 pork carcasses with pH 5.55-5.70 chosen at random at the abattoir were used for the experiment. The loins were vacuum packed, aged for 4 days and frozen at

-20°C. Before the sensory analysis, the loins were thawed for approximately 20 hours at 4°C. The loins were sliced in 20 mm thick chops. The centre temperature of the chops was 10-15°C before frying.

Frying

A cast iron pan was used for frying. The pan was heated to 200°C before the fat was added. Two tablespoons of oil or 20 g solid fat or one tablespoon of oil/10 g solid fat were used. The chops were turned every two minutes and fried to a centre temperature of 65-68°C.

Triangle test

A triangle test (eight assessors, three replicates) was carried out to investigate whether the assessors were able to register a difference between chops fried in different types of frying fat. All samples in the same serving came from the same loin. The following fat types were compared:

- Butter/olive oil (cold pressed) – Olive oil (cold pressed)
- Olive oil (cold pressed) – Rapeseed oil (cold pressed)

- Rapeseed oil (warm pressed) – Grapeseed oil (warm pressed)
- Rapeseed oil (warm pressed) – Rapeseed oil (cold pressed)
- Butter - Margarine

Sensory Profiling analysis

Based on the results of the triangle test it was decided to perform a sensory profiling analysis (eight assessors and five replicates) on chops fried in the following frying fats:

Butter, Margarine, Butter/Olive oil (cold pressed), Rapeseed oil (cold pressed), grapeseed oil (warm pressed).

The intensity of the sensory attributes was assessed on a 15 cm unstructured line scale ranging from nothing to very much for the attributes: Piggy odour, Fried meat odour, Oily odour, Buttery odour, Sweet odour, Sour odour, Uneven fried surface, Bitter taste, Piggy flavour, Fried meat flavour, Metal taste, Oily flavour, Buttery flavour, Sweet taste, Sour taste, Tenderness, Juiciness, Total after taste. Only the attributes of the different frying fats that differ significantly will be mentioned in the section on results.

Results & Discussion

Triangle test

The triangle test showed that when frying in butter, the butter was the main cause for variation (Table 1). There was a significant difference between chops fried in butter or butter/olive oil compared to frying in margarine and olive oil respectively. The assessors could not detect a difference between frying in the two cold pressed oil types – olive oil and rapeseed oil – or between the two warm pressed oil types – rapeseed oil and grapeseed oil. The comparison between the two pressing methods for the same oil type – cold versus warm pressed rapeseed oil – showed a tendency towards a significant difference. One of the servings was different from the other two as only 2 assessors selected the right sample. Had it not been for this serving, the difference would have been significant ($P < 0.05$).

Using butter for frying resulted in the largest difference in eating quality. The main difference between the oils was caused by the pressing methods (warm and cold pressed) and not the type of oil used (olive, rapeseed or grapeseed).

Sensory profile

The sensory profile analysis compared warm pressed and cold pressed types of oil. As grape seed oil is used as standard in our sensory laboratory, this oil type was chosen as the warm pressed oil. Rapeseed oil is a Danish product with a high amount of polyunsaturated fatty acids. The fatty acid composition is important in nutrition, but at the same time it is a source of potential off-flavour. It was therefore chosen as the cold pressed oil. Since the consumers in the previously mentioned consumer survey used olive oil most frequently, olive oil was chosen as the oil type to be mixed with butter.

The difference between the various types of frying fat was larger in relation to odour than to flavour (Table 2). The oiliest odour was 4.1 whereas the oiliest flavour was only 2.6 compared to 0.7 as the least oily odour and flavour. Similarly, the most buttery odour

was 4.7 whereas the most buttery flavour was only 3.4. The least buttery odour and flavour was 1.4 and 1.2, respectively.

As to the triangle test, the main difference in sensory profile was between butter and the other types of frying fats. Frying in butter gave a butterier odour and flavour but also a more fried meat flavour and a higher juiciness. In contrast, frying in warm pressed grape seed oil gave a more oily odour and flavour.

In a PCA-plot with each replicate being one object, it can be seen that the chops fried in butter lie close and opposite the chops fried in oil and margarine (Figure 1). This shows that the chops fried in butter are alike and that they are markedly different from the other samples. In contrast, chops fried in the two oil types and in the margarine are much more overlapping. The margarine samples lie close to piggy flavour and opposite oily flavour with one single sample being an outlier and close to meat flavour. The rapeseed oil samples lay as close as the butter samples showing that they too are very similar. They seem relatively neutral – being near 0 for PC2, and opposite butter and fried meat flavour with respect to PC1. In contrast, the samples fried in grape seed oil have a large distribution with respect to PC2 showing a larger variation between replications. With the butter samples in one part of the plot and the oil and margarine samples in the other part the combination of oil and butter lie between the two groups a little towards the butter samples. Combining oil and butter seems to give an intermediate result and not a special flavour on its own.

Conclusion

During frying on a pan with frying fat the meat only absorbs a small percentage of the frying fat (Clausen & Ovesen, 2005). The absorbed fat is probably situated as a layer around the surface of the meat and not absorbed deeply into the meat. This may explain why the odour is more affected by the type of frying fat than the flavour.

Butter is the frying fat that differentiates the most from the other types of fat. A study of the volatile compounds formed during deep frying has shown that frying in butter gives more long chained ketones than frying in oil (Ramirez, Estevez, Morcuende & Cava, 2004). The two oil types in this study – olive oil and sunflower oil –give a more similar profile of the volatile compounds with more aldehydes, especially hexanal, than when chops are fried in butter.

The effect of frying oil on odour seems more to be a question of how the oil has been produced (warm or cold pressed) than of the origin of the oil. We did not analyze the fatty acid composition of the oils but we assumed that the difference in fatty acid composition is larger between oils of different origin than between oil of same origin though produced by different methods (warm versus cold pressing). The differences in flavour between frying oils might therefore be a result of other substances in the oil than the fatty acid composition giving rise to different volatile compounds.

Consumers use a variety of fat types for frying. The main reason for choosing a fat type can be health, flavour and tradition. Some chefs in Denmark recommend to use a combination of oil and butter for frying. This study shows that a combination gives an odour and flavour intermediate between butter and oil for both buttery odour, oily odour and fried meat odour. The frying fat you choose as a consumer will therefore reveal

whether you prefer the buttery odour and flavour, the oily odour and flavour, an intermediate odour and flavour or a more neutral odour and flavour.

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Tables and Figures

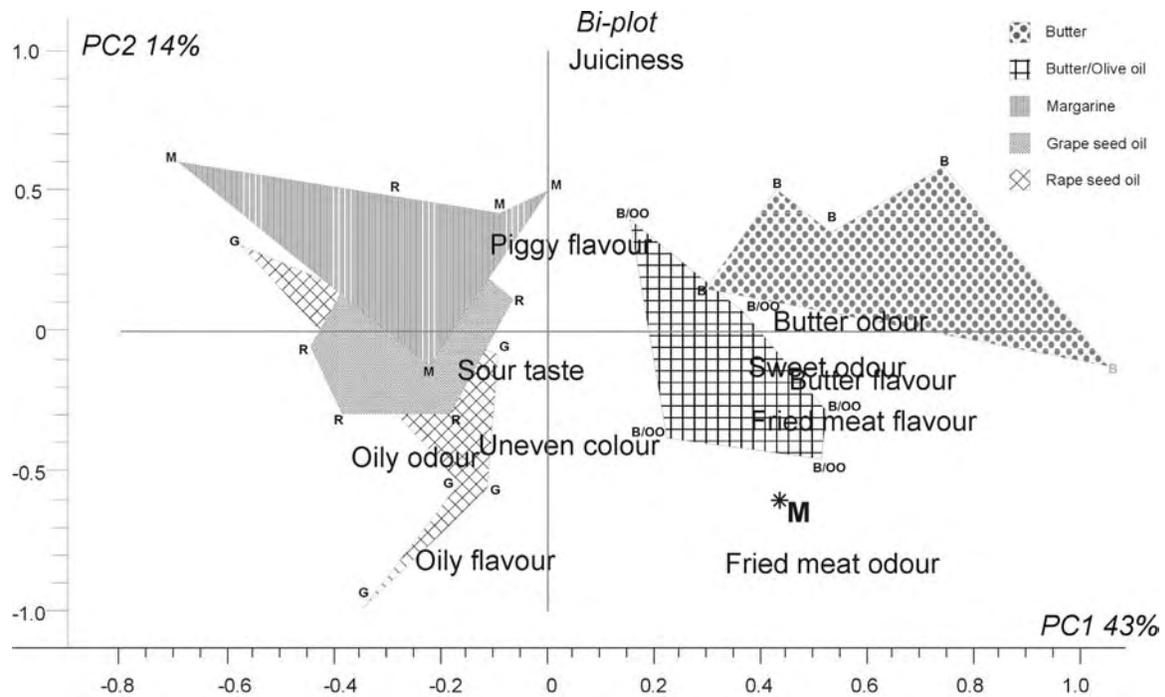
Table 1. The difference in eating quality of chops when fried in various frying fats.

Comparison	P(difference)
Butter/Olive oil (cold pressed) – Olive oil (cold pressed)	0.003**
Olive oil (cold pressed) – Rapeseed oil (cold pressed)	0.259
Rapeseed oil (warm pressed) – Grapeseed oil (warm pressed)	0.254
Rapeseed oil (warm pressed) – Rapeseed oil (cold pressed)	0.068 ^a
Butter – Margarine	0.003**

^aThe P values of the three replicates were 0,09, 0,09 and 0,81.

Table 2. Sensory profile of chops fried in various frying fats assessed on a 15 cm unstructured line scale.

	Butter	Margarine	Butter/Olive oil	Rapeseed Cold pressed	Grapeseed Cold pressed	P ^{*1}	Std.err
Fried meat odour	3.4 ^a	1.3 ^b	2.3 ^b	1.2 ^c	1.7 ^{bc}	*	1.0
Oily odour	0.7 ^d	2.4 ^{bc}	1.5 ^{cd}	2.6 ^b	4.1 ^a	***	0.7
Buttery odour	4.7 ^a	2.0 ^b	2.8 ^b	1.9 ^{bc}	1.4 ^c	***	0.8
Sweet odour	4.0 ^a	2.9 ^b	3.3 ^{ab}	2.8 ^b	2.6 ^b	**	0.7
Uneven fried surface	5.4 ^b	5.5 ^b	4.2 ^c	5.5 ^b	6.5 ^a	***	0.8
Piggy flavour	3.8 ^a	3.2 ^a	2.2 ^b	3.9 ^a	3.4 ^a	**	1.0
Fried meat flavour	7.5 ^a	6.5 ^{bc}	7.4 ^a	6.1 ^c	7.1 ^{ab}	**	0.8
Oily flavour	0.7 ^c	1.4 ^{bc}	1.3 ^c	2.1 ^{ab}	2.6 ^a	***	0.6
Buttery flavour	3.4 ^a	1.3 ^b	2.3 ^b	1.2 ^c	1.7 ^{bc}	***	0.6
Sour taste	5.4 ^b	5.5 ^b	4.2 ^c	5.5 ^b	6.5 ^a	***	0,8
Juiciness	7.9 ^a	6.3 ^{bc}	5.9 ^c	6.9 ^b	6.8 ^{bc}	***	0.8



*M outlier

Figure 1. Principal Component Analysis of the sensory profile of fried pork chops pan fried in various frying fats. B – butter, B/OO - butter and olive oil, M – Margarine, R – rapeseed oil, G – grapeseed oil

**THE EFFECT OF ENHANCEMENT WITH SALT, PHOSPHATE AND MILK
PROTEINS ON THE PHYSICAL AND SENSORY PROPERTIES OF PORK
LOIN**

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Key Words: Enhanced pork; Sensory; Phosphate; beta-lactoglobulin enriched fraction; Whey protein concentrate.

Introduction

Tenderness and juiciness rank as two of the top attributes in most consumer surveys (Rust, 1998). However, a lot of fresh pork today is often overcooked producing a tough and dry product (Rust, 1998). The pork industry has taken advantage of injection technology to provide consumers with enhanced, more tender and juicy products (Brewer et al., 2002; Prestat et al., 2002a; Sheard et al., 1999). Non-meat ingredients are used to improve juiciness and/or tenderness, enhance flavour, improve and stabilise colour, increase shelf life or increase water- holding capacity (WHC) in the final product. Additionally, some of these ingredients work synergistically with others to further enhance their functionality. Phosphates, the most common ingredient group used for enhancement, modify the charge environment of the myofibrillar protein, leading to increased WHC, decreased purge loss, improved flavour, colour stability (Prestat et al., 2002b; Sutton et al., 1997, Keeton, 1983; Ockerman et al., 1978) and juiciness (Sheard et al., 1999). Enhancement is not a method for improvement of low quality pork, but a means for the industry to improve the overall quality of fresh pork in the retail display (Miller, 1998).

Objectives

The objective of this study was to determine the effects of enhancement using salt, sodium tripolyphosphate (STPP), beta-lactoglobulin (beta-Ig) enriched fractions and whey protein concentrate 80% (WPC80) on the physical and sensory attributes of fresh pork loins and to evaluate the use beta-Ig enriched fractions and WPC80 as possible replacement ingredients for STPP, which may lead to the potential reduction of phosphate in the enhancement solution.

Methodology

Three brine solutions were formulated to contain different concentrations of ingredients for comparison with unpumped control loins (Table 1). Fresh pork loins (72 hours *post mortem*) were pumped to 110% of original weight with a brine solution using

a Dorit Model No. PSM-21-4.5 multi-needle brine injector. The effects of enhancement using salt, STPP, beta-Ig and WPC80 were analysed in terms of cook loss, drip loss, purge loss and Warner Bratzler shear force (WBSF). Pork loins were weighed immediately after injection (pumping), vacuum packaged and were then allowed to equilibrate at 4°C for 24 hr and re-weighed for % purge determination (Sutton et al., 1997). A sample (2.5cm thick) weighing ~80g, was removed from each sample chop used for drip loss determination and stored under atmospheric pressure at 4°C for 48 hours. The drip loss was expressed as a percentage of the original weight (Honikel, 1987). An 8-member trained panel was employed to evaluate the sensory characteristics of pork chops from each treatment. Results were analysed using The SAS system (SAS, 1985). One-way analysis of variance (ANOVA) was carried out and the mean values were separated using Tukey's Family Error rate. A consumer study (n= 60) comparing enhanced to non-enhanced pork chops was carried out. Two samples were given to each consumer (one enhanced and one non-enhanced) with a questionnaire per pair of samples. The attributes tested for were tenderness and juiciness. The individuals were also asked which sample they preferred and based on their preference, would they be willing to purchase the pork chops. Results for the consumer study were analysed using the software program Statistics Package for Social Sciences (SPSS® Base) Version 9.0 for Windows.

Results & Discussion

The moisture content of the enhanced pork loins increased ($p < 0.001$) in comparison to the control as expected (Table 2). The enhancement process had no effect ($p > 0.05$) on cook loss (Table 2). This agrees with studies carried out by Brashear et al., (2002) and Sheard et al., (1999). The differences in purge losses were significant but small. Salt/beta-Ig recorded the highest purge loss of 1.8% while salt/STPP had the lowest purge loss of 1.2%. Sutton et al., (1997) also reported that STPP decreased purge loss in fresh injected loins. All of the WBSF values decreased by ~13N ($p < 0.001$) in the enhanced pork chops compared to the control. All of the enhanced products were similar ($p > 0.05$) in peak force values. Prestat et al., (2002b) found that enhancement with 0.4% STPP and 0.4% salt significantly ($p < 0.05$) decreased WBSF values. Smith et al., (1984) also noted lower shear force values for pork loins enhanced with 0.48% STPP. However, Sutton et al., (1997) found no differences between controls and pork chops enhanced with 0.4% STPP. Redness (a^*) decreased significantly ($p < 0.05$) in chops enhanced with salt/STPP at day 1 (Table 1). However redness (a^*) at day 7 was increased significantly in chops enhanced with salt/beta-Ig and salt/ WPC80. Redness (a^*) decreased in all treatments between day 1 and day 7.

Tenderness and juiciness were significantly increased ($p < 0.001$) with enhancement (Table 3). The enhanced pork chops had a slightly higher overall flavour and overall acceptability to the control pork chops. The differences in overall flavour were small but significant ($p < 0.024$). Pork loins enhanced with phosphate and salt had a higher overall flavour rating in comparison to the control ("very good" vs "good"). No difference was recorded between the salt/beta-Ig enhanced and the salt/WPC80 enhanced ($p > 0.01$). All the treatments were found to have either good or very good overall flavour rating. Sensory analysis results showed that beta-Ig and WPC80 could be used as potential replacement ingredients to reduce the amount of phosphate used in enhancement

solutions, as they were comparable to salt/STPP enhancement solution. Enhancement adds value to pork loins, but only if consumers perceive them to be as or more acceptable than control loins (Brewer et al., 2002). Glaeser et al., (2003) found that the overall eating quality of pork was markedly improved by needle injection of a brine solution, containing STPP and salt.

In the consumer study, consumers rated the tenderness, juiciness and taste of the enhanced chops significantly higher than the control chops. Sixty percent of the consumers preferred the enhanced pork chops (Figure 1). Sixty-two percent stated that they preferred the enhanced sample, sixty per cent stating that they would buy the product, while only 2 per cent stating that they would not buy the product (Figure 2).

Conclusions

Beta-Ig enriched fractions and WPC80 can be used as potential replacement ingredients to reduce the amount of phosphate used in enhancement solutions, as they were comparable to salt/STPP enhancement solution. In the consumer study, consumers rated the tenderness, juiciness and taste of the enhanced chops significantly higher than the control chops. These findings indicate the potential of pork enhanced meat products to the pork industry.

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Tables and Figures

Table 1: Brine formulation for pork loin enhancement

Treatment	Water	Nitrite Salt (%)	STPP	β-Ig	WPC80
Control	-	-	-	-	-
STPP/Salt	92.2	5.5	3.3	-	-
Salt/Beta-Ig fraction	89.0	5.5	-	5.5	-
Salt/WPC80	89.0	5.5	-	-	5.5

Table 2: The effect of enhancement on the physical properties of pork loins

Treatment	Moisture (%)	Drip Loss (%)	Purge Loss (%)	Cook Loss (%)	Day 1 a*	Day 7 a*
Control	74.5 ^a	1.7 ^{ab}	0.0 ^a	31.5 ^a	5.3 ^b	1.8 ^a
STPP/Salt enhanced	77.0 ^c	0.9 ^a	1.2 ^b	30.7 ^a	3.2 ^a	2.2 ^a
Salt/Beta-Ig fraction enhanced	76.4 ^{cb}	2.8 ^c	1.8 ^c	31.4 ^a	5.5 ^b	3.7 ^a
Salt/ WPC80 enhanced	76.0 ^b	2.0 ^{bc}	1.6 ^{bc}	30.6 ^a	5.2 ^{ab}	3.8 ^a
SED	0.19	0.24	0.19	0.62	0.41	0.33

^{a, b} Means in the same column with unlike superscripts are different (p<0.001).

SED: standard of the difference of the means error

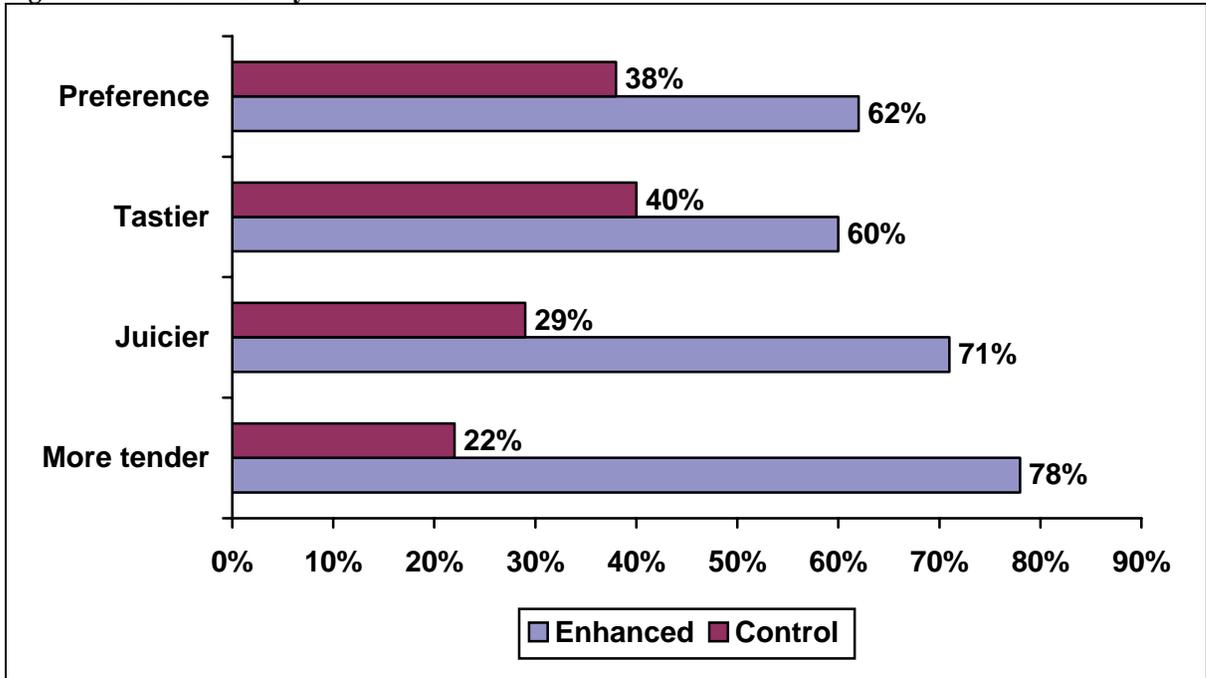
Table 3: Enhancement effects on the sensory characteristics of pork loins

Treatment	WBSF (N)	Tenderness	Juiciness	Overall Flavour	O/F	O/A
Control	38.5 ^b	5.3 ^a	4.8 ^a	4.1 ^a	6.3 ^b	4.0 ^a
STPP/Salt	24.7 ^a	6.7 ^c	6.7 ^c	4.6 ^b	5.4 ^a	4.7 ^b
Salt/Beta-Ig fraction	27.7 ^a	6.1 ^b	6.0 ^b	4.3 ^a	5.6 ^a	4.3 ^a
Salt/WPC80	24.9 ^a	6.7 ^c	6.8 ^c	4.3 ^a	5.3 ^a	4.4 ^a
SED	1.63	0.12	0.12	0.09	0.08	0.09

^{a, b} Means in the same column with unlike superscripts are different (p<0.001).

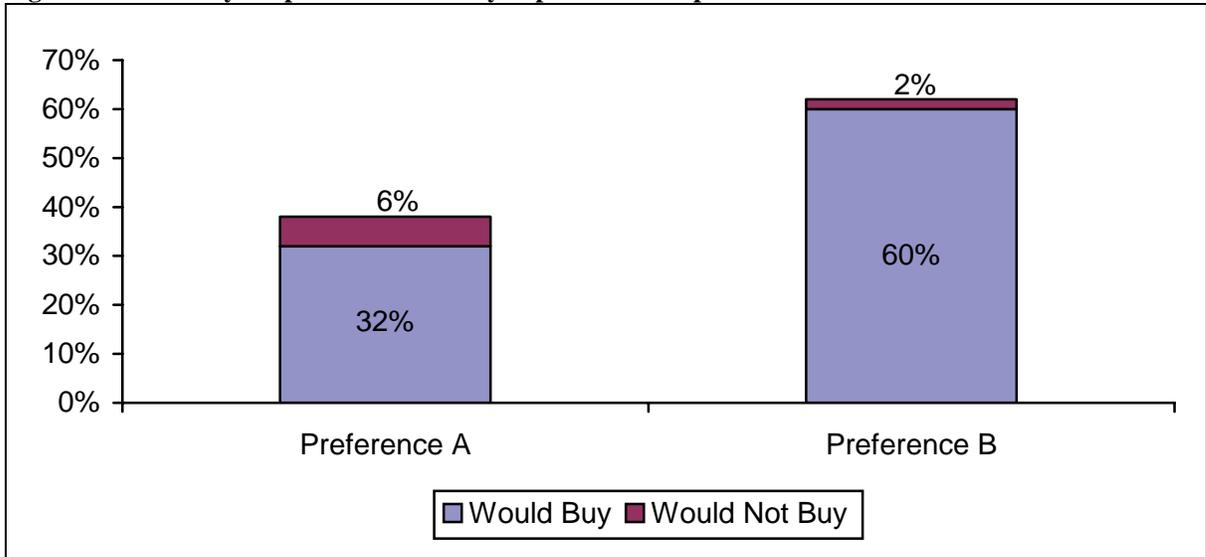
O/F= Overall Firmness, O/A= Overall Acceptability, SED: standard of the difference of the means error

Figure 1: Consumer Study on Pork Enhancement



Base: 63 Respondents

Figure 2: Based on your preference would you purchase this product?



Base: 63 Respondents
Preference A: Control (Control); Preference B: Enhanced

EFFECT OF ENHANCEMENT ON FLAVOR VOLATILES OF VARIOUS BEEF MUSCLES

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Key Words: beef, enhancement, aging, SPME

Introduction

Consumers continue to demand high quality, consistent meat products at a reasonable price. Historically, aging of meat products has been used to improve quality (Dransfield, 1994; Mottram, 1998). The enhancement of poultry and in more recent years, pork has been used to ensure that a consistent product reaches the consumer (Grey et al, 1978, Brewer et al., 2002). This same technology can be applied to beef in order to ensure consumers receive consistent, quality products. Tenderness and flavor are the two sensory traits that affect consumer acceptance of beef and therefore influence repeat purchases (Robbins et al., 2003). Enhancement of meat products could alter the volatile flavor compounds found in different muscles (Gasser and Grosch, 1988). By gaining a better understanding of which muscles would profit from enhancement, the beef industry will benefit economically.

Objectives

The objectives of this project were (1) to profile the flavor changes and to evaluate Warner-Bratzler shear and color changes and (2) to identify and quantify the compounds, which change during aging, of ten beef muscles enhanced prior to aging.

Methodology

Phase 1

The *Gluteus medius*, *Infraspinatus*, *Psoas major*, *Rectus femoris*, *Teres major*, *Complexus*, *Serratus ventralis*, *Vastus lateralis*, *Vastus medialis* and *Longissimus dorsi* were removed from 20 USDA Select carcasses 48 h after slaughter. Muscles from one side of each carcass were divided into two sections (1/aging period), vacuum-packaged, stored at 4 °C and used as the unenhanced control. Muscles from the remaining side were enhanced to 108% of original weight for a final concentration of 0.3% salt and 0.4% sodium tripolyphosphate for comparison with unenhanced samples. Enhancement solution was injected at 0 °C, 1.5 Barr and 52 strokes/min through injection needles spaced 2.5 cm apart using a multi-injector system (Model N50, Wolf-tec Inc., Kingston, NY). Muscles were weighed before and after pumping to determine the solution uptake.

Each enhanced muscle was divided into two sections (1/aging period), vacuum-packaged, and stored at 4 °C. Sections, both enhanced and control, were aged 7 or 14 d. Sections were removed from vacuum bags, weighed for purge loss determination, measured for pH, faced, sliced into 2.5 cm steaks for sensory evaluation, proximate analysis, cook loss and Warner-Bratzler shear force. Steaks were allowed to bloom for color determination (Minolta L*, a*, b*, Konica-Minolta, Japan), vacuum-packaged and stored for 18 hrs at 4 °C until sensory evaluation.

A 10-member trained sensory panel evaluated raw color (red, brown, green) prior to cooking on a semi-structured 15-cm line scale where 0 = light and 15 = dark. Cooked (70 °C) steaks were evaluated for flavor (tenderness, juiciness, saltiness, beef flavor, oily mouthfeel, rancid off-flavor, liver off-flavor) on a semi-structured 15-cm line scale where 0 = none and 15 = intense. Standards were provided for both color and flavor attributes. For cook loss and shear force, raw steaks were weighed, cooked (70 °C), re-weighed, cooled for 1 h at 4 °C, cored, and evaluated using an Instron Universal testing machine for Warner-Bratzler shear force. Moisture and fat content were determined following each aging period. Samples were trimmed of fat and connective tissue, homogenized, and oven-dried (10-g duplicate samples) for 48 h at 110 °C (AOAC, 1990). Moisture content was determined by weight difference between wet and dry sample. Fat content was determined by extraction with an azeotropic mixture of warm chloroform and methanol (4:1) (Novakofski et al., 1989).

Phase 2

Volatile flavor components were determined using solid-phase microextraction-gas chromatography (SPME) coupled with a sulfur-selective detector. Homogenized cooked meat samples (5 g) were placed in a 22-mL glass headspace vial and sealed with a Teflon-lined septum. Vials were preincubated for 20 minutes at 50 °C, then headspace volatiles were sampled for 10 min using a Carboxen/PDMS SPME fiber and subsequently thermally desorbed in the injection port of the GC (Zhu et al. 2001). Levels of sulfur-containing compounds were determined by selective detection using a flame photometric detector (Zhu et al. 2001). Parallel detectors (mass spectrometer and flame ionization detector) were used to monitor non S-containing flavor components. Free amino nitrogen was measured as an index of liberated free amino acids (and peptides) as a function of aging or enhancement using the NOPA method (Shively and Henick-Kling, 2001).

Analysis

Data were analyzed as a repeated measures (carcass), factorial design using PROC MIXED (SAS, 2002) with the model accounting for the effects of muscle, aging time, enhancement and appropriate interactions. Effects were considered significant at $p < 0.05$. Least squares means were separated using probability of difference. Changes in compounds were correlated with sensory flavor profiles determined in phase 1 of the study.

Results & Discussion

Enhancement generally increased positive sensory attributes (tenderness, juiciness, beef flavor, saltiness, pH and moisture content) and decreased negative attributes (purge loss and off-flavors). Enhancement decreased instrumental color values (L^* , a^* , b^* , chroma) by varying degrees for different muscles. Increasing pH often increases water-holding capacity which increases moisture content, juiciness and tenderness and decreases shear values. The salt in the enhancement solution would be expected to impact flavor by increasing saltiness and beef flavor which in turn may have masked low levels of off-flavors originally present or after aging.

Aging had positive effects on tenderness, flavor and shear values, negative effects on instrumental color values (L^* , a^* , b^* , chroma), and minimal effects on beef flavor. Flavor-active volatiles affected by enhancement and aging in the various muscles include nonanal, 2,3-octanedione, pentanal, 3-hydroxy-2-butanone, 2-pentyl furan, 1-octen-3-ol, butanoic acid, pentanal and hexanoic acid. Enhancement decreased hexanal and hexanoic acid. Aging decreased butanoic acid. Pentanal content varied among muscles depending on enhancement and aging. Some variation in responses may be explained by the original variation (tenderness, moisture, fat content and color) in the 10 muscles.

Conclusions

Enhancement increased quality characteristics of muscles however some benefited more than others. Aging had smaller effects. Volatile compounds known to affect flavor varied due to the specific muscle that was subjected to enhancement and aging.

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FLAVOR AND TEXTURE PROFILING OF ENHANCED BEEF LONGISSIMUS

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Key Words: beef, sensory, enhanced, principal component analysis, packaging and storage

Introduction

Enhanced beef (ie. with added water, salt, phosphate and other ingredients) has become more popular in the marketplace (Robbins and others 2003). Currently, there are a number of enhanced raw beef steaks available at retail, and a few roasts are available as fully cooked products. Historically, meat scientists have limited sensory evaluation of fresh meats to 6-8 product attributes. However, using only a few descriptors such as “tenderness” or “overall flavor” may not fully describe the sensory experience for enhanced products.

Objectives

The objective of this research was to characterize the flavor and texture of enhanced beef *longissimus* muscle using a highly-trained descriptive panel.

Methodology

Twelve beef loin samples were selected that represented different quality, processes and packaging (Table 1). Three were fully cooked (prime rib) while three represented various quality grades (USDA Prime, Certified Angus Beef [CAB], USDA Select) of intact loin. There were four commercially “enhanced” steaks. Two additional treatments were included (CAB dry-aged steaks and a USDA Select loin roast). Three replications of the experiment were performed. Steaks packaged in high oxygen were generally evaluated 3 to 4 days before the “sell by” date.

Steaks were grilled (14 to 22 min) to a final internal temperature of 71°C (removed from the electric grill at 69°C). The USDA Select loin roast was heated in a conventional oven at 177°C to an internal temperature of 63°C and held for three minutes. The precooked prime rib roasts were cut into 2.5 cm-thick slices and reheated in a microwave oven to 60°C. Steaks (and roast slices) were weighed before and after cooking (reheating), and the percentage of cooking (reheating) loss was calculated.

Descriptive profiling was conducted by a 5-member professional panel in the Sensory Analysis Center (SAC), Kansas State University. The panelists evaluated six treatments during each 90 min session. These panelists had completed 120 h of training in all aspects of sensory techniques, had a minimum of 1000 h of general sensory testing, and had

experience evaluating meat products. Selection and refinement of terms was carried out during four preliminary sessions. Cooked samples were trimmed of fat and connective tissue so that only the center portion of the *longissimus lumborum* was served to the sensory panel. Panelists rated flavor and textural properties on 15-point scales.

Six to 8 cores (1.27 cm in dia) were removed parallel to muscle fibers from each sample and were sheared once perpendicular to the fiber direction using a Warner Bratzler shear (WBS) attachment on an Instron (Model 4201, Canton, MA) with a 25 kg load cell and a crosshead speed of 250 mm/min. Expressible moisture (EM) was determined by measuring moisture loss following centrifugation (750 x g; Shand 2000). A sample of the *longissimus* muscle of each cooked steak was analyzed for pH, moisture, protein, crude fat, ash and sodium chloride using standard methods.

Analysis of variance (ANOVA), principal component analysis (PCA) and cluster analysis (CA) were conducted using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA).

Results & Discussion

Proximate composition: Proximate composition of cooked non-enhanced steaks was as expected, with the USDA Prime steak containing the most fat (12.9%) and the USDA Select steak the least (3.7%). The E1 steaks with 8.9% fat were from USDA Choice beef, while E2 to E4 were from USDA Select beef (fat content 3.8 to 4.2%). Precooked roasts had similar and higher sodium chloride content, moisture content and pH, and lower protein and fat content than intact and enhanced steaks.

Cook yield and doneness: Cook yields of enhanced steaks were similar at 77-78% and slightly higher than that of the intact USDA Prime steaks at 72%. All steaks were cooked to 71°C (160°F) with most appearing to be of medium to medium-well doneness. Two products, E2 and E3, were distributed in high oxygen packaging and may have undergone premature browning resulting from oxidation of the myoglobin during storage (Seyfert and others 2004). Reheating yields in the microwave for precooked roast slices were 83-86%.

Textural properties: The non-enhanced USDA Select steak was noticeably tougher with higher Warner-Bratzler shear values than other intact and enhanced steaks (Table 2). It also had low juiciness and high chewiness, fiber awareness and residual connective tissue scores. There was some variability in textural properties of the four enhanced steaks. The E3 steaks were generally tougher than the others and were similar to the non-enhanced Select steaks. E1, E2 and E3 had similar textural properties to the non-enhanced CAB and USDA Prime steaks. While E1 might be expected to be similar to CAB (as E1 was from USDA Choice beef), the enhancement of E2 and E4 clearly improved eating properties above that expected for USDA Select beef. Since we only replicated each treatment three times, E3 steaks may not be typical of that supplier. However, these results may indicate that enhancement cannot overcome the inherent toughness of some USDA Select beef. No atypical ham-like textures were noted. In general, the precooked roasts were more tender and juicy than the non-enhanced USDA Select roasts, but were considered to be more “mealy” in texture than the grilled steaks.

Flavor properties: Non-enhanced steaks and some of the enhanced steaks (E1 and E4) had high beef flavor ID and brown roasted notes with some “bloody serummy” flavor

(Table 3). Both enhanced steaks previously stored in high oxygen (E2 and E3) had slight warmed-over flavor with less beef flavor ID and less bloody serummy flavor, while E1 (stored in carbon monoxide/carbon dioxide) and E4 (under vacuum) did not. The E2 steaks had an atypical brothy note. Other flavor notes, such as metallic and rancid, were of low intensity. While all steaks had similar sour and bitter flavors, enhanced steaks (especially E2) were slightly more salty than the intact steaks.

All precooked prime rib products had brothy/boullion-like notes and were somewhat salty. These also tended to have some organ meat notes. As expected, the prime rib samples (cooked in a bag) did not have browned roasted notes. Even though P1 to P3 were precooked and were stored under vacuum for several weeks, “warmed-over” flavor was almost undetectable. The USDA Select roast and P1 to P3 roasts had more metallic flavor than the steaks. Since the roasts appeared rare in doneness, the presence of a metallic note was not unexpected.

Principal component (PCA) and cluster analysis (CA): PCA was used to investigate underlying similarities and differences among products and degree of correlation among descriptive terms. The first two principal components (PCs) accounted for 90% of the total variability (Figure 1). In plots of PCs, terms that are correlated with each other group together (if positive) or move in the opposite direction (if negative) and objects close together have similar characteristics. PC1 accounted for 74% of the variation. Terms such as brown roasted and beef flavor identity had high positive loadings for PC1, while brothy and salty had moderately negative loadings. PC2 accounted for much less of the variability (14%) and seemed to be driven by textural properties. PC2 had high positive loadings for initial firmness, fiber awareness and residual connective tissue and a high negative loading for tenderness.

Beef *longissimus* samples were further categorized into 3 clusters by CA (Figure 1). Products in Cluster 1 were high in beef flavor ID and browned roasted notes, but varied in tenderness, with CAB, Prime and E1 and E4 showing a negative loading for PC2 and CABdry, E4 and the Select loin steak a positive loading for PC2. All of the precooked roasts grouped together in Cluster 2. In general, they lacked browned flavor notes and were higher in brothiness and saltiness. The Select Roast and Enhanced steak 2 formed cluster 3. This group was intermediate in some properties. The Select Roast lacked the browned notes of the steaks, but had more fiber awareness than the precooked roasts. The E2 enhanced steak was very different than its enhanced counterparts for PC1 and PC2. It was much more salty and brothy, which cannot be explained from the ingredient listing. Both E2 and E3 also had slight warmed-over flavor, which differentiated these products from the others when PC3 was plotted (data not shown). E4 (from Select beef) clustered in the same region as products from higher grades (E1, Prime, CAB), while the non-enhanced USDA select steak and roast were much tougher.

Conclusions

Overall, enhancement of beef loin steaks resulted in greater changes in flavor properties than textural properties. Generally, enhanced steaks were very lean but more tender and juicy than the non-enhanced USDA Select steaks, showing the importance of enhancement to improving quality of USDA Select beef in particular. However, some enhanced steaks were still relatively tough. No atypical textural properties were reported.

The flavor differences between intact and enhanced steaks ranged from “slight” to “major”, depending on the composition of the enhancement solution and packaging method. Enhancement generally increased the perception of saltiness. Some enhanced steaks also had atypical brothy flavors and were quite salty. Furthermore, packaging of enhanced steaks in a high oxygen environment, unlike those in the carbon monoxide masterpack, likely caused oxidation and led to several flavor changes after cooking, including increases in warmed over flavor and decreases in bloody serummy and beef flavor ID. Premature browning during cooking also was evident for steaks packaged in high oxygen.

Precooked roasts were distinctly different in textural and flavor properties from steaks. In addition, these three products varied widely in composition, ingredients, pH and fat level. Even though the P1 roasts were not injected, the marination and/or cooking method resulted in a similar flavor profile to the other two products. As ready-to-eat products represent a potential growth area, it is also important to better understand drivers for consumer acceptability of this type of product.

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Table 1. Steak and roast samples selected for this study.

Sample	Grade	Added Ingredients	Packaging
Prime strip steak, aged for 22-29 days	USDA Prime	None	Vacuum packaged
Certified Angus Beef strip steak, aged for 22-29 days	USDA Choice	None	Vacuum packaged
Dry Aged CAB strip steak, dry aged 17-22 days, total of 22-29 days	USDA Choice	None	Mostly aerobic storage
Select strip steak, aged 23-30 days	USDA Select	None	Vacuum packaged
E1 - Enhanced strip steak #1, supplier 1	USDA Choice	Contains up to 12% of a patented solution of beef broth, potassium lactate, sodium phosphate, salt, sodium diacetate, flavoring	Tray with O ₂ -permeable over-wrap. Previously in CO / CO ₂ master pack
E2 - Enhanced strip steak #2, supplier 2	USDA Select	Contains up to a 10% solution of water, sodium phosphate, salt and natural flavor	Rigid tray with high oxygen
E3 - Enhanced strip steak #3, supplier 3	USDA Select	Same as E2	Rigid tray with high oxygen
E4 - Enhanced strip steak #4, supplier 3	USDA Select	Same as E3	Vacuum packaged
Select strip loin roast (freshly cooked)	USDA Select	None	Vacuum packaged
P1 - Fully cooked prime rib roast, not enhanced (foodservice)	USDA Choice	Rubbed with salt, dextrose, hydrolyzed soy protein, corn syrup solids, onion powder, caramel color, garlic powder, spice, soybean oil, natural flavor	Vacuum packaged
P2 - Fully cooked prime rib roast, enhanced (retail)	USDA Select	Marinated up to 10% with a solution of water, sodium phosphate, salt, hydrolyzed corn protein, flavoring. Coated with salt, dextrose, hydrolyzed corn protein, caramel color, dried beef stock, spices	Vacuum packaged
P3 - Fully cooked prime rib roast, enhanced (retail)	Not known	Contains up to 10% of water, sodium phosphate, salt, hydrolyzed corn protein, spice extractives and onion extractives. Coated with salt, dextrose, hydrolyzed corn protein, dried beef stock, black pepper, flavorings	Vacuum packaged

Table 2. Sensory properties¹ (texture) and Warner-Bratzler shear values of control and enhanced beef loin steaks and precooked prime rib roast samples².

Product	Initial Firmness	Tender-ness	Juiciness	Chewi-ness	Rubberi-ness	Mealy	Fiber Awareness	Residual Connect-ive Tissue	WB shear, kg
USDA Prime Steak	5.3	8.4	8.5	6.7	0.1	0.8	4.2	2.5	2.6
CAB Steak	5.6	8.6	7.4	7.8	0.0	1.2	3.9	2.7	3.2
CAB dry-aged Steak	7.1	7.4	7.4	8.4	0.2	0.4	4.8	3.4	3.6
USDA Select Steak	8.9	6.0	5.8	9.6	0.2	0.6	6.7	4.8	5.1
E1 Steak	4.6	8.7	7.4	7.4	0.1	1.2	4.4	2.4	2.7
E2 Steak	5.8	8.9	7.7	7.5	1.3	0.9	3.7	3.0	2.5
E3 Steak	8.1	6.5	6.2	8.9	0.1	1.4	5.9	3.1	4.0
E4 Steak	4.2	9.6	7.9	7.9	0.1	1.8	3.5	2.0	2.8
USDA Select Roast	5.6	7.1	6.8	8.2	0.5	1.4	5.6	3.2	4.6
P1 Roast	4.1	9.8	7.7	7.0	0.1	3.5	3.2	1.5	2.4
P2 Roast	3.2	11.3	9.4	6.5	0.4	3.6	3.3	1.4	1.8
P3 Roast	6.3	8.6	9.0	7.7	0.2	2.4	4.9	3.4	2.6
<i>LSD</i> ³	2.6	2.7	1.3	1.5	0.7	1.4	1.7	1.4	1.4

¹ Highest possible score = 15; 15= extremely firm, tender, juicy, chewy, rubbery, mealy, increased fiber awareness and high amount of perceptible connective tissue.

² Based on analysis of three steaks or roasts per product by five expert panelists. E=enhanced steaks P=precooked roasts

³ LSD= Least significant difference.

Table 3. Sensory properties¹ (flavor) of control and enhanced beef loin steaks and precooked prime rib roast samples².

Product	Beef										
	Flavor ID	Brown Roasted	Organ Meat	Bloody Serummy	Brothy	WOF	Metallic	Rancid	Sour	Salty	Bitter
USDA Prime Steak	11.5	12.1	0.6	1.3	0.0	0.7	1.7	0.9	2.0	3.0	2.5
CAB Steak	11.5	11.9	0.6	1.1	0.0	0.1	1.6	0.4	1.9	3.2	2.7
CAB dry-aged Steak	11.5	11.7	0.2	1.3	0.0	0.4	1.8	0.8	2.4	3.3	2.6
USDA Select Steak	10.4	11.5	0.1	0.5	0.0	0.5	1.7	1.2	2.2	3.3	3.1
E1 Steak	11.7	12.7	0.4	0.5	0.0	0.4	1.6	0.2	2.4	4.4	2.7
E2 Steak	7.8	9.8	0.1	0.1	2.8	2.3	1.7	0.8	2.5	6.4	2.8
E3 Steak	9.9	10.8	0.0	0.1	0.1	3.2	1.4	0.6	2.5	4.0	2.7
E4 Steak	11.1	11.7	0.1	1.3	0.2	0.4	1.7	0.9	2.3	3.7	2.8
USDA Select Roast	5.5	3.0	1.0	2.8	1.0	1.3	2.6	1.6	2.6	3.3	2.9
P1 Roast	3.7	3.4	2.3	0.5	5.3	0.7	2.6	1.1	2.8	6.8	3.3
P2 Roast	3.6	2.8	1.5	1.7	3.7	0.6	2.4	0.2	2.8	6.7	2.9
P3 Roast	3.1	2.4	0.4	1.0	6.6	0.1	2.8	0.3	2.7	7.1	3.3
LSD³	<i>1.5</i>	<i>1.2</i>	<i>1.0</i>	<i>1.5</i>	<i>0.7</i>	<i>1.0</i>	<i>0.9</i>	<i>ns</i>	<i>0.5</i>	<i>0.7</i>	<i>0.5</i>

¹Highest possible score = 15; 15= extremely intense, 0= not detectable.

² Based on analysis of three steaks or roasts per product by five expert panelists. E= Enhanced steaks, P= precooked roasts

³LSD= Least significant difference.

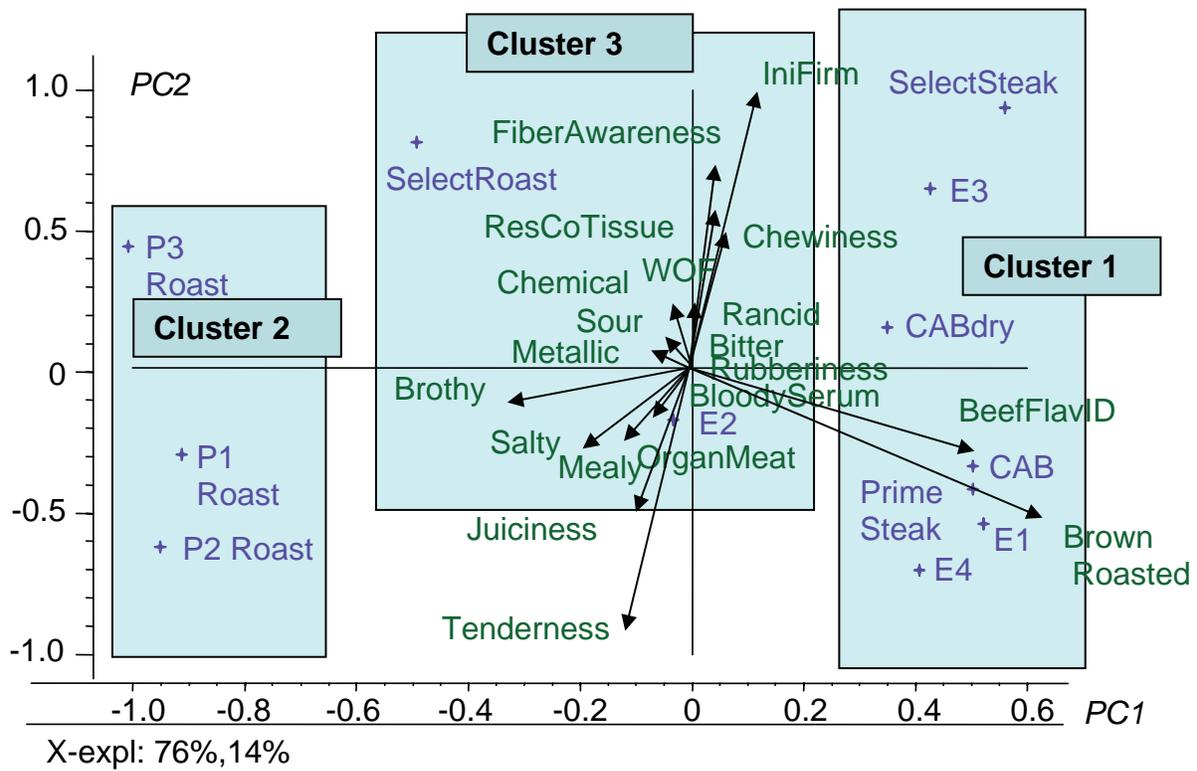


Figure 1: Sensory map of the first two principal components for describing the flavor and texture characteristics of enhanced beef *longissimus* steaks and roasts.

INFLUENCE OF LACTATE, PHOSPHATE, SALT AND ACETATE ON THE PROPERTIES OF INJECTION-ENHANCED BEEF

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Key Words: Lactate, Acetate, Enhanced beef, Gloss, Steaks.

Introduction

Tenderness, juiciness, and flavor are the three most important components of meat palatability. Tenderness is regarded as more important than flavor by consumers because consumers feel they may have a greater impact on flavor than tenderness (Patterson, 1998). Beef tenderness may be enhanced or improved in a number of ways, including electrical stimulation, aging carcasses or cuts, mechanical tenderization, marination, or injection enhancement.

Common ingredients in enhanced pork include phosphates, salt, sodium or potassium lactate, sodium diacetate, and flavoring, as well as water (Miller, 1998). These ingredients have positive impacts on the tenderness and juiciness of pork products and may also be applicable to beef cuts. Moeller and Courington (2002) found that consumers had interest in an economical line of marinated beef products that would have improved tenderness and juiciness.

Little research has been published concerning injection-enhanced whole muscle beef products, though it is commonplace in industry. Ingredients included in injection-enhancement solutions may impact various traits of beef, including color and flavor.

Objectives

The objectives of this study were to determine the influence of lactate, phosphate, salt and acetate on the visual and sensory properties of injection-enhanced beef.

Methodology

Seven USDA Select beef ribs and four USDA Select beef strip loins (8 d postmortem) were used to evaluate injection-enhancement treatments. Five treatments were applied to the beef ribs and four treatments were applied to the strip loins. Treatments were as follows: No Lactate or Acetate - 0.3% salt, 0.3% phosphate, 0% lactate and 0% acetate; Lactate Control - 1.5% potassium lactate, 0.3% salt, 0.3% phosphate; Lactate + High Salt - 1.5% potassium lactate, 0.6% salt, 0.3% phosphate; Lactate + Acetate - 1.5% potassium lactate, 0.3% salt, 0.3% phosphate, 0.1% acetate; Lactate + Acetate (No phosphate) - 1.5% potassium lactate, 0.3% salt, 0% phosphate, 0.1% acetate. All treatments were the same for the ribs and strip loins, except the Lactate + Acetate (No phosphate) treatment was not applied to strip loins.

All treatments were applied to a 7.62-cm thick section of seven paired beef ribs. Three 2.54-cm thick steaks were cut from each section and evaluated on d 2, 9, and 14 post-injection after storage at 2°C. Steaks were evaluated on a 1 to 7 scale for visual color, muscle darkening, discoloration (metmyoglobin formation), and shine, where 1 was the best and 7 was the worst for each trait. Also, on d 2, 9, and 14, steaks were evaluated for instrumental CIE color, odor, microbial growth, and oxidation (TBARS). Odor was evaluated on a 1 to 5 scale with 1 being no odor and 5 being extremely objectionable.

Four treatments were applied to each section of each beef strip loin. Three 1.91-cm thick steaks and one 2.54-cm steak were cut from each section. The 1.91-cm steaks were used to evaluate surface shine and a battery of sensory attributes on d 2, 9 and 14 post-injection. The 2.54-cm steaks were used for Warner-Bratzler shear force (WBSF) evaluation.

Results & Discussion

The lactate + high salt treatment steaks had the darkest ($P < 0.05$; Table 1) visual color each day of all treatments throughout the trial. All treatments had higher visual color scores on d 9 than on d 2 ($P < 0.05$), indicating color deterioration. The no lactate or acetate treatment steaks had less muscle darkening ($P < 0.05$) on d 2 than steaks from the lactate control, lactate + high salt, and lactate + acetate treatments. High-salt treatment steaks showed the most darkening ($P < 0.05$) each day of evaluation and for the longissimus thoracis and spinalis dorsi. Treatment did not affect discoloration (percent surface metmyoglobin), but the spinalis muscle discolored faster and to a greater extent than the longissimus. The lactate + high-salt treatment steaks had less ($P < 0.05$) shine on d 2 than the lactate control steaks.

CIE L* values showed the lactate + high salt steaks were the darkest (Table 2). Steaks on d 9 were significantly darker than on d 2 or d 14. Steaks from all treatments were more red ($P < 0.05$) on d 2 than on d 9. The CIE color value b* indicated that no differences ($P > 0.05$) existed among treatments or among days after injection. On d 9, no lactate or acetate treatment steaks had less intense ($P < 0.05$) color (lower chroma value) than lactate + acetate and the lactate + acetate without phosphate treatment steaks. Chroma values decreased for all treatments over time indicating decreasing color intensity. All steaks were the most ($P < 0.05$) red (lower hue angle values) on d 2 ($P < 0.05$), but were not different among treatments. Objectionable off-odor scores were lowest on d 2, and increased significantly on d 9 and d 14. Mean objectionable off odor scores for any day did not exceed 2.5, which is typically considered unacceptable on the given scale. TBARS values on d 2 (0.280 mg malonaldehyde/kg meat) were less ($P < 0.05$) than d 9 (0.540), which were less ($P < 0.05$) than d 14 (0.990). Microbial growth was not affected by treatment or day in MAP, contrary to expectations. Microbial counts were very low throughout the trial, with means for treatment and day never exceeding 1 log.

Lactate + acetate steaks were more tender ($P < 0.05$) than the no lactate or acetate and the lactate control steaks (Table 3). Gloss generally lessened with increasing time (Table 4). Rancid flavor development was greater in the no lactate or acetate steaks and lactate + high salt steaks on d 14 than for the lactate control and lactate + acetate steaks (Table 5).

Brown/roasted and beef flavors were greatest on d 9 (Table 6 and 7). As time increased, oxidized meat and stale flavors increased (Table 7). However, meat protein flavor decreased over time (Table 7). Salty flavor increased in lactate + high salt treatment steaks when compared to other treatments (Table 8).

Conclusions

The lactate control showed the least darkening from d 2 to d 9, indicating lactate's ability to stabilize color. Lactate appeared to reduce shine of the steaks, prevent rancid flavor development, and increase brown/roasted flavor. Lactate also impacted flavor to a greater degree at d 9 post-injection, indicating that lactate may require dwell time to impact flavor development.

Increasing the salt level had detrimental impacts on visual color, darkening, and flavor. The lactate + high salt steaks had the most darkening of all treatments. Generally, the lactate + high salt steaks had the least shine, possibly due to its dark appearance. Steaks with the increased salt level also had greater red color deterioration from d 9 to d 14 than all other treatments as well as increased rancid flavor development and increased salty flavor.

Acetate may have aided the reduction of shine as the no lactate or acetate steaks generally had the greatest shine. Acetate may have increased color intensity because steaks from the two acetate containing treatments had greater chroma values than the no lactate or acetate steaks. Acetate generally had a tenderizing effect. However, the mechanism through which this takes place is unknown.

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Tables and Figures

Table 1. Least squares means of trained visual panel values during a 14-day trial of beef rib steaks enhanced with five different solutions

Trait/Trt/Muscle	Days in MAP							SEM
	2	9	10	11	12	13	14	
Visual Colorⁱ								
No Lac or Ace	2.6 ^{a,z}	3.2 ^{b,z}	3.6 ^{c,z}	3.9 ^{d,z}	3.9 ^{cd,z}	4.4 ^{e,z}	4.5 ^{e,z}	0.183
Lac Con	3.0 ^{a,y}	3.4 ^{b,z}	4.2 ^{cd,y}	4.0 ^{e,zy}	4.3 ^{de,y}	4.5 ^{e,z}	4.5 ^{e,z}	
Lac + High Salt	3.7 ^{a,x}	4.6 ^{b,x}	5.3 ^{c,x}	5.5 ^{cd,w}	5.3 ^{cd,x}	5.5 ^{cd,x}	5.6 ^{d,y}	
Lac + Ace	3.1 ^{a,y}	4.1 ^{b,y}	4.5 ^{c,y}	4.7 ^{c,x}	4.6 ^{c,y}	5.0 ^{d,y}	4.7 ^{cd,z}	
Lac + Ace(no Phos)	2.8 ^{a,zy}	3.8 ^{b,y}	4.4 ^{d,y}	4.3 ^{cd,y}	4.5 ^{d,y}	4.5 ^{d,z}	4.8 ^{e,z}	
Muscle								
Longissimus	3.1 ^{a,z}	3.6 ^{b,z}	4.2 ^{c,z}	4.2 ^{c,z}	4.3 ^{c,z}	4.5 ^{d,z}	4.6 ^{d,z}	0.148
Spinalis	3.0 ^{a,z}	4.0 ^{b,z}	4.6 ^{c,y}	4.8 ^{c,y}	4.8 ^{c,y}	5.0 ^{d,y}	5.0 ^{d,y}	
Darkening^j								
No Lac or Ace	2.4 ^{a,z}	2.8 ^{b,z}	3.1 ^{bc,z}	3.3 ^{cd,z}	3.3 ^{cd,z}	3.7 ^{de,z}	3.9 ^{e,z}	0.242
Lac Con	3.4 ^{ab,x}	3.2 ^{a,zy}	3.8 ^{cd,y}	3.5 ^{abc,z}	3.7 ^{bcd,zy}	3.8 ^{cd,z}	3.9 ^{d,z}	
Lac + High Salt	3.9 ^{a,w}	4.6 ^{b,w}	5.0 ^{c,x}	5.1 ^{c,x}	5.0 ^{c,w}	5.1 ^{c,x}	5.1 ^{c,y}	
Lac + Ace	3.1 ^{a,yx}	3.9 ^{b,x}	4.2 ^{bc,y}	4.4 ^{c,y}	4.2 ^{bc,x}	4.3 ^{bc,y}	4.2 ^{bc,z}	
Lac+ Ace(no Phos)	2.7 ^{a,zy}	3.4 ^{b,y}	4.1 ^{cd,y}	3.8 ^{bc,z}	4.0 ^{cd,yx}	3.8 ^{cd,zy}	4.2 ^{d,z}	
Muscle								
Longissimus	3.1 ^{a,z}	3.3 ^{a,z}	3.8 ^{b,z}	3.8 ^{b,z}	3.9 ^{b,z}	4.0 ^{bc,z}	4.1 ^{c,z}	0.192
Spinalis	3.0 ^{a,z}	3.9 ^{b,y}	4.3 ^{c,y}	4.2 ^{c,y}	4.2 ^{c,y}	4.3 ^{c,y}	4.4 ^{c,z}	
Discoloration^k								
Muscle								
Longissimus	1.0 ^{a,z}	1.0 ^{a,z}	1.0 ^{a,z}	1.1 ^{a,z}	1.1 ^{a,z}	1.3 ^{b,z}	1.3 ^{b,z}	0.079
Spinalis	1.0 ^{a,z}	1.2 ^{ab,z}	1.2 ^{b,y}	1.6 ^{c,y}	2.0 ^{d,y}	2.2 ^{e,y}	2.4 ^{f,y}	
Shine^l								
No Lac or Ace	2.7 ^{a,zy}	3.7 ^{c,x}	3.1 ^{a,x}	3.0 ^{a,y}	2.7 ^{ab,y}	2.2 ^{b,z}	2.5 ^{b,y}	0.196
Lac Con	2.7 ^{bc,y}	3.0 ^{c,y}	2.7 ^{bc,yx}	2.5 ^{ab,zy}	2.0 ^{a,z}	2.0 ^{a,z}	2.1 ^{a,zy}	
Lac + High Salt	2.2 ^{ab,z}	2.3 ^{ab,z}	2.6 ^{b,yx}	2.3 ^{ab,z}	2.2 ^{ab,zy}	2.0 ^{a,z}	2.1 ^{a,zy}	
Lac + Ace	2.5 ^{b,zy}	2.3 ^{ab,z}	2.4 ^{b,zy}	2.2 ^{ab,z}	2.0 ^{ab,z}	1.9 ^{a,z}	1.9 ^{a,z}	
Lac + Ace(no Phos)	2.3 ^{b,zy}	1.9 ^{ab,z}	2.0 ^{ab,z}	2.1 ^{ab,z}	1.8 ^{ab,z}	1.8 ^{ab,z}	1.7 ^{a,z}	

^{abcde} Means in a row with different superscript letters differ ($P < 0.05$).

^lShine scale: 1 = no shine, 2 = slight amount of shine, 3 = small amount of shine.

^jDarkening scale: 2 = very slightly dark, 3 = slightly dark, 4 = modestly dark, 5 = moderately dark.

ⁱVisual color scale: 2 = bright red, 3 = dull red, 4 = slightly dark red, 5 = moderately dark red or brown, 6 = dark red to brown.

^kDiscoloration scale: 1 = no discoloration (0%), 2 = slight discoloration (1-19%), 3 = small discoloration (20-39%).

^{wxyz} Means in a column within a trait or muscle with different superscript letters differ ($P < 0.05$).

Table 2. Least Squares means of instrumental color valuesⁱ of beef rib steaks injection-enhanced with five treatment solutions and evaluated on d 2, 9, and 14 post-injection.

Trait/Trt	Days in MAP			trt mean	SEM
	2	9	14		
L*					
No Lac or Ace	40.3	37.0	40.6	39.3 ^b	0.440
Lac Con	39.6	37.1	39.9	38.9 ^b	
Lac + High Salt	37.4	37.2	38.0	37.6 ^a	
Lac + Ace	38.6	38.4	39.3	38.8 ^b	
Lac + Ace (no Phos)	39.8	38.7	40.3	39.6 ^b	
Mean	39.1 ^b	37.7 ^a	39.6 ^b		
a*					
No Lac or Ace	31.9 ^{b,z}	27.8 ^{a,z}	28.6 ^{a,y}		0.575
Lac Con	31.8 ^{b,z}	29.1 ^{a,yz}	29.0 ^{a,y}		
Lac + High Salt	31.5 ^{c,z}	29.4 ^{b,yz}	26.0 ^{a,z}		
Lac + Ace	30.8 ^{b,z}	29.8 ^{ab,y}	28.3 ^{a,y}		
Lac + Ace (no Phos)	31.8 ^{b,z}	29.6 ^{a,y}	28.3 ^{a,y}		
b*					
No Lac or Ace	24.7	23.4	24.3		0.747
Lac Con	24.7	24.1	24.6		
Lac + High Salt	24.1	23.9	22.9		
Lac + Ace	23.8	24.6	24.5		
Lac + Ace (no Phos)	24.9	24.5	24.5		
Chroma^j					
No Lac or Ace	40.34 ^{b,z}	36.39 ^{a,z}	37.55 ^{a,y}		0.747
Lac Con	40.24 ^{b,z}	37.78 ^{a,yz}	37.99 ^{a,y}		
Lac + High Salt	39.68 ^{b,z}	37.87 ^{b,yz}	34.63 ^{a,z}		
Lac + Ace	38.93 ^{a,z}	38.63 ^{a,y}	37.47 ^{a,y}		
Lac + Ace (No PO4)	40.33 ^{b,z}	38.41 ^{ab,y}	37.45 ^{a,y}		
Hue Angle^k					
No Lac or Ace	37.66 ^{b,z}	40.02 ^{a,z}	40.33 ^{a,y}		0.307
Lac Con	37.89 ^{b,z}	39.51 ^{a,yz}	40.34 ^{a,y}		
Lac + High Salt	37.42 ^{c,z}	39.14 ^{b,y}	41.49 ^{a,z}		
Lac + Ace	37.65 ^{c,z}	39.54 ^{b,yz}	40.98 ^{a,yz}		
Lac + Ace (No PO4)	38.05 ^{c,z}	39.53 ^{b,yz}	40.85 ^{a,yz}		
Muscle					
Longissimus	37.59 ^{c,z}	39.18 ^{b,y}	40.06 ^{a,y}		0.194
Spinalis	37.88 ^{c,z}	39.91 ^{b,z}	41.54 ^{a,z}		

^{abc} Means in a row with different superscript letters differ ($P < 0.05$).

ⁱL* - greater values are lighter, a*- greater values are more red, b*- greater values are more yellow, chroma- greater values are more intense, hue angle- greater values are less red.

^jCalculated using the equation: chroma = $[(a^{*2} + b^{*2})^{1/2}]$.

^kCalculated using the equation: hue angle = $[(b^*/a^*)^{tan^{-1}}]$.

^{yz} Means in a column within a trait or muscle and trait with different superscript letters differ ($P < 0.05$).

Table 3. Warner-Bratzler shear force and sensory panel tenderness and juiciness least squares means for strip loin steaks injection-enhanced with four solutions

	Treatment				Day Mean	SEM
	No Lac or Ace	Lac Con	Lac + High Salt	Lac + Ace		
Warner-Bratzler shear force, kg	2.6 ^a	2.6 ^a	2.2 ^{ab}	1.9 ^b		0.384
Panel tenderness^c						
d 2	9.6	9.7	10.1	10.0	9.8 ^y	0.150
d 9	9.6	8.9	9.1	9.1	9.2 ^z	
d 14	9.1	8.7	8.9	9.3	9.0 ^z	
Mean	9.5	9.1	9.4	9.4		
Panel juiciness^c						
d 2	6.1	5.7	6.5	6.0	6.1 ^y	0.146
d 9	5.5	5.1	5.9	5.6	5.5 ^z	
d 14	5.1	5.0	5.4	5.7	5.3 ^z	
Mean	5.6	5.3	6.0	5.8		

^{ab}Means with a different superscript letter differ ($P < 0.05$).

^cScored on a 15 point scale.

^{yz}Means within a column and trait with a different superscript letter are different ($P < 0.05$).

Table 4. Instrumental gloss and trained sensory panel shine least squares means for strip loin steaks injection-enhanced with four solutions

	Days in MAP			SEM
	2	9	14	
gloss at 20°				
No Lac	0.5	0.4	0.7	
Lac Con	0.5	0.5	0.6	
Lac + Hi Salt	0.5	0.5	0.6	
Lac + Ace	0.7	0.5	0.7	
Mean	0.6	0.5	0.6	0.094
gloss at 60°				
No Lac	2.95	1.95	1.53	
Lac Con	3.08	2.85	2.10	
Lac + Hi Salt	3.93	2.63	2.08	
Lac + Ace	4.05	2.40	2.10	
Mean	3.50 ^a	2.45 ^{ab}	1.95 ^b	0.525
gloss at 85°				
No Lac	0.8	0.4	2.1	
Lac Con	1.2	0.4	5.8	
Lac + Hi Salt	1.8	1.2	6.1	
Lac + Ace	1.8	1.4	13.5	
Mean	1.4	0.8	6.9	2.414
shine scoreⁱ	8.67 ^a	8.18 ^a	6.52 ^b	0.256

^{ab} Means within a row within a trait with a different superscript letter differ ($P < 0.05$).

ⁱShine scale: 1 = no shine, 15 = extremely shiny.

Table 5. Least squares means for the day x rancid flavor^c interaction of beef strip loin steaks injection-enhanced with four treatment solutions evaluated on d 2, 9, and 14 post-enhancement

	Day in MAP			SEM
	D 2	d 9	D 14	
No Lac or Ace	0.40 ^{a,y}	0.59 ^{a,yz}	2.06 ^{b,z}	0.336
Lac Con	0.19 ^{a,y}	1.19 ^{b,z}	0.75 ^{ab,y}	
Lac + High Salt	0.65 ^{a,y}	0.86 ^{a,yz}	2.36 ^{b,z}	
Lac + Ace	0.48 ^{a,y}	0.19 ^{a,y}	0.96 ^{a,y}	

^{ab}Means within a row with different superscript letters differ ($P < 0.05$).

^cRancid flavor scored on a 0 to 15-pt scale.

^{yz}Means within a column with different superscript letters differ ($P < 0.05$).

Table 6. Least squares means for brown/roasted flavor^d for beef strip loin steaks injection-enhanced with four solutions and evaluated on d 2, 9, and 14 post-enhancement

Day/Treatment	Brown/Roasted Flavor	SEM
Day		
d 2	6.05 ^a	0.156
d 9	6.87 ^c	
d 14	6.47 ^b	
Treatment		
No Lac or Ace	5.92 ^a	0.138
Lac Con	6.72 ^b	
Lac + High Salt	6.43 ^b	
Lac + Ace	6.81 ^b	

^{abc}Means with different superscript letters differ ($P < 0.05$).

^dBrown/roasted flavor scored on a 0 to 15-pt scale.

Table 7. Least squares means for flavor^c attributes x day interactions for injection-enhanced strip loin steaks evaluated on d 2, 9, and 14 post-enhancement

	Day in MAP			SEM
	d 2	d 9	d 14	
Beef flavor ^c	4.95 ^a	6.35 ^b	5.35 ^a	0.325
Bloody/serumy flavor ^c	0.20 ^a	0.42 ^b	0.30 ^{ab}	0.064
Meat protein flavor ^c	1.65 ^c	1.06 ^b	0.90 ^a	0.202
Oxidized flavor ^c	0.39 ^a	0.28 ^a	1.01 ^b	0.106
Metallic flavor ^c	2.64 ^b	2.31 ^a	2.75 ^b	0.136
Stale flavor ^c	0.93 ^a	0.90 ^a	2.63 ^b	0.194

^{ab}Means with different superscript letters differ ($P < 0.05$).

^cFlavor scored on a 0 to 15-pt scale.

Table 8. Least squares means for salty flavor^c for strip loin steaks injection-enhanced with four solutions

Treatment	Mean	SEM
No Lac or Ace	4.79 ^a	0.138
Lac Con	4.86 ^a	0.138
Lac + High Salt	5.56 ^b	0.138
Lac + Ace	4.63 ^a	0.138

^{ab} Means with different superscript letters differ ($P < 0.05$).

^c Salty flavor scored on a 0 to 15-pt scale.

USING PH-ENHANCEMENT OF BEEF STEAKS TO IMPROVE CUSTOMER SATISFACTION.

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Key Words: Beef, Enhancement, Consumer

Introduction

The palatability of beef is one of the most important attributes in determining customer satisfaction. Beef consumers are willing to pay higher prices for more palatable beef than for less palatable beef (Platter et al., 2005). Therefore, methods of improving beef palatability would likely increase customer satisfaction.

Numerous researchers have shown that the pH of beef is related to tenderness and overall palatability (Purchas, 1990; Watanabe et al., 1995; Wulf et al., 2002). Meat pH can affect palatability by affecting postmortem protein degradation as well as water-holding capacity. Dransfield (1981) reported that beef with an abnormally high ultimate pH (dark, firm and dry beef) was considerably more tender than normal beef. Consequently, increasing meat pH has the potential to improve beef palatability and customer satisfaction.

Freezing Machines, Inc. (FMI) of Dakota Dunes, SD has developed and patented the use of ammonium hydroxide as a processing aid in the production of foodstuffs, including beef products (the technology is the subject of various issued and pending patents, and is validated to reduce or eliminate potential pathogens in meat products processed using the technology; other patent pending technology also includes dissolved carbon oxide in the injection process). Ammonium hydroxide has the potential to increase the pH of meat and meat products and may therefore also be useful in improving beef palatability. Ammonium hydroxide is a GRAS (“Generally recognized as safe” according to U.S. Food and Drug Administration) food additive commonly used as a leavening and/or pH control agent.

Objectives

Hypothesis: Increasing pH of fresh beef with FMI technology improves cooked beef palatability and customer satisfaction.

Objective: Determine the effect of pH enhancement with FMI technology on consumer acceptability of beef steaks.

Methodology

A 2 x 2 x 2 factorial design was used with two beef sources: Limousin cattle (LIMO) and Certified Angus Beef (CAB), two muscles: longissimus lumborum (LL) and semitendinosus (ST), and two treatments: control (CON) and pH-enhanced (PHE). The LIMO beef was obtained from cattle from a purebred Limousin breeder, and the CAB beef was obtained as boxed beef. Twelve subprimals were used for each source by muscle by treatment combination. Muscles designated for PHE were injected with a patent pending solution containing water, ammonium hydroxide, carbon dioxide, and salt.

pH was measured on raw steaks by homogenizing 10 g of muscle tissue with 90 g of water. Steaks from each subprimal and treatment combination were cooked on electric broilers to a target internal temperature of 71 degrees C. After cooling cooked steaks to room temperature, six 1.3-cm-diameter cores were removed from each steak, parallel to the muscle fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation, on a Warner-Bratzler shear machine. The average peak force of six cores was calculated for each steak as Warner-Bratzler shear force (WBS). Dividing the cooked weight by the raw weight and multiplying by 100 determined cooking loss.

Consumer panels were conducted 1 to 5 days after enhancement. Panelists were recruited from the Brookings, SD area using fliers and newspaper advertising. Two hundred eighty-eight consumers participated in the study over 12 different panel times. Steaks were cooked on gas grills, turning every 2.5 minutes, to a target internal temperature of 71 degrees C. Immediately following cooking, steaks were cut into uniform 1.3 by 2.5 cm samples using a sample sizing guide, placed into styrofoam bowls with holes punched in the bottom to allow juices to drain, covered with aluminum foil, and held in a 60 degree C warming oven until served. Panels were conducted in booths preventing panelist interaction. Prior to the start of the panel, panelists were given brief instructions about panel procedure and were asked to sign a notice of informed consent. All samples were served under red lights to limit differences in visual appearance. One sample of each treatment combination was served in a random order to the panel. The first sample was always a longissimus steak obtained from the SDSU Meat Lab and was used as a warm-up sample to prevent first-sample bias; this data was not included in the analysis of data nor were any conclusions drawn from that sample. Samples were coded with a random code to blind consumers to treatment combinations.

Results & Discussion

Carcass trait means for the 12 LIMO carcasses were 385 kg hot weight, 0.7 cm fat thickness, 107 sq. cm ribeye area, 1.54 USDA yield grade, and Slight 23 marbling score (data not presented in tabular form). These carcass traits indicated that the LIMO carcasses were lean, muscular, and high cutability with low marbling scores.

A wide range of consumer demographics were represented in the consumer panel (Table 1). A higher-than-normal proportion of young (18 to 29) consumers were sampled.

Steaks from LIMO had a higher pH (5.87 vs. 5.68) than steaks from CAB (Table 2). Meat pH was raised from 5.39 for CON to 6.16 for PHE. Steaks from LL had less cooking loss (25.2 vs. 34.6%) than ST steaks. Control steaks had less cooking loss (28.3 vs. 31.4%) than PHE steaks. A significant source by muscle interaction for WBS

indicated that the tenderness difference between LL and ST was greater for CAB (LL = 2.41 kg, ST = 4.17 kg) than for LIMO (LL = 3.29 kg, ST = 3.31 kg). A significant source by treatment interaction for WBS indicated that pH enhancement had greater effects on LIMO (CON = 4.13 kg, PHE = 2.47 kg) than on CAB (CON = 3.68 kg, PHE = 2.90 kg). A significant muscle by treatment interaction for WBS indicated that pH enhancement had a greater effect on LL (CON = 3.69 kg, PHE = 2.01 kg) than on ST (CON = 4.12 kg, PHE = 3.36 kg).

A significant source by muscle interaction for consumer “overall like”, consumer “like of tenderness”, consumer “like of juiciness”, consumer “like of flavor”, and consumer intent-to-purchase indicated that the differences between LL and ST were greater for CAB (LL vs. ST = 7.25 vs. 5.78 for “overall like”, 7.59 vs. 5.30 for “like of tenderness”, 7.18 vs. 5.52 for “like of juiciness”, 6.88 vs. 5.81 for “like of flavor”, 71 vs. 42% for intent-to-purchase) than for LIMO (LL vs. ST = 6.70 vs. 6.24 for “overall like”, 6.97 vs. 6.55 for “like of tenderness”, 6.53 vs. 6.32 for “like of juiciness”, 6.49 vs. 5.82 for “like of flavor”, 62 vs. 52% for intent-to-purchase). A significant source by treatment interaction for “overall like”, “like of tenderness”, and “like of juiciness” indicated that pH enhancement had greater effects on LIMO (CON vs. PHE = 5.56 vs. 7.27 for “overall like”, 5.61 vs. 7.90 for “like of tenderness”, 5.41 vs. 7.43 for “like of juiciness”) than on CAB (CON vs. PHE = 5.83 vs. 7.19 for “overall like”, 5.62 vs. 7.27 for “like of tenderness”, 5.62 vs. 7.07 for “like of juiciness”). pH enhancement resulted in higher “like of flavor” ratings (CON = 5.51, PHE = 7.00) and more “Yes” responses to the question, “Would you be likely to purchase this steak?” (CON = 43%, PHE = 71%).

Overall, treatment explained 68%, muscle explained 28%, and source explained 4% of total explained variation in “overall like” ratings (data not presented in tabular form). The effect of pH enhancement on palatability traits was very large. pH enhancement lowered WBS by 46% in LL steaks and by 18% in ST steaks. Overall, pH enhancement increased “overall like” ratings by 1.58 units. This 1.58 unit increase in “overall like” from pH enhancement was much greater than the muscle effect (CON LL – CON ST = 0.86 units) and the source effect (CON CAB – CON LIMO = 0.27 units). Consumers rated PHE beef higher than CON beef for all three palatability traits – tenderness, juiciness, and flavor. Based on these observations, it appears that pH enhancement offers great potential for enhancing beef steak palatability and improving beef customer satisfaction.

Conclusions

pH enhancement using the FMI technology was very effective at improving palatability of beef steaks. pH enhancement lowered Warner-Bratzler shear force and increased consumer ratings for “overall like”, “like of tenderness”, “like of juiciness”, and “like of flavor”. pH enhancement increased consumer intent-to-purchase from 43% to 71%. pH enhancement offers great potential for enhancing beef steak palatability and improving beef customer satisfaction, in addition to its other favorable attributes as a processing aid.

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Tables and Figures

Table 1. Demographic profile of 288 consumer panelists.

	Number of consumers	% of consumers
<u>Age</u>		
18 to 29	174	60
30 to 39	29	10
40 to 49	41	14
50 to 59	27	9
60 and older	17	6
<u>Household Annual Income</u>		
Under \$20,000	159	56
\$20,000 to \$39,000	58	20
\$40,000 to \$59,000	35	12
\$60,000 and higher	33	12
<u>Working Status</u>		
Not employed	29	10
Part-time	39	14
Full-time	125	44
Student	93	33
<u>Gender</u>		
Male	136	47
Female	151	53
<u>Times per week beef is consumed</u>		
0	8	3
1 to 2	94	33
3 to 5	110	38
6 to 8	53	18
9 or more	22	8
<u>Times per month steak is consumed</u>		
0	29	10
1	66	23
2	65	23
3 to 5	95	33
6 or more	32	11

Table 2. Least-squares means for meat pH, cooking loss, and Warner-Bratzler shear force (WBS) by source (LIMO = Limousin cattle, CAB = Certified Angus Beef), muscle (LL = longissimus lumborum, ST = semitendinosus), and treatment (CON = control, PHE = pH-enhanced).

Source	Muscle	Treat- ment	Meat pH	Cooking Loss, %	WBS, kg
LIMO	LL	CON	5.41	24.0	4.47
LIMO	LL	PHE	6.30	25.9	2.11
LIMO	ST	CON	5.47	33.4	3.80
LIMO	ST	PHE	6.28	35.4	2.82
CAB	LL	CON	5.36	23.0	2.90
CAB	LL	PHE	6.13	28.0	1.91
CAB	ST	CON	5.32	33.0	4.45
CAB	ST	PHE	5.91	36.5	3.89
Pooled SEM			0.08	1.9	0.22
<u>Probability of no effect</u>					
Source (S)			0.002	0.728	0.952
Muscle (M)			0.336	<0.001	<0.001
Treatment (T)			<0.001	0.022	<0.001
S X M			0.185	0.953	<0.001
S X T			0.135	0.404	0.005
M X T			0.243	0.794	0.005
S X M X T			0.671	0.773	0.128

Table 3. Least-squares means for consumer ratings (10 = like extremely, 1 = dislike extremely) by source (LIMO = Limousin cattle, CAB = Certified Angus Beef), muscle (LL = longissimus lumborum, ST = semitendinosus), and treatment (CON = control, PHE = pH-enhanced).

Source	Muscle	Treat- ment	Overall Like	Like of Tender- ess	Like of Juiciness	Like of Flavor	Would Purchase, %
LIMO	LL	CON	5.74	5.63	5.47	5.62	46
LIMO	LL	PHE	7.66	8.30	7.59	7.36	78
LIMO	ST	CON	5.39	5.60	5.35	5.02	36
LIMO	ST	PHE	7.08	7.51	7.28	6.63	67
CAB	LL	CON	6.52	6.79	6.45	6.17	59
CAB	LL	PHE	7.98	8.38	7.91	7.58	82
CAB	ST	CON	5.15	4.45	4.79	5.21	29
CAB	ST	PHE	6.40	6.15	6.24	6.42	55
Pooled SEM			0.13	0.17	0.13	0.14	3
<u>Probability of no effect</u>							
Source (S)			0.630	0.007	0.424	0.059	0.822
Muscle (M)			<0.001	<0.001	<0.001	<0.001	<0.001
Treatment (T)			<0.001	<0.001	<0.001	<0.001	<0.001
S X M			<0.001	<0.001	<0.001	0.047	<0.001
S X T			0.017	0.006	0.003	0.066	0.102
M X T			0.258	0.172	0.614	0.422	0.698
S X M X T			0.951	0.062	0.626	0.850	0.675

**BLENDED LIPID SOLUTIONS AS A FUNCTIONAL INGREDIENT TO
ENHANCE LOW QUALITY BEEF**

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Keywords: marbling, beef tallow, safflower oil

Introduction

USDA beef carcass quality grades are based on carcass maturity and the amount of marbling or intramuscular fat present in the exposed surface of the *longissimus dorsi* muscle at the 12th-13th rib interface (USDA-AMS, 1997). Smith et al. (1984) reported minute, but statistically significant differences in meat palatability (juiciness, tenderness, and flavor) as the degree of marbling decreased from Moderately Abundant (USDA Prime) to Practically Devoid (USDA Standard).

The palatability of whole muscle cuts fabricated from lower quality (less than USDA Choice) beef carcasses may be improved through innovative non-meat ingredient and processing technologies. Development of a blended lipid solution that can be directly injected into lower quality whole muscle beef cuts (USDA Select or lower) may enhance its overall palatability by mimicking the organoleptic properties of fat and have an appearance similar to that of marbling.

Quinlan and Osburn (2004a) determined optimal combinations of non-meat ingredients that resemble the appearance and functional properties of marbling to determine their feasibility in development of an injectable “modified marbling” solution. A solution of calcium alginate, iota carrageenan, whey protein isolate and modified potato starch was deemed feasible. However, the authors noted that gel solution firmness and hydrophobicity must be improved to enhance gel particle definition and minimize the absorption of the meat pigments within the meat matrix.

A subsequent study (Quinlan and Osburn, 2004b) compared the effect of injecting a solution containing sodium alginate (1%), iota carrageenan (0.4375%), whey protein isolate (1.5%) modified food starch (0.375%), beef tallow (3%) and beef flavoring (0.25%) on the quality attributes of USDA Select ribeye rolls. Injected ribeyes were higher ($P<0.05$) compared to the control (USDA Select, no injection) in beef fat flavor. However a slight sensory off-flavor was found ($P<0.05$) in the injected ribeye which corresponded to higher TBARS values ($P<0.05$). The authors concluded that improvements must be made to minimize the off-flavor. Additionally, the evaluated solution continued to hydrate and become more viscous after mixing and during injection, which created problems in attaining the desired targeted percent injection level and obtaining proper dispersion of the solution into the meat matrix.

Blends of fatty acids may be manufactured as a “modified lipid” solution that could be incorporated into meat products to mimic the functional and organoleptic properties of

intramuscular fat (marbling) in whole muscle meat subprimals. As an added benefit, meat products enriched with oleic acid may be legitimately promoted as a functional food.

Objectives

Hypothesis: Development of a blended lipid solution containing beef tallow and safflower oil can mimic the organoleptic properties of intramuscular fat and enhance the quality of lower quality beef subprimals.

Objective: To develop an injectable lipid solution that is similar to intramuscular fat in appearance and functionality, but superior in nutritional quality.

Materials and Methods

Preliminary Study

A preliminary study determined that a blend of 30-60% of beef tallow and 40-70% safflower oil could produce blended lipid solutions that could be pumped through a hand held brine pump at 4.4, 7.2 and 10°C. When injected into boneless beef inside round muscles the injected solution created fat-like particles that looked like marbling. These results indicated that blended lipid solutions could be manufactured with varying solidification points and acceptable viscosity for mechanical injection systems.

Lipid Solution Formulation and Manufacture

Based on the results of the preliminary study, varying percentages of rendered beef tallow (BT, Proliant, Inc, Ames IA) and high oleic safflower oil (HOSO, Montola Growers Inc, Culbertson, MT) were formulated to produce 1000g lipid solutions containing 50/50, 53/47, 57/43 and 60/40 percent of BT and HOSO respectively, and then blended at either 22° or 32°C. Lipid solutions blended at 22°C were formulated by weighing appropriate amounts of refrigerated (7.2°C) BT and HOSO to achieve the desired percentage of each ingredient and placed into 1000 mL beakers, covered with plastic and aluminum foil and tempered at 22°C for 72 h.

Lipid solutions blended at 38°C were formulated as previously described with the exception that the samples were kept in refrigerated storage (7.2°C) for 72 h prior to blending. At Day 0 each BT/HOSO x temperature treatment combination (n=8) was blended in a randomly assigned order. Lipid solutions manufactured at 22°C were blended by pouring the appropriate amount of HOSO into a Waring blender, followed by addition of one-third of the required amount of BT. The lipids were blended for 30 sec. on a high speed setting until all the tallow was thoroughly mixed.

After the initial mixing, the remaining BT was added and the lipid solution mixed an additional 30 sec. In producing the 32°C blended lipid solution treatments, the pre-weighed refrigerated HOSO was poured into a 2000 mL beaker, placed on a heated stir plate (set on medium heat and medium stirring speed) and heated for 7 min until the oil reached 26°C. One-half of the required BT was then added to the HOSO and the mixture heated to 26°C (13 min). The remaining BT was added and blended an additional 5 min until the solution reached 32°C (25 min total).

Temperature of the lipid solution was continually monitored using a copper constantan thermocouple (Omega Engineering Inc; Stamford, CT). Immediately following mixing of either treatment, the appropriate amounts of each lipid solution blend were weighed into various containers, placed in a refrigerated cooler until the solutions reached 7.2°C and then analyzed for pH, viscosity and fatty acid composition (Day 0) and for color (L*, a* and b*) and thiobarbituric acid reactive substances (TBARS); (Day 0, 3 and 7).

pH

Lipid solution samples (10g) were homogenized using a Polytron homogenizer (Kinematica, Switzerland) with 90 mL of dd H₂O and readings taken using an Accumet pH meter. Readings were measured at 22°C.

Color

A Minolta Colorimeter (CR-300, Minolta Co., Ramsey, NJ) with a 10° standard observer and an 8 mm reading orifice was used to measure the L* (lightness), a* and b* values of the exterior surface color of each lipid solution. On each day of manufacture lipid solution samples (50 g) were placed in petri dishes (n=2) covered with Saran™ wrap and refrigerated (2°C) to allow the solutions to cool and solidify (7°C) before measurements were taken on Day 0. After color measurements were taken, all samples were placed in a retail display case at (2°C; 1200 lx) and color readings taken on Day 3 and 7 of storage.

Viscosity

The viscosity of each lipid solution (50 g) was measured using a Brookfield Viscometer (Model DV-II, Brookfield Engineering, Co., Stoughton, MA) at speed setting 12 and a temperature of (12.7°C). The selected spindle (4) was lowered into the geometric center of the lipid solution until the indented ring on the spindle was level with solution surface. Viscosity readings were recorded in centipoise (cPs) once the displayed reading was stabilized.

Thiobarbituric Acid Reactive Substances Analysis (TBARS)

Thiobarbituric acid reactive substance (TBARS) analysis was conducted on Day 0, 3 and 7 to monitor oxidative rancidity. Four replicates were run for each 30g lipid solution sample according to methods established by Tarladgis and others (1960) and Zipser and others (1962) as modified by Rhee (1978).

Fatty Acid Analyses

The total lipid of each sample was extracted and the fatty acids measured with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler (Varian Inc., Walnut Creek, CA). Separation of fatty acid methyl esters was conducted on a silica capillary column CP-Sil88 (Chrompack Inc. Middleburg, The Netherlands) with helium

as the carrier gas (1.2mL/min). After 32 min at 180°C, the oven temperature was increased to 20°C/min to 225°C and held for 13.75 min.

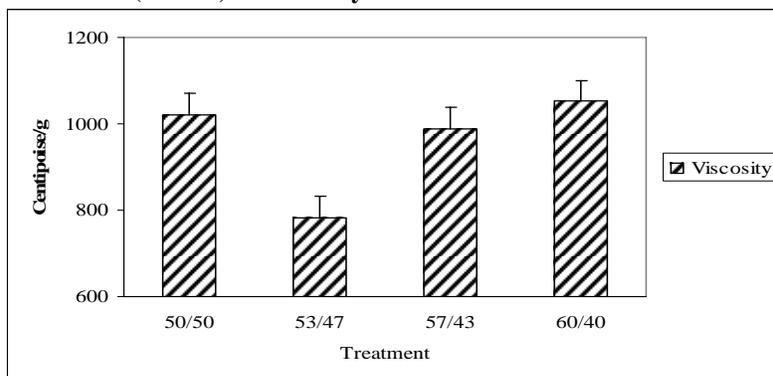
Experimental Design and Statistical Analysis

A two-way analysis of variance with four BT/HOSO combinations and two blending temperatures (n=8) was replicated twice (N=16). For color determinations data was analyzed as a repeated measures design using sample x BT/HOSO x day as the error term. For viscosity measurements, sample temperature was used as a covariate. A predetermined level of significance (P<0.05) was used and Tukey's Least Significant Difference determined differences between attribute means (SAS, Version 9.0).

Results and Discussion

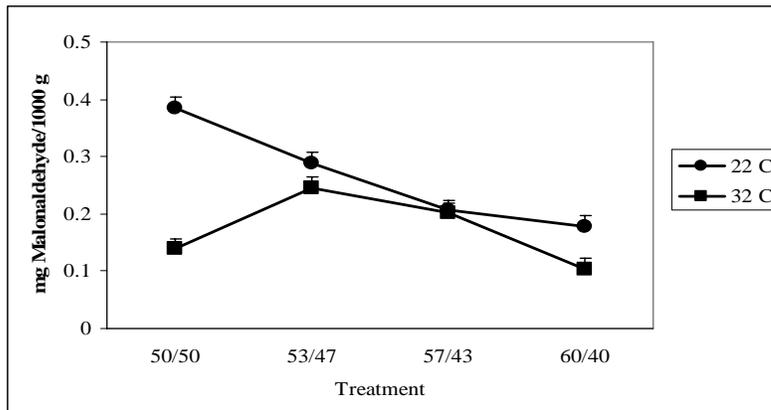
The viscosity of the lipid solution blends were similar among the 50/50, 57/43, and 60/40 BT/HOSO solutions but was considerably lower (P < 0.01) for the 53/47 solution (Fig. 1). Temperature at mixing had no effect (P = .66) nor did the temperature of the lipid solution blend at the time of measurement affect overall viscosity of the lipid solutions. Solutions blended at 22°C averaged 992.54 cPs/g versus those blended at 32°C which averaged 930.50 cPs/g. Solution temperatures ranged from 12.9 to 14.8°C. As the percent BT increased, the viscosity increased for all solutions with the exception of the 53/47 blend. The decrease in viscosity may be due to differences in mixing (Waring blender for 22°C solutions versus a stir bar for the 32°C solutions).

Figure 1. Least squares means for main effect of percent BT/HOSO (P<0.05) on viscosity values



Sample pH values were not different (P>0.05) ranging in value from 6.18 to 6.58. A BT/HOSO x day (P<0.05) and BT/HOSO x temperature (P<0.01) interaction was observed for lipid solution TBARS values. As length of storage increased, TBARS values tended to decrease (Fig. 2a), particularly for solutions containing greater percentages of BT. This same trend was observed with respect to blending temperatures (22 or 32°C; Fig. 2b). The BT contains a higher percentage of saturated fatty acids and is less susceptible to lipid oxidation.

Figure 2b. Least squares means of percent BT/HOSO x temperature interaction for TBAR values among lipid solutions



Lipid solution L* values (Fig. 3a and 3b) showed a BT/HOSO x day ($P < 0.05$) and temperature x day interaction ($P < 0.01$) was observed. As length of storage increased, L* values tended to increase. Solutions blended at 22°C tended to have constant L* values throughout the 7 day storage period while solutions blended at 32°C exhibited lower L* values on Day 0 but exhibited an increase in L* values throughout storage. This could be attributed to the yellow color observed when beef tallow and safflower oil were heated during blending while solutions blended at 22°C remained white in color. The a* and b* mean values for all lipid solutions were affected by a temperature x day interaction ($P < 0.05$). The solution a* values ranged from -5.10 to -2.36 while the b* values ranged from 4.26 to 9.82.

Figure 3a. Least squares means of BT/HOSO x day for L* values of lipid solutions

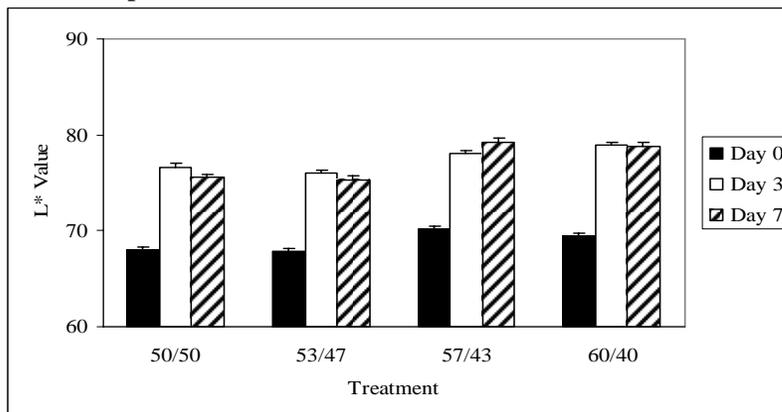
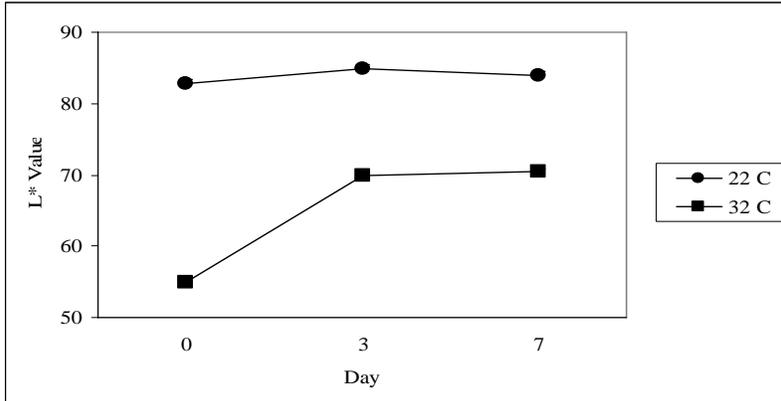


Figure 3b. Least squares means of temperature x day interaction for L* values among lipid solutions



The fatty acid composition of BT, HOSO and lipid solutions are shown in Table 1. Additionally intramuscular fat (marbling) from Angus based cattle fed a commercial corn-fed diet was analyzed. As the percent of BT increased the amount of stearic acid (18:0) increased, while the amount of oleic acid (18:1) and linoleic acid (18:2) decreased. However, when compared to intramuscular fat, although not statistically analyzed, the lipid solutions were much higher in oleic and linoleic acid and lower in stearic acid. Additionally the ratio of saturated to unsaturated fatty acids tended to increase as the amount of BT increased, but was at least 2.5 times lower compared to intramuscular fat. These results indicate that the blended lipid solutions manufactured in this study may be a healthy and nutritious “marbling substitute” if injected into less marbled whole muscle beef subprimals.

Table 1. Least squares means for fatty acid composition among lipid solutions

Item	Lipid Solutions ^d						IM ^e
	BT	HOSO	50/50	53/47	57/43	60/40	
14:0	2.20	0.63	1.16c	1.25b	1.34a	1.40a	3.50
16:0	23.1	3.90	13.8c	14.6bc	15.1ba	15.8a	29.3
16:1	2.30	0.25	1.32c	1.37c	1.46b	1.56a	2.91
18:0	17.5	1.45	9.07b	9.45ba	10.1ba	10.4a	21.7
18:1	38.2	70.5	61.5a	60.9ba	58.6ba	57.9b	38.1
18:2	2.20	12.6	8.63a	8.38ba	7.79bc	7.54c	2.90
Ratio ^f	1.00	0.07	0.34c	0.36bc	0.39ba	0.41a	1.33

^{abc}Least square means without common superscript differ (P<0.02)

^dLipid Solutions = blends (%) of beef tallow/safflower oil

^eIM = average composition of dissected intramuscular fat (marbling) from two Angus steers fed a corn-based diet

^fRatio = saturated vs. unsaturated fatty acids

Conclusions

This study investigated the feasibility of developing an injectable lipid solution that is similar to intramuscular fat in appearance and functionality, but superior in nutritional quality. We conclude that a blend of BT and HOSO may possess the desired functional (viscosity, color) and nutritional (high oleic acid content, low saturated to unsaturated

fatty acid ratio) attributes to improve the quality of lower marbled beef cuts. Further studies will determine if these solutions can be injected into beef subprimals via an automatic injection system to create fat particles resembling marbling when fabricated into steaks.

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**EFFECT OF THAWING RATE ON DISTRIBUTION OF AN INJECTED SALT
AND PHOSPHATE BRINE IN BEEF**

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Key Words: moisture enhancement, beef, brine, brine distribution, thaw rate

Introduction

The problem of striping in injected products has been noted in the literature repeatedly over the decades (Voyle et al., 1986; Knight and Parsons, 1988; Gooding et al., 2004). It was seen first in cured products and was solved using post-injection tumbling. Today, striping is a problem in moisture enhanced meats. These are injected at low levels (108-112%), and generally do not undergo a tumbling treatment because the product is marketed raw and must still appear desirable in the retail case. There have been some suggestions made and equipment constructed to change the manner in which meat is injected, in order to minimize striping (Freixenet, 1993), yet the problem still exists. Possibilities for the effect that non-nitrite/ate containing brines have on meat colour have been postulated (Swatland, 2004), and preferential brine movement from the injection site, along muscle fiber long axes, has recently been reported (Gooding et al., 2004), confirming speculation by Swatland (2004, and in Voyle et al., 1986). Since there is evidently something about the internal structure of the meat which restricts brine flow, a pre-injection treatment which disturbs this structure but which minimally changes the external appearance would be useful. Freezing and thawing rates have been known to affect internal meat structure to varying degrees through cellular disruption by ice crystal formation (Gonzalez-Sanguinetti, 1985; Judge et al., 1989; Ambrosiadis, et al., 1994; Mandigo and Osburn, 1996).

Objectives

There were two objectives in this research, firstly, to determine the brine distribution path after injection. Secondly, to determine if the beef packers' practice of freezing excess boxed meat for a time may be beneficial to brine distribution once thawed meat is injected.

Methodology

Ribeyes (*longissimus thoracis*, LT) and eye of rounds (*semitendinosus*, ST) were collected from the right side of chilled carcasses of marketweight Angus-cross steers. Meat was trimmed of all fat, vacuum packed and aged at 3 °C for a week. Two thirds of

the samples of each muscle type were then frozen (-35 °C) for a month. The other third was injected on an InjectStar® “New Twist” BI-72 with two rows (50% offset) of single 4mm-diameter needles spaced 2.5cm apart. Brine pressure was 1.5-2 bar and head speed was 60 strokes/min for LT’s and 44 strokes/min for ST’s. LT’s were oriented dorsal surface upward for injection, and ST’s were placed rounded surface upward. Injection was to 108-110% of initial muscle weight. Brine composition was 4.8% salt, 4.8% sodium tripolyphosphate, 200ppm FDC Blue #1, and reverse osmosis water. Injected samples were stored on trays at 3 °C overnight, then cut to expose four faces and photographed for image analysis to determine the percentage of blue area. Faces were: 1) parallel to (and along) injection needle sites, 2) perpendicular to needles, 3) parallel to fasciculi, and 4) perpendicular to fasciculi. The ST is a muscle with a parallel structure therefore one cut exposed both faces 1 and 4, and a second cut exposed faces 2 and 3. Of the frozen samples, half were thawed slowly at 3°C in air over two days, the other half were thawed quickly in 12-17°C water over 5 hours. All then underwent the same process as the fresh samples.

Results & Discussion

Table 1 summarizes brine injection levels by muscle and treatment. There were no significant differences.

Subjective observations of exposed faces revealed a number of consistent features for each muscle type across all treatments. On Face 1 (parallel to needles) of the LT, brine was found to be deposited in the top 2/3 to 3/4 of the muscle, and in many cases there were blue stripes with the same spacing as needles. These were fairly clearly visible near the center of the face indicating poor lateral brine distribution here, while nearer to the edges they became blurred, indicating better brine distribution. Brine appeared to be fairly consistently injected throughout the needle path as was evidenced by uniform thickness of the blue bands. Faces 3 (parallel to fasciculi) and 4 (perpendicular to fasciculi) very clearly showed preferential movement of brine in fibre direction. Face 3 showed individual penetration sites by blue dots often near the center of the face, indicating minimal brine deposition, whereas the edges to either side appeared quite blue, often in somewhat blurred fine stripes that followed fibre direction. Face 4 showed areas of blue limited by intramuscular connective tissue, likely the perimysium. This applied to brine movement both within and between fasciculi. In the former case, brine appeared to be restricted within the fasciculi whereas in the latter case brine between fasciculi appeared to continue to move between groupings preferentially to moving far into adjacent fasciculi. In several samples this movement along fibres was particularly well-illustrated through the contrast between Faces 1 and 3, for although needle entry depth was clearly only 2/3 of meat depth on Face 1, Face 3 showed dyed brine along the full length of the fibres. Face 2 (perpendicular to needles) consistently showed that the best brine distribution was along muscle edges, while the poorest was at the center of the cranial end.

Faces 1 & 4 (parallel to needles and perpendicular to fasciculi) were exposed by the same cut on the ST. Needle penetration depth was similar to the LT, and lateral brine movement (ie across fasciculi) was clearly shown to be limited. Injection sites in the form of clear blue lines were obvious, particularly near the center of the sample. Faces 2 & 3

(perpendicular to needles and parallel to fasciculi) were also exposed by one cut. Best brine distribution was at the edges of the muscle, and the poorest, as evidenced by blue dots or even just holes where needles entered, was generally in a band just off-center of the longitudinal axis. Preferential movement of brine along muscle fibres was evident. There is a transverse band of connective tissue in the ST, and in a number of cases, this was clearly a barrier to longitudinal brine movement. In both the LT and ST, fat was unaffected, with little evidence of opportunistic brine movement along the edges of fat deposits. In cases where needles pierced vascular tissues, brine entered, but did not seem to continue into muscle tissues at distant points, possibly because of insufficient pressure.

Strongly characteristic of both muscles, although to differing degrees, was the center portions which were often all but free of brine. This is likely due to the inevitably uneven pressure exerted by the stripper bar (a single piece, on this equipment) on a meat sample of non-uniform height. A greater intramuscular pressure builds under the high points of the sample where the bar is pressing, than under the low points where it is not. Internal meat pressure under the high points appears to be greater than brine pressure so little or no brine can enter the tissue. Equipment does exist in which the stripper bar is reduced to a series of stripper blocks, possibly with the aim to minimizing this problem.

The effect of thawing rate is presented in Table 2. Lack of significance in the percentage of area dyed blue across treatments within a face shows that neither a fast thaw nor a slow thaw treatment affected brine distribution. Since thaw treatment would be expected to disrupt the intrafascicular structure, this null effect offers evidence that lateral brine movement is restricted in some manner by the connective tissue.

Conclusions

In LT and ST samples injected to approximately 110%, direction of brine movement appeared to be governed by intramuscular connective tissue, causing brine to run lengthwise within or between muscle bundles more readily than across them. Also, in areas where pressure within the muscle could have exceeded brine pressure, brine was unable to penetrate. The cellular disruption caused by different thawing rates did not affect the extent of brine distribution, providing evidence that intramuscular connective tissue may play a significant role in restricting brine movement.

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Tables and Figures

Table 1: LSMeans LT and ST % pump

Treatment	n	LT		n	ST	
		(% pump)	SE		(% pump)	SE
Non-frozen	6	109.5	0.603	12	109.5	0.973
Fast Thaw	9	108.93	0.492	13	110.8	0.935
Slow Thaw	12	108.98	0.426	12	109.99	0.973

Table 2: LSMeans LT and ST % blue by face, across treatments

Treatment	n	LT					SE	ST			
		Face*						Face			
		1	2	3	4	SE		n	1 & 4	2 & 3	SE
% Blue											
Non-frozen	6	29.4	50.7	42.7	50.8	3.4	12	28.2	54.2	3.5	
Fast Thaw	9	34.0	46.9	41.6	43.7	2.9	13	34.1	51.4	3.4	
Slow Thaw	12	35.5	46.9	49.0	46.4	2.4	12	32.2	50.5	3.5	

* Face 1= parallel to needles; 2 = perpendicular to needles; 3 = parallel to fasciculi; 4 = perpendicular to fasciculi

EARLY POST-MORTEM ENHANCEMENT COUPLED WITH ACCELERATED CHILLING TO IMPROVE PORK QUALITY

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Key Words: Pork, Early Post-Mortem, Accelerated Chilling, Enhancement

Introduction

With the incidence of pale, soft, and exudative (PSE) pork still prevalent in the pork supply and causing significant losses (Benchmarking Value in the Pork Supply Chain, 2003), ways to combat this problem both ante- and post-mortem are being explored. One such method is the use of accelerated chilling (i.e.: blast chill, spray chills, emersion in super chilled solutions, etc.). While this method has shown mixed results, the increased rate of temperature decline can lead to improved loin quality (Crenwelge, et. al., 1984; Weakley, et. al., 1986; Ohene-Adjei, et. al., 2003)

Enhancement technology in whole muscle cuts has seen an increase since initial work done in the 1980's (Smith, et. al., 1984). Initially brines would include salt and phosphates, but today lactates, flavorings, and other compounds are common. These brine solutions have been shown to help improve color, decrease purge loss, and improve sensory attributes (Banks, et. al., 1998; Detlenne and Wicker, 1999; Sheard, et. al., 1999; Prestat, et. al., 2002).

While both accelerated chilling and enhancement have been studied, the coupling of these two technologies has minimal research.

Objectives

The purpose for this study was to evaluate the effects of enhancement early post-mortem, coupled with accelerated chilling and to determine if these treatments will have an additive effect when evaluating a variety of quality measurements.

Methodology

A total of 16 pigs were harvested over two days. At 1 h post-mortem (accelerated chilling) the loin from the right side was excised and cut into two sections (anterior and posterior). These loin sections were alternately assigned to enhancement with a 0.4% salt, 0.4% phosphate, and 2.5% lactate in the finished product at 112% of green weight (Brine Temp $\approx 4^{\circ}\text{C}$). Once the loin section was enhanced, both sections were vacuum packaged and submerged in a super-chilled solution (starting temp. of -27°C) for 1 h. At 24 h post-mortem, the loin from the left side was removed and cut into two sections. The

corresponding section to the right side was enhanced with the same brine. All sections were vacuum packaged and stored at 4°C until 10 d post-mortem.

At 10 d post-mortem, vacuum bags were opened and loin sections removed to determine percent purge loss. From each loin section, a chop was removed from the face of the loin to expose a fresh surface for evaluation. After 15 min of “bloom”, subjective scores for color and marbling (NPPC, 1999), firmness (NPPC, 1991), and striping (Gooding, 2003) were evaluated. Objective measurements for color using a Minolta Chromameter CR-300 (Minolta Camera Co., Japan, illuminant D65 and 0° observer) were recorded. Lastly, pH was measured using an SFK Star Probe (SFK Technologies, Cedar Rapids, IA).

After evaluation, 2.5 cm chops were cut for Warner-Bratzler shear force determination and trained sensory analysis. Once cut, these chops were vacuum packaged and frozen at -30°C until the aforementioned tests could be conducted. Chops for the trained sensory panel were allowed to thaw at 4°C, trimmed to a uniform size, and cooked on a Farberware open hearth grill (Model 455N, Walter Kidde, Bronx, NY). Chops were cooked to an internal temperature of 70°C, before being served to a six member trained panel. Panelists evaluated samples for juiciness, tenderness, and off-flavor using a 15 cm anchored unstructured line scale (0 = extremely tough, extremely dry, and no off-flavor; 15 = extremely tender, extremely juicy, and extreme off-flavor).

Chops for Warner-Bratzler shear force were treated in the same fashion as that for sensory analysis. After cooking, chops were allowed to cool to 25°C before four, 1.3 cm cores were removed parallel to the orientation of the muscle fibers. Cores were sheared on an Instron® universal testing machine (Model 112). The shear force from each of the four cores was averaged together to give one shear value for each chop.

Data were analyzed utilizing the MIXED procedure in SAS (1999). The experiment was designed as a 2 x 2 factorial arrangement with chilling method: accelerated (AC) vs. conventional (CC) and enhancement: enhanced (EN) vs. non-enhanced (NE) as treatments. The model included the fixed effects of harvest day, chilling method, enhancement, and chilling*enhancement. Pig nested within harvest day as a random variable.

Results & Discussion

With the absence of many chill*enhancement interactions, the main effects for chill and for enhancement were evaluated. As indicated in Table 1, accelerated chilling caused an increase ($P \leq 0.05$) in the amount of purge loss after 10 d post-mortem. During the accelerated chilling process, loin sections became partially frozen while in the -27°C solution. Upon the removal of the loin sections, the freeze/thaw process may have caused this increase in the amount of purge present. With the use of accelerated chilling, there was a decrease ($P \leq 0.05$) in the $L^*a^*b^*$ values (darker, less red, less yellow). Subjective scores for color and striping increased ($P=0.049$ and $P=0.032$ respectively), while scores for marbling and firmness did not change ($P=0.054$ and $P>0.05$ respectively). Although significant differences ($P \leq 0.05$) were indicated by the subjective evaluations, accelerated chilling resulted in values that were only 0.2 units higher for both color and striping. The ability to visually detect these differences would be difficult. Accelerated chilling resulted in a lower ($P \leq 0.05$) percent cook loss and higher ($P \leq 0.05$) juiciness scores. There

was no difference ($P>0.05$) for tenderness and off-flavor. Using accelerated chilling, loin sections underwent a very rapid decrease in the internal temperature of the meat. A rapid decrease in temperature, would help protect proteins from the detrimental low pH/high temperature phenomenon, and would result in the improved quality.

As presented in Table 1, the use of enhancement resulted in a difference ($P\leq 0.05$) for all traits measured except for subjective marbling scores ($P>0.05$). Percent purge at 10 d post-mortem and percent cook loss were lower ($P\leq 0.05$), objective color scores ($L^*a^*b^*$) were lower ($P\leq 0.05$), and subjective scores for color, firmness, and striping were all higher ($P\leq 0.05$). While enhancement did result in increased striping scores, (1.5 vs. 1.1) these differences were minimal. As described by Gooding (2003) a score of 1.0 (1-5) indicated no striping and 2.0 indicated faint striping. With a score of 1.5, the striping in the enhanced sections would be difficult for an untrained person to observe. Trained taste panel evaluation indicated that enhanced pork was juicier, more tender, and had more off-flavor than non-enhanced pork ($P\leq 0.05$). The off-flavor that was detected by the trained taste panel may have been the result of some panelist detecting an excessive amount of salty taste that can be associated with enhanced pork. However, while the panel was able to detect more of an off-flavor in the enhanced pork, the mean value was 0.9 vs. 0.3 on a 15 point scale. Both of these values indicate minimal off-flavor. With the brine pH around 7.2, the use of enhancement was able to buffer up the pH in the enhanced loin sections (Table 2). With the increased pH, this would result in the binding of more water by the proteins found in the meat. This would lead to the improvement in percent purge and corresponding increase in juiciness. In addition, with more bound water, there would be less light reflectance at the surface of the loin, resulting in the darker color.

The addition of harvest day to the model was used to help control any day to day variation that would have been present. The exploration of the effect of harvest day indicated that during the second harvest day, the accelerated chilling sections were enhanced to a higher percentage than on the first day. This may have resulted in the significant interactions for 10 d pH (Table 2). In addition, the significant interaction for shear value may be explained by the aforementioned freezing of the accelerated chilling loin sections. As indicated in Table 2, the non-enhanced accelerated chilled section had the highest mean shear value (not significantly different ($P>0.05$) than the non-enhanced conventional chilled section), while the enhanced accelerated chilled section has the lowest mean shear value (not significantly different ($P>0.05$) than the enhanced conventional chilled section). Through the freezing that occurred in the accelerated chilling sections, the non-enhanced section may have undergone more freezing than the enhanced loin due to the absence of the brine solution. This extra freezing, may have damaged more of the proteolytic enzymes and hence less tenderization.

Conclusions

With the prevalence of pale, soft, and exudative pork still a problem in the pork industry, ways to prevent lower quality pork are advantageous. In this experiment, the use of enhancement was coupled with accelerated chilling in an effort to improve quality. The main effects of accelerated chilling and enhancement independently improved quality, but when coupled together, the improvement in quality was greater than the

individual treatments alone. Hence, if a processor was able to couple these technologies together, they may be able to improve quality and increase consumer satisfaction.

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Tables and Figures

Table 1. Main Effects of Chilling Treatment and Enhancement Treatment

	Chill		SEM	P-value	Enhancement		SEM	P-value
	AC	CC			EN	NE		
10d Purge, %	3.42	2.74	0.33	0.0368	2.61	3.56	0.33	0.0046
Minolta L*	47.99	50.48	0.65	<.0001	47.02	51.45	0.65	<.0001
Minolta a*	7.80	8.45	0.19	0.0003	7.23	9.02	0.19	<.0001
Minolta b*	4.98	5.75	0.24	<.0001	4.35	6.39	0.24	<.0001
Color ^a	3.4	3.2	0.13	0.0491	3.6	3.1	0.13	0.0002
Marbling ^b	2.2	2.4	0.12	0.0544	2.4	2.3	0.12	0.4017

Firmness ^c	3.4	3.3	0.11	0.6846	3.5	3.2	0.11	0.0181
Striping ^d	1.4	1.2	0.08	0.032	1.5	1.1	0.08	0.0003
Cook Loss, %	19.70	22.49	0.94	0.0154	18.18	24.00	0.94	<.0001
Juiciness ^e	8.7	8.0	0.25	0.0125	9.4	7.2	0.25	<.0001
Tenderness ^f	9.2	8.7	0.33	0.0937	10.2	7.7	0.33	<.0001
Off-Flavor ^g	0.6	0.6	0.33	0.684	0.9	0.3	0.33	0.0002

Means are significantly different at $P \leq 0.05$

^a NPPC, 1999 (1-6)

^b NPPC, 1999 estimated % lipid

^c NPPC, 1991(1-5)

^d Gooding, 2003 (1=None 5=Severe)

^e 0 = Extremely Dry, 15 = Extremely Juicy

^f 0 = Extremely Tough, 15 = Extremely Tender

^g 0 = None, 15 = Extremely Intense

Table 2. Chill * Enhancement Treatment Interactions

	Enhanced		Non-Enhanced		SEM	Interaction <i>P</i> -value
	AC	CC	AC	CC		
pH @ 10d	5.84 ^a	5.73 ^b	5.55 ^c	5.54 ^c	0.02	0.0032
WBS, kg	2.27 ^a	2.54 ^a	3.13 ^b	2.93 ^b	0.11	0.0277

^{abc}Means within the same row with different superscripts are different at $P \leq 0.05$

**THE EFFECT OF HOT BONING AND REDUCED PHOSPHATE ON THE
PROCESSING AND SENSORY PROPERTIES OF CURED BEEF PREPARED
FROM TWO FOREQUARTER MUSCLES**

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Background

Hot boning (HB) involves the removal of muscles or cuts before the onset of rigor mortis. It allows muscles to be treated optimally according to glycolytic behaviour patterns with the aim of producing consistent beef tenderness (West, 1983). Other advantages include reduced weight loss, reduced drip, lower capital and operating costs. A disadvantage of hot boning is that muscles disconnected from the framework of the carcass and exposed to low temperatures (chilling) and rapid pH decline are more prone to contract and toughen than muscle held in a stretched state by the carcass (Pisula & Tyburcy, 1996). The addition of phosphates to cured meat products results in improvements in water holding capacity (WHC) and binding by synergistically working with the salt to extract myofibrillar proteins. A reduction in phosphate is designed to address customer concerns about excess additives in processed food.

Objectives

The objective of this study was to examine the effect of different boning methods and phosphate levels on the quality of cured beef produced from two forequarter muscles.

Materials and Methods

Beef cuts from two forequarter muscles, *M. infraspinatus* (IS) and *M. pectoralis profundus* (PP) were hot boned within 1.5h *post mortem*. Cold boning was carried out 24h *post mortem*. Brines containing normal levels of phosphate (to give 0.3% in meat) and reduced levels of phosphate (0.15% in meat) were prepared, which resulted in a total of 4 treatments per muscle. Muscles were injected to 115% of green weight and tumbled for 2h. Tumbled muscles were enclosed in elastic netting, vacuum packed and steam cooked to a core temperature of 72 °C. Cooked samples were analysed for moisture, fat and protein content (Bostian *et al.*, 1985; Sweeney & Rexford, 1987). Cook loss and yield were calculated. Texture Profile Analysis (TPA) and Warner-Bratzler Shear Force (WBSF) measurements were determined using Instron models 4464 and 5543 respectively. Sarcomere length was determined by laser diffraction as described by Cross *et al.* (1980) using a helium neon laser (Uniphase Ltd., Stevenage, Herts, UK). Mohr's method was used to determine salt content and a colourimetric molybdenum blue method

(ISO 13730) was used with a Foss FIAstar 5000 flow-rate (Ruzika *et al.* 1981) phosphate analyser (Foss Tecator AB, Sweden) to determine phosphate content. An 8 member trained panel was employed to evaluate sensory quality of sample treatments (AMSA, 1995). Results were analysed using a two-way analysis of variance (ANOVA).

Results and Discussion

Boning method (Table 1 and 2) affected yield ($P \leq 0.05$ and $P \leq 0.01$ respectively). Hot-boned IS muscles retained 3.4% less yield than cold-boned IS muscles. Similarly, hot-boned (PP) retained 3.7% less yield than cold-boned PP muscles. Boning method had an effect ($P \leq 0.05$) for cook loss in PP formed beef only giving higher losses. Previous studies showed higher yields and reduced cook losses due to hot boning (Pisula & Tyburcy, 1996; Van Laack *et al.*, 1990; Cecchi *et al.* 1988. etc.). However, West (1983) reported that advantages in moisture loss for hot-boned primals may be seen in the early stages of processing but that these advantages are often offset in later processing such as cooking. Jeremiah *et al.* (1985) found that hot-boned LD muscles had higher total cooking losses than their conventionally chilled counterparts. Phosphate level had an effect ($P \leq 0.001$ and $P \leq 0.001$ respectively) on % STPP in the final meat products for IS and PP muscles, as expected. Colour was affected by phosphate level ($P \leq 0.001$, $P \leq 0.001$ and $P \leq 0.001$ respectively) across all the colour variables (L^* , a^* , b^*) for both IS and PP products. Samples containing normal phosphate levels were much redder (higher a^* values) in colour than the reduced phosphate samples. Claus *et al.* (1994) state that polyphosphates contribute to colour stability. There were no significant interactions ($P \geq 0.05$) between boning method and phosphate level for cook loss, yield, STPP and colour in both products.

Phosphate level (Table 3 and 4) did not effect ($P \geq 0.05$) the sensory quality, instrumental texture and sarcomere length of both IS and PP samples. Phosphate levels affected ($P \leq 0.05$) TPA hardness and springiness for PP joints. A reduction in hardness (111.2 vs. 138.9) and springiness (5.7 vs. 6.0) would be expected at a lower phosphate level, due to reduced extraction of myofibrillar proteins (Claus *et al.* 1994). PP cold-boned products were more tender than their hot-boned counterparts. TPA results for hardness were higher ($P \leq 0.001$); Warner-Bratzler Shear Force (WBSF) were found to be higher ($P \leq 0.05$) and sarcomere length was shorter ($P \leq 0.05$) for PP products. These results are supported by the work of Cecchi *et al.* (1988), who reported that WBSF values were lower for cold-boned samples than hot-boned. However, hot-boned IS samples were rated more tender ($P \leq 0.05$) than cold-boned by taste panelists. These results were not supported by other texture measurements. TPA hardness was not affected ($P \geq 0.05$) as well as WBSF ($P \geq 0.05$). Sarcomere length contradicted taste panel results for boning method, which found hot-boned were shorter than cold-boned ($P \leq 0.001$) thus tougher. A previous study showed that the sarcomere length of hot-boned muscles were longer than that of cold-boned muscles (Nakamura *et al.* 1986). A significant interaction ($P \leq 0.001$) between boning method and phosphate level occurred in the sarcomere lengths of both IS and PP products.

Conclusions

The results indicate that hot boning did not give an expected increase in yield and reduction in cook losses in the processed products. Hot-boned joints were shown to yield tougher final products than the conventional cold-boned joints, especially in the case of PP products. Although reduced phosphate levels gave a small increase in tenderness, it had detrimental effects on binding and forming during processing and on cured colour formation. Intervention techniques such as low voltage electrical stimulation and special packaging measures may be required to reduce the toughening of the meat as well as natural binding alternatives to increase binding in low-phosphate brines.

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Table 1: Effect of boning method and phosphate level on % cook loss, % yield, % STPP and colour of cured beef (*M. infraspinatus*)

Treatment	% Cook Loss	% Yield	% STPP	L*	a*	b*
<i>A: Boning Method</i>						
HB	18.8	94.9	0.24	41.6	15.0	8.4
CB	16.5	98.3	0.25	42.6	15.4	8.5
SL	Ns	*	ns	ns	Ns	ns
<i>B: Phosphate level</i>						
0.3%	16.9	97.8	0.35	44.4	16.1	9.6
0.15%	18.5	95.4	0.14	39.8	14.3	7.3
SL	Ns	ns	***	***	***	***
<i>Interactions A x B</i>						
SL	Ns	ns	ns	ns	Ns	ns
<i>Samples</i>						
HB 0.3% Phos	17.4	96.0	0.35	44.0	15.9	9.6
HB 0.15% Phos	20.2	93.7	0.13	39.1	14.1	7.1
CB Phos	16.4	99.6	0.36	44.7	16.3	9.6
CB 0.15% Phos	16.7	97.0	0.15	40.5	14.4	7.4
SEM	0.52	0.83	0.02	0.38	0.14	0.17

SL – significance level; *, **, *** = significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively; ns – not significant; SEM – Standard Error of Means.

Table 2: Effect of boning method and phosphate level on % cook loss, % yield, % STPP and colour of cured beef (*M. pectoralis profundus*)

Treatment	% Cook Loss	% Yield	% STPP	L*	a*	b*
<i>A: Boning Method</i>						
HB	15.0	91.8	0.30	45.0	15.3	9.0
CB	12.3	95.5	0.35	46.2	15.2	9.7
SL	*	**	ns	**	Ns	ns
<i>B: Phosphate level</i>						
0.3%	13.7	95.4	0.41	48.0	16.2	10.1
0.15%	13.6	93.6	0.24	43.2	14.3	7.5
SL	Ns	ns	***	***	***	***
<i>Interactions A x B</i>						
SL	Ns	ns	ns	ns	Ns	ns
<i>Samples</i>						
HB 0.3% Phos	13.9	93.4	0.38	47.6	16.3	10.3
HB 0.15% Phos	16.1	90.1	0.22	42.3	14.3	7.7
CB Phos	13.3	97.5	0.45	48.5	16.2	9.9
CB 0.15% Phos	16.0	93.5	0.26	44.0	14.2	7.4
SEM	0.74	1.14	0.02	0.31	0.14	0.15

SL – significance level; *, **, *** = significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively; ns – not significant; SEM – Standard Error of Means.

Table 3: Effect of boning method and phosphate level on sensory quality^a, instrumental texture and sarcomere length of cured beef (*M. infraspinatus*)

Treatment	Tenderness	Overall Texture	Hardness	Springiness	Warner-Bratzler Shear Force	Sarcomere Length
<i>A: Boning Method</i>						
HB	6.4	4.0	77.8	5.8	15.7	2.0
CB	5.9	4.0	77.5	6.4	15.6	2.2
SL	*	ns	ns	***	Ns	***
<i>B: Phosphate level</i>						
0.3%	6.1	3.9	80.7	6.1	16.0	2.1
0.15%	6.2	4.1	74.7	6.1	15.3	2.1
SL	Ns	ns	ns	ns	Ns	ns
<i>Interactions A x B</i>						
SL	Ns	ns	ns	ns	ns	***
<i>Samples</i>						
HB 0.3% Phos	6.3	3.9	84.3	5.9	16.5	2.0
HB 0.15% Phos	6.4	4.1	71.4	5.7	14.9	2.0
CB Phos	5.9	3.8	77.0	6.3	15.5	2.2
CB 0.15% Phos	5.9	4.1	77.9	6.4	15.7	2.3
SEM	0.09	0.08	2.59	0.07	0.48	0.01

^a Tenderness and overall texture were evaluated by means of eight-point scales (8 = Extremely tender/good, 7 = Very tender/good, 6 = Moderately tender/good, 5 = Slightly tender/good, 4 = Slightly tough/poor, 3 = Moderately tough/poor, 2 = Very tough/poor, 1 = Extremely tough/poor).

SL – significance level; *, **, *** = significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively; ns – not significant; SEM – Standard Error of Means.

Table 4: Effect of boning method and phosphate level on sensory quality^a, instrumental texture and sarcomere length of cured beef (*M. pectoralis profundus*)

Treatment	Tenderness	Overall Texture	Hardness	Springiness	Warner-Bratzler Shear Force	Sarcomere Length	
<i>A: Boning Method</i>							
HB	4.4	3.5	144.7	5.8	30.0	2.0	
CB	5.3	3.9	105.4	5.9	26.3	2.5	
SL	***	**	***	ns	*	***	
<i>B: Phosphate level</i>							
0.3%	4.9	3.7	138.9	6.0	27.7	2.2	
0.15%	4.8	3.7	111.2	5.7	28.6	2.2	
SL	Ns	ns	*	*	Ns	ns	
<i>Interactions</i>							
<i>A x B</i>							
SL	Ns	ns	ns	ns	Ns	***	
<i>Samples</i>							
HB	0.3%	4.5	3.5	163.8	5.9	30.2	2.1
HB	0.15%	4.3	3.5	125.5	5.7	29.3	1.9
CB	Phos	5.4	3.9	114.0	6.1	25.2	2.4
CB	0.15% Phos	5.3	3.9	98.5	5.8	27.3	2.3
SEM		0.10	0.07	6.06	0.06	0.77	0.01

^a Tenderness and overall texture were evaluated by means of eight-point scales (8 = Extremely tender/good, 7 = Very tender/good, 6 = Moderately tender/good, 5 = Slightly tender/good, 4 = Slightly tough/poor, 3 = Moderately tough/poor, 2 = Very tough/poor, 1 = Extremely tough/poor.

SL – significance level; *, **, *** = significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively; ns – not significant; SEM – Standard Error of Means.

EFFECT OF PARTICLE SIZE AND FAT LEVELS ON THE PHYSICO-CHEMICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF LOW-FAT GROUND PORK PATTIES

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Key words: low-fat ground pork patties, low cholesterol meat product, particle size, fat levels

Introduction

Dietary fat and cholesterol content are considered to be controllable factors in multifactorial etiology of heart diseases and obesity. Meat and meat products are maligned with a fact of high- fat, high cholesterol and high calorie diet. This has lead to increase in demand of low-fat meat products. Pork patties generally contain 30-40% fat and warrants reduction in fat contents. However rheological, structural, processing, nutritional and sensory characteristics of comminuted meat products are directed by the fat content in the formulation and particle size of ground meat system (Kregel et al, 1986; Manish Kumar and Sharma, 2003)

Objectives

The present study was envisaged with a twin objectives to standardize the level of particle size and added fat levels in the processing of low-fat ground pork patties(LFGPP) on the basis of physico-chemical, textural and sensory characteristics. The effect of varying fat levels on cholesterol and calorie content was also evaluated.

Methodology

The study involved two experiments each with three trials. Experiment I was carried out to investigate the influence of three particle sizes viz 3,4 and 6 mm at constant fat level of <10%. (4 added fat level). The experiment II was carried out to examine the influence of fat levels viz 3, 4 and 5% at a constant grind size of 3 mm on the quality of low-fat pork patties.

Formulation and Processing of pork patties

Market age crossbred (Landrace × Local) hogs (N=3) weighing 60-70kg were humanely slaughtered at Divisional Experimental Abattoir. Prerigor raw materials were obtained with in 1hr post mortem by fabricating each carcass into boston butt, picnic shoulder, loin and ham. All skin, external fascia, subcutaneous and seam fat and all adhering connective tissues were removed. Hand deboned lean meat and back fat free from adhering skin were stored separately at $-18\pm 2^{\circ}\text{C}$ in low density polyethylene

(LDPE) packs and were used after partial thawing at 5°C for 12-15hr. The spice mixture, condiments and other additives were purchased from local market. The refined wheat flour used as binder has composition 18.73±2.11 moisture, 74.43±0.85 carbohydrates percent.

Meat and back fat were cut into small cubes and minced separately through 3mm, 4mm and 6 mm grind plate in Electrolux meat mincer (Model 9512) as per requirements of Experiment I.

The formulation and processing of control and low-fat patties were standardized by preliminary trials (Manish Kumar and Sharma 2004). The formulation consist of added fat 4.0%, binder 4.0%, added water 15%, condiment mix 3%, table salt and spice mix 1.5%. All the ingredients and minced meat constituents were thoroughly mixed along with slowly adding chilled water by electrically operated meat mixer (Hobart Paddle Mixer, N-50) for 3min. Thereafter, 75g of each mix was moulded into patties with the help of a Petri dish of defined size (75mm×15mm). The moulded patties were cooked in preheated hot air oven at 190±5°C to an internal end point temperature of 75±2°C recorded at geometrical center of each patty using probe thermometer. The patties were turned upside down twice at 5min interval for better appearance, color and texture. Samples from each batch were analyzed on the same day.

The moisture, fat and protein content of buffalo meat and patties was determined by methods of AOAC (1995). Cooking yield was calculated from raw and cooked weights of 9 patties for each treatment. The dimensional parameters of cooked patties were recorded using vernier callipers minimum at three different positions respectively to obtain the mean values. The percent shrinkage, moisture and fat retention of patties were determined as per El-Magoli *et al*, 1996.

The shear force value of 1cm² of the sample was recorded as per Berry and Stiffler (1981) using Warner- Bratzler Shear press (Model: 810310307 G.R Elect. Mfg. Co. USA) and expressed as kg/cm². An experienced sensory panel consisting of seven scientists and post graduate students evaluated the sensory characteristics of warmed product viz., appearance and color, flavor, juiciness, texture and overall acceptability using 8- point objective scale (Keeton, 1983), where 8- denoted extremely desirable and 1 denoted extremely undesirable.

The textural profile was determined on Instron Universal Testing Machine (Model 4464) following the procedure described by Brady *et al* (1985). The fat content of the samples were extracted adopting the method described by Folch *et al.*, (1957) and Total lipids were determined gravimetrically. The different components of lipids included total phospholipids, total cholesterol, glycolipids and free fatty acids were measured by standard procedures described by Marinetti (1962), Hanel and Dam (1955), Roughan and Batt (1968) and Koniecko (1979) respectively, whereas total glycerides were indirectly calculated by subtracting all these from total lipid values.

Gross energy of sample was determined by Gallenkamp and Ballistic Bomb Calorimeter (Haque and Murarilal, 1999) using Benzoic Acid as a standard and expressed as Kcal/100g. Total calorie estimates of raw and cooked sausages were calculated on the basis of 100g portion using Atwater values for fat (9.0 kcal/g), protein (4.02 kcal/g) and carbohydrates (4.0 kcal/g) calories. Since analysis of per cent carbohydrates in the meat samples was not performed, the calorie values were estimates and not actual values.

The data obtained from various trials under each experiment was pooled and processed at Institute's computer centre. The statistical design of the study was 4(treatment) × 3 (replication) randomized block design. All chemical and physical determinations were conducted in triplicate. There were seven sensory determinations (judges) for each treatment × replication combination. Data were subjected to one way analysis of variance. Duncan's Multiple Range test and critical difference were determined at 5% significance level (Snedecor and Cochran, 1989).

Result and Discussion

Particle Size:

The effect of different particle size on physico-chemical properties of cooked low-fat ground pork patties are presented in table 1. Percent cooking yield, moisture and moisture retention were significantly ($P < 0.05$) better at lower particle size (3mm) than higher particle size (6mm). It could be due to more compact binding at lower particle sizes which do not allow the release of moisture. Lin and Keeton (1994) and Small et al. (1995) also reported the increase in cooking losses with the increase in particle size. The shear force value increased significantly ($P < 0.05$) with an increase in particle size. Mean sensory scores (table 2) were significantly ($P < 0.05$) better in juiciness, texture and overall acceptability for particle size 3mm than higher particle size. It appears that smaller particle size provided increased binding and compactness to low-fat ground pork patties. Our results are in conscience of Small et al. (1995) and Berry et al. (1999). Texture Profile showed an increase in hardness and springiness with the increase in particle size. Since cooking determinants and sensory attributes were better with 3mm particle size, hence it was adopted as optimum particle size for further studies.

Fat levels:

The fat percent increased linearly with the increase in added fat levels (table 3). Cooking yield and moisture retention were recorded maximum at 4% added fat level. The percent gain in height increased significantly ($P < 0.05$) at 4 and 5% added fat levels. However shear force value decreased significantly ($P < 0.05$) with the increase in fat levels. These findings are in accordance with Trout et al. (1992). There was marginal decrease in the fat retention of the product with increasing fat levels. Mean sensory scores (table 4) are significantly ($P < 0.05$) better for 5% added fat level than 3% added fat level whereas patties with 4 % added fat were comparable to patties with 5 % added fat level. the juiciness scores of the product were marginally higher for increased fat levels.

Perusal of table 5 showed that in raw product total lipid content, phospholipids, cholesterol content and calorific value (wet weight basis) increased significantly ($P < 0.05$) with increasing added fat levels. However on dry weight basis, cholesterol content increased significantly ($P < 0.05$) only at 5% added fat level. As for calorific value, the relationship was linear with fat levels in raw patties. In cooked product the total lipid content increased significantly ($P < 0.05$) with increasing added fat levels, whereas phospholipids and cholesterol content showed only a marginal increase. The cholesterol retention did not show any trend in the present study. Calorific value increased significantly ($P < 0.05$) with increasing added fat levels. It is obvious because fat

contributes 9.2 kcal/g energy which is about 2.25 times more than carbohydrates and proteins.

Conclusions

Cooked patties with 5 % added fat levels have mean total fat level more than 10, which does not fit into the definition of low-fat meat products (Keeton, 1994). Since cooking determinants and sensory attributes are better for 3mm particle size and 4% added fat level. Therefore the optimum level of particle size 3mm and added fat level 4% are recommended for formulation of low-fat ground pork patties.

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Table 1. Effect of particle size on physico-chemical properties of cooked low-fat ground pork patties. (Mean±S.E.)*

Parameters	Particle size (mm)		
	3	4	6
pH	6.14±0.007	6.15±0.009	6.15±0.009
Moisture (%)	61.63±0.24 ^a	60.44±0.27 ^b	57.53±0.55 ^c
Fat (%)	9.12±0.13	9.21±0.09	9.03±0.10
Protein (%)	18.57±0.04	18.51±0.09	18.51±0.1
Moisture Protein Ratio	3.32±0.01 ^a	3.27±0.04 ^a	3.10±0.04 ^b
Cooking Yield (%) ^A	75.86±0.21 ^a	73.95±0.38 ^b	67.82±0.30 ^c
Cooking Loss (%) ^B	24.47±0.25 ^c	26.05±0.38 ^b	32.18±0.30 ^a
Decrease in Diameter (%)	15.04±0.27 ^c	18.74±0.17 ^b	22.80±0.44 ^a
Gain in Height (%)	21.46±0.99	20.68±0.28	20.28±0.17
Shrinkage (%) ^C	10.79±0.24 ^c	12.10±0.15 ^b	15.55±0.44 ^a
Moisture Retention (%) ^D	46.76±0.27 ^a	44.09±0.24 ^b	39.02±0.35 ^c
Fat Retention (%) ^E	77.98±0.32 ^a	76.24±0.38 ^a	70.47±0.49 ^b
Shear Force Value (kg/cm ²)	0.37±0.007 ^c	0.40±0.006 ^b	0.47±0.005 ^a

*Mean± S.E with different superscripts in a row differ significantly (P<0.05)

N=6 for each treatment

A per cent yield= (raw weight-cooked weight/raw weight) ×100

B cook loss= per cent cook loss: 100-per cent yield

C Shrinkage %= (Raw thickness- cooked thickness) + (raw diameter- cooked diameter)/ (raw thickness+ raw diameter)

D % Moisture retention= % yield × % moisture in cooked patties/100

E % Fat Retention= (cooked weight × %fat in cooked patties/raw weight×% fat in raw patties) ×100

Table 2. Effect of particle size on sensory attributes of low- fat ground pork patties.
(Mean \pm S.E.)*

Attributes	Particle size (mm)		
	3	4	6
Appearance	6.98 ± 0.08	6.93 ± 0.12	6.81 ± 0.12
Flavor	7.00 ± 0.077	6.95 ± 0.048	6.91 ± 0.074
Texture	7.29 ^a ± 0.095	7.10 ^a ± 0.074	6.58 ^b ± 0.14
Juiciness	6.93 ^a ± 0.079	6.83 ^{ab} ± 0.072	6.62 ^b ± 0.097
Overall Acceptability	6.98 ^a ± 0.064	6.77 ^b ± 0.056	6.43 ^c ± 0.093

* Mean \pm S.E. with same superscript in a row do not differ significantly (P<0.05)
Means are scores given by sensory panelists on 8-point scale where
1: extremely poor and 8: extremely desirable
N=21 for each treatment

Table 3. Effect of added fat levels on physico-chemical properties of cooked low-fat ground pork patties. (Mean±S.E.)*

Parameters	Added fat levels (%)		
	3	4	5
pH	6.14±0.008	6.16±0.004	6.16±0.015
Moisture (%)	61.27±0.18 ^a	61.73±0.27 ^a	60.32±0.22 ^b
Fat (%)	8.18±0.11 ^c	9.08±0.07 ^b	10.64±0.08 ^a
Protein (%)	19.04±0.04	18.87±0.09	18.64±0.04
Moisture Protein Ratio	3.22±0.009 ^b	3.27±0.015 ^a	3.24±0.01 ^b
Cooking Yield (%) ^A	74.14±0.22 ^b	75.34±0.28 ^a	74.87±0.38 ^{ab}
Cooking Loss (%) ^B	25.87±0.22 ^a	24.66±0.28 ^b	25.13±0.38 ^{ab}
Decrease in Diameter (%)	24.74±0.15	25.13±0.28	25.19±0.14
Gain in Height (%)	18.61±0.48 ^b	20.28±0.48 ^a	21.12±0.17 ^a
Shrinkage (%) ^C	17.51±0.14	17.56±0.29	17.40±0.11
Moisture Retention (%) ^D	45.42±0.25 ^b	46.51±0.21 ^a	45.62±0.32 ^{ab}
Fat Retention (%) ^E	79.46±0.50	79.22±0.38	79.01±0.39
Shear Force Value (kg/cm ²)	0.55±0.009 ^a	0.50±0.004 ^b	0.47±0.006 ^c

*Mean± S.E with different superscripts in a row differ significantly (P<0.05)

N=6 for each treatment

A per cent yield= (raw weight-cooked weight/raw weight) ×100

B cook loss= per cent cook loss: 100-per cent yield

C Shrinkage %= (Raw thickness- cooked thickness) + (raw diameter- cooked diameter)/ (raw thickness+ raw diameter)

D % Moisture retention= % yield × % moisture in cooked patties/100

E % Fat Retention= (cooked weight × %fat in cooked patties/raw weight×% fat in raw patties) ×100

Table 4. Effect of added fat levels on sensory attributes of low- fat ground pork patties.
(Mean \pm S.E.)*

Attributes	Added fat levels (%)		
	3	4	5
Appearance	6.88 ± 0.06	6.91 ± 0.04	6.98 ± 0.04
Flavor	6.74 ^b ± 0.09	6.93 ^{ab} ± 0.05	6.98 ^a ± 0.04
Texture	6.74 ^b ± 0.08	6.93 ^a ± 0.05	6.95 ^a ± 0.03
Juiciness	6.88 ± 0.06	6.95 ± 0.06	7.00 ± 0.05
Overall Acceptability	6.74 ^b ± 0.08	6.93 ^a ± 0.05	7.02 ^a ± 0.05

* Mean \pm S.E. with same superscript in a row do not differ significantly (P<0.05)
Means are scores given by sensory panelists on 8-point scale where
1: extremely poor and 8: extremely desirable
N=21 for each treatment

Table5. Effect of added fat levels on total lipids, phospholipids, cholesterol and calorific value of raw low-fat ground pork patties.

Parameters (mg/g)	Added fat levels (%)					
	3		4		5	
	Mean	SEM	Mean	SEM	Mean	SEM
Total Lipids	41.36 ^c	0.96	58.50 ^b	0.60	76.55 ^a	0.57
Phospholipids	13.75 ^c	0.13	15.02 ^b	0.19	17.42 ^a	0.15
Cholesterol (wet weight basis)	1.15 ^c	0.02	1.21 ^b	0.01	1.28 ^a	0.004
Cholesterol (dry weight basis)	362.82 ^b	7.23	371.55 ^{ab}	3.03	378.81 ^a	2.71
Calorific Value* (Kcal/100g)	128.60 ^c	0.35	162.82 ^b	0.64	178.98 ^a	0.81

N=6 for each treatment

*Calorific value measured by Gallenkamp and Ballistic Bomb calorimeter

Table 6. Effect of added fat levels on total lipids, phospholipids, cholesterol and calorific value of cooked low-fat ground pork patties.

Parameters (mg/g)	Added fat levels (%)					
	3		4		5	
	Mean	SEM	Mean	SEM	Mean	SEM
Total Lipids	42.97 ^c	0.34	61.55 ^b	1.02	71.28 ^a	1.09
Phospholipids	19.75 ^b	0.32	21.96 ^a	0.35	21.92 ^a	0.23
Cholesterol (wet weight basis)	1.34 ^b	0.18	1.45 ^a	0.15	1.49 ^a	0.01
Cholesterol (dry weight basis)	346.46 ^b	4.98	378.46 ^a	3.86	376.00 ^a	2.81
Cholesterol Retention (%)	86.38 ^b	1.41	90.43 ^a	0.93	87.35 ^b	0.25
Calorific Value* (Kcal/100g)	171.52 ^c	1.93	184.49 ^b	1.01	208.96 ^a	0.67

N=6 for each treatment

*Calorific value measured by Gallenkamp and Ballistic Bomb calorimeter

Table 6 Effect of refrigerated storage on physico-chemical, microbiological and sensory characteristics of aerobically packaged low-fat ground pork patties. (Mean \pm S.E.)*

Treatments	Storage Period (Days)			
	0	7	14	21
Physico-Chemical Characteristics				
	TBA Value (mg malonaldehyde/kg)			
Control	0.41 \pm 0.02 ^{d1}	0.52 \pm 0.01 ^{c1}	0.78 \pm 0.02 ^{b1}	0.98 \pm 0.01 ^{a1}
Low-Fat Patties	0.28 \pm 0.007 ^{d2}	0.39 \pm 0.01 ^{c2}	0.59 \pm 0.07 ^{b2}	0.67 \pm 0.01 ^{a2}
	pH			
Control	6.13 \pm 0.009 ^c	6.20 \pm 0.007 ^{bc}	6.27 \pm 0.004 ^b	6.38 \pm 0.008 ^a
Low-Fat Patties	6.12 \pm 0.002 ^c	6.17 \pm 0.004 ^b	6.24 \pm 0.004 ^{ab}	6.33 \pm 0.012 ^a
Microbiological Characteristics				
	Total Plate Count (log cfu/g)			
Control	1.65 \pm 0.03 ^{d2}	1.94 \pm 0.02 ^{c1}	2.29 \pm 0.05 ^b	2.86 \pm 0.04 ^a
Low-Fat Patties	1.73 \pm 0.11 ^{d1}	2.13 \pm 0.04 ^{c2}	2.24 \pm 0.08 ^b	2.74 \pm 0.07 ^a
	Psychrophilic Count (log cfu/g)			
Control	ND	ND	1.15 \pm 0.02 ^b	1.40 \pm 0.09 ^a
Low-Fat Patties	ND	ND	1.19 \pm 0.01 ^b	1.42 \pm 0.05 ^a
	Coliform Count (log cfu/g)			
Control	ND	ND	ND	ND
Low-Fat Patties	ND	ND	ND	ND
Sensory Characteristics**				
	Appearance			
Control	7.12 \pm 0.11 ^a	6.86 \pm 0.10 ^{ab}	6.76 \pm 0.12 ^b	6.69 \pm 0.12 ^b
Low-Fat Patties	7.18 \pm 0.06 ^a	7.05 \pm 0.12 ^{ab}	6.89 \pm 0.08 ^b	6.72 \pm 0.14 ^b
	Flavor			
Control	6.97 \pm 0.07 ^a	6.88 \pm 0.09 ^a	6.71 \pm 0.10 ^{ab}	6.63 \pm 0.11 ^b
Low-Fat Patties	6.94 \pm 0.10 ^a	6.91 \pm 0.11 ^a	6.78 \pm 0.09 ^{ab}	6.72 \pm 0.12 ^b
	Juiciness			
Control	7.12 \pm 0.09 ^a	6.94 \pm 0.07 ^{ab}	6.71 \pm 0.10 ^{bc}	6.67 \pm 0.11 ^c
Low-Fat Patties	6.99 \pm 0.08 ^a	6.96 \pm 0.05 ^a	6.81 \pm 0.09 ^{ab}	6.70 \pm 0.13 ^b
	Texture			
Control	7.07 \pm 0.09 ^a	7.00 \pm 0.09 ^a	6.86 \pm 0.09 ^{ab}	6.74 \pm 0.08 ^{b1}
Low-Fat Patties	7.00 \pm 0.09	6.96 \pm 0.08	6.89 \pm 0.09	6.81 \pm 0.11
	Overall Acceptability			
Control	7.05 \pm 0.09 ^a	6.98 \pm 0.08 ^{ab}	6.76 \pm 0.09 ^{bc}	6.64 \pm 0.12 ^c
Low-Fat Patties	7.02 \pm 0.09 ^a	6.93 \pm 0.04 ^a	6.81 \pm 0.07 ^{ab}	6.69 \pm 0.07 ^b

*Mean \pm S.E. with different superscripts row wise (alphabet) and column wise (numeral) differ significantly (P<0.05)

ND= Not Detected

N=21 no. of observations for sensory parameters and N=6 for other parameters

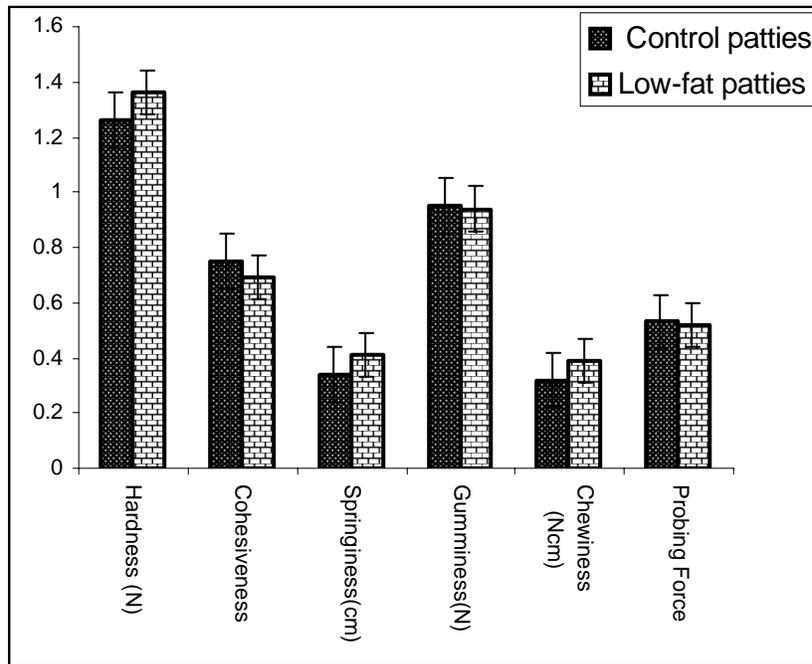
** Means are scores given by sensory panelists on 8-point scale where
1: extremely poor and 8: extremely desirable

Table 7. Effect of refrigerated storage on physico-chemical, microbiological and sensory characteristics of vacuum packaged low-fat ground pork patties. (Mean \pm S.E.) *

Treatments	Storage Period (Days)					
	0	7	14	21	28	35
Physico-Chemical Characteristics						
	TBA Value (mg malonaldehyde/kg)					
Control	0.41 \pm 0.018 ^{b1}	0.44 \pm 0.007 ^{b1}	0.47 \pm 0.08 ^{ab1}	0.49 \pm 0.012 ^{a1}	0.54 \pm 0.012 ^{a1}	0.56 \pm 0.004 ^{a1}
Low-Fat Patties	0.28 \pm 0.007 ²	0.32 \pm 0.04 ²	0.31 \pm 0.007 ^{d2}	0.34 \pm 0.019 ^{c2}	0.37 \pm 0.01 ^{b2}	0.37 \pm 0.009 ^{a2}
	pH					
Control	6.13 \pm 0.009 ^a	6.12 \pm 0.01 ^a	6.09 \pm 0.005 ^{a1}	6.00 \pm 0.01 ^{b1}	5.95 \pm 0.02 ^{bc1}	5.82 \pm 0.07 ^c
Low-Fat Patties	6.12 \pm 0.002 ^a	6.12 \pm 0.007 ^a	6.08 \pm 0.002 ^{ab}	6.00 \pm 0.007 ^b	5.89 \pm 0.011 ^{bc}	5.81 \pm 0.02 ^c
Microbiological characteristics						
	Total Plate Count (log cfu/g)					
Control	1.65 \pm 0.03 ^f	1.86 \pm 0.008 ^e	2.10 \pm 0.02 ^d	2.41 \pm 0.04 ^c	2.80 \pm 0.018 ^b	3.26 \pm 0.016 ^a
Low-Fat Patties	1.74 \pm 0.01 ^f	1.94 \pm 0.15 ^e	2.21 \pm 0.015 ^d	2.49 \pm 0.004 ^c	2.78 \pm 0.013 ^b	3.16 \pm 0.04 ^a
	Psychrophilic Count (log cfu/g)					
Control	ND	ND	ND	1.75 \pm 0.03 ^c	1.95 \pm 0.018 ^b	2.25 \pm 0.04 ^a
Low-Fat Patties	ND	ND	ND	1.84 \pm 0.02 ^b	2.02 \pm 0.03 ^{ab}	2.13 \pm 0.03 ^a
	Anaerobic Plate Count (log cfu/g)					
Control	ND	ND	ND	ND	1.26 \pm 0.013 ^b	1.48 \pm 0.05 ^a
Low-Fat Patties	ND	ND	ND	ND	1.22 \pm 0.012 ^b	1.43 \pm 0.03 ^a
	Lactic Acid Bacteria Count (log cfu/g)					
Control	ND	ND	ND	ND	1.05 \pm 0.024 ^b	1.18 \pm 0.007 ^a
Low-Fat Patties	ND	ND	ND	ND	1.15 \pm 0.007 ^b	1.22 \pm 0.008 ^a
	Coli form Count (log cfu/g)					
Control	ND	ND	ND	ND	ND	ND
Low-fat Patties	ND	ND	ND	ND	ND	ND
Sensory Characteristics						
	Appearance					
Control	7.12 \pm 0.11 ^a	7.00 \pm 0.08 ^{ab}	6.95 \pm 0.08 ^{ab}	6.81 \pm 0.11 ^{bc}	6.67 \pm 0.10 ^c	6.67 \pm 0.12 ^c
Low-Fat Patties	7.18 \pm 0.05 ^a	7.11 \pm 0.07 ^a	7.02 \pm 0.09 ^{ab}	6.93 \pm 0.09 ^{bc}	6.79 \pm 0.09 ^c	6.81 \pm 0.07 ^{bc}
	Flavor					
Control	7.02 \pm 0.07 ^{ab}	7.02 \pm 0.08 ^a	6.98 \pm 0.07 ^a	6.83 \pm 0.09 ^{abc}	6.71 \pm 0.07 ^{bc}	6.60 \pm 0.13 ^{c2}
Low-Fat Patties	6.95 \pm 0.10 ^{ab}	6.98 \pm 0.04 ^a	7.00 \pm 0.12 ^a	6.86 \pm 0.09 ^{ab}	6.81 \pm 0.06 ^{ab}	6.76 \pm 0.07 ^{b1}
	Juiciness					
Control	7.12 \pm 0.10 ^a	7.00 \pm 0.07 ^{ab}	6.90 \pm 0.09 ^{abc}	6.86 \pm 0.09 ^{abc}	6.76 \pm 0.06 ^c	6.53 \pm 0.12 ^d
Low-Fat Patties	6.99 \pm 0.08 ^a	7.00 \pm 0.05 ^a	6.98 \pm 0.09 ^a	6.88 \pm 0.08 ^b	6.71 \pm 0.07 ^b	6.65 \pm 0.13 ^b
	Texture					
Control	7.07 \pm 0.07 ^a	7.05 \pm 0.07 ^a	6.95 \pm 0.07 ^{ab}	6.79 \pm 0.08 ^{bc}	6.81 \pm 0.07 ^{bc}	6.67 \pm 0.11 ^c
Low-Fat Patties	7.00 \pm 0.08 ^a	7.00 \pm 0.05 ^a	6.93 \pm 0.09 ^{ab}	6.84 \pm 0.08 ^{ab}	6.78 \pm 0.06 ^b	6.71 \pm 0.10 ^b
	Overall Acceptability					
Control	7.05 \pm 0.07 ^a	7.02 \pm 0.07 ^a	6.93 \pm 0.08 ^{ab}	6.87 \pm 0.08 ^{ab}	6.71 \pm 0.07 ^b	6.59 \pm 0.12 ^b
Low-Fat Patties	7.02 \pm 0.09 ^a	7.00 \pm 0.08 ^a	7.00 \pm 0.05 ^a	6.83 \pm 0.09 ^{ab}	6.79 \pm 0.09 ^{ab}	6.72 \pm 0.09 ^b

*Mean±S.E. with different superscripts row wise (alphabet) and column wise (numeral) differ significantly (P<0.05)
 ND= Not Detected
 N=21 no. of observations for sensory parameters and N=6 for other parameters
 ** Means are scores given by sensory panelists on 8-point scale where
 1: extremely poor and 8: extremely desirable

Fig.1. Comparative instrumental Texture Profile of control and formulated low-fat patties.



**PROXIMATE COMPOSITION AND FAT RETENTION OF BEEF STEAKS AS
INFLUENCED BY TISSUE COMPOSITION, USDA QUALITY GRADE, AND
COOKING METHOD**

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Key Words: Broiling; Seam fat; Oven broiling; Separable lean; Surface fat; Waste

Introduction

Various factors are known to affect lipid content, and consequently, the overall proximate composition of meat. Some of these factors include retail cut (Jones et al., 1992b; Wahrmund-Wyle et al., 2000b), cooking method (Jones et al., 1992b; Renk et al., 1985, Morgan et al., 1988), USDA quality grade (Jones et al., 1992b; Wahrmund-Wyle et al., 2000b), and the extent of fat trimming and fat retention (Coleman et al., 1988; Jones et al., 1992b). While several researchers have reported that increasing amounts of surface fat left on retail cuts before cooking increases fat retention (Coleman et al., 1988; Morgan et al., 1988; Jones et al., 1992c) and the corresponding fat concentration of the separable lean (Jones et al., 1992c; Coleman et al., 1988; Morgan et al., 1988), others have disregarded such claims (Novakofski et al., 1989) or found mixed results (Goihl et al., 1992; Wahrmund-Wyle et al., 2000a) in beef cuts. Evidenced by the relatively high amount of chemical fat retained in some retail cuts trimmed of surface fat, it has been suggested that fat migration can be also attributed to the seam fat (Jones et al., 1992c). Therefore, further studies are needed to elucidate the effect of the relative proportion of separable fat depots left in retail cuts on fat retention and nutrient content of the edible portion across cooking methods and quality grades.

Objectives

a) To examine the variation in raw tissue composition, cooking yield, fat retention, and proximate composition of the edible portion of four retail cuts differing in seam and surface fat. b) To identify the influence of cooking method and quality grade on the same response variables.

Methodology

Carcass selection and fabrication

The right side of six USDA Choice and six USDA Select carcasses was fabricated into the following four subprimals as described by their USDA (1996) Institutional Meat

Purchase Specifications (IMPS) Number: Ribeye (IMPS# 112); Shoulder Clod (IMPS# 114); Top round (IMPS# 168); and Tenderloin (IMPS# 190). Steaks, 2.54 cm thick, were cut from each listed subprimal, assigned a cooking method, vacuum packaged, and frozen for subsequent cooking and proximate composition analysis. Ribeye steaks were trimmed of any surface fat while top round and shoulder clod steaks were trimmed to 0.32 cm surface fat. Tenderloins were defatted (no surface fat present) but not trimmed of any seam fat.

Dissection and Cooking

All retail cuts were vacuum packaged, grouped according to cooking method, and stored frozen at -23°C until needed for cooking and dissection. Steaks were analyzed as a broiled, oven broiled, or raw sample. Samples that were treated as raw were thawed and dissected into separable tissue components (separable lean, surface fat, seam fat, and waste) that were weighed and converted to percentages. Broiled steaks were cooked to an internal temperature of 70°C in an electric Farberware Open-Hearth Broiler. Steaks designated as oven broiled were cooked to an internal temperature of 65°C in a preheated broiler. Raw, cooked, and cold weights (used to determine percentage yield) along with on and off temperatures were recorded. Cooked weights of beef retail cuts taken after cuts were chilled and were used to calculate cooking yields as follows:

$$\text{Percentage cooking yield} = (\text{cooked weight})/(\text{raw weight}) \times 100$$

Proximate composition

Separable lean from raw and cooked steaks was homogenized in a Cuisinart® food processor. Protein, moisture, and ash analyses were conducted by AOAC methods (AOAC, 1990). Fat analysis was determined by the Modified Folch Method (Folch et al., 1957).

Fat retention

Fat retention values were determined for each sample using raw versus cooked data as described by Jones et al. (1992c), and expressed as follows:

$$\text{Percentage fat retention} = (\% \text{ fat in cooked lean})/(\% \text{ fat in raw lean}) \times \% \text{ cooking yield}$$

Statistical analysis

Data were analyzed using SAS (2002) PROC GLM. Main effects and interactions were analyzed. Independent variables not significant for the interactions were pooled into the error term. A significance level of $P < 0.05$ was used, and means were separated using the PDIFF option of SAS.

Results & Discussion

Raw tissue composition

Percentage waste was the only dissectible component affected ($P < 0.01$) by USDA quality grade (results not shown in tabular form). Choice steaks yielded 3% more ($P < 0.05$) waste than their Select counterparts (percentage LS Means and SE were 7.4 ± 0.8 vs. 4.3 ± 0.8 , respectively). Conversely, Wahrmund-Wyle et al. (2000a) did not find any differences in percent waste between USDA Select and Choice.

Retail cuts varied significantly in tissue components (Table 1). Ribeye and shoulder clod steaks produced the lower amounts of separable lean ($P < 0.05$) whereas top round

Table 1
Least squares means and standard errors for percentages of raw tissues components of four retail steaks

	Retail cut			
	Ribeye steaks (n=12)	Tenderloin Steaks (n=12)	Shoulder clod steaks (n=10)	Top round steaks (n=12)
Separable lean, %	77.5±1.8a	82.1±1.8ab	77.8±2.0a	85.9±1.8b
Surface fat, %	0.0±1.1a	0.0±1.1a	4.4±1.2b	6.0±1.1b
Seam fat, %	15.2±1.8a	12.0±1.8ab	10.5±1.9ab	2.9±1.8c
Waste, %	6.7±1.1	4.9±1.1	6.9±1.2	4.6±1.1

Means in the same row lacking a common letter differ ($P < 0.05$) steaks ranked highest in lean yield.

Cooking yield.

USDA grade or cooking method did not affect cooking yield ($P > 0.05$). Lack of variation in cooking yield due to USDA grade has been reported previously (Jones et al., 1992c; Wahrmond-Wyle et al., 2000b). Retail cuts trimmed to zero surface fat (i.e., ribeye and tenderloin) had lower ($P < 0.05$) cooking losses and exhibited the higher yields (Table 2). This finding contradicts that of Jones et al. (1992c) where cooking yields were not significantly affected by surface trim levels and an inverse trend to our results was observed for most cuts (i.e., lower numerical values for predicted cooking yields were found in those retail cuts trimmed to zero before cooking).

Table 2
Least squares means and standard errors for cooking yield and related variables of four retail cuts

	Retail cut			
	Ribeye steaks (n=24)	Tenderloin steak (n=24)	Shoulder clod steak (n=23)	Top round steak (n=24)
Raw weight, g	263.5 ± 12.4a	188.1 ± 12.4b	515.3 ± 12.6c	333.8 ± 12.4d
Cooked weight, g	209.8 ± 10.4a	146.9 ± 10.4b	394.5 ± 10.7c	251.5 ± 10.4d
Cook loss, g	42.7 ± 2.9a	34.8 ± 2.9a	79.4 ± 3.0b	62.3 ± 2.9c
Cook yield, %	75.9 ± 0.8a	74.3 ± 0.8a,b	73.5 ± 0.8b	72.7 ± 0.8b

Means in the same row lacking a common letter differ ($P < 0.05$)

Proximate composition and fat retention of the separable lean.

Chemical fat. Cooked samples had higher ($P < 0.05$) fat contents (7.6% and 6.8% for broiled and oven broiled steaks, respectively) than their raw (4.5%) counterparts (Figure 1). The higher fat extraction in cooked samples is widely supported (Renk et al., 1985; Smith et al., 1989; Goihl et al., 1992; Jones et al., 1992c), but Wahrmond-Wyle et al. (2000b) found the opposite. The significant variation in fat content due to broiling method was not found by Renk et al. (1985) in pork samples.

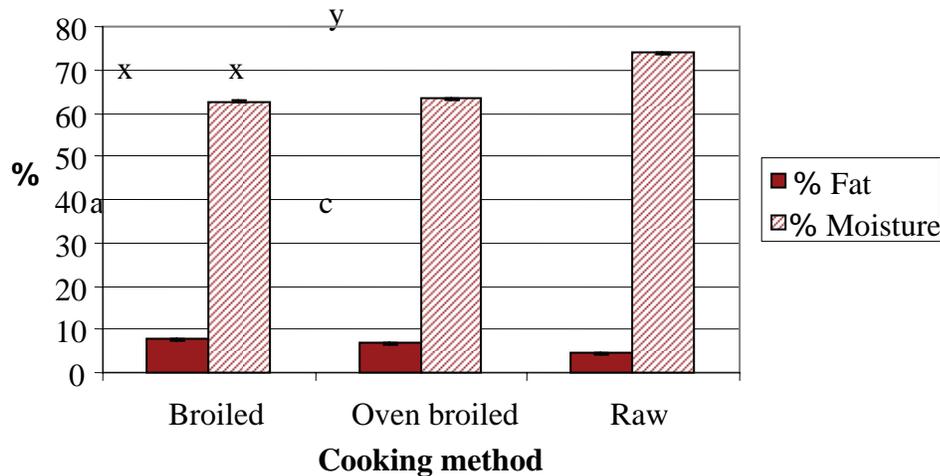


Figure 1. Least squares means and standard errors for percentages of chemical fat and moisture from proximate analysis of broiled, oven broiled and raw retail cuts. Columns with different letters differ ($P < 0.05$).

The general trend reported previously by Wahrmond-Wyle et al., (2000b) and Jones et al. (1992b) was that Choice steaks had significantly more percentage chemical fat than their Select counterparts. However, there was an interaction ($P < 0.0001$) between retail cut and USDA quality grade (Figure 2). Whereas fat content progressively decreased as seam fat proportion in Choice retail cuts decreased (in the order: Ribeye > Tenderloin > Shoulder clod > Top round), an irregular pattern in fat content was observed among retail cuts of the Select grade.

Moisture. Raw samples had higher ($P < 0.05$) moisture contents compared to counterparts of broiled or oven broiled steaks (Figure 3). The lower percentage moisture in the cooked steaks has been reported previously (Smith et al., 1989; Goihl et al., 1992; Jones et al., 1992b). Comparison of quality grades shows a similar tendency to that reported by Wahrmond-Wyle et al. (2000b) and Jones et al. (1992b) in that separable lean of Select steaks had more moisture than their Choice counterparts. However, there was an interaction ($P = 0.0028$) between retail cut and USDA quality grade (Figure 3) that shows an opposite trend to that described for the fat content. Whereas moisture content progressively increased as seam fat in Choice retail cuts decreased (in the order: Ribeye > Tenderloin > Shoulder clod > Top round), an irregular pattern in moisture content was observed among retail cuts of the Select grade.

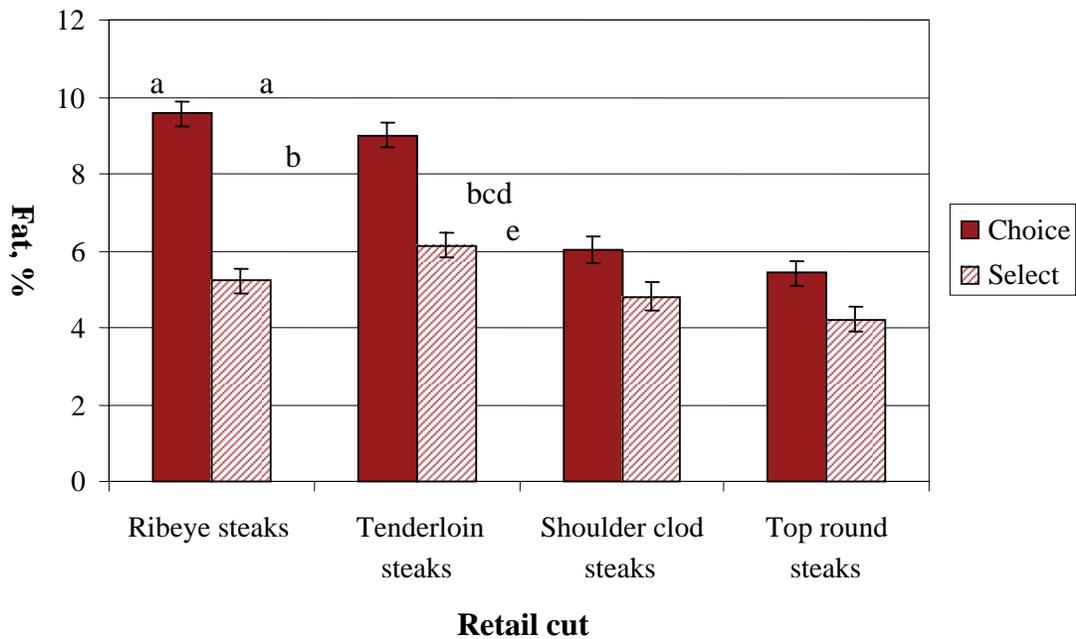


Figure 2. Least squares means and standard errors for percentages of fat from proximate analysis as affected by retail cut and USDA quality grade. Columns with different letters differ ($P < 0.05$).

Protein. There were two significant two-way interactions for protein: cooking method \times USDA quality grade ($P = 0.0217$) and retail cut \times cooking method ($P = 0.0043$).

Figure 4 shows that broiled steaks of the Select grade had more ($P < 0.05$) protein than their Choice counterparts ($P < 0.05$) but such differences (if any) in grade were of lesser magnitude and not significant for oven broiled or raw steaks (Figure 5). Cooked steaks did not differ in percent protein within the same USDA quality grade whereas protein contents of raw steaks regardless of USDA grade were lower than Choice and Select cooked steaks ($P < 0.05$).

Figure 5 depicts the relationship between retail cut and cooking method, showing that variations in protein content due to cooking treatments were of different magnitudes across retail cuts although the protein content was always lowest in the raw samples.

Ash. Percent ash of the separable lean was only affected by retail cut ($P = 0.0109$), but differences were minor among cuts (results not shown in tabular form). Top round steaks exhibited the highest ash percentage (1.09 ± 0.03), different ($P < 0.05$) from ribeye (0.96 ± 0.03) and tenderloin (0.99 ± 0.03) steaks but similar to shoulder clod (1.01 ± 0.03) steaks ($P = 0.0554$).

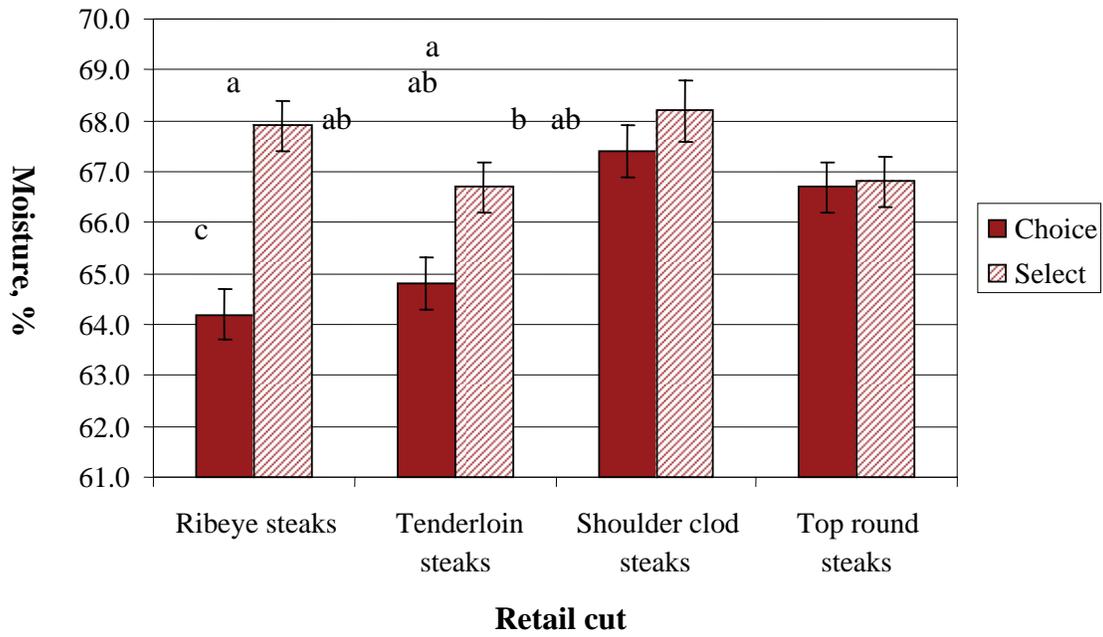


Figure 3. Least squares means and standard errors for percentages of moisture as affected by retail cut and USDA quality grade. Columns with different letters differ ($P < 0.05$).

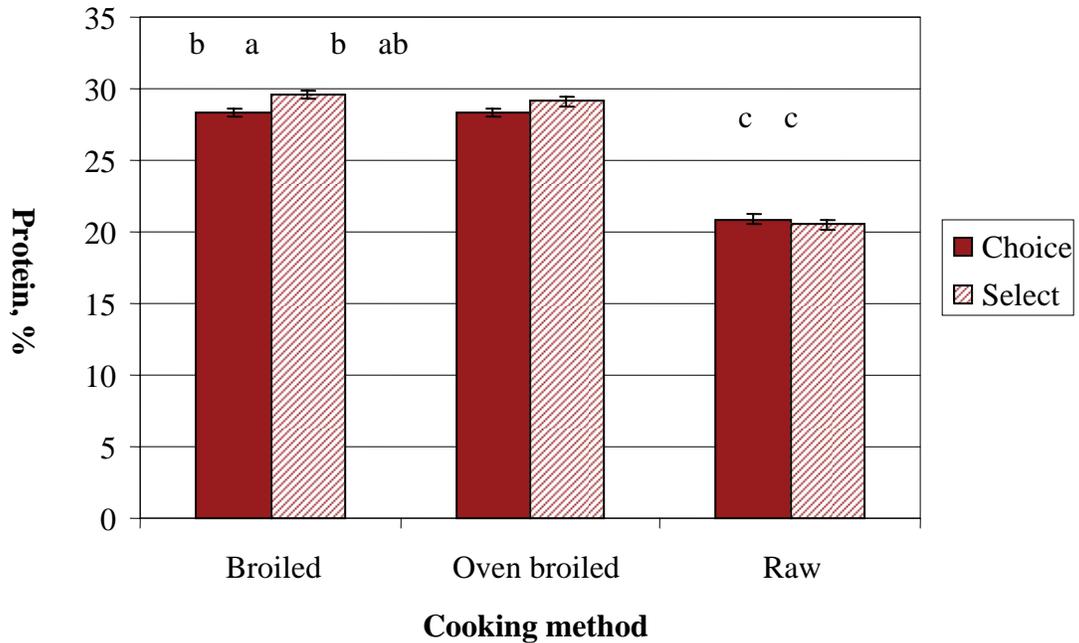


Figure 4. Least squares means and standard errors for percentages of protein as affected by USDA quality grade and cooking method. Columns with different letters differ ($P < 0.05$).

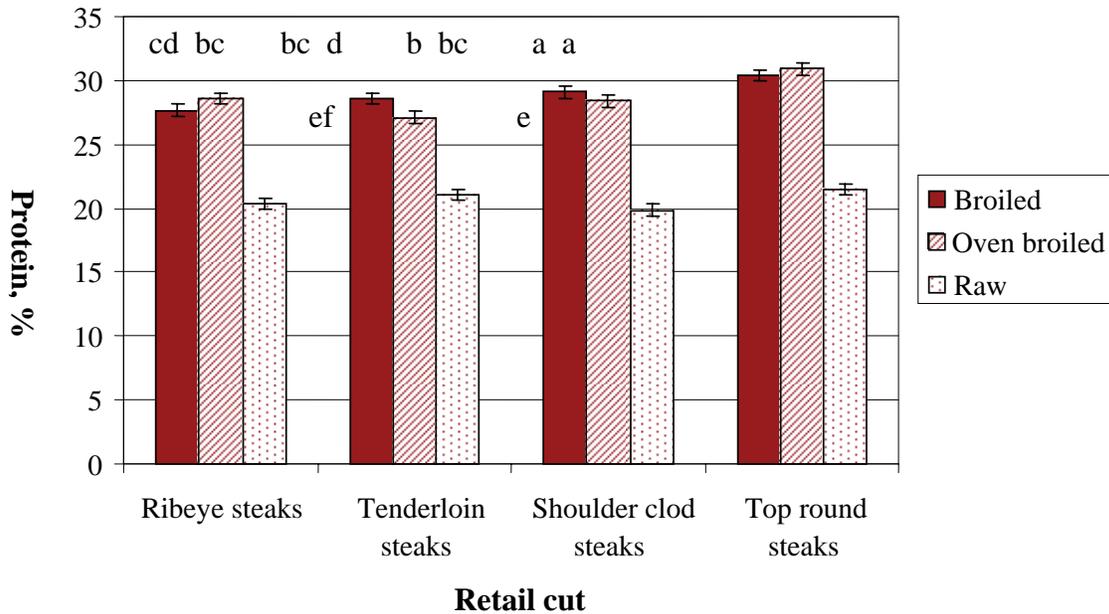


Figure 5. Least squares means and standard errors for percentages of protein as affected by retail cut and cooking method. Columns with different letters differ ($P < 0.05$).

Fat retention. Retention of fat within the lean after cooking was only affected significantly by USDA quality grade ($P = 0.0128$) and cooking method ($P = 0.0053$). The non-significant variation in fat retention due to retail cut (Figure 6) does not agree with the findings of Morgan et al. (1988) and Jones et al. (1992c). It also contradicts our hypothesis that fat retention would be affected by retail cut due to the wide range of seam and surface fat present in the selected retail cuts. Adjusted means for fat retention in the lean portion exceeded 100% in all selected retail cuts (Figure 6), supporting the common suspicion that lipids from other depots migrated into the lean. However, lack of significant variation in fat retention among retail cuts differing in proportion or seam fat does not contribute to support the influence of rendered lipids migrating from the seam fat depots into the lean edible portion as suggested by Jones et al. (1992c) or Renk et al. (1985).

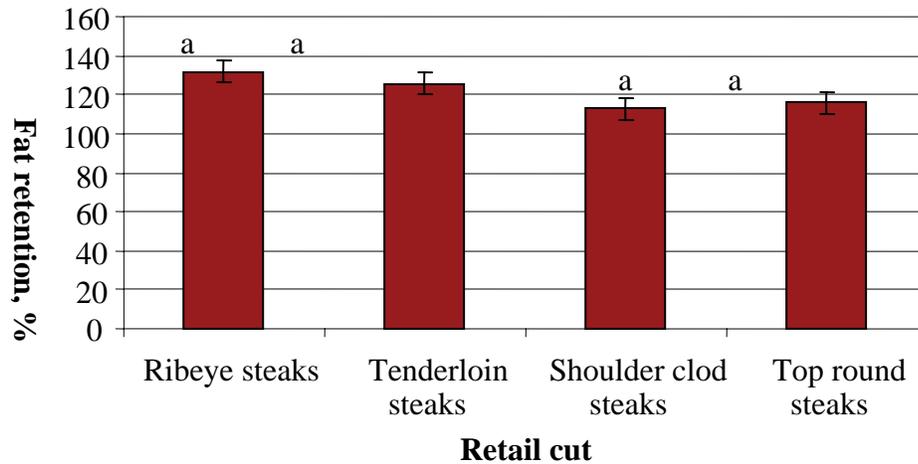


Figure 6. Least squares means and standard errors for fat retention percentages of ribeye, tenderloin, shoulder clod, and top round steaks. Columns with different letters differ ($P < 0.05$).

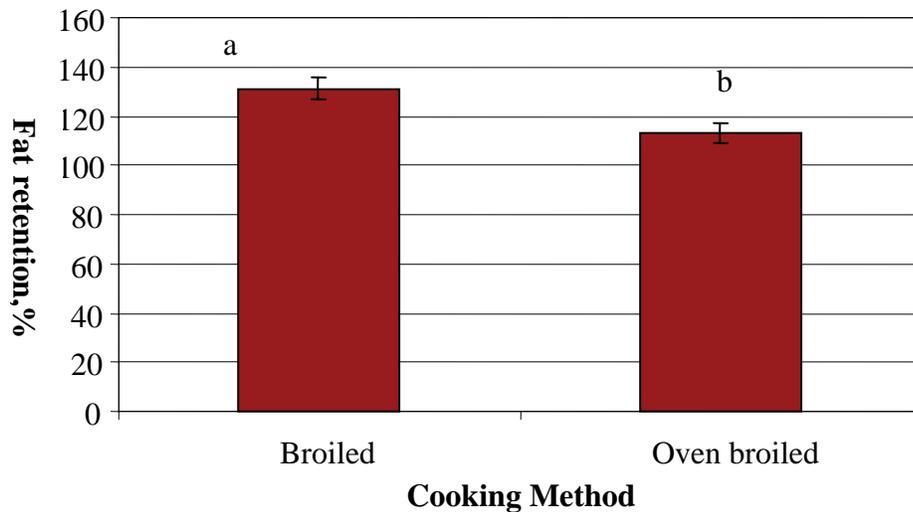


Figure 7. Least squares means and standard errors for percentage of fat retention of broiled and oven broiled steaks. Columns with different letters differ ($P < 0.05$).

Percentage fat retention was higher ($P = 0.0026$) for broiled steaks (Figure 7). Conversely, Renk et al. (1985) reported that cooking method did not affect fat retention. Fat retention was higher in separable lean of Select steaks than in Choice steaks (Figure 8). However, Renk et al. (1985) reported that degree of marbling did not have an effect on fat retention.

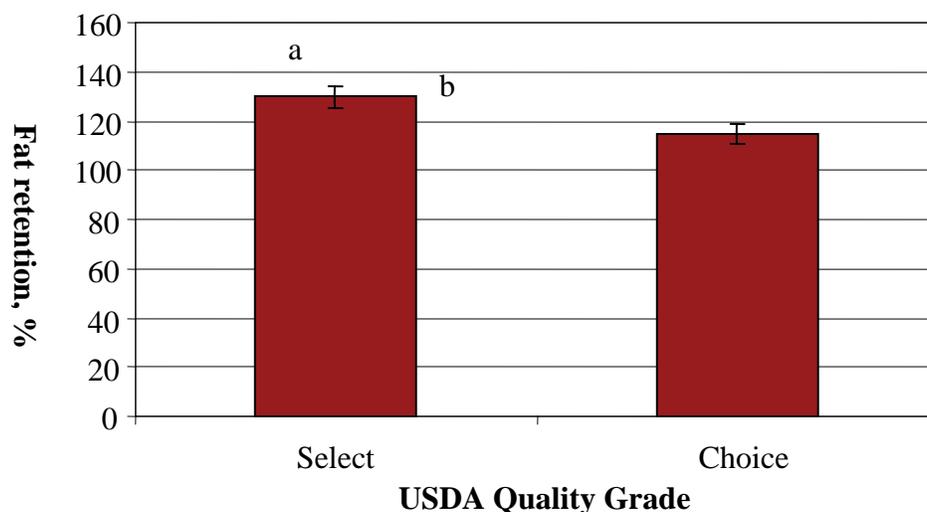


Figure 8. Least squares means and standard errors for fat retention percentages of Select and Choice steaks. Columns with different letters differ ($P < 0.05$).

Conclusions

Retail cuts, due to their anatomical location, are physically composed of different percentages of lean, surface fat, and seam fat. Retail cut had a direct effect on dissection yields and an indirect effect on proximate composition values simply because of the amount of fat deposition and fat trimming level. Cooking method did alter the percentage of proximate composition with regards to percentage chemical fat and moisture. Migration of fat throughout the lean is affected by cooking method. However, the question regarding the original source of these migrating lipids remains unresolved.

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**DETERMINATION OF ZINC PROTOPORPHYRIN IX IN A NEW MODEL
SYSTEM WITHOUT ADDITION OF MYOGLOBIN**

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Key Words: Parma ham, Zinc protoporphyrin IX (ZPP), Heme, Model system

Introduction

Nitrite or nitrate is usually added to meat products as a curing ingredient, and it reacts with myoglobin to yield stable-red nitrosylmyoglobin, MbFe(II)NO. The north Italian traditional dry-cured ham “Parma ham” is made only from the leg of a fattened pig by salting with sea salt, drying, and maturing over period of one year. Although nitrite or nitrate is not added to Parma ham, the color of the ham is an extremely stable bright red and is hardly changed by exposure of the ham to light. Morita *et al.* (1996) reported that the red pigment of Parma ham is a myoglobin derivative that can be easily extracted with 75% acetone, and they suggested that the formation of the red myoglobin derivative in Parma ham is caused by the action of microorganisms. It was later shown that the red pigment extracted with 75% acetone from Parma ham is Zn protoporphyrin IX (ZPP) (Wakamatsu *et al.*, 2004a). Wakamatsu *et al.* (2004b) established a model experiment system in which ZPP is formed by incubation of myoglobin, pork homogenate and antibiotics in the absence of oxygen. However, effect of myoglobin added to the model system on the ZPP formation is suspicious, and it is therefore necessary to establish a simpler model system without addition of myoglobin. Studies of ZPP in Parma ham will be useful for manufacturing meat products being desirable color without addition of either nitrite or nitrate.

Objectives

The objectives of this study were to establish a simpler model system in which ZPP is formed without addition of myoglobin and to obtain a clue for elucidating the mechanism of ZPP formation by using the established model.

Methodology

Model experiment: About 20 g of pork loin was homogenized with 30 ml distilled water at 10,000 rpm for 1 min. Model solutions consisted of pork homogenates (final meat concentration of 20%) and antibiotics (final concentrations of 100 units/ml for penicillin G potassium, 0.1 mg/ml for streptomycin sulfate and 0.05 mg/ml for gentamicin sulfate). The solutions were put into gas-impermeable bags and incubated for

5 days at 25 °C anaerobically. An anaerobic condition was obtained by using an oxygen absorber (A-500HS, I.S.O. Inc., Yokohama, Japan).

Experiments using the model: In experiments on the effect of temperature, model solutions were incubated at 4, 15, 25 and 37 °C. In experiments on the effect of ion intensity, ion intensities of solutions were adjusted to 0 – 1 M by using sodium chloride. In experiments on the effect of pH, pH values of solutions were adjusted to 3 – 8 by using hydrochloric acid and sodium hydroxide. In experiments on the effect of pork concentration, pork concentrations of solutions were adjusted to 0 – 50% by adding pork homogenate.

Extraction of ZPP: Incubated model solutions (each 1.5 g) were mixed well with 4.5 ml of ice-cold acetone, and the mixtures were held on ice for 30 min. After filtration through filter paper (No. 2, Toyo Roshi Co., Ltd., Tokyo, Japan), the fluorescence intensity (excitation: 420 nm, emission: 590 nm) of the extracts was measured by using a Model RF-53000PC fluorescence spectrophotometer (Shimadzu Co., Kyoto, Japan).

Quantitative analysis of ZPP and heme in the model and Parma ham: Parma ham (10 g) was homogenized with 40 ml distilled water at 10,000 rpm for 1 min. Model solutions (each 1.5 g) (before and after incubation) or Parma ham homogenates (each 1.5 g) were mixed well with 4.5 ml of ice-cold acetone. The mixtures were placed on ice for 30 minutes and were centrifuged (3,000 rpm, 10 min, 4 °C). The supernatants were diluted up to 50 ml with 75% acetone, and fluorescence intensity (Ex/Em: 420/590 nm) was analyzed by using a fluorescent spectrophotometer. The pellets were mixed with hydrochloric acid (final percentage of 0.7%) and acetone (final percentage of 75%). The mixtures were placed on ice for 30 minutes and were centrifuged (3,000 rpm, 10 min, 4 °C). The supernatants were diluted up to 50 ml with 0.7% hydrochloric acid-75% acetone, and absorbance at 383 nm was analyzed by using a Model U-3310 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The concentrations of ZPP and heme were calculated using a standard curve from a standard reagent.

Results & Discussion

Model solutions consisting of only pork homogenate and antibiotics were incubated for 5 days at 25 °C anaerobically, and the fluorescence intensity (Ex/Em: 420/590 nm) of 75% acetone extracts of model solutions showed that the amount of ZPP was considerably increased after incubation (Fig. 1). This result indicated that ZPP was formed from pork in the absence of myoglobin and microorganisms. Next, we examined the effects of temperature, ion intensity, pH and pork concentration on ZPP formation (Fig. 2). The amount of ZPP increased with increase in incubation temperature. The amount of ZPP showed a maximum at ion intensity of 0.05 M and thereafter decreased. The amount of ZPP showed a maximum at around pH 5.5 and increased with increase in pork concentration. These results indicate that ZPP formation depends on each condition, suggesting that components of pork are involved in ZPP formation. Next, we carried out quantitative analysis of ZPP and heme in the model solution and Parma ham. The concentration of ZPP was analyzed by measuring fluorescence intensity (Ex/Em: 420/590 nm) of 75% acetone extracts, and the concentration of heme was analyzed by measuring absorbance at 383 nm of 0.7% hydrochloric acid-75% acetone extracts. As shown in Table 1, the proportions of ZPP were 7.9% in the model solution after incubation and

6.9% in Parma ham. Thus, the percentage of ZPP formed in the model solution is almost the same as that in Parma ham. This suggests that the model system is useful for determining the mechanism by which ZPP is formed. Next, we carried out quantitative analysis in the model solution before and after incubation. As shown in Fig. 3, the amount of ZPP increased after incubation. Additionally, since the total amount of ZPP and heme increased after incubation, it was speculated that ZPP and heme were newly formed during incubation. ZPP and heme in Parma ham may also be newly formed during processing. Elucidation of the mechanism by which ZPP is formed in Parma ham awaits further studies.

Conclusions

We established a simple model system in which ZPP is formed by incubation of pork and antibiotics anaerobically. ZPP formation depended on various conditions, suggesting that components of pork are involved in ZPP formation. The results also indicated the possibility that ZPP and heme were newly formed during incubation. ZPP equal in proportion to that in Parma ham was formed only from pork in the model established without microorganisms, this model system will be useful for determining the mechanism by which ZPP is formed in Parma ham.

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Tables and Figures

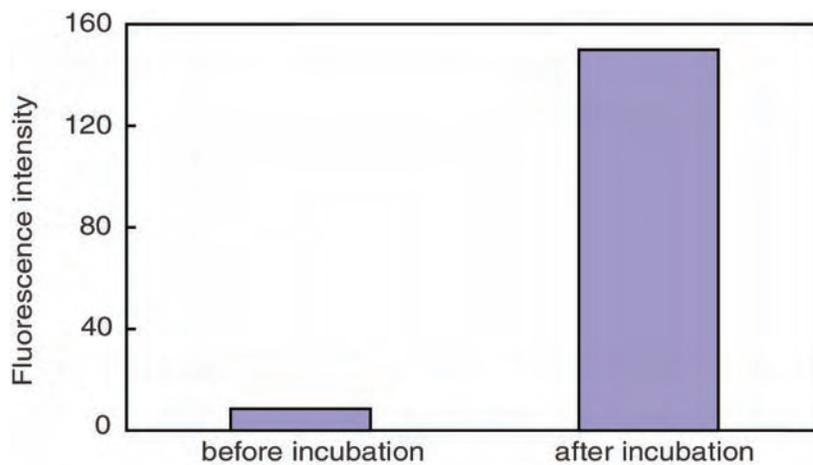


Fig. 1. Fluorescence intensity of ZPP in model solutions before and after incubation (Ex/Em: 420/590 nm).

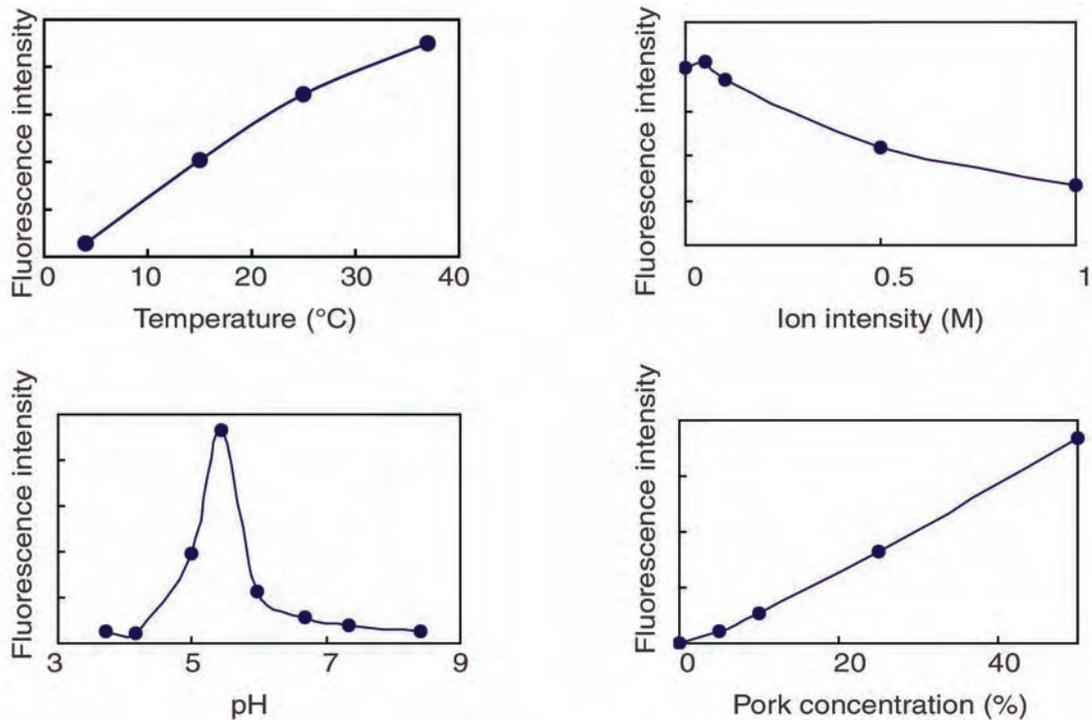


Fig. 2. Effects of incubation temperature, ion intensity, pH and pork concentration on the formation of ZPP (Ex/Em: 420/590 nm).

Table 1. Concentrations and percentages of ZPP and heme in the model solution after incubation and in Parma ham.

	ZPP	Heme
	(mmol/g)	
Model solution	5.5 ± 0.1	64.7 ± 1.2
	(7.9%)	(92.1%)
Parma ham	10.5 ± 0.2	142.7 ± 1.8
	(6.9%)	(93.1%)

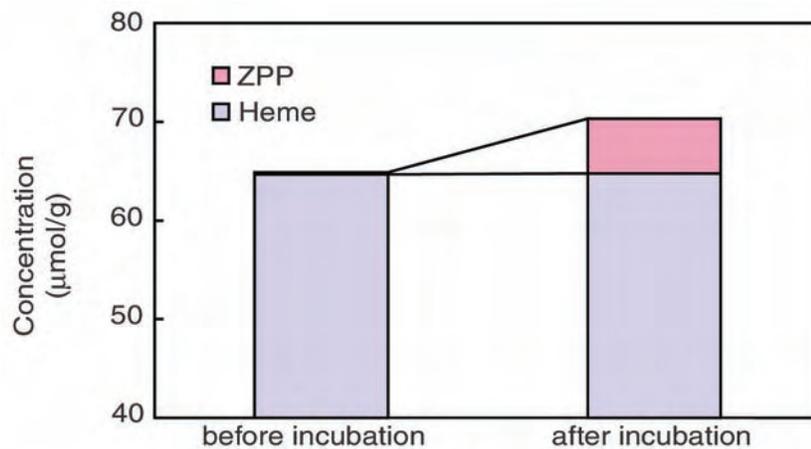


Fig. 3. Concentrations of ZPP and heme in the model solution before and after incubation.

QUANTIFICATION OF CALPASTATIN USING AN OPTICAL SURFACE PLASMON RESONANCE BIOSENSOR

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Key Words: biosensor, calpastatin, tenderness

Introduction

There is a large body of evidence indicating that μ -calpain is the major cause of postmortem tenderization of skeletal muscle through degradation of structural proteins (for reviews see Koohmaraie, 1996; Geesink et al., 2000). The activity of μ -calpain in postmortem muscle is controlled by calpastatin, its endogenous inhibitor. Post rigor calpastatin activity (1-2 days p.m.) accounts for a greater portion of the variation in tenderness of aged beef longissimus (~40%) than any other single measure (Whipple et al., 1990; Shackelford et al., 1994). For this reason calpastatin quantification in skeletal muscle is commonly performed in research on meat tenderness.

Most frequently, calpastatin quantity, measured as m-calpain inhibitory activity, is performed on samples subjected to ion-exchange chromatography to separate calpastatin from the calpains (Koohmaraie, 1990). To facilitate quantification of a large number of samples in a breed evaluation program, muscle extracts were heated to 95°C instead of subjected to chromatography, making use of the fact that calpastatin retains its activity under these conditions (Shackelford et al., 1994). Methods based on immunological quantification include an ELISA (Doumit et al., 1996) and Western blotting (Sensky et al., 1999). Although the ELISA method lends itself to automation, all of these methods are rather labor intensive and require at least several hours to complete.

An alternative to these methods is the use of a biosensor, which is a measurement system that uses biological ligands as a part of a biotransducer that transforms chemical information into an analytically useful signal. A number of recent publications have addressed the suitability of surface plasmon resonance (SPR) based biosensors for analyses in the food industry (see Karlsson, 2004 for a review). From these studies it appears that SPR biosensors offer a valuable alternative to other techniques because it is both rapid and real-time.

Objectives

The purpose of the present study was to explore the suitability of a SPR biosensor system (Biacore Q) for quantification of calpastatin.

Methodology

Samples

Samples were obtained in three separate experiments. The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in the first two experiments. The animals were slaughtered humanely and dressed using typical procedures. Carcasses were chilled at 0°C.

Experiment 1

Twelve sheep (12 months of age) were used. From 5 animals, a portion of the longissimus of one carcass side was dissected after dressing for determination of calpastatin activity. At 2 days p.m. the longissimus of the intact carcass side was dissected and a portion was removed for determination of calpastatin activity. After vacuum storage at 4°C, the remainder of the muscle was sampled for determination of calpastatin activity at 10 days p.m.

Experiment 2

From thirty steers (14-16 months of age), a portion of the longissimus of one carcass side was sampled at 2 days p.m. for determination of calpastatin activity.

Experiment 3

Thirty longissimus samples from female cattle (300-400 kg carcass weight) were obtained at 1 day p.m. from a commercial slaughterplant. The muscles were sampled at 1 day p.m. for determination of calpastatin activity. Samples were stored at -80°C until quantification of calpastatin. The remainder of the muscle was vacuum packed and aged at 2°C till 14 days p.m.. After the aging period a 2 cm thick steak was vacuum packaged and stored at -20°C until determination of the shear force.

Preparation of samples for quantification of calpastatin

Samples (25 g) were homogenized in three volumes of cold 50 mM Tris (pre rigor) or 100 mM Tris (post rigor), 10mM EDTA, pH 8.3, containing 0.05% β -MCE, 100 mg/L ovomucoid, 2 mM PMSF, and 6 mg/L leupeptin. The homogenates were centrifuged at 16,000 x g for 2 hours at 4°C. The supernatant was collected, heated to 95°C in a waterbath, and maintained at this temperature for 15 min. Samples were chilled on ice and clarified by centrifugation at 37,500 x g for 1 hour at 4°C. The supernatants were dialyzed overnight against 40 mM Tris, 5 mM EDTA, 0.05% β -MCE, pH 7.35 at 4°C. Calpastatin activity of the heated extracts was determined as described by Koohmaraie (1990).

In experiment 1 and 2, aliquots (2 mL) of the extracts were frozen at -80°C and shipped on dry ice to CCL research. Samples were stored at -80°C until use. For the beef samples, aliquots were also collected before dialysis. Extracts from the third experiment were assayed directly for calpastatin content as described below.

Preparation of the biosensor chip

This assay was developed on a BIACORE Q biosensor system (Biacore, Uppsala, Sweden) controlled by a BIACORE Q Software package version 3.01.

Human erythrocyte calpastatin (Calbiochem, San Diego, CA) was used to coat the biosensor chip and for the calpastatin standard curve.

Prior to coating the CM5 biosensor chip (Biacore, Uppsala, Sweden), calpastatin was diluted to 40 µg/mL in 10 mM sodium acetate pH 4.0. The carboxymethylated dextran layer on the sensor chip surface was activated by derivatization with N-hydroxysuccinimide (NHS) mediated by N-ethyl-N'-(3-diethylaminopropyl)carbodiimide (EDC) according to Fagerstam et al. (1992). Activation, coupling of calpastatin and blocking of reactive groups were performed at a flow rate of 10 µL/min and 25°C. Activation was carried out at through injection of 70 µL 200 mM EDC, 50 mM NHS. Covalent coupling of calpastatin was achieved through injection of 70 µL of the calpastatin solution. Remaining dextran-conjugated NHS-esters were deactivated with 70µL 1 M ethanolamine pH 8.5. Non-covalently bound proteins were washed from the surface with running buffer (HBS-EP: 0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4).

Assay for quantification of calpastatin

For this assay monoclonal anti-calpastatin antibody 3B9 was used (Doumit & Koohmaraie, 1999). The antibody was purified from hybridoma cell supernatant using Immunopure immobilized protein G (Pierce, Rockford, IL), according to the manufacturers instructions. The protein concentration of the purified antibody was 4.6 mg/mL. Prior to use, the antibody was diluted 20-fold with HBS-EP.

The heated muscle extracts were filtered over 0.45 µm filters. Samples with a calpastatin content higher than covered by the standard curve were diluted with dialysis buffer. To all samples 0.3 g/L carboxymethylated dextrane from a stock solution of 3 g/L was added to prevent non-specific interaction with the dextrane matrix of the chip. Subsequently, samples were transferred to 96-wells microtitre plates and placed in the biosensor's autosampler compartment. For each series of 12 samples a standard curve of calpastatin (0.05 – 2.0 µg/mL) was included.

The system was operated at a flow rate of 20 µL/min. Before injection of the sample, HBS-EP was passed through the flow cell during 200 s. Prior to injection of the sample, the autosampler mixed 1 part of antibody solution with 9 parts of sample. This mixture was passed through the flow cell during 120 s, followed by HBS-EP during 80 sec. The chip surface was regenerated by injection of 8 mM NaOH during 120 s. followed by HBS-EP during 20 s. The difference in response 5 s before and 5 s after injection of the sample was recorded and expressed as µg calpastatin/mL using the standard curve.

Shear force

Frozen vacuum packaged steaks were tempered for 48 hours at 2°C, cooked during 1 hour in a waterbath heated at 75°C and chilled under running tapwater. Of each sample 10 rectangular subsamples of 1 cm² were cut parallel with the fibre direction. Shear force (N/cm²) was determined perpendicular to the fibre direction using a draw bench (Adamel Lhomargy DY 30; Division d'Instruments S.A., Paris, France) equipped with a triangular shearing blade. The average shear force of the 10 measurements per sample is reported.

Statistical analysis

Significance of correlation coefficients between parameters was determined with Pearson's test using the SAS statistical package.

Results & Discussion

Results from the biosensor method correlated well with the activity measurements, but the intercept of the linear relationship did not pass through zero (Figures 1). The reason for this is that the immunological detectable amount of calpastatin decreases faster during postmortem storage than the amount detectable in the enzymatic assay (Figure 2). A similar observation, using Western blotting, was reported by Geesink & Koohmaraie (1999). The explanation for this is that calpastatin is degraded postmortem, but some proteolytic fragments retain their inhibitory activity (Mellgren & Carr, 1983; Imajoh et al., 1984; Doumit & Koohmaraie, 1999). Thus, the epitope recognized by the antibody is degraded faster than the inhibitory sites of calpastatin. As a consequence, the biosensor assay is not suitable to study the evolution in calpastatin activity during postmortem storage of muscle.

Since post rigor and not pre rigor calpastatin activity is related to tenderness of aged longissimus (Whipple et al., 1990) the calpastatin activity was determined in 30 beef muscle extracts (2 days p.m.) and they were shipped to CCL.

The procedure to produce the extracts includes a dialysis step. Omission of this step would save a considerable amount of time and labor. To test whether this step can be omitted without a detrimental effect on the results, samples of the beef extracts were taken before and after dialysis. Omission of dialysis resulted in a decreased response in the biosensor assay with a mean value of 0.42 $\mu\text{g/mL}$. However, the correlation with the measurements on the dialyzed samples was good ($r = 0.82$; data not shown), and correlation with the activity measurements was significant ($p < 0.01$; Figure 3). Thus, the dialysis step can be omitted from the extraction procedure without a detrimental effect on the results.

In the third experiment we tested whether post rigor calpastatin content, as determined using the biosensor assay, is related to the shear force of aged beef. In agreement with results of Whipple et al. (1990), post rigor calpastatin content was significantly correlated ($p < 0.01$) to the shear force of aged longissimus (Figure 4).

The Biacore Q system contains four flow channels, allowing for the sequential analysis of four different compounds in a single sample. The stability of the chip surface has not been fully tested, but we have performed more than 700 assays using a single flow channel. Thus one chip should allow for the measurement of at least 2800 samples, including standard curves.

The repeatability and reproducibility were tested on twelve beef samples which were measured in duplicate on different dates. The mean intra-assay CV's were 3.2% and 5.6% on the different dates. The mean inter-assay CV was 5.8%. This performance is similar to the ELISA for calpastatin developed by Doumit et al. (1996). In comparison, the ELISA showed a stronger correlation with activity measurements than the biosensor assay. The correlation between activity measurements and biosensor measurements can probably be improved by using the same polyclonal antibodies as used in the ELISA. Another important difference between the ELISA and biosensor assays is that with the ELISA a large number of samples can be processed simultaneously whereas the biosensor assay processes them sequentially. However, Biacore has developed a parallel 8-channel system and array systems are in development. With this development, high-throughput

applications appear possible in the near future. One of these possibilities would be on-line screening for calpastatin content as a predictor of beef tenderness. Given the fact that the sample preparation procedure is simple (extraction, heating, clarification), it should also lend itself to automation with sufficient speed to process the number of samples generated at production speed in beef plants.

Conclusions

At present, the calpastatin biosensor assay appears suitable for research purposes where large amounts of samples need to be processed like breed evaluation or selection programs.

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Tables and Figures

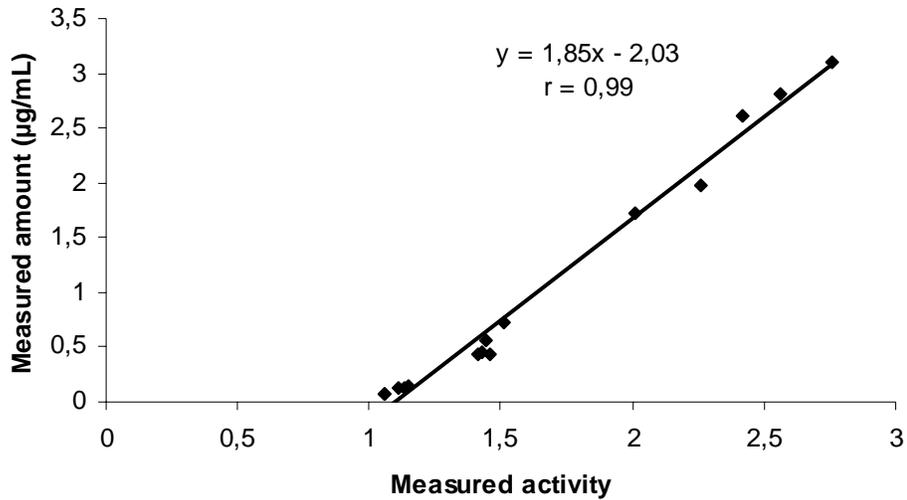


Figure 1. The relation between the activity and immunologically detectable amount of calpastatin in ovine longissimus muscle sampled at 0, 2 and 10 days postmortem.

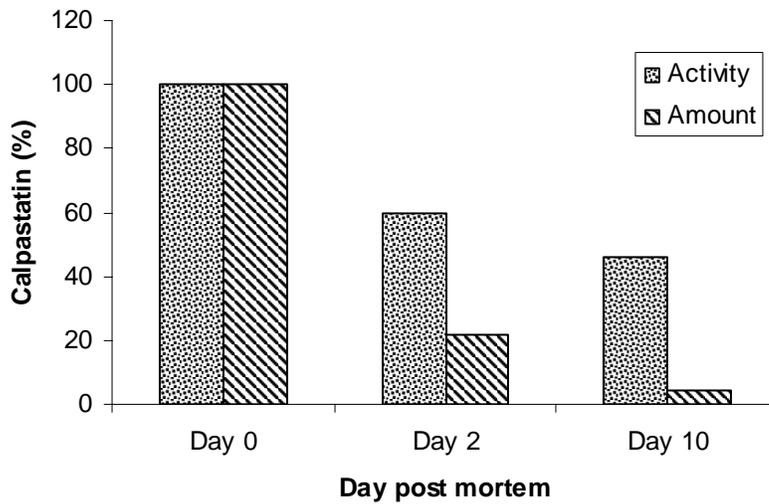


Figure 2. Relative amount of calpastatin in ovine muscle at 0, 2 and 10 days postmortem.

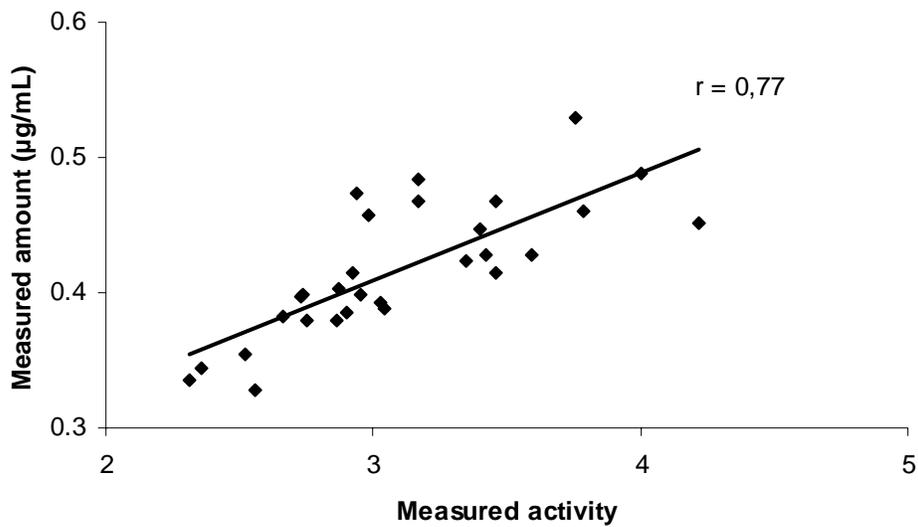


Figure 3. The relationship between the activity and immunologically detectable amount of calpastatin in bovine longissimus sampled at 2 days p.m.

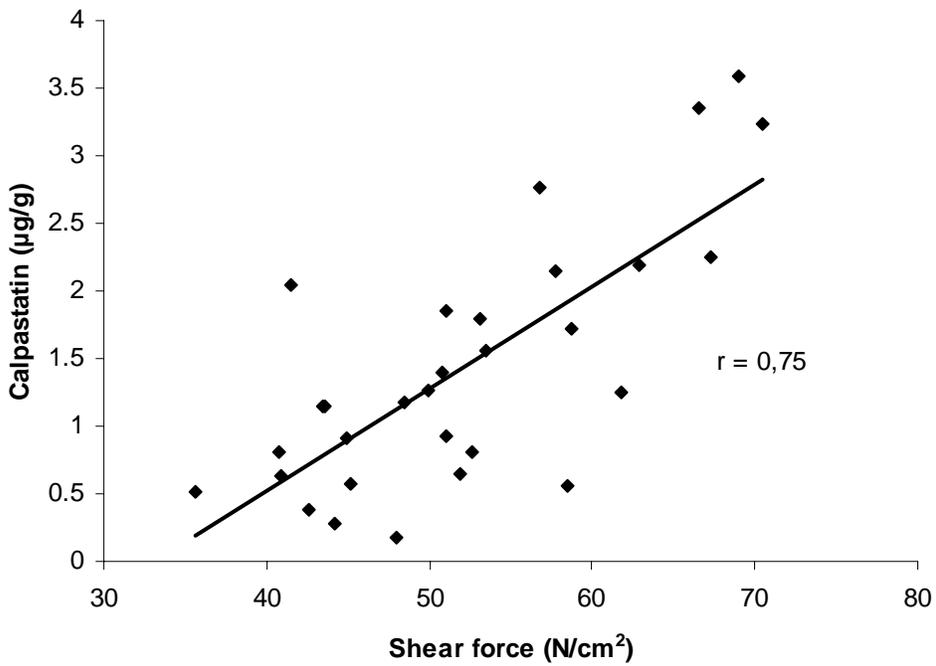


Figure 4. The relationship between the immunologically detectable amount of calpastatin at 1 day p.m. and shear force at 14 days p.m. of bovine longissimus.

**GENERATION OF ACE INHIBITORY DIPEPTIDES BY PORK MUSCLE
DIPEPTIDYL PEPTIDASES I AND III**

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Key Words: Dipeptidyl peptidases; dry-cured ham; dipeptides; angiotensin-I converting enzyme; fluorimetric assay; hypertension

Introduction

Dipeptidyl peptidases (DPP) are a group of enzymes belonging to different families and catalytic classes but that have in common the ability to liberate dipeptides from the N-terminus of larger peptides. Their action in the living cells has been related with many physiological processes such as regulation of bioactive peptides, protein turnover or implication in some pathological conditions [Rawlings et al., 2004]. Concerning the biochemistry of postmortem muscle, DPP are assumed to contribute to the intense proteolysis of muscle proteins occurring during meat aging but specially during the processing of meat products. This phenomenon gives rise to an intense breakdown of the myofibrillar structure and the generation of important amounts of free amino acids and small peptides (lower than 1200 Da), which are directly related with the development of the typical flavor characteristics of these products [Toldrá and Flores, 1998]. However, little is known about the physiological function of these peptides generated during the ripening of dry-cured ham. This physiological, or tertiary function, can be defined as the property of some food components to exert direct beneficial effects on health by regulating different biochemical, biological and/or physiological processes. One of the most interesting tertiary functions is the property of some peptides to inhibit the activity of angiotensin-I converting enzyme (ACE; EC 3.4.15.1), an ectoenzyme implicated in the renin-angiotensin system. ACE catalyzes the conversion of inactive angiotensin I into angiotensin II, which is a potent vasoconstrictor and also inactivates the vasodilator bradykinin, giving rise to a hypertensive effect [Houston, 2002]. By this way, substances inhibiting ACE activity are able to reduce blood pressure.

Objectives

The present work has the objective to investigate if the activity developed by dipeptidyl peptidases I and III along the processing of dry-cured ham could contribute to the generation of small peptides having antihypertensive activity, which could be especially positive for helping in the regulation of blood pressure through diet. For that purpose, some dipeptide sequences known to be products of the activity of DPP I and III on different substrates were assayed for the inhibition of ACE activity.

Methodology

Preparation of enzyme solutions.

Stock solution: Commercial angiotensin-I converting enzyme (0.51 mg) was diluted in 3.3 mL of a 50 % glycerinated solution with 0.15 M tris buffer, pH 8.3, containing 1 μM ZnCl_2 . This solution was kept at -20°C until use.

ACE working solution. Stock solution was diluted 1/20 with 0.15 M tris buffer, pH 8.3, having a concentration of 7.2 $\mu\text{g mL}^{-1}$ of ACE corresponding to 3 mU mL^{-1} of enzyme activity.

Inhibition of ACE activity by addition of dipeptides: Inhibition of angiotensin-I converting enzyme activity by dipeptides shown in figure 2 at different concentrations, ranging from 0.5 to 200 μM , was determined through the development of a fluorimetric assay using *o*-aminobenzoylglycyl-*p*-nitrophenylalanylproline (Abz-Gly-Phe(NO_2)-Pro) as substrate. The assay was optimized until the following conditions: 50 μL of each peptide concentration were added to wells of a microtiter plate, then adjusting to 100 μL by addition of ACE working solution. The reaction was initiated by the addition of 200 μL of 0.45 mM Abz-Gly-Phe(NO_2)-Pro dissolved in 150 mM tris buffer, pH 8.3, containing 1.125 M NaCl. The reaction mixtures (300 μL /well) were immediately shaken and incubated at 37°C for 45 minutes and the generated fluorescence was measured in a multiscan fluorometer (Fluoroskan Ascent, Labsystems, Finland) using excitation and emission wavelengths of 355 and 405 nm, respectively. ACE activity obtained in the presence of each assayed peptide concentration was referred to controls, which were simultaneously measured in the absence of any added peptide, taking the value of 100 %.

Assay of DPP I and DPP III enzyme activity on different peptide substrates. Study of substrate specificity of DPP I and III on the release of N-terminal dipeptides from both synthetic and natural peptides, together with determination of optimum pH, was described in previous works [Sentandreu and Toldrá, 1998 and 2000].

Results & Discussion

Table 1 shows some of the N-terminal dipeptide sequences generated due to proteolytic action of DPP I and III on both synthetic and natural peptides. DPP I hydrolyzed well peptide substrates having alanine or arginine in N-penultimate position under acidic conditions. On the contrary, peptides having a basic amino acid in N-terminal position, or those containing a proline residue in any side of the peptide bond were resistant to attack. According to this, Met-Ala, Gly-Arg, Ala-Arg and Ala-Ala could be split from different substrates due to porcine muscle DPP I. These two latter dipeptides were also generated due to DPP III activity at basic pH, together with Arg-Arg, Arg-Phe, Phe-Gly and Arg-Gly. Even if DPP I and DPP III share some common substrate specificity, each one differs from the other in terms of optimum pH, reducing conditions, concentration of some divalent cations and subcellular location. A remarkable difference is the fact that DPP I was able to hydrolyze peptides as short as tripeptides, whereas DPP III was unable, being tetrapeptides the minimum length required for activity [Sentandreu and Toldrá, 1998 and 2000].

It has been shown that dipeptidyl peptidases I and III remain active during the whole processing period of dry-cured ham. In the case of DPP I its optimum pH range is coincident with muscle pH during dry-curing, and so an important dipeptide generation due to DPP I activity would be expected from the early stages of dry-curing. In the case of DPP III its optimal pH is higher than muscle pH and consequently a relevant dipeptide generation would be expected mainly after a long time of action, at the end of the process [Sentandreu and Toldrá, 2001]. We considered of interest, from the nutritional aspects of dry-cured ham intake, to evaluate if dipeptides that can be generated by the proteolytic action of DPP I and III during the whole processing of Spanish dry-cured ham could be effective angiotensin-I converting enzyme inhibitors. For that purpose, we have developed a fluorimetric assay for a rapid and accurate determination of ACE activity which is based on the hydrolysis of the internally quenched fluorescence substrate Abz-Gly-Phe(NO₂)-Pro developed by Carmel and Yaron (1978). Hydrolysis of this substrate by the action of ACE generates the fluorescent product *o*-aminobenzoylglycine (Abz-Gly), which can be continuously monitored in a microtiter-plate fluorescent reader, using excitation and emission wavelength of 355 and 405 nm, respectively. Then, we have established the optimal conditions for its linearity, sensitivity and precision. This assay has several advantages with respect to other methods actually in use such as the simplicity to carry on and the possibility to process a high number of samples in a short time. As can be appreciated in figure 1, the method showed a very good correlation ($r = 0.9925$) with that of Cushman and Cheung (1971), which is the assay commonly employed for the study of ACE inhibitors in foods. As can be observed, a higher hydrolysis rate of Abz-Gly-Phe(NO₂)-Pro is obtained with respect to hydrolysis of hippuryl-His-Leu (Hip-His-Leu) at a given enzyme concentration (figure 1).

Different concentrations of the dipeptides generated by the activity of DPP I and III (see table 1) were incubated with angiotensin-I converting enzyme before the addition of the substrate in order to evaluate if these dipeptides can be effective ACE inhibitors able to positively influence the control of blood pressure. As can be seen in figure 2, Ala-Ala, which can be liberated by the action of both DPP I and DPP III, exerted an important ACE inhibitory activity since 50 % inhibition was achieved at 50 μ M peptide concentration. Ala-Arg, also generated by the two exopeptidases, and Arg-Phe, generated by DPP III, proved to be also effective ACE inhibitors. Met-Ala and Gly-Arg, both of them generated by DPP I activity, were less inhibitory, requiring a concentration of 150 μ M to achieve 50 % ACE inhibition. The rest of the assayed dipeptides, Arg-Gly, Phe-Gly and Arg-Arg, did not exert a remarkable inhibition of ACE activity (figure 2).

Dry-cured ham processing can vary depending on the different types and qualities of hams but, in all cases, an intense proteolysis takes place on both sarcoplasmic and myofibrillar proteins during this long period. As it has been reported, this intense protein degradation is mainly due to the coordinated action of endogenous muscle enzymes [Toldrá and Flores, 1998]. So, muscle endopeptidases such as calpains, cathepsins and proteasome would be responsible of the initial breakdown of proteins to generate large polypeptides, a phenomenon directly related with the development of meat tenderness [Sentandreu, Coulis and Ouali, 2002]. Then, these polypeptides would serve as substrates for the action of different groups of exopeptidases like DPP I and III. The ability of these enzymes to generate dipeptides able to inhibit ACE activity (see figure 2), together with their good stability during dry-curing [Sentandreu and Toldrá, 2001] and the long

duration of the process, would make feasible the generation of sufficient amounts of antihypertensive peptides that, in some way, would help to balance the effect of NaCl content of these products on hypertension.

Conclusions

The proteolytic action of dipeptidyl peptidases I and III along the processing of dry-cured ham can generate dipeptides which, some of them, are able to considerably inhibit the activity of angiotensin-I converting enzyme. This could help to maintain an adequate systolic blood pressure even if dry-cured ham is present in the diet, which it would be specially positive for hypertensive population from countries like Spain, having great tradition in consumption of this traditional food.

Acknowledgements

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Tables and Figures

Table 1: Proteolytic action of Dipeptidyl peptidases I and III on different natural and synthetic peptides

Enzyme ¹	Classification ²	Location	pH range (Optimum pH)	Released dipeptides due to their enzyme activity ³
DPP I (EC 3.4.14.1)	Cystein peptidase Family C1, clan CA (Papain family)	Lysosome	5 – 6.5 (5.5)	Gly-Arg-↓AMC Ala-Arg-↓AMC Ala-Ala-↓pNa Met-Ala-↓Ser
DPP III (EC 3.4.14.4)	Metallopeptidase Family M49, clan MA	Cytosol	7.5 –8.5 (8.0)	Arg-Arg- ↓AMC Ala-Arg-↓AMC Ala-Ala-↓pNa Arg-Arg-↓Lys-Ala-Ser-Gly-Pro Arg-Phe-↓Arg-Ser Phe-Gly-↓Gly-Phe Arg-Gly-↓Asp-Ser

¹: Data from NC-IUMB (<http://www.chem.qmw.ac.uk>)

²: Data from MEROPS database (<http://www.merops.co.uk>)

³: Red arrow indicate cleavage of the corresponding peptides bond

Figure 1: Correlation between the fluorimetric assay using Abz-Gly-Phe(NO₂)-Pro as substrate for ACE (ordinate) with that of Cushman and Cheung (abscissa).

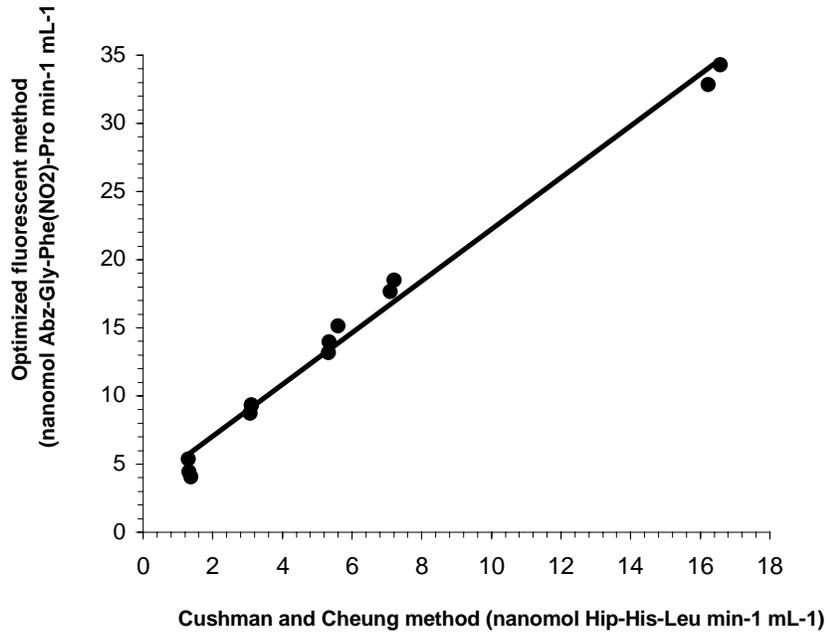
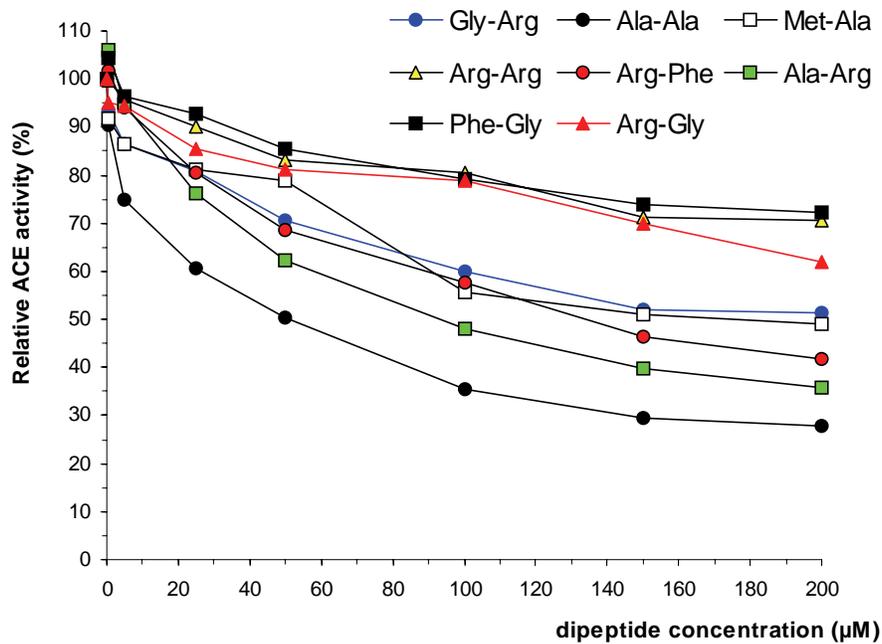


Figure 2: Effect of dipeptides generated by proteolytic action of DPP I and DPP III on the activity of angiotensin-I converting enzyme. Activity in the absence of added peptide was taken as 100 %.



**STEAROYL COENZYME A DESATURASE GENE EXPRESSION MEDIATES
FATTY ACID COMPOSITION OF ADIPOSE TISSUE FROM ANGUS AND
WAGYU STEERS FED TO U.S. AND JAPANESE ENDPOINTS**

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Key Words: Bovine, Fatty acid, SCD.

Introduction

Zembayashi (1994) demonstrated that totally trimmed *M. longissimus lumborum* of Japanese Black (Wagyu) cattle fed in Japan may contain as much as 20% extractable lipid. A subsequent report from this laboratory showed that the *M. longissimus dorsi* from Wagyu steers fed a corn and barley-based diet for 552 d contained nearly 19% extractable lipid, even though the steers were a mixture of 3/4 and 7/8 crossbred Wagyu steers (Lunt *et al.*, 1993). *M. longissimus dorsi* of Angus steers fed the same diet for the same period of time contained 14.5% extractable lipid. Although USDA quality grades did not differ between the Angus and Wagyu steers of the previous study (Prime¹⁹ vs Prime⁴⁸, respectively), the Wagyu steers had a greater beef marbling score (7.30 vs 4.50) and quality grade (4.40 vs 3.40) than the Angus steers, based on the Japanese grading system. Although Japanese Black and American Wagyu steers produce carcasses with higher quality grades than Angus steers when fed to a typical Japanese endpoint (650 kg), it is less clear whether Japanese cattle will produce higher quality carcasses if fed to a typical U.S. live weight endpoint. Japanese Black cattle fed in Japan typically are fed diets low in concentrate and high in fiber, but the concentration of intramuscular lipid (IML) in the *M. longissimus thoracis* appears to increase throughout their extended feeding periods (Zembayashi *et al.*, 1995). It is not known if Angus steers can deposit intramuscular lipid throughout extended feeding or if they can produce high quality carcasses if fed high roughage diets for extended periods.

Meat from Wagyu cattle is characterized by a higher amount of monounsaturated fatty acid (MUFA) in adipose tissues than is observed in adipose tissue of breed types typically produced in the U.S. (Sturdivant *et al.*, 1992). An early investigation (Cameron *et al.*, 1994) indicated no difference in subcutaneous (s.c.) adipose tissue stearoyl coenzyme A desaturase (SCD) enzyme activity or gene expression between Angus and American Wagyu cattle fed to the Japanese endpoint, in spite of significantly greater MUFA in Wagyu adipose tissue. We hypothesized that differences in fatty acid composition between Wagyu and Angus cattle may be due to greater SCD activity over the feeding period, which would have resulted in increased MUFA deposition at some point before slaughter. Therefore, we compared Angus and Wagyu cattle fed a corn-based finishing diet or a hay-based diet to 525 kg (U.S. endpoint) or 650 kg (Japanese endpoint).

Objectives

We hypothesized that USDA quality grades of Angus and Wagyu steers would not differ if the steers were fed to a typical U.S. endpoint (approximately 525 kg), but that Wagyu steers would have greater quality grades than Angus steers when fed to a Japanese endpoint (650 kg). We hypothesized that differences in fatty acid composition between Wagyu and Angus cattle are due to greater SCD activity earlier in production.

Methodology

Animal and Diets: Sixteen Wagyu crossbred (7/8 Wagyu or higher) and 16 Angus steers were purchased as calves at weaning (approximately 8 mo of age). The diet was designed to achieve an average gain of 1.36 kg/d, and was fed free choice for 8 or 16 mo after weaning ($n = 4$ per breed and time on feed). The remaining 8 steers of each breed type were offered coastal bermuda grass hay free choice, supplemented with non-protein nitrogen, and fed daily an amount of the corn-based diet estimated to achieve a targeted rate of gain of 0.9 kg/d. The hay-fed steers were fed for 12 or 20 mo after weaning ($n = 4$ per breed and time on feed). Targeted final body weights were 525 kg for steers fed for either 8 mo on corn or 12 mo on the hay-based diet, and were 650 kg for steers fed for either 16 mo on corn or 20 mo on the hay-based diet. After being fed for their respective time periods, the steers in each group were slaughtered on two consecutive days. The 5th-8th thoracic rib section of the *M. longissimus thoracis* and overlying fat was removed immediately after hide removal.

Carcass Characteristics: Carcasses were chilled at 4°C for 48 h and quality and yield grade factors were evaluated by trained personnel (USDA, 1997). USDA quality grade factors include overall maturity score and marbling score, whereas USDA yield grade was calculated based on adjusted fat thickness, *M. longissimus dorsi* cross-sectional (ribeye) area, carcass weight, and percentage of kidney, pelvic, and heart fat.

Fats and Moistures: A 100-g portion of the *M. longissimus thoracis*, completely trimmed of s.c. adipose tissue, was homogenized. Fat and moisture content were determined by standard methods (AOAC, 1990).

Total Lipid Extraction: Total lipid was extracted from muscle and s.c. adipose tissue by a modification of the methods of Folch *et al.* (1957).

SCD Enzyme Activity and Gene Expression: The activity of SCD enzyme activity was determined as described by St. John *et al.* (1991). SCD RNA was measured by Northern blot analysis using SCD RNA probes.

Fatty Acid Composition Analysis: Fatty acid methyl esters (FAME) of s.c. adipose tissue were analyzed using a Varian gas chromatograph (model CP-3800) by the method of Smith *et al.* (2002).

Statistical Analyses: Data were analyzed using the GLM as a two-factor design with the SAS version 8.1 (SAS Inst. Inc., Cary, NC). Means were compared by three-factor ANOVA. Main effects were breed type, diet, and endpoint (U.S. or Japanese), and the model tested all 2- and 3- way interactions. Interaction means were separated using the probability statement of GLM in the significant difference ($P < 0.05$).

Results & Discussion

Marbling scores and USDA quality grades were not different between breed types ($P \geq 0.30$; Table 1). However, most of the carcasses in the 16-mo and 20-mo groups were up into the USDA prime grade. Under the USDA grading system, it is difficult to discern differences between such highly marbled carcasses.

The interaction between breed and time-on-feed was significant for IML% of the *M. longissimus thoracis* ($P < 0.03$); the Wagyu carcasses in the 20-mo group contained more than 20% IML as compared to 12% for the Angus at the same endpoint. Intramuscular lipid increased in Angus steers until 16 mo on feed and did not increase thereafter. In contrast, IML continued to increase in the Wagyu cattle until the end of the study.

There was sufficiently greater s.c. fat thickness in the Angus steers ($P = 0.01$) to cause a significant difference in yield grade ($P = 0.01$; Table 1). Endpoint also had a significant effect on ribeye area, adjusted fat thickness, and USDA yield grade ($P = 0.01$). The higher yield grade of the Angus steers indicates the greater carcass adiposity of this breed type, compared to Wagyu steers (Zembayashi, 1994).

Wagyu contained a lower ($P < 0.01$) percentage of palmitate than Angus at all slaughtering time. Palmitoleate (16:1) was increased ($P < 0.01$) in s.c. adipose tissue when steers were finished to the Japanese endpoint compared to those finished to the U.S. endpoint. Stearate (18:0) remarkably ($P < 0.01$) decreased in Japanese endpoint steers compared with U.S. endpoint steers, and oleate (18:1) percentages were greater ($P < 0.01$) in Japanese endpoint steers than in U.S. endpoint steers. Wagyu steers had higher ($P < 0.03$) concentrations of oleate than Angus steers. The sum of MUFA in steers fed to the Japanese endpoint increased significantly ($P < 0.01$) compared to MUFA in steers fed to the U.S. endpoint.

SCD enzyme activity was not affected by breed type ($P = 1.00$) or diet ($P = 0.40$; Table 2), but was higher ($P = 0.01$) in Japanese endpoint steers than in U.S. endpoint steers. The diet x endpoint interaction ($P = 0.08$) suggested that SCD activity was greater in hay-fed cattle than corn-fed cattle, but only at the U.S. endpoint. The pattern of SCD gene expression was similar to that for enzyme activity (Table 2). Also, the significant breed x endpoint interaction indicated that Angus steers had peak expression at the U.S. endpoint, whereas SCD gene expression was highest at the Japanese endpoint for Wagyu steers. The pattern SCD enzyme activity and gene expression were similar to changes in fatty acid composition (Table 2). Waldman *et al.* (1968) and Huerta-Leidenz *et al.* (1996) previously indicated that concentration of MUFA increases in s.c. adipose tissue of U.S. cattle with age and carcass weight. Certainly, the age of the Wagyu and Angus steers affected MUFA concentrations in the cattle of this study.

Conclusions

We conclude that Wagyu cattle should be fed a high roughage diet for a relatively lengthy feeding period in order to reach their genetic potential to deposit maximum levels of marbling. This investigation showed no differences in SCD gene expression between Wagyu and Angus steers, but SCD activity peaked earlier in Angus steers than in Wagyu steers.

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Tables and Figures

Table 1. Carcass characteristics and longissimus proximate composition for Angus and Wagyu steers fed corn or hay-based diets for 8, 12, 16, or 20 mo

Item	Months on feed/diet								SE	<i>P</i> -values		
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			Breed	Time	B x T ^x
	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu				
Carcass weight, kg	323.4	252.3	307.7	283.0	407.8	357.2	403.	353.1	37.6	0.01	0.01	0.70
Skeletal maturity ^a	133.3 ^z	140.0 ^z	165.0 ^{yz}	140.0 ^z	167.5 ^{yz}	172.5 ^y	185.0 ^y	185.0 ^y	11.3	0.42	0.01	0.03
Lean maturity ^a	160.0	147.5	160.0	150.0	170.0	160.0	170.0	177.5	12.4	0.17	0.01	0.37
Overall maturity ^a	146.6	142.5	162.5	146.2	168.7	165.0	178.7	181.2	8.3	0.08	0.01	0.18
Marbling ^b	673.3	612.5	580.0	572.5	802.5	897.5	672.5	762.5	135. ₃	0.55	0.01	0.62
Quality grade ^c	483.3	462.5	443.7	468.7	531.2	562.5	487.3	518.7	44.4	0.30	0.01	0.63
No. steers grading USDA Prime, %	2/3	0/4	0/4	1/4	3/4	4/4	1/4	3/4				
Adjusted fat thickness, cm	1.44	0.95	1.30	1.05	2.51	1.53	1.90	1.30	0.45	0.01	0.01	0.44
Ribeye area, cm ²	78.3	68.4	71.8	68.9	76.0	87.3	85.2	82.6	8.8	0.75	0.01	0.15
KPH, %	3.00	2.88	2.63	3.13	2.75	3.00	2.50	3.25	0.51	0.07	0.99	0.42
Yield grade	3.33 ^c	2.75 ^c	3.33 ^c	3.08 ^c	5.17 ^a	3.27 ^c	4.04 ^b	3.29 ^c	0.56	0.01	0.01	0.03
Lipid, %	9.3 ^z	6.1 ^z	8.3 ^z	7.8 ^z	14.7 ^{yz}	14.1 ^{yz}	12.0 ^z	20.4 ^y	3.84	0.47	0.01	0.03
Moisture, %	67.7 ^y	70.6 ^y	68.7 ^y	68.1 ^y	62.9 ^{yz}	62.1 ^{yz}	67.2 ^y	59.6 ^z	3.35	0.24	0.01	0.04
Initial body weight, kg	208.7	169.1	207.5	175.1	218.6	174.3	205.5	175.3	6.9	0.01	0.98	0.98
Final body weight, kg	525.0	427.9	528.4	479.4	662.8	573.3	663.1	603.4	16.8	0.01	0.01	0.82
Cumulative ADG, kg	1.29	1.05	0.89	0.83	0.90	0.81	0.75	0.70	0.56	0.06	0.01	0.26

^aA = 100; B = 200; C = 300; D = 400; E = 500.

^bPractically Devoid = 100; Traces = 200; Slight = 300; Small = 400; Modest = 500; Moderate = 600; Slightly Abundant = 700; Moderately Abundant = 800; Abundant = 900.

^cStandard = 200; Select = 300; Choice = 400; Prime = 500.

^xBreed x diet interaction. Interaction means with different ^{yz} superscripts differ.

Table 2. Percentage of total fatty acids in subcutaneous adipose tissue of Wagyu and Angus steers fed U.S. and Japanese endpoints.

Item	Months on feed/diet										P-values		
	U.S. endpoint				Japanese endpoint				SE	Breed	Diet	End point	DxE ^b
	8 mo/Corn		12 mo/Hay		16 mo/Corn		20 mo/Hay						
	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu					
16:0	27.6	26.1	28.5	26.3	25.9	25.7	26.8	24.4	1.69	0.01	0.79	0.03	0.54
16:1n-7	2.95	4.02	2.31	2.44	6.54	6.55	5.87	4.98	1.39	0.88	0.04	0.01	0.99
18:0	16.2	14.2	20.4	19.3	7.25	4.17	9.11	8.32	4.50	0.55	0.07	0.01	0.35
18:1n-9	37.5	39.3	32.1	35.4	41.7	42.8	39.9	44.4	3.10	0.03	0.04	0.01	0.05
18:2n-6	2.58	2.49	1.56	2.07	2.41	2.71	1.72	1.90	0.30	0.05	0.01	0.91	0.92
18:3n-3	0.00	0.09	0.23	0.30	0.05	0.19	0.14	0.18	0.11	0.05	0.01	0.71	0.03
SFA	47.9	43.7	52.8	48.8	36.9	36.8	40.3	35.9	4.91	0.09	0.09	0.01	0.29
MUFA	43.6	46.2	37.7	40.3	51.5	52.7	49.2	52.3	4.12	0.12	0.02	0.01	0.14
PUFA	2.91	3.24	2.29	3.00	3.27	3.46	2.58	3.09	0.46	0.01	0.01	0.16	0.77
MUFA:SFA	0.91	1.10	0.72	0.87	1.42	1.44	1.22	1.47	0.22	0.07	0.07	0.01	0.43
Index ^y	0.84	1.04	0.65	0.80	1.39	1.39	1.17	1.41	0.22	0.08	0.06	0.01	0.46
SCD activity ^a	4.92	6.32	8.57	7.91	13.3	10.4	9.88	12.0	2.69	1.00	0.40	0.01	0.08
SCD gene expression ^c	0.15	0.06	0.54	0.26	0.17	0.62	0.29	0.50	0.20	0.34	0.06	0.07	0.05

^a nmol palmitoyl-CoA converted to palmitoleoyl-CoA per 7 min of incubation per mg protein.

^b Diet x endpoint interaction. There was a significant breed x endpoint interaction for SCD gene expression ($P = 0.01$).

^c SCD/28S RNA ratio.

^y Desaturation index = $(14:1 + 16:1 + 18:1_{cis-9} + 18:2_{cis-9,trans-11}) / (14:0 + 16:0 + 17:0 + 18:0 + 18:1_{trans-11})$.

EFFECT OF TWO COOKING METHODS – HOT WATER AND STEAM – ON THE QUALITY PARAMETERS OF *SEMITENDINOSUS* MUSCLES

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Key words: thermal treatment, quality, tenderness, shearing force, cooking methods

Abstract

Cooking methods such as in hot water and steam at 70 °C and 80°C were evaluated to eliminate *Clostridium botulinum* type E. This is a risk-pathogen for cooked, vacuum packed and chilled products. Non-aged standard weight, *Semitendinosus* beef cuts were 1) steamed with direct vapor injection in incubators, and 2) heat-treated in a hot water cooker. Internal temperature of the samples was measured via a thermocouple attached to a sensor during the experiment. Data obtained based on the D values for this pathogen, D (82.2°C) and Z (8.8 min), and pasteurization (P) were estimated for each method tested. Cooking loss and objective tenderness attributes were analyzed to measure the quality of the cooked meat cuts. Results showed that the pasteurization of the products occurred at 80°C; the objective tenderness evaluated through shearing force was not affected by either of the cooking methods used and levels of temperature. Significant difference between these two cooking methods (which greater in hot water than in steam), temperature levels (which were greater at 80°C than at 70°C), and interaction between temperature and type of heating for cooking losses. Sensorial evaluation revealed that juiciness had a positive influence, while the presence of collagen had a negative effect on the tenderness of the meat cuts.

Introduction

Cooked meat cuts undergo strict processing controls during industrial production. This control must analyze various aspects of pathogenic microorganisms and the positive consequences of the treatment process on the yield of the meat and on the final product, thus resulting in proper sensorial characteristics for the consumer. *Clostridium botulinum* type E is a microorganism that is associated with microbiological risks inherent in cooked, chilled and vacuum packed meat products, since it is able to grow and produce toxin at 3°C. The cooking process causes the denaturation of insoluble proteins and is time-dependent. The resulting denaturation is shown by the low measurement values given by the Warner- Bratzler Shear Force machine (Powell, Dikeman & Hunt, 1999). The objectives of this study were to evaluate 1) the effectiveness of thermal treatments utilized in meats for antimicrobial purposes with *C. botulinum* type E as a target pathogen; 2) evaluate the measurement results for the cooking loss variables (%) and Warner-Bratzler shear values (kg) when comparing to the cooking methods and

temperature levels, and 3) at which stage flavor, presence of collagen and juiciness of samples cooked at 70 and 80°C in water or steam influences the subjective tenderness.

Material and Methods

Vacuum packed samples were cooked at 70°C and 80°C in water by utilizing two different heat-treatment methods: a hot water cooker and a vapor injection incubator. Controls and the monitoring of the internal temperatures of the samples were conducted with thermocouples attached to sensors. The effectiveness of the cooking process was evaluated based on thermobacteriology principles with *Clostridium botulinum* E as a reference-microorganism. The D (82.2°) and Z (8.8 min) concept values were utilized for this microorganism. The pasteurization value P was estimated for each treatment conducted. The objective tenderness was measured through the shear force analysis by utilizing the Warner- Bratzler Shear Force device. Percentage values for cooking loss were estimated as follows: [(weight of the raw sample – weight of the cooked sample)/weight of the raw sample] x 100. The sensorial evaluations were conducted with 13 trained panelists by utilizing quantitative tests for tenderness, juiciness, presence of collagen, and flavor attributes.

Analysis of variance, Tukey test, correlation analysis and multiple regression analysis were used to evaluate the data obtained (Montgomery, 1991, Montgomery and Peck, 1992).

Results and Discussion

Results from Table 1 show that the reduced decimal values found for the samples treated at 70°C were not sufficient to obtain an inactivated *C. botulinum* E safety product, since adequate pasteurization occurs only when the decimal reduction values are between 5 and 10. These findings corroborate with those reported by Lawlor et al. (2000), who conducted experiments with cooked turkey meat followed by the inoculation with *Clostridium botulinum* B, and found that 72.2°C was inadequate to eliminate this microorganism. The D value for this pathogen is between 0.1-0.2, which is lower than that necessary to eliminate *C. botulinum* E (D value = 0.3–3.0) (Gonçalves and ; Germer, 1992). Therefore, the cooking temperature at 70°C was considered too low to obtain satisfactory pasteurization indexes for *C. botulinum* even when applied for longer periods.

With the objective to evaluate two factors together, the cooking method and the temperature utilized, in relationship to the Warner-Bratzler shear values and Cooking loss indexes, the following factorial model for each index was adopted: $Y_{ij} = \mu + X_{2i} + X_{3j} + (X_2 * X_3)_{ij} + E_{ij}$, where Y was the dependent variable, i.e., for each of the measurements (Warner-Bratzler shear values and Cooking Loss); X_{2i} corresponded to the cooking method tested ($i=2$); X_{3j} was the temperature tested ($j=2$), and $X_{2ij} * X_{3ij}$ corresponded to the interaction between these parameters (Tables 2 and 3). Statistic differences were found for temperature levels (which were greater at 80°C than at 70°C), cooking method (which were greater in hot water than in steam), and interaction between temperature and type of heating for the cooking loss attribute. Analyses conducted by Palka and Daun (1999) with *Semitendinosus* beef showed an increase of 10.3 and 9.7% in the loss of

fluids during the cooking period at 50°C-60°C and between 70°C-80°C, respectively. According to these authors, the loss of fluids was due to the two basic alterations in the protein structure of the meat: the actomyosin complex and collagen. No significant differences ($P>0.05$) were observed for the cooking methods and levels of temperature at the Warner-Bratzler shear values (Tables 4 and 5).

The interaction between the following parameters was observed during the sensorial evaluation on the presence of conjunctive tissue, tenderness, flavor and juiciness. The tenderness parameter was considered the variable that greatly influenced the quality of the product, and was correlated with other sensorial attributes. To estimate how the presence of collagen, flavor and juiciness attributes influenced the tenderness, a regression multiple linear model was developed to understand which variables were positively or negatively affecting its quality. The model used was: $Y_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i3} + E_i$, where Y_i = tenderness; X_{i1} = succulence/juiciness; X_{i2} = collagen; X_{i3} = flavor, and E_i = the random error associated with the model. The estimated model for the steamed samples was: $Y_i = 0.683 X_{i1} + 0.204 X_{i2} + 0.0507 X_{i3} + 2.470$. The presence of collagen influenced negatively, while the succulence/juiciness attribute showed a positive effect on the tenderness of the samples ($P < 0.01$). For samples heat-treated in the hot water cooker, the estimated model was: $Y_i = 0.657 X_{i1} + 0.136 X_{i2} + 0.0191 X_{i3} + 2.721$. In this case, juiciness was the only variable that had a significant effect on this model ($P < 0.01$). Flavor did not show a correlation with any other attribute analyzed, thus agreeing with results reported by Otremba et al. (2000), who evaluated the succulence/juiciness, firmness, fibrosis, tenderness, and the easiness of chewing attributes of cooked cuts of *Semitendinosus* muscles. On the other hand, Wheeler et al. (1998) conducted a study for this type of muscle utilizing belt grilled and open hearth electric broiler methods and found differences for the belt grill-cooked samples had lower ($P < 0.01$) percentage of cooking losses (21.5 vs 25.8%) and higher ($P < 0.01$) shear force values (4.6 vs 4.3 kg) than electric broiler-cooked samples.

Table 1. Decimal reduction values obtained for cooking, cooking loss and objective tenderness of *Semitendinosus* muscle heat-treated in hot water or steamed at 70°C and 80°C.

Treatment	Decimal reductions values observed
Water at 70°C	0.51
Steam at 70°C	0.73
Water at 80°C	11.29
Steam at 80°C	10.12

* Each treatment had two repetitions

Table 2. Variations observed in the cooking loss for *Semitendinosus* muscle heat-treated in hot water or steamed at 70°C and 80°C.

Source	Sum of Squares	Df	Mean	F	Sig.
Corrected Model	2442,241 ^a	3	814,080	79,380	0,000
Intercept	109978,891	1	109978,891	10723,880	0,000
X2	732,203	1	732,203	71,396	0,000
X3	1704,534	1	1704,534	166,207	0,000
X2 * X3	126,150	1	126,150	12,301	0,001
Error	1189,639	116	10,256		
Total	137839,977	120			
Correct Total	3631,880	119			

Table 3: Overall means for cooking loss (%)

Temperature	Cooking method		Total
	Steam	Water	
70°C	24.40	31.80	28.11
SE	1.27	0.16	
80°C	34.57	37.64	36.11
SE	0.52	0.28	
Total	29.49	34.72	

SE = standard error

Table 4. Variations in the Warner-Bratzler shear values (kg) observed for *Semitendinosus* muscle heat-treated in hot water or steamed at 70°C and 80°C.

Source	The sum of squares (Type III)	Df	Mean Square	F	Sig.
Corrected Model	12,423 ^a	3	4,141	0,848	0,471
Intercept	4183,350	1	4183,350	856,385	0,000
X2	7,073	1	7,073	1,448	0,231
X3	0,267	1	0,267	0,055	0,816
X2 * X3	1,536	1	1,536	0,314	0,576
Error	566,648	116	4,885		
Total	5260,323	120			
Corrected Total	579,071	119			

Table 5: Overall means for Warner-Bratzler shear values (kg)

Temperature	Cooking method		Total
	Steam	Water	
70°C	6.45		6.31
SE	0.28	0.28	
80°C	6.50		6.17
SE	0.52	0.28	
Total	6.48	6.00	

SE = Standard error

Table 6: Sensorial evaluation scores given for *Semitendinosus* muscle after heat-treatments in hot water or steamed at 80°C.

Treatment	Tenderness	Juiciness	Collagen	Flavor
Water at 80°C	5.91 ^a	4.63 ^b	1.75 ^c	5.05 ^d
Steam at 80°C	5.47 ^a	4.51 ^b	2.63 ^c	5.41 ^d

Note: The results correspond to an evaluation conducted by utilizing a non-structured scale from 0 to 10, with 0 equal to the lowest scores and 10 to the highest scores according to the perception of each panelist given for each sensorial attribute tested.

Conclusion

The complete pasteurization of the products occurred at 80°C. The objective tenderness evaluated through shearing force analysis was not affected by the cooking methods tested. A significant difference ($P < 0.01$) was found between the cooking methods in relationship to the cooking loss variable. The sensorial evaluation showed that juiciness had a positive correlation ($P < 0.01$), while collagen had a negative correlation with tenderness. Flavor was not influenced by either of the cooking method tested.

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**EFFECTS OF SALTING ON MICROSTRUCTURE AND PROTEIN
SECONDARY STRUCTURE AS MEASURED BY
FT-IR MICROSPECTROSCOPY IN PORCINE M. SEMITENDINOSUS OF
DIFFERENT QUALITY**

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Key Words: salting, FT-IR microspectroscopy, microstructure, protein Amide I region, PCA

Introduction

Meat muscle that is immersed in salt solution will swell first (NaCl > 1%) and again shrink, when the salt concentration in the muscle exceeds 6% NaCl. This is mainly due to a swelling and shrinking of muscle cells. Cross-sectional areas of myofibers will therefore display microstructural changes depending on existing salt levels and the degree of swelling or dehydration of the myofibrils. Swelling and water expelling will depend on the post mortem status of the meat as well as the type of muscle and fibres selected for the experiment (Offer et al., 1989; Knight et al., 1989; Egelanddal et al. 1995).

FT-IR microspectroscopy, i.e., the combination of infrared spectroscopy and microscopy, has great potential for chemical analysis of tissue on microscopic scale. Chemical changes, as changes in secondary structure of proteins (e.g. from native to aggregated proteins) can be studied spatially resolved on microscopic level, e.g., for connective tissue and myofiber cells, separately. Conventional histological techniques do not allow the direct detection of these types of changes. In addition the chemical methods commonly used to identify altered protein structures give no information about their spatial distribution necessary to explain salt uptake/water holding properties. Kirschner et al. (2004) applied FT-IR microspectroscopy to follow denaturation during heating in beef muscle tissue.

A combination of both, histological and microspectroscopic techniques, enables us to relate chemical changes to structural changes.

Objectives

The scope of this study was to investigate effects of salting on microstructure and protein secondary structure of pork muscle tissue by light microscopy and FT-IR microspectroscopy with respect to different raw meat qualities.

Methodology

A variation in meat quality was introduced by an experimental design including different pre-slaughter treatments of the animals. The pre-slaughter treatment was as follows: (1) treadmill and electronic stunning (Bertram et al. 2004) (2) injection of adrenaline 15 h prior slaughter (Henckel et al. 2000) and (3) a control animal without special treatment. Samples of *M. semitendinosus* of 4 cm x 4 cm x 4 cm were excised and placed into brine solutions of different concentrations: 0.9% ("1"), 3% ("2"), 6% ("3") and 9% ("4") (10 mM Na-acetate buffer pH 5.5 with 0.05% NaN₃ to prevent microbial growth). They were kept in the brine for 8 days at 4 °C. Samples for microspectroscopy were excised, embedded in O.C.T. compound (Tissue-Tek, Electron Microscopy Sciences, Hatfiles, USA) and frozen in liquid nitrogen before ("0"-samples) and after salting. They were kept at -80 °C until sectioning, which was performed transversely to fiber direction on a cryostat (Leica Germany). 8 µm thick sections were prepared and thaw-mounted on infrared transparent 2 mm thick CaF₂ slides for FT-IR microscopic measurements and parallel sections were collected for light microscopy. The latter sections were stained according to a standard procedure with Hematoxylin Erythrosine.

FT-IR spectra were acquired with an IRScope II coupled to an Equinox 55 (BRUKER OPTICS, Germany). The spectra were scatter-corrected using extended multiplicative signal correction (EMSC). EMSC is a pre-processing method that allows the separation of physical light-scattering effects (e.g. sample thickness) from chemical absorbance effects in spectra (Kohler et al. 2005).

The Unscrambler[®] version 9.1 and in-house developed algorithms written in C++ (CAMO Process AS, Norway, 2004) were used for spectral processing and multivariate analysis of the data.

Results & Discussion

Fig.1 shows selected light microscopic images of Hematoxylin Erythrosine stained-sections (differences in color intensity may be due to differences in pH value and salt content of the samples). The selected regions shown in the images correspond to areas in which FT-IR spectra were collected. The upper panel presents sections from samples of the control animal, the middle from the treadmill-treated pig, and the lower from the adrenaline-treated animal. The images in the left column represent samples taken 24 hours after slaughter followed to the right by columns for salted samples with the salt concentration indicated in the figure. The unsalted samples already reveal structural differences among the treatments: In the control and adrenaline treated samples the myofibres are well attached, whereas in the treadmill-sample the myofibres are detached and the extracellular area is extended. The adrenaline-sample has a more bluish color than the two other samples. This may be due to the higher pH value of this sample. During salting the fibers first swelled (diameter increases) and thereafter shrunk at NaCl ≥ 6 %. At the highest salt concentration (images the column furthest to the right) the control and treadmill samples appear shrunken and more edgy in shape. In contrast, the adrenaline samples seemed to keep an apparently intact microstructure. However, at the high salt concentration it was difficult to distinguish between individual fibers and the fibres were amorphous.

The Amide I band at the frequency region from 1700-1600 cm⁻¹ is the most prominent feature in the FT-IR spectrum of the muscle fibers. This band is mainly due to the carbonyl stretching vibration with minor contribution of C-N stretching and N-H bending vibrations and it mostly depends on the secondary structure of the protein backbone. Therefore it is commonly used for secondary structure analysis of proteins (Barth and Zscherp 2002). To gain a better resolution the second derivative was applied to the spectra. In Fig. 2 the second derivative spectrum of the Amide I region is shown exemplary for the “0”-sample of the control animal. The band at 1653 cm⁻¹ is most likely referring to α -helical structures in the myofibrillar proteins. Jackson and Mantsch (1995) assign bands from 1610 cm⁻¹ to 1628 cm⁻¹ to denatured aggregated β -sheet components, while they relate the band between 1630-1640 cm⁻¹ to antiparallel β -sheet structures. In Fig. 2 bands that are possibly related to denatured aggregated β -sheet structures are found at 1619 cm⁻¹ and 1628 cm⁻¹ and the band at 1638 cm⁻¹ could be related to antiparallel β -sheets. Due to transition dipole coupling in β -conformational structures, the bands found at the higher wavenumbers, 1682 cm⁻¹ and 1693 cm⁻¹, may be the weaker components of the antiparallel β -sheet structures and the denatured aggregated β -sheet components, respectively.

In order to analyze the FT-IR spectra a principal component analysis (PCA) was carried out with the second derivative spectra (average spectra resulting from 12 spectra taken from two areas on the tissue section) in the spectral region from 1700-1600 cm⁻¹. One sample outlier was identified and removed.

The score plot of the first two principal components (PCs) is presented in Fig.3a. The first PC results from variations in the raw material showing differences between adrenaline (red), control (blue) and treadmill (green) animal. Compared to the other two groups the treadmill group reveals a much larger variation over PC1. PC2 gives the variation in the salt content of the samples (labels “1” to “4” indicate the salt concentration). The explained variance for PC1 and PC2 is 64% and 23%, respectively. By comparing the x-loading plots (Fig. 3b) with the score plot for PC1 and PC2, one can find which samples are related to certain wavenumbers. For example the frequency range around 1628 cm⁻¹, which might be corresponding to denatured aggregated β -sheet, could be associated with the treadmill samples. The band at 1652 cm⁻¹ (α -helix) might be related to samples low in salt content and adrenaline samples show correlation to antiparallel β -sheet components. These results indicate that raw quality of pork muscle as well as salt concentration leads to differences in the protein secondary structure as measured by FT-IR spectroscopy.

Conclusions

Pork muscle samples displayed microstructural differences related to raw material and salt content. FT-IR spectroscopy revealed corresponding changes in the protein secondary structure. Corresponding differences were found. This suggests that the microscopic changes are inherent with a change at the molecular level.

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Acknowledgement

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Tables and Figures

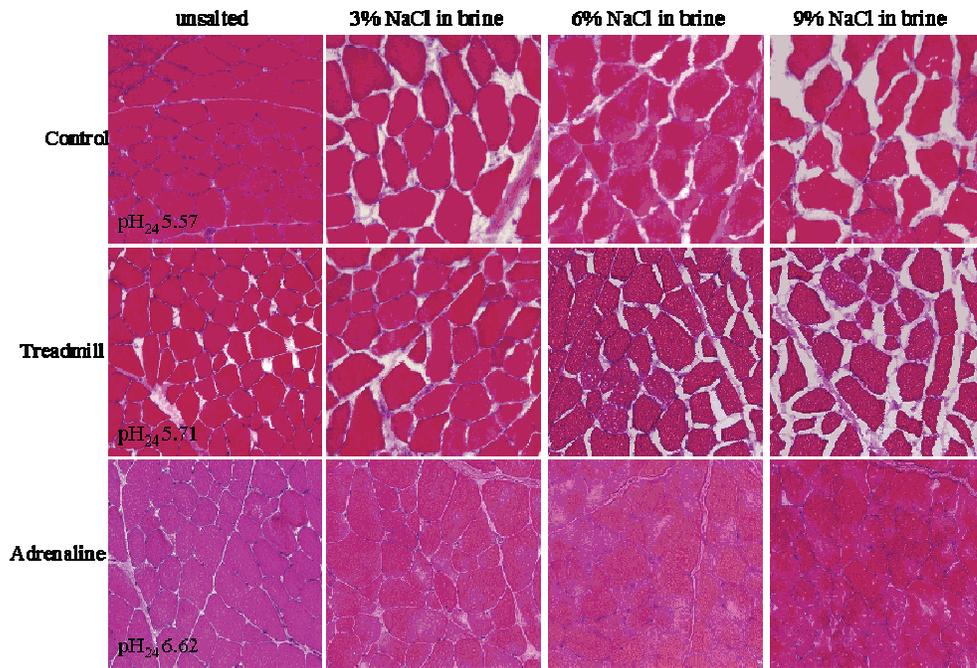


Fig. 1: Selected Hematoxylin Erythroline images of porcine *M. semitendinosus*. FT-IR spectra were collected in the same areas.

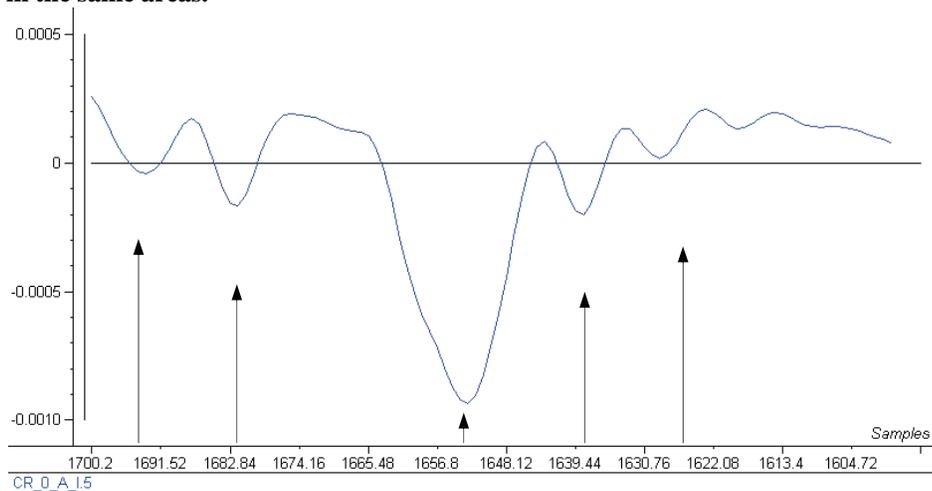


Fig. 2: 2nd derivative spectrum (average of 12 spectra) of unsalted control sample in the wavenumber range 1700-1600 cm^{-1} (shown on the x-axis). The arrows indicate wavenumbers described in the text.

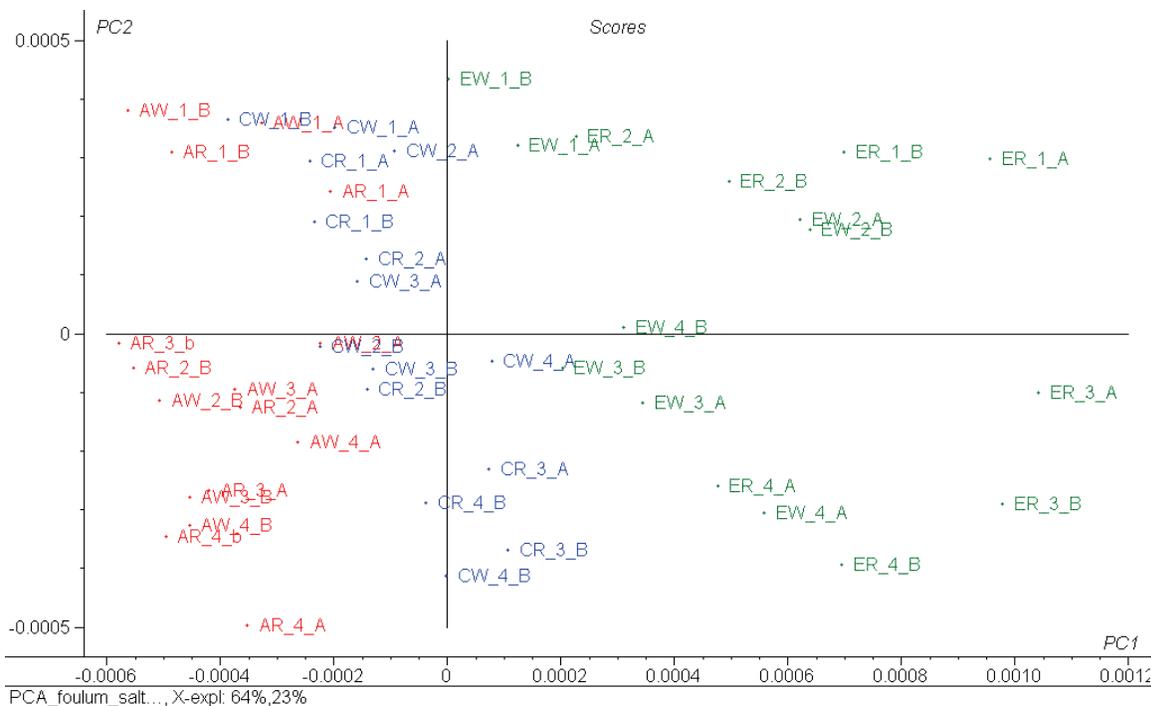


Fig. 3a: PCA score plot (PCA run for 1700-1600 cm⁻¹) with 64% and 23% explained variance for PC1 and PC2 respectively.

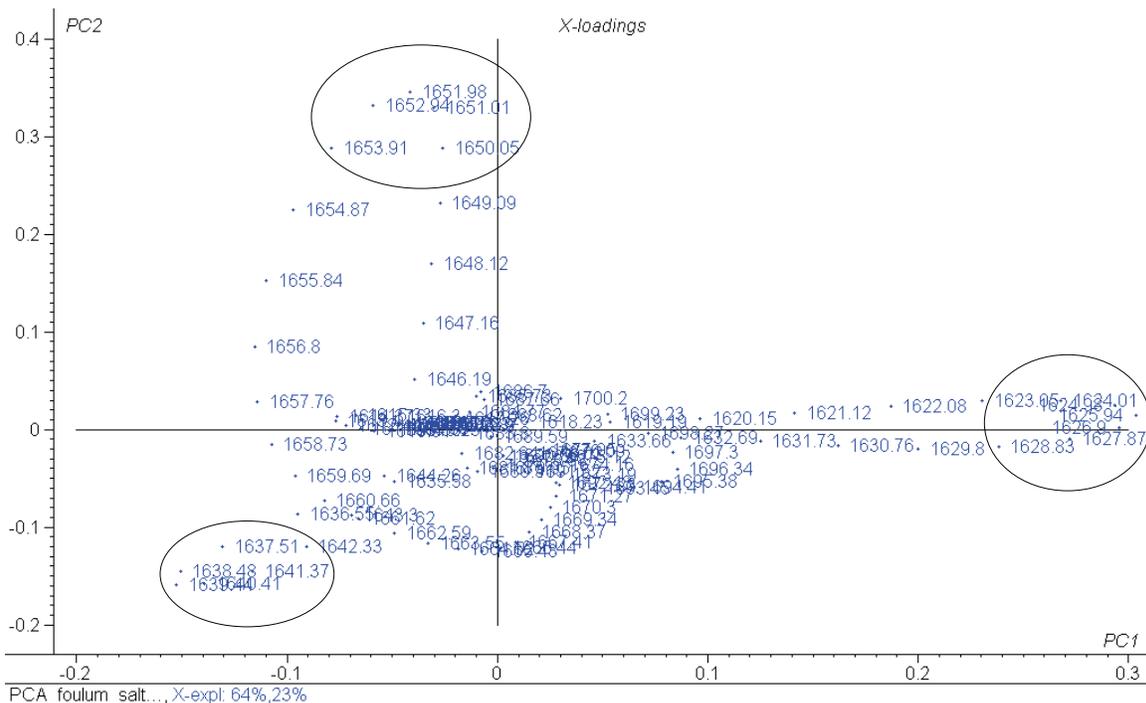


Fig. 3b: x-loading plot corresponding to score plot shown Fig. 3a. Wavenumber ranges indicated are described in the text.

EFFECTS OF MUSCLE FIBER TYPES ON GEL PROPERTY OF SURIMI-LIKE MATERIALS FROM CHICKEN, PORK AND BEEF

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Key Words: Surimi-like materials, Gel property, Muscle fiber type,

Introduction

Muscle fiber type, fiber areas and composition of specific muscles are important factors influencing many of the pre- or post-mortal biochemical processes. There are marked differences in fiber type composition of different muscles, both within and between animals, which may influence meat quality. Myofibrillar protein functionality, particularly gelation, in relation to muscle food quality, has been extensively researched. It has been suggested that the gelation properties of myofibrillar proteins are influenced by the distribution of specific fiber types in the muscles from which myofibrillar proteins are extracted. The literature concerning unfolding, aggregation and gelation of myofibrillar proteins with respect to muscle fiber type, pH and heating conditions has been reviewed by Lesiów and Xiong (2001). It was stressed that under dynamic conditions aggregation plays a major role in producing gel elasticity differences between white and red myofibrillar proteins. While many efforts have been made to elucidate effects of muscle fiber type on processed meat quality, however, there is a little information on gel properties of surimi-like materials (SLM) derived from meat animals such as chicken, pork and beef. An understanding of the characteristics of SLM from meat animals will assist in determining recommendations for how to handle the animal muscles during processing of surimi products.

Methodology

Fresh chicken, pork and beef were purchased at a local meat market, and the surimi block of Alaskan Pollock (AP) was supported from a commercial company in Korea. All muscle samples were removed external fat tissue, and the lean muscles were diced into approximately 2 cm cubes, and ground through a 4.7 mm diameter orifice with a mincer to manufacture a surimi-like material (SLM). The SLM manufacture procedure was modified to method of Kang et al. (2004). pH of cooked SLM were measured using a pH-meter (MP230, Mettler Toledo, Swiss) and gel strength of cooked SLM was measured with a Sun Rheo Meter (COMPAC-100, Japan). The microstructure changes in muscle and gel of cooked SLM were evaluated using a field emission scanning electron microscope (FE SEM; XL30S, Philips, Netherlands). SDS-PAGE was applied to investigate changes in sarcoplasmic and myofibrillar proteins of muscles. Amino acids composition was determined using an automatic amino acid analyzer (Biochrom 20, Pharmacia, USA).

Results & Discussion

Results showed that gel strength and hardness of cooked SLM were increased as decrease of pH (Fig. 1). The lower pH in beef-surimi speculated lower moisture, resulted in hard and strong gel strength. Different pH of AP and animal muscles might be related to difference from muscle protein composition and species. Also, the different pH had not effects on only remaining of sarcoplasmic protein in SLM against washing procedure, but also functionality of myofibrillar protein. This result confirmed reports of Lan et al. (1995) who suggested that ultimate pH differences in fish, beef and pork muscles had effects on protein extractability but that on an equal protein basis, gelation properties differed among species.

The differences in thickness and diameter of muscle fibers from chicken breast, *semimembranosus* of porcine and bovine were observed by the SEM micrographs (Fig. 2). Pork had thicker muscle fibers whereas beef had smaller diameter of the muscle fibers. This present was related with gel strength and microstructure of cooked SLM. The differences in the pattern and size of the voids in the SEM micrographs (Fig. 3) indicated much difference in the microstructure for gels by muscle fibers. As decrease of muscle fiber thickness, gel of cooked SLM showed many smaller particles of amorphous proteins that might be related with hard and strong gel structure. The gel from beef muscle showed a structure of coarse aggregates and a rough surface, and had many of smaller conglomerates. These conglomerates seem more interconnected than larger structure particles of others cooked SLM, which may explain the higher strength of the structure. Contrary, the structures of gel from chicken and pork presented larger pockets than beef gel structures in which water may have been held. This feature might be associated with water content and gel strength. Small spaces within the gel network showed the lower water content of cooked SLM derived from beef muscle. This also explains the low expressible fluid of cooked SLM. These results implied that gel texture of SLM was depending upon muscle fiber types from animal species.

Myosin ratio in myofibrillar proteins was lower in SLM from beef than those from AP, chicken and pork (Fig. 4). The myosin ratio was increased as decrease of red muscle fiber ratio, i.e. beef>pork>chicken>AP. This data suggested that the myosin ratio in SLM depended on muscle fiber types in raw muscles, resulted in gel forming ability and texture of cooked SLM. Result was agree with report of Xiong (1997) that myofibrillar proteins play the most critical role during meat processing as they are responsible for cohesive structure and the firm texture of meat products. Also, gel-forming ability differences among animal species were observed by Lan et al. (1995) and Park et al. (1996). The differences in gel texture may be related to pH of raw muscle, protein extractability and gelation, and to gel forming differences in the various myosin isoforms present. Amino acid composition analysis showed significantly ($p<0.05$) lower proline ratio of hydrophobic amino acids in pork compared with chicken and beef (Table 1). It was postulated that a three-dimensional network was established during heating process through linkages in the tail portion of the myosin molecule via hydrophobic interactions. Therefore, the structure of gel from pork presented larger pockets in which water may have been held might be due to the higher proline ratio compared with chicken or beef.

Conclusions

The lower pH of SLM from beef muscle was related with hard and strong gel strength of cooked SLM. Chicken and pork muscles showed thicker myofibrils and larger diameter of fibers, and formed larger particles of amorphous protein in cooked SLM. Gel-forming ability and texture of cooked SLM were affected by myosin ratio in myofibrillar proteins. Ratio of myosin heavy chain was lower in SLM of beef, resulted in many of smaller conglomerates in gel and harder texture of surimi.

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Tables and Figures

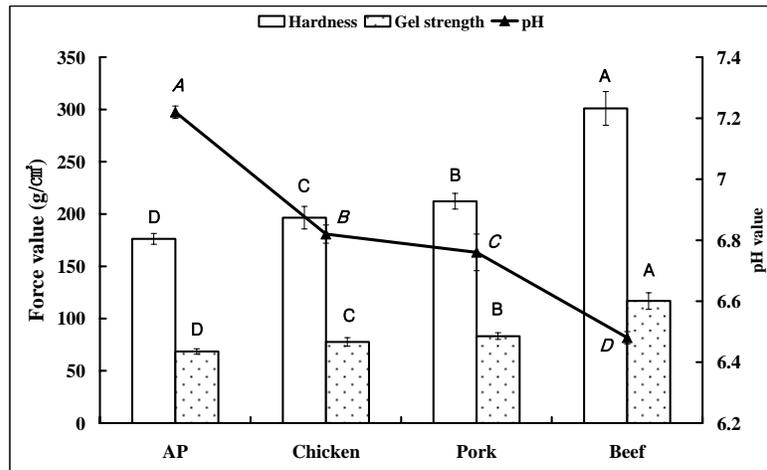


Figure 1. pH and texture measurements of surimi-like materials from Alaska Pollack (AP), chicken, pork and beef. ^{A-D}Means±S.D with different superscripts within a variable differ (p<0.05).

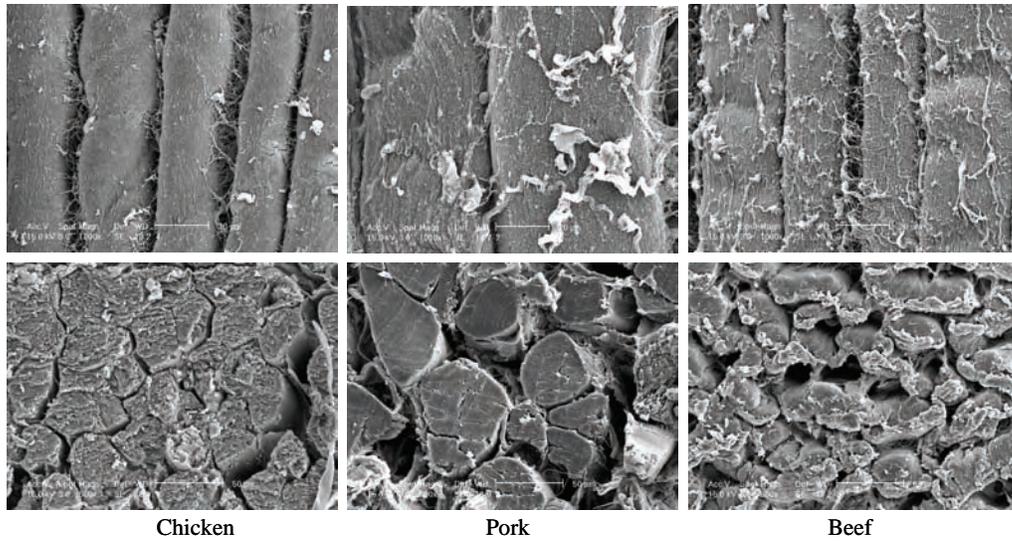


Figure 2. SEM photographs of muscle fiber thickness (*upper*) and diameter (*lower*) from chicken, pork and beef.

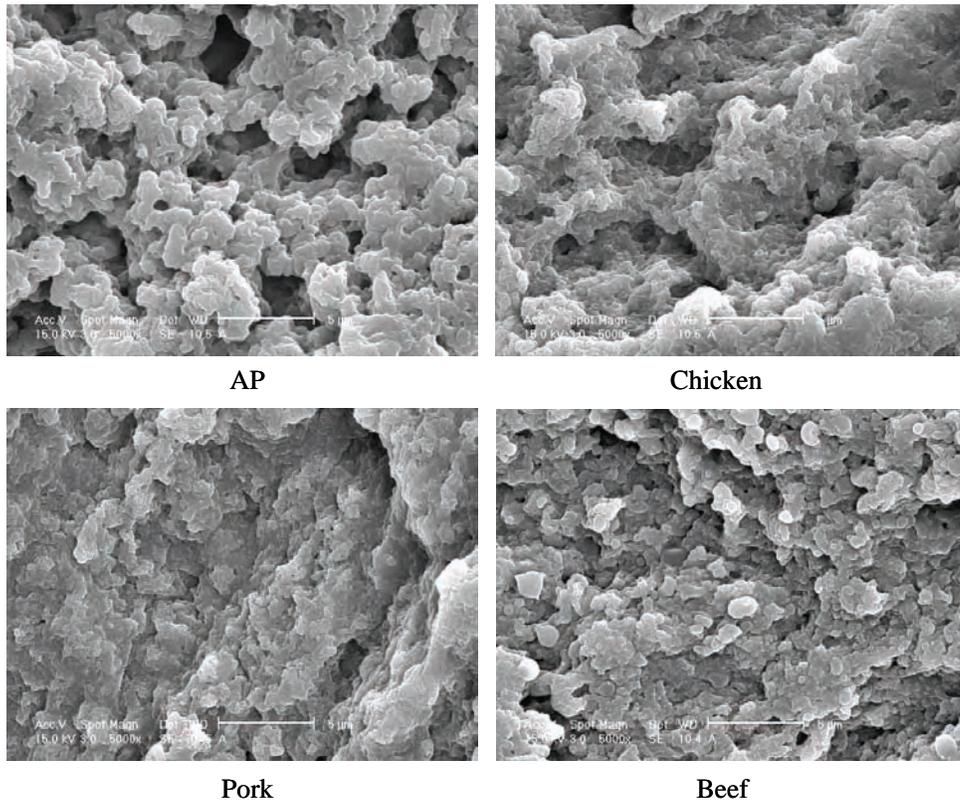


Figure 3. The microstructures of cooked surimi-like materials from Alaska Pollack (AP), chicken, pork and beef.

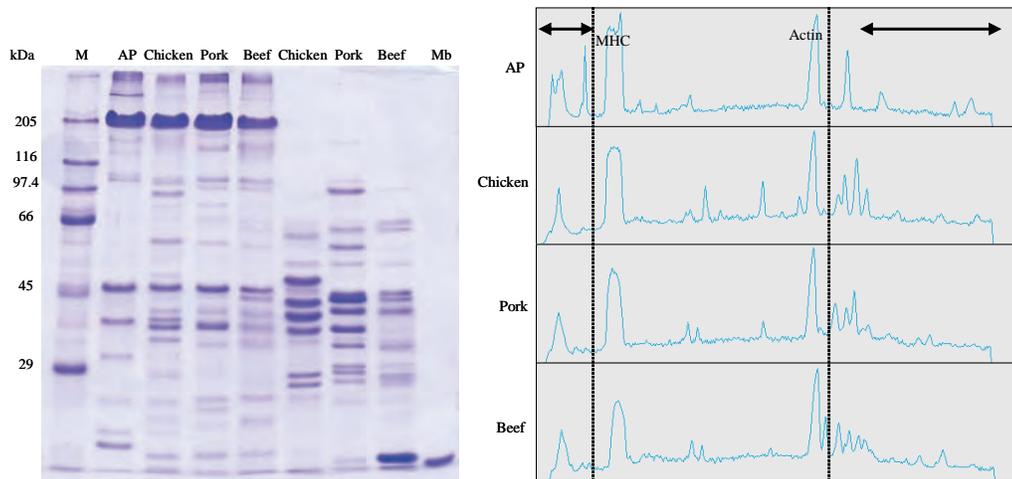


Figure 4. SDS-PAGE patterns (left) and band intensity (right) of myofibrillar and sarcoplasmic protein from chicken breast, *semimembranosus* muscle porcine and bovine. The myofibrillar (lanes 2-5) and sarcoplasmic (lanes 6-8) protein fractions (left), which were defined as the sediment and supernatant after the first water-washing and centrifugation of the water-washed pork from chicken breast, SM of pork and beef. M and Mb denote protein molecular mass standards and horse myoglobin, respectively. AP; Alaska Pollock, MHC; myosin heavy chain.

Table 1. Amino acid composition of surimi-like materials from chicken, pork and beef

Amino acids	Chicken	Pork	Beef
Aspartic acid	9.57±0.12	9.84±0.47	9.58±0.18
*Threonine	5.27±0.05	5.30±0.03	5.31±0.05
Serine	4.53±0.25	4.37±0.09	4.46±0.15
Glutamic acid	15.07±0.38	15.15±0.60	15.14±0.60
Proline	4.61±0.11 ^A	3.83±0.52 ^B	4.51±0.32 ^{AB}
Glycine	3.97±0.06 ^{AB}	3.90±0.05 ^B	4.03±0.03 ^A
Alanine	5.97±0.17	5.89±0.08	6.00±0.12
Cysteine	0.55±0.12	0.57±0.06	0.58±0.04
* Valine	5.19±0.28	5.53±0.16	5.26±0.15
* Methionine	3.05±0.09 ^A	2.84±0.07 ^B	2.79±0.08 ^B
* Isoleucine	5.30±0.36	5.48±0.06	5.48±0.04
* Leucine	8.85±0.17	9.08±0.16	8.79±0.10
Tyrosine	4.32±0.03	4.18±0.29	4.23±0.04
* Phenylalanine	4.55±0.11	4.52±0.23	4.57±0.03
* <i>Histidine</i>	2.87±0.10 ^A	2.74±0.07 ^{AB}	2.71±0.04 ^B
* <i>Lysine</i>	8.91±0.23	9.33±0.48	9.02±0.14
* <i>Arginine</i>	7.42±0.21	7.45±0.16	7.54±0.06
Total	100	100	100
<i>Basic amino acids</i>	19.20±0.35	19.52±0.36	19.28±0.22
Hydrophobic amino acids	37.53±0.46	37.17±0.71	37.40±0.23

^{A,B}Means±S.D with different superscripts within row differ (p<0.05).

*Essential amino acids.

GEL PROPERTIES OF SURIMI-LIKE MATERIALS FROM CARDIAC AND SKELETAL MUSCLES OF PORCINE

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Key Words: Gel property, Myofibrillar protein, Pig heart, Surimi-like materials

Introduction

Interest in utilizing edible meat by-products as value-added products has increased in recent years. The use of beef heart muscle as an ingredient in restructured meat products has been limited by low protein functionality and less desirable flavor compared with skeletal muscle. Also, it was suggested that heart muscle had a problem because of higher fat, heme pigment and collagen content in processing surimi-like material (SLM) (Park et al., 1996). Contrary, McKeith et al. (1988) demonstrated that SLM prepared from beef hearts had improved textural properties when compared with fish surimi. Kenney et al. (1992) also showed that incorporation of washed beef cardiac muscle into restructured beef enhanced sensory and instrumental texture traits. Considerable work investigating the role of various proteins in thermal gelation of muscle foods has indicated that myosin and actomyosin are superior to sarcoplasmic proteins. Cardiac muscle, which is composed mainly of red fibers having two isoforms of myosin, is considered to be relatively poor in functional properties, possibly as a consequence of the low solubility of its myofibrillar proteins and its high connective tissue content. However, there is a little information on gel properties of SLM derived from cardiac and skeletal muscle of porcine. An understanding of the characteristics of porcine muscles will assist in determining recommendations for how to handle the SLM from pork muscles for manufacture of surimi products.

Objectives

The objectives of this study were to investigate the characteristics of cardiac and skeletal muscles and to evaluate the gel properties of SLM from porcine muscles.

Methodology

Fresh pig heart (PH), and psoas major muscle (PM) and semimembranosus muscle (SM) of porcine were obtained at Meat Plant of Gyeongsang National University in Korea. After removal of caps, vessels and external fat tissue, the lean muscle was diced into approximately 2 cm cubes, and ground through a 4.7 mm diameter orifice with a mincer to manufacture SLM. The SLM manufacture procedure was modified to method of Kang et al. (2004). The all minced muscles were chopped in a homogenizer with ten volumes (v/w) of cold 25 mM phosphate buffer (NaH₂PO₄/Na₂HPO₄), pH 7.0. The

resulting slurry was filtered through a 1 mm-mesh metal screen to remove connective tissues. The filtrate was centrifuged and the supernatant containing fat and water-soluble proteins was discarded. The sediment was mixed with 25 mM phosphate buffer, the volume of which was equal to that of the supernatant discarded in the previous step, homogenized again and filtered through a 500 μ m-mesh metal screen and centrifuged (15 min/2,220 \times g). A final wash was done in five volumes (v/w based on original weight of mince) of iced water. The washing procedure was repeated by third times. The resulting residue was centrifuged at 2,220 \times g for 15 min at 4 $^{\circ}$ C and the supernatant discarded. Finally, sediment (SLM) concentration was adjusted 5% protein by adding solution containing 3% NaCl, 0.5% tripolyphosphate (TPP) and 4% sorbitol. The adjusted SLM were mixed with 3% NaCl, 0.5% TPP and 4% sorbitol. Gel of cooked SLM was prepared by heating 15 mL of SLM in capped 1.5 cm diameter tubes for 20 min in a water bath at a constant 75 $^{\circ}$ C. After heating, tubes were cooled (\approx 15 min) to room temperature in an ice bath. Gel characteristics were determined by proximate composition, color, cooking loss, water-holding capacity, pH, firmness and SDS-PAGE.

Results & Discussion

SLM from SM had significantly ($p < 0.05$) higher moisture content and lower crude protein content compared with PH and PM (Table 1). Crude fat was removed almost ($< 0.02\%$) by water-washing procedure. This was expected since the repeated water washing, the centrifugation and the lower density of the fat result the fat to float off and be removed. Although pH of PH was higher than those of PM and SM, there were no significant differences in pH among SLMs from all muscles (Fig. 1). No difference in pH among SLMs might be due to addition of 3% NaCl, 0.5% TPP and 4% sorbitol. Also, there was no significant difference in pH of cooked SLM gels from all muscles.

There was significant difference in color measurements of cooked SLM (Table 2). The cooked SLM of PH was darker than those of PM and SM. Gel of PH had significantly ($p < 0.05$) lower L^* and hue values, and higher b^* and chroma values compared to gels of PM and SM. Results suggested that some of sarcoplasmic proteins including heme pigments and enzymes in PH would not be excluded enough by water-washing procedure, resulted in remaining in SLM. SDS-PAGE clearly showed many of sarcoplasmic protein bands in myofibrillar protein fractions of PH sample (Fig. 3). Also it was speculated that the sarcoplasmic proteins remained in SLM would affect on not only color measurements but also functionality of cooked SLM gel.

The cooked SLM of PH had poor water-holding capacity (WHC) resulting in higher cooking loss (Table 3). WHC of cooked SLM is mainly a function of protein-protein interaction results in an open matrix allowing a higher proportion of total water to be immobilized than in meat proteins with strong protein interactions. It was suggested that cardiac muscle of porcine had strong protein interactions compared with skeletal muscles. Addition of salt and phosphate into SLM dissociates actomyosin, reducing interactions of meat proteins and opening the protein matrix in gel of cooked SLM. Although protein content in SLM of PH was higher than that of SM, however, moisture content in SLM was less in PH (Table 1) because of its poor WHC (Table 3). The poor WHC probably affected on gel-forming ability of cooked SLM of PH. Gel of PH had significantly ($p < 0.05$) lower gel strength and hardness compared with gels of PM and SM (Fig. 2).

These results suggested that proteins of cardiac muscle was not dissociated enough with salt and phosphate, resulted in higher cooking loss compared with both skeletal muscles of PM and SM.

Myofibrillar proteins play the most critical role during meat processing as they are responsible for cohesive structure and the firm texture of meat products (Xiong, 1997). PH sample showed significantly different SDS-PAGE gel pattern of myofibrillar and sarcoplasmic proteins compared to SM and PM samples (Fig. 3). Especially, in myofibrillar protein fractions, the bands of myosin and TM/TN (tropomyosin/troponin) had reduced staining intensity in PH sample, and some of unidentified bands that were not in PM and SM samples, were observed in PH samples. SDS-PAGE indicated that the most notable difference between cardiac and skeletal muscles was the intensity of myosin and TM/TN bands. This result implied that compositions of muscle protein in SLM had effects on gel properties of cooked SLM.

Conclusions

SLM from cardiac muscle of porcine had a poor WHC resulted in much cooking loss compared with those from skeletal muscles. Also SLM of cardiac muscle made a dark gel of cooked SLM because of sarcoplasmic enzymes and heme pigments remained in SLM. Proteins of cardiac muscle did not produce a good gel for surimi because they had strong interaction each other resulted in reducing gel matrix for water, and had less myosin and TM/TN compared with skeletal muscle.

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Tables and Figures

Table 1. Proximate composition of surimi-like materials from pig heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of porcine

Treatments	Moisture %	Crude protein %	Crude fat %
PH	73.66±1.75 ^B	28.71±1.79 ^A	<0.02
PM	72.75±1.65 ^B	29.52±1.58 ^A	<0.01
SM	77.05±0.80 ^A	25.03±0.84 ^B	<0.01

^{A,B}Means±S.D with different superscripts within a variable differ (p<0.05).

Table 2. Color measurements of surimi-like materials from pig heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of porcine

Treatments	CIE values			Chroma	Hue
	L*	a*	b*		
PH	59.43±1.80 ^B	-1.06±0.16 ^A	11.03±0.92 ^A	11.08±0.91 ^A	95.53±1.22 ^C
PM	73.68±0.92 ^A	-3.70±0.06 ^B	3.13±0.18 ^B	4.84±0.13 ^B	139.89±1.64 ^B
SM	72.52±1.41 ^A	-3.80±0.07 ^B	-2.56±0.20 ^C	4.58±0.11 ^B	213.96±2.26 ^A

^{A-C}Means±S.D with different superscripts within a variable differ (p<0.05).

Table 3. Cooking loss and water-holding capacity (WHC) of surimi-like materials from pig heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of porcine

Treatments	Cooking loss %	WHC %
PH	20.52±0.46 ^A	67.63±2.34 ^C
PM	17.33±1.05 ^B	84.82±3.11 ^A
SM	19.97±0.72 ^A	74.30±3.46 ^B

^{A-C}Means±S.D with different superscripts within a variable differ (p<0.05).

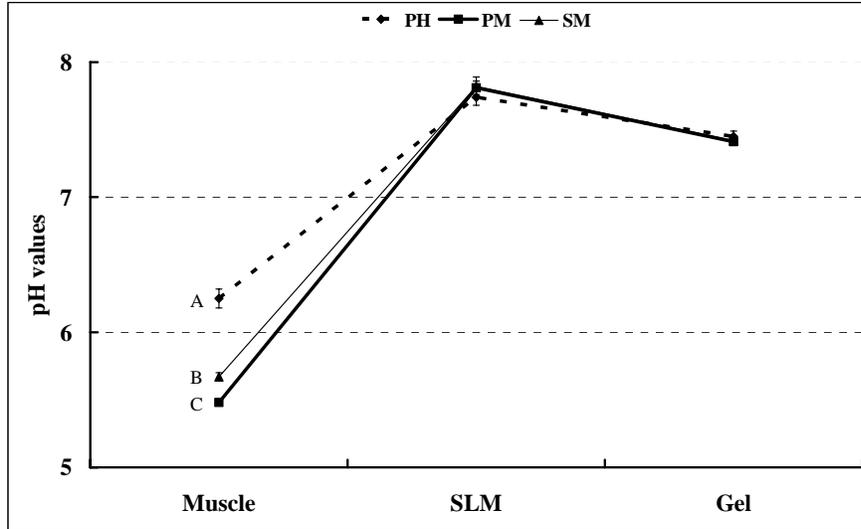


Figure 1. Changes in pH during processing of surimi-like materials from pig heart (PH), *psaos major* muscle (PM) and *semimembranosus* muscle (SM) of porcine. ^{A-C}Means±S.D with different superscripts differ (p<0.05).

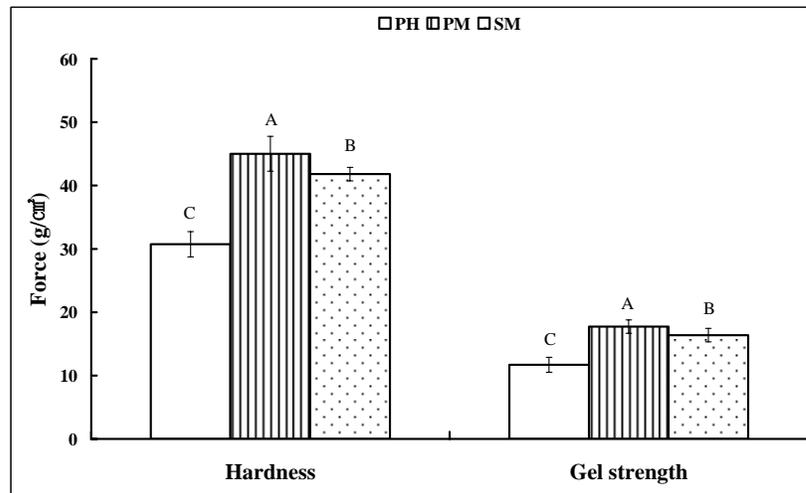


Figure 2. Hardness and gel strength of cooked surimi-like materials from pig heart (PH), *psaos major* muscle (PM) and *semimembranosus* muscle (SM) of porcine. ^{A-C}Means±S.D with different superscripts within a variable differ (p<0.05).

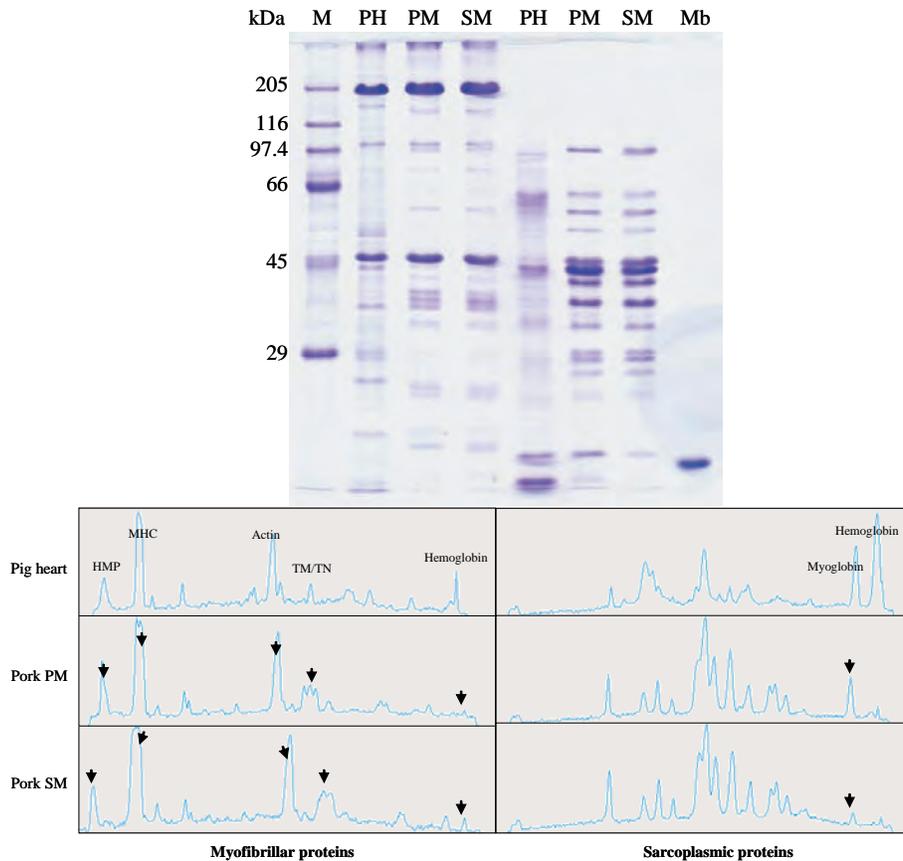


Figure 3. SDS-PAGE patterns (upper) and band intensity (lower) of myofibrillar and sarcoplasmic protein from pig heart (PH), psoas major muscle (PM) and semimembranosus muscle (SM) of porcine. The myofibrillar (lanes 2-4) and sarcoplasmic (lanes 5-7) protein fractions (upper), which were defined as the sediment and supernatant after the first water-washing and centrifugation of the water-washed pork from heart, PM and SM of porcine. M and Mb denote protein molecular mass standards and horse myoglobin, respectively. TM/TN P is tropomyosin/troponin.

**ANTIMICROBIAL ACTIVITY OF SEVERAL HERBAL AND SPICE
EXTRACTS AND THEIR ROLE IN THE PRESERVATION OF VACUUM-
PACKAGED PORK**

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Key Words: pork, antimicrobial, herb, spice, sensory

Introduction

Many plant-derived antimicrobial compounds exhibit a wide spectrum of activity against bacteria and fungi, and this has led to the suggestion that they could be used as natural preservatives in food (Farag et al., 1989; Djenanea et al., 2002, 2003). In particular, many kinds of oriental spice plants have long been known to possess antimicrobial effects. Among them are cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, scutellaria, and forsythia suspensa (Thunb) (Shelef et al., 1980; Yildirim et al., 2000). Many plant oils have also been shown to be effective in inhibiting foodborne pathogens, including *Escherichia coli* O157:H7 and *Salmonella enterica* (Friedman et al., 2004).

Because of its high nutrient density, meat can be an excellent reservoir for food-borne pathogens and other infectious agents, letting alone various spoilage microorganisms. *Escherichia coli*, including the *E. coli* O157:H7 strain, and *Pseudomonas fluorescens*, are potential pathogens that can contaminated animal-derived foods, including ground beef, hamburger patties, poultry, milk, and even ham and cheese sandwiches (Zottola and Smith, 1991; Eriksson et al., 1995). Lactic acid bacteria are the predominant group of microorganisms isolated from vacuum-packaged meat and meat products (Hitchener et al., 1982; Shaw & Harding, 1984; Kato et al., 2000; Sakala et al., 2002). Although the antimicrobial effects of some of herbs and spices have been well documented, few studies have been conducted to investigate the feasibility of using herbal and specie extracts as potential antimicrobial agents for the preservation of chilled meat packaged under vacuum conditions.

Objectives

In the present study, the growth response of three representative bacteria that commonly exist in vacuum-packaged chilled meat and meat products, including *E. coli*, *P. fluorescens* and *L. plantarum*, to the extracts of several herbs and spices was investigated. Specifically, the effect of honeysuckle, scutellaria, forsythia suspensa (Thunb), cinnamon, rosemary and clove water or 70% ethanol extracts, alone and in

combination, on growth of these three bacteria on agar media was examined. The optimum antimicrobial conditions (extract concentrations, types and combinations) established were subsequently used to inhibit microbial growth in vacuum-packaged chilled meat.

Methodology

Preparation of herb and spice extracts

Water extracts of honeysuckle, scutellaria, forsythia suspensa (Thunb), cinnamon, and rosemary were prepared by boiling (100°C) the pulverized dry herbs/spices (50 g each) for 2 h and then filtration with a filter paper. The filtrates were concentrated on a rotary evaporator with a vacuum pump to a final 50 mL volume. The concentration of each of the extracts was assigned a '1 g/mL' unit (based on the herb/spice weight). Ethanol (75%) extracts were prepared by mixing 50 g of pulverized and herbs and spices with 250 mL of 75% ethanol for 48 h with constant agitation. After filtration with a filter paper, the residue was re-extracted with an additional 100 mL of ethanol for another 24 h and then filtered. The combined filtrates were subsequently concentrated on a rotary evaporator with a vacuum pump to 50 mL, and the concentration of the extracts was assigned a '1 g/mL' unit (based on the herb/spice weight).

For both the water and the ethanol extracts, a serial dilution was done to obtain the following concentrations: 1, 0.5 0.25 0.125, and 0.063 g/mL. Olive oil was diluted to 1.0, 0.75, 0.50, and 0.25% with 75% ethanol. Sterilized water and 75% ethanol alone (0 g/mL herb or spice extract) was used as controls. These solutions were used singly or in various combinations to inhibit bacterial growth as described later.

Antimicrobial activity test

The antimicrobial activity of herb/spice extracts was examined using the disk diffusion test (Kim et al., 1995). Briefly, 1 mL of bacterial cultures (Escherichia coli strain ATCC 25922, Pseudomonas fluorescens strain AS1.1802, and Lactobacillus plantarum, 10^6 - 10^8 CFU/mL) was inoculated into 100 mL of sterile agar (30-37°C) and gently mixed. The mixture was poured into sterile plates and cooled to 20°C to allow solidification. Sterile micro steel cups (0.78d ×1.0h cm) were vertically set on the agar in the plates, and 0.5 mL of the extractives from each of the herbs or spices at each dilution (0.063-1.0 g/mL) or the diluted olive oil (0.25-1.0%) was then pipetted into the steel cups. With the lid on, the plates were incubated at 30°C for 24 h for P. fluorescens and at 37°C for 24 h for E. coli and L. plantarum. The inhibitory effect was assessed by measuring the diameter of the clear zone (circle) around the extract-filled steel cup by means of a vernier caliper.

Combination antimicrobial tests in agar media

In addition to testing the antimicrobial activity of the water or ethanol extracts individually (singly), combination antimicrobial tests were also conducted using three herbs/oil (cinnamon, rosemary and clove oil) or four herbs (scutellaria, cinnamon,

honeysuckle, and forsythia suspensa), which was arranged as an orthogonal experiment of three and four factors, respectively, each at three levels (0.125, 0.25, and 0.5 g/mL). Antimicrobial effects in chilled meat

Four ethanol extracts of herbs and three ethanol extracts of spices that exhibited the strongest antimicrobial effects as demonstrated in the agar medium experiment were selected for use as antimicrobial preservatives in chilled meat. Longissimus dorsi muscles were aseptically obtained from pork carcasses 12 h after harvest and divided into 200-250 g chops. Samples were immersed into herb/spice preservatives for 20 s, then vacuum packaged in plastic trays sealed with BOPA/PE films (water and O₂ transmission rates lesser than of 8 and 35cm³.m⁻².24h.atm⁻¹, respectively). The products were subsequently stored at 4°C and examined weekly for microbial growth for up to 4 weeks. The microbial analysis was done by means of aerobic plate count (APC) and sensory panel evaluation. The APC counts were recorded by colony forming units per gram of meat sample (CFU/g), and the sensory evaluation was performed with a 6-member train panel that evaluated the meat color using a 5-point scale (5 = bright purplish red, to 1 = brown), and off-odor also using a 5-point scale (5 = intense, to 1 = none).

Statistical analysis

The significance of differences among samples was determined by analysis of variance using the least square difference method of the General Linear Model procedure. Differences were considered significant at the $P < 0.05$ levels.

Results & Discussion

Antimicrobial effects of herbal and spice extracts in agar medium

Both the water and the ethanol extracts of all the herbs/spices examined (honeysuckle, scutellaria, forsythia suspensa, cinnamon, and rosemary) were inhibitory of the growth of the three bacteria (*E. coli*, *P. fluorescens*, and *L. plantarum*) as evidenced by the size enlargement of the diffusion disc. However, the water extract of scutellaria showed the strongest antimicrobial activity especially against *E. coli* – e.g., the disc diameter (DD) was 17.0 mm at the concentration of 0.5 mg/mL. The same extract also had a strong inhibition to *P. fluorescens* and *L. plantarum*, showing a DD of 14.4 mm and 10.3 mm, respectively, at the concentration of 0.5 mg/mL. Water extracts of forsythia and honeysuckle also exhibited considerable antimicrobial activities, especially to *E. coli*. The water extract of rosemary had a weak inhibition against *E. coli*, but was suppressive to *L. plantarum*.

The antimicrobial effects of the ethanol extracts were overall similar to those of water extracts at equal dosages, suggesting that the same type of bioactive components were likely extracted with both solvent systems. The antimicrobial substance of cassia bark has been identified to be trans-cinnamic aldehyde, which showed insecticidal and fumigant activities against *Mechoris ursulus* (Park et al., 2000). Cinnamic aldehyde has also been isolated from cinnamon shoot (Kim et al., 2004). Zhang et al. (1997) found that the main components of *Scutellaria* are flavonoids, which can inhibit a variety of bacteria.

Clove oil, which was dissolved in ethanol before application, did produce a remarkable inhibition even at low concentrations against all the three bacteria. However, an inverse relationship was observed between the concentration of the oil and the

microbial growth inhibition, probably due to the poor diffusivity of the oil in the agar, a water-based medium.

The combination of three or four different ethanol extracts resulted in significantly enhanced ($P < 0.05$) antimicrobial capability essentially for all the herbs and spices against the three bacteria evaluated, with the improvements typically in the 30-50% range (Table 1; Table 2). The best composite herbal antimicrobial system appeared to be 0.125 g/mL scutellaria + 0.25 g/mL cinnamon + 0.5 g/mL honeysuckle + 0.125 g/mL forsythia (Table 1). The R value calculated from the orthogonal experiment results indicated that the relative contributions of the extracts followed that order of cinnamon > honeysuckle > scutellaria > forsythia. The mixed extracts were especially effective against *E. coli*, surpassing the activity of potassium sorbate at 2-5 mg/mL concentration level. Djenanea et al. (2002) has previously reported that the mixture of rosemary and vitamin C significantly reduced the rates of metmyoglobin formation and lipid oxidation, as well as microbial growth, and it extended the display life of beef steaks from about 10 to about 20 days. Similarly, the combination of three spice ethanol extracts yielded a high antimicrobial activity, with the 0.25 mg/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil producing the highest inhibition of microbial growth (Table 2). From the calculated R value, it can be established that the relative importance of the three spices was rosemary > cinnamon > clove oil.

Antimicrobial effect of herbal/spice extracts in refrigerated pork

The aerobic plate counts throughout display of chilled pork are shown in Figure 1. The initial bacterial population was $3.16 \log_{10}$ CFU/g. From day 5 to day 28, the aerobic plate counts gradually increased to $7.4 \log_{10}$ CFU/g for control, but for treated meat samples, the values were between 5 and $6.2 \log_{10}$ CFU/g, which reflected 1.2 to 2.4 log reductions ($P < 0.05$).

The antimicrobial effect of the following combined spice preservative treatment showed the greatest of all: 0.25 g/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil, which lowered the \log_{10} CFU/g value by 2.32 when compared with the control after 28 days of storage. The effects of combined all-spice ethanol extracts, 0.5g/mL scutellaria + 0.25 g/mL cinnamon + 0.25 g/mL honeysuckle + 0.25 g/mL forsythia suspense, was also quite significant, lowering the \log_{10} units by 0.45, 1.48, 1.93 and 1.57 CFU/g on days 7, 14, 21 and 28 during storage when compared with control.

The effect of herbal/spice extracts on sensory characteristics of refrigerated pork

The Hunter color L^* values of samples treated with herb/spice preservatives were higher than that of control throughout the 28 days of storage ($P < 0.05$). The Hunter color a^* value was also higher than that of control, indicating that the herbal/spice extracts contained antioxidant activities. Lee and Shibamoto (2001) showed that extract of clove buds had considerable antioxidant activity.

Djenanea et al. (2003) showed that the use of the antioxidant mixture of rosemary and vitamin C for fresh beef steak packaged in high oxygenation conditions significantly reduced the rate of metmyoglobin formation and lipid oxidation. Mancini et al. (2005) used rosemary and lactate in beef for improving strip lion steak color stability during display in modified atmosphere package, and showed that steaks with rosemary retained more red color during storage. Results of sensory analysis of stored meat showed no difference between samples treated with preservatives and control ($P > 0.05$), although all the meat samples, regardless of herb/spice treatments, showed an increased off-flavor

(from 2 – low to 4 – high) and a decreased red color (from 4.5 – red to 2.5 – brown) during storage. The results supported the notion that the addition of herb/spice preservatives had no adverse effects on sensory characteristics of chilled meat, which was somewhat consistent with the reports of Djenanea et al. (2003). The sensory color evaluation did exactly agree with the Hunter color values, probably due to the variation in the scores assigned by the individual panel members despite the training provided.

Conclusions

The study demonstrated antimicrobial activities of selected herbs and spices in both agar and meat systems, suggesting that they may serve as potential, natural antimicrobial agents for the inhibition of spoilage and pathogenic microorganisms in muscle foods. Although many of the herbs and spices exhibit antibacterial effect, the combination of several herbal/spice extracts would produce stronger microbial inhibition. The lack of side effects of these antimicrobial food ingredients on the color and flavor further indicates the commercial feasibility of this alternative meat quality preservation technique.

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Tables and Figures

Table 1: Factors and levels scheme and results of orthogonal experiment of ethanol extracts from four herbs.

No.	Scutellaria (g/mL)	Cinnamon (g/mL)	Honeysuckle (g/mL)	Forsythia (g/mL)	Diameter of the diffusion zone (mm)			Average
					<i>Escherichia coli</i>	<i>Pseudomonas fluorescens</i>	<i>Lactobacillus plantarum</i>	
1	0.500	0.500	0.500	0.500	20.58	19.96	17.67	19.40
2	0.300	0.25	0.25	0.25	20.41	11.05	23.97	18.48
3	0.500	0.125	0.125	0.125	18.83	10.40	16.10	15.11
4	0.250	0.500	0.25	0.125	17.40	12.23	20.57	16.73
5	0.250	0.250	0.125	0.500	17.19	12.72	18.22	16.04
6	0.250	0.125	0.500	0.250	16.82	16.14	19.23	16.04
7	0.125	0.250	0.125	0.250	17.12	15.17	22.59	18.29
8	0.125	0.250	0.500	0.125	17.02	19.29	20.42	18.91
9	0.125	0.125	0.25	0.5	14.54	16.68	15.72	15.65
k ₁	17.66	18.14	18.12	17.03				
k ₂	16.27	17.81	16.95	17.60				
k ₃	17.62	15.60	16.48	16.92				
R value	1.39	2.54	1.64	0.68				

Table 2: Factors and levels scheme and results of orthogonal experiment of ethanol extracts of three spices.

Number	Cinnamon (g/mL)	Rosemary (g/mL)	Clove oil (%)	Diameter of the zone of inhibition of bacterial growth (mm)			Average
				<i>Escherichia coli</i>	<i>Pseudomonas fluorescens</i>	<i>Lactobacillus plantarum</i>	
1	0.500	0.500	0.500	9.66	1285	13.74	11.26
2	0.500	0.250	0.250	8.58	1169	15.85	12.04
3	0.500	0.125	0.125	8.24	1390	18.90	13.68
4	0.250	0.50	0.125	8.43	1634	11.86	12.21
5	0.250	0.250	0.500	1.363	154	13.03	14.02
6	0.250	0.125	0.250	1/838	1668	12.97	16.01
7	0.125	0.500	0.250	8.56	1356	11.72	11.28
8	0.125	0.250	0.125	9.99	1640	13.01	13.13
9	0.125	0.125	0.500	1.465	1491	12.89	14.15
k ₁	13.33	11.60	13.47				
k ₂	14.07	13.03	13.30				
k ₃	12.83	14.60	12.77				
R value	1.74	3.00	0.70				

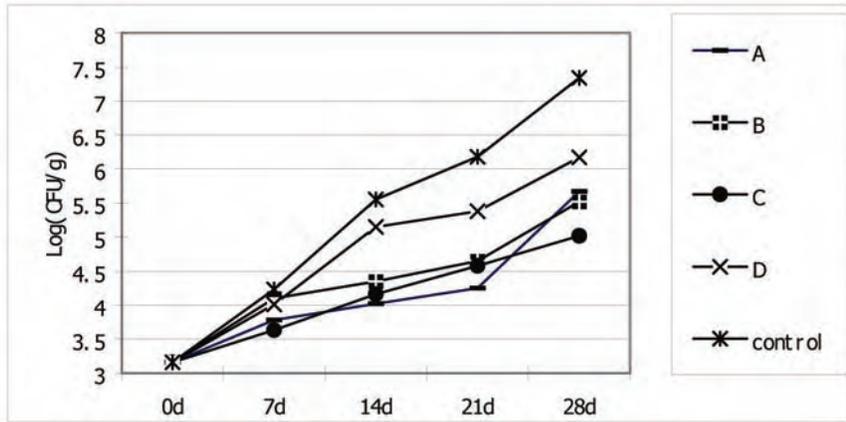


Figure 1: Aerobic plate count numbers in vacuum-packaged pork, treated with combined herbal/spice ethanol extracts, during refrigerated storage (4°C).

A = 0.5 g/mL scutellaria + 0.25 g/mL cinnamon + 0.25 g/mL honeysuckle + 0.25 g/mL forsythia suspensa; B = 0.125 g/mL scutellaria + 0.25 g/mL cinnamon + 0.5 g/mL honeysuckle + 0.125 g/mL forsythia suspense; C = 0.25 g/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil; D = 0.125 g/mL cinnamon + 0.125 g/mL rosemary + 0.5% clove oil; Control = 75% ethanol only.

**MICROBIAL QUALITY OF GROUND BEEF WITH ADDED LACTIC ACID
BACTERIA AT ABUSIVE AND REFRIGERATED TEMPERATURES
PACKAGED IN MODIFIED ATMOSPHERE AND TRADITIONAL PACKAGING**

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Key Words: Lactic acid bacteria, ground beef, spoilage, interventions, packaging

Introduction

Currently, there are few food-safety interventions for ground beef processors. Research conducted has shown that lactic acid bacteria (LAB) reduced food-borne pathogens (3). LAB is GRAS (1) and can create a competitive environment through several different mechanisms including production of hydrogen peroxide, bacteriocins, and weak acids. However, for LAB to be approved by the FDA and USDA-FSIS, it must be determined if LAB addition would cause the product to be adulterated or misbranded by making the ground beef of greater value than untreated products or masking spoilage (2).

Objectives

The effect of LAB on the microflora of ground beef in traditional and MAP packaging displayed under refrigerated or abusive temperatures.

Methodology

Patty Preparation and Temperature Treatments

A total of 209.1 kg of coarse-grind ground beef was obtained from a commercial processing facility over a 6 week period, further processed under simulated industry conditions through a 0.32 cm grinder plate, and formed into 145.2 g patties. Treated ground beef was inoculated with 10^9 cfu LAB/g solution suspended in sterile distilled water. Control samples contained a similar volume (500 mL) of sterile distilled water to standardize added water. Equipment was cleaned thoroughly between treatments and rinsed with 180°C water. The LAB cocktail consisted of *Lactobacillus acidophilus* NP 51, *Lactobacillus crispatus* NP 35, *Pediococcus acidilactici*, and *Lactobacillus lactic* spp. *Lactic* (Culture Systems, Inc.; Mishawaka, IN). Samples were stored in retail display cases maintained at either 0°C or 10°C. Packages were randomly identified and assigned to coffin-style retail cases that were illuminated with fluorescent lighting specially designed to mimic retail grocery outlets. The intensity of lighting at the surface of the package averaged 1900 lux.

Packaging

Traditional Packaging. Ground beef was divided randomly into two treatment groups: control and LAB inoculated. Patties were placed on foam trays and wrapped with a gas permeable polyvinylchloride (PVC) film.

Modified Atmosphere Packaging. To mimic industry practice, the addition of rosemary oleoresin was included as an additional treatment in MAP portion of the study. Ground beef was divided randomly into four treatment groups: control; control + rosemary; LAB inoculated; and LAB + rosemary. Rosemary oleoresin was added at a level of 0.01% (w/w; 1000 ppm) using Herbalox® Type HT-W rosemary oleoresin (Kalsec, Inc.; Kalamazoo, MI). Beef patties were placed in #10 plastic lidded trays (Cryovac; Duncan, SC) and sealed with LID 1050 film (oxygen transmission rate: less than 20.0 cc, 24 h, m² at 40°F and 100% RH). Packages were flushed with a targeted 80% O₂ and 20% CO₂. Headspace was analyzed on random packages during processing and all were monitored for leaks.

Microbial Analysis

For traditional replications, 2.5 g of 4 patties representing 1 treatment were combined (10 g total) in a sterile filtered homogenizer bag and 99 mL of buffered peptone water was added. For MAP replications, 5 g of 2 patties of 1 treatment were combined. The bag was processed at normal speed for 1 min in a laboratory blend stomacher. Serial dilutions were made and samples were automatically plated using the Spiral Biotech Autoplate® (Spiral Biotech, Norwood, MA). The following media were used to isolate and enumerate microorganisms present: Trypticase Soy Agar, Pseudomonas F Agar, YM Agar, Violet Red Bile Agar, Lactobacilli MRS Agar, and STAA Agar with supplement SR151E. Duplicate plates were incubated at appropriate temperatures for each media and were counted using the Spiral Biotech Q Count (Version 2.0, Spiral Biotech). Only colonies exhibiting a greenish-yellow fluorescent pigment on Pseudomonas F plates under short wavelength UV light (254 nm) were counted.

Statistical Analysis

All experiments were performed in triplicate for each of the packaging types. Microbial counts were transformed into log₁₀ units for analysis. The Mixed Model of SAS (SAS Institute, Inc., Cary, NC) was used to evaluate the effect of treatments on microbial loads with replication as a random effect. The dependent variables were the population of bacteria (log transformed). Independent variables included treatment, presence of resin, and sampling time. Treatment*replication was a repeated measure and treatment and time were fixed effects. Significant main effects and interactions were analyzed using the least squares means (LSM) method and separated using the PDIF option of SAS. All tests were considered significant at $P < 0.05$.

Results & Discussion

TRADITIONAL PACKAGING

At 0°C Temperatures

Microbial data can be seen in Tables 1 and 2. There was a significant interaction between treatment and time for total APC. APC counts for uninoculated patties significantly increased after 72 and 84 h of display. However, LAB-inoculated beef APC populations remained constant throughout display. Control patties had significantly lower APC counts at times 0, 24, and 48 h as compared to LAB-inoculated ground beef. This would be expected because the LAB would be counted as part of the aerobic plate count. After 72 and 84 h of display, there were no significant differences between APC counts for treatments indicating that the LAB inhibited some of the naturally occurring aerobic organisms. LAB counts were significantly higher in LAB-inoculated samples than controls. This was expected because LAB was added at approximately 10^9 cfu/g of meat so these differences were not a concern. Significant increases in LAB populations were observed during display (data not shown). YM counts in LAB-inoculated patties were significantly lower than controls, which indicated that LAB inhibited YM growth at refrigeration temperatures. YM populations significantly increased over time (data not shown). No significant differences were found in coliform, *B. thermosphacta*, and pseudomonad counts between treatments, but significant increases in populations over time were seen in both coliforms and *B. thermosphacta*, while pseudomonad counts did not significantly differ during display (data not shown).

At 10°C Temperatures

Microbial results can be seen in Tables 3 and 4. APC counts were significantly higher for treatment groups inoculated with LAB. This was expected as the TSA media would allow for growth of the LAB which was added to the ground beef. APC counts significantly increased over time (data not shown). The bacteria were present in an environment conducive to growth over time, which is a concern for product that has been temperature abused prior to or after the consumer has purchased the product. There was a significant interaction between treatment and time for LAB counts. The controls increased by approximately 3 log cfu/g after 36 h; however, LAB-treated samples showed no significant increase in growth and there were no differences between control and treated samples after 36 h. No significant differences were found in populations of yeast and molds, coliforms, *B. thermosphacta*, and pseudomonad counts between treatments, but significant increases in populations over time occurred for yeast and molds, *B. thermosphacta*, and coliforms. No significant differences were found over time for pseudomonad populations.

MAP PACKAGING

At 0°C Temperatures

Results can be seen in Table 5. Those treatments inoculated with LAB had significantly higher APC and LAB counts than those samples without added LAB. These results were expected as the medias used allowed the growth of LAB. Significant differences were also found in *B. thermosphacta* counts between treatments. However,

yeast and molds, coliform and Pseudomonad counts did not significantly differ between treatments. Over time, significant increases occurred for yeast and mold, coliform, *B. thermosphacta*, and Pseudomonad counts (data not shown).

At 10°C Temperatures

Microbial results can be seen in Table 6 and Table 7. APC counts were significantly higher for treatment groups inoculated with LAB. During display, APC counts significantly increased over time (data not shown). There was a significant interaction between treatment and time for LAB counts. No significant increases in LAB populations were observed in either LAB-inoculated treatment group over time. However, uninoculated treatment groups had significant increases in LAB counts as display progressed. At each interval, LAB treatment groups had significantly higher populations of LAB as compared to control groups. These results were expected since the ground beef was inoculated. No significant differences were observed between treatments for yeasts and molds, *B. thermosphacta*, coliforms, and Pseudomonads. Significant increases over time for yeasts and mold, *B. thermosphacta*, and coliforms counts occurred, while the pseudomonad population did not increase significantly (data not shown).

Conclusions

Previous research conducted has shown that the addition of certain strains of LAB to ground beef does inhibit the growth of *E. coli* O157:H7 and *Salmonella* spp. at a minimum inoculation level of 10^9 cfu/g of meat (3). It is important that proposed food additives not mask factors consumers associate with a spoiled product. While samples inoculated with LAB had significantly higher APC and LAB populations, microbiological and sensory data indicate the addition of LAB does not mask spoilage. Moreover, the populations of LAB from inoculated samples did not increase over time. Rosemary oleoresin, however, did not have a significant effect on growth of the microorganisms isolated in this study, but its addition did not interact with LAB and affect the spoilage characteristics or microbiological properties of the ground beef. Therefore, LAB can be added to ground beef as a processing intervention without masking spoilage and did not result in a misbranded or adulterated product as required by USDA-FSIS (2).

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Tables and Figures

Table 1 Aerobic plate counts (log cfu/g) for ground beef patties in traditional packaging at 0°C.

Treatment	Time (h)				
	0	24	48	72	84
Control	4.00 ^{az}	4.18 ^{az}	4.35 ^{az}	5.46 ^{bz}	5.74 ^{bz}
LAB	5.61 ^{ay}	5.49 ^{ay}	5.68 ^{ay}	5.75 ^{az}	6.14 ^{az}

^{a,b,c}Means within rows lacking same superscript differ ($P < 0.05$)

^{y,z}Means within columns lacking same superscript differ ($P < 0.05$)

Table 2 Bacteria counts (log cfu/g) enumerated from ground beef patties in traditional packaging at 0°C.

Microorganisms	Treatment	
	Control	Control w/resin
Lactic acid bacteria	4.10 ^a	6.88 ^b
Yeasts and molds	3.03 ^a	2.36 ^b
<i>B. thermospatcha</i>	2.04	2.02
Coliforms	4.13	4.18
Pseudomonads ^a	1.22	1.07

^aFluorescin producing pseudomonads

^{b,c,d,e}Means within rows lacking same superscript differ ($P < 0.05$)

Table 3 Bacteria counts (log cfu/g) enumerated from ground beef patties in traditional packaging at 10°C.

Microorganisms	Treatment	
	Control	Control w/resin
Aerobic plate count	5.09 ^a	5.88 ^b
Yeasts and molds	3.16	3.27
<i>B. thermospatcha</i>	2.29	2.11
Coliforms	4.27	4.20
Pseudomonads ^a	1.15	1.10

^aFluorescin producing pseudomonads

^{b,c,d,e}Means within rows lacking same superscript differ ($P < 0.05$)

Table 4 Lactic acid bacteria counts (log cfu/g) for ground beef patties in traditional packaging at 10°C.

Treatment	Time (h)			
	0	12	24	36
Control	3.34 ^{az}	4.28 ^{abz}	4.85 ^{bcz}	6.23 ^{cz}
LAB	6.66 ^{ay}	6.88 ^{ay}	7.05 ^{ay}	7.10 ^{az}

^{a,b,c}Means within rows lacking same superscript differ ($P < 0.05$)

^{y,z}Means within columns lacking same superscript differ ($P < 0.05$)

Table 5 Bacteria counts (log cfu/g) enumerated from ground beef patties in MAP packaging at 0°C.

Microorganisms	Treatment			
	Control	Control w/resin	LAB	LAB w/resin
Aerobic plate count	3.92 ^b	3.57 ^c	5.41 ^d	5.17 ^e
Lactic acid bacteria	1.59 ^b	3.12 ^c	6.19 ^d	6.70 ^d
Yeasts and molds	2.27	1.86	1.85	2.21
<i>B. thermospacha</i>	2.64 ^c	2.00 ^b	1.87 ^b	2.36 ^{bc}
Coliforms	3.83	3.54	3.64	3.60
Pseudomonads ^a	1.19	1.15	1.21	1.36

^aFluorescin producing pseudomonads

^{b,c,d,e}Means within rows lacking same superscript differ ($P < 0.05$)

Table 6 Bacteria counts (log cfu/g) enumerated from ground beef patties in MAP packaging at 10°C.

Microorganisms	Treatment			
	Control	Control w/resin	LAB	LAB w/resin
Aerobic plate count	4.26 ^b	3.93 ^b	5.26 ^c	5.59 ^c
Yeasts and molds	3.23	2.73	3.00	3.50
<i>B. thermospacha</i>	3.01	2.34	2.52	2.70
Coliforms	4.20	3.86	3.98	3.94
Pseudomonads ^a	1.17	1.23	1.02	1.29

^aFluorescin producing pseudomonads

^{b,c,d,e}Means within rows lacking same superscript differ ($P < 0.05$)

Table 7 Lactic acid bacteria counts (log cfu/g) for ground beef patties in MAP packaging at 10°C.

Treatment	Time (h)			
	0	12	24	36
Control	0.87 ^{ax}	2.49 ^{by}	3.90 ^{cy}	4.48 ^{cy}
Control w/resin	2.87 ^{aby}	2.79 ^{ay}	3.58 ^{bcy}	4.33 ^{cy}
LAB	3.40 ^z	6.41 ^z	6.35 ^z	6.50 ^z
LAB w/resin	6.85 ^z	6.76 ^z	6.75 ^z	6.20 ^z

^{a,b,c}Means within rows lacking same superscript differ ($P < 0.05$)

^{x,y,z}Means within columns lacking same superscript differ ($P < 0.05$)

**APPLICATION OF NISIN AND LACTOPEROXIDASE SYSTEM TO IMPROVE
THE MICROBIOLOGICAL QUALITY OF MARINATED CHICKEN
DRUMSTICKS**

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Key Words: Nisin; Lactoperoxidase system; Marination; Microbiological quality

Introduction

Currently, marination is practiced to improve poultry product's physical and sensory attributes (Young & Buhr, 2000; Zheng *et al.*, 2000), but this process is usually not intended to improve the microbial quality of the product. Nisin, which is a natural, nontoxic, heat stable polypeptide produced by *Lactococcus lactis*, and has been shown to inhibit many microorganisms. The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN⁻), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk, and has been shown to be inhibitory against some pathogenic and spoilage microorganisms, and has been mainly studied for the application in the milk and dairy products (Zapico *et al.*, 1998). Limited information on the contribution of marination, nisin, and LPS to the microbial quality of treated products was available.

Objectives

The objective of this study was to evaluate the effects of nisin, LPS and storage time on the microbial quantity of marinated chicken drumsticks.

Methodology

A solution containing 1% acetic acid and 3% salt with pH adjusted to 4 was applied as a standard marinade. Adding nisin at levels of 0, 50 or 100 IU/ml with 20mM EDTA, the marinade solutions were adjusted to pH 4, autoclaved for 121°C for 15 min, and then stored at 4°C. A LPS consisted of 1 µg/ml of LP, 5.9mM KSCN, and 2.5mM H₂O₂. The LP and H₂O₂ were prepared in distilled water, and filter sterilized separately using a .45µm filter, and the KSCN solution was autoclaved. The individual components were then added to the marinade solution no earlier than 5 min before marinating. The contents of 1 and 2 units of LPS were 1, and 2 µg/ml LP, 5.9, and 11.8 mM KSCN, and 2.5, and 5.0 mM H₂O₂, respectively. Drumsticks were aseptically placed and marinated in a plastic bag with autoclaved marinade solution so that all the drumsticks could be covered

completely by the marinade solution and the drumsticks were marinated at 4°C for 18 hr. After marinating, the drumsticks were aseptically removed and drained for 2.5 min, rotated, and drained an additional 2.5 min in a walk-in cooler maintained at 4°C, and packaged individually in sterile plastic bags and storage under refrigeration at 4°C. At specified sampling times of 0, 2, 4, or 7 day, using a rinse procedure, each drumstick was placed in a bag containing 20 ml of 0.1% peptone water and manually shaken for 2 min. Duplicate plates using the pour plate method and plate count agar were prepared for enumeration of bacteria in each bacteria group. Total microflora and psychrotrophs were incubated at 35°C for 48 hours and 7°C for 10 days, respectively and were expressed as log₁₀ CFU per ml of peptone rinse. Least square mean was analyzed using the GLM of SAS Procedures at a 5% level of significance. A complete three-way GLM model was first used to analyze each measurement. Then, a new two-way GLM reduced model was conducted by SAS after the three-way interaction was removed from the model if the three-way interaction was not significant at the 0.05 level.

Results & Discussion

In this study, there was no significant three-way and two-way interactions among the three factors of nisin added level, LPS added level and storage time for the total microflora counts of the samples with different levels of nisin and LPS added during refrigerated storage at 4°C. Table 1 illustrates the nisin level effect on the microbial counts of the treated samples. Adding nisin at level of 50 IU/ml resulted in significantly lower total microflora counts of 7.09 log CFU/ml, when compared with the samples without adding any nisin, which had higher count of 7.46 log CFU/ml. Adding even more nisin to the level of 100 IU/ml resulted in a further significant lower microbial count of 6.83 log CFU/ml. The results imply that the more nisin added up to at level of 100 IU/ml the less total microflora counts obtained. The psychrotrophs counts of the samples exhibited similar patterns. A low concentration of nisin (either 50 or 100 IU/ml) was chosen in this study, because nisin alone was not intended to be the only hurdle treatment. In addition, even though higher levels of nisin may result in an increased effect, a lower concentration of nisin might be appropriate due the economic concern for the cost of nisin. Adding LPS at level of 1 unit resulted in significantly lower total microflora counts of 6.97 log CFU/ml, when compared with samples without adding any LPS which had a higher count of 7.71 log CFU/ml (Table 2). Adding even more LPS to the level of 2 units resulted in a further significant lower microbial count of 6.69 log CFU/ml. The results imply that the more LPS added up to at level of 2 units the less total microflora counts obtained. Similar patterns could be also observed for the psychrotrophs counts of the samples. No significant interactions among the nisin-added level, and LPS-added level on the microbial qualities of the treated samples were observed in this study. Table 3 illustrates the storage effect on the total microflora counts of the samples. The microbial counts significantly decreased from the 7.34 log CFU/ml of the day 0 samples to the 7.10 log CFU/ml of the day 2 samples. No significant difference of the microbial counts was found of the samples after 2 days refrigerated storage at 4°C up to 7 days. Typically, spoilage could be detected when bacterial numbers exceed 10⁸ log CFU/g (Jay, 1996). In this study, even though a few total microflora counts of some samples exceeded

this “log 8 criteria”, no off-odors and slime formation was detected in any of the samples in this study when evaluated by sensory evaluation within 7 days of refrigerated storage.

Conclusions

In conclusion, adding nisin up to the level of 100 IU/ml, and adding LPS up to 2 units consisted of 2 µg/ml LP, 11.8 mM KSCN, and 5.0 mM H₂O₂, respectively, significantly decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks during refrigerated storage up to 7 days.

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Tables and Figures

Table 1. Effects of nisin-added level on the total microflora and psychrotrophs counts of marinated chicken drumsticks

Nisin added level (IU/ml)	Total microflora count ¹ (log CFU/ml)	Psychrotrophs count ² (log CFU/ml)
0	7.46 ^a	8.09 ^a
50	7.09 ^b	7.68 ^b
100	6.83 ^c	7.49 ^c

^{abc} Means within a column with different superscript are significantly different (p<0.05).

¹Total microflora count: incubated at 35°C for 48 hrs.

²Psychrotrophs count: incubated at 7°C for 10 days.

Table 2. Effects of LPS-added level on the total microflora and psychrotrophs counts of marinated chicken drumsticks

LPS added level (unit) ¹	Total microflora count (log CFU/ml)	Psychrotrophs count (log CFU/ml)
0	7.71 ^a	8.42 ^a
1	6.97 ^b	7.63 ^b
2	6.69 ^c	7.21 ^c

^{abc} Means within a column with different superscript are significantly different (p<0.05).

¹LPS unit: 1 unit = 1 µg/ml LP, 5.9 mM KSCN, and 2.5 mM H₂O₂; 2 unit = 2 µg/ml LP, 11.8 mM KSCN, and 5.0 mM H₂O₂.

Table 3. Effects of storage time on the total microflora and psychrotrophs counts of marinated chicken drumsticks

Refrigerated storage time (day)	Total microflora count (log CFU/ml)	Psychrotrophs count (log CFU/ml)
0	7.34 ^a	7.83 ^a
2	7.10 ^b	7.82 ^a
4	6.94 ^b	7.66 ^a
7	7.12 ^b	7.69 ^a

^{ab} Means within a column with different superscript are significantly different (p<0.05).

**EFFECT OF GAMMA IRRADIATION ON THE ENZYMATIC ACTIVITY AND
THE HAEMOSTATIC FUNCTION OF FIBRINOGEN, THROMBIN AND
FACTOR XIII**

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Key Words: Clotting agent, enzymatic activity, gamma-irradiation

Introduction

Plasma contains fibrinogen, thrombin and factor XIII which are blood coagulant factors. It has been found there are many valuable biomedical materials. In 2003, we extracted fibrinogen and thrombin from porcine blood as a haemostat solution and investigate its effectiveness of clotting. We found it had very effective haemostatic action (Chen *et al.*, 2003). Next year, we tried to use fibrinogen and thrombin to prepare haemostatic powder and film. The result revealed that the freeze-dried film was more porous and could arrest the bleeding effectively (Chen *et al.*, 2004). This year, we study the additory effect of factor XIII on the haemostatic function of fibrinogen and thrombin plus calcium chloride. In order to obtain aseptic these coagulants, so the gamma irradiation is employed to sterilize the solution of fibrinogen, thrombin and factor XIII.

Objectives

This study is to investigate the additory effect of factor XIII on the haemostatic function of fibrinogen and thrombin plus calcium chloride, and the effect of gamma irradiation on the enzymatic activity and haemostatic action of the clotting agents.

Methodology

Fibrinogen, thrombin and factor XIII (transglutaminase) were extracted from porcine blood by the methods of Futami *et al.* (1984), Divakaran (1982) and Tseng (1999), respectively. These coagulating factors were freeze-dried to make powder which was irradiated by the dose of 3 Kgy gamma ray (China Biotech Co.). The powder (without irradiation as the control) and the irradiated powder were diluted into solution by the ratio of 1:25 (powder : dist. water) for enzymatic activity analysis. The activities of thrombin and factor XIII of the control and the irradiated samples were determined by the methods of Abe (1961) and Folk (1970), separately. The solutions of thrombin and fibrinogen mixture (1:20) or plus factor XIII were dropped on the wound surface of ears of New Zealand white rabbit, then added with 0.25M calcium chloride to form a clot and

recorded its clotting time to indicate the haemostatic function. The effectiveness of irradiation on microbial counts was also determined.

Results & Discussion

The total microbial counts were found 2.16, 1.56 and 0.47 log cfu/g for the control samples of fibrinogen, thrombin and factor XIII, and zero for the irradiated samples, respectively (table 1). The result revealed that the dose of 3 KGy of gamma ray could kill the microorganisms presented in/on the powder of coagulating factors. Table 2 showed the effects of irradiation on enzymatic activities of thrombin and factor XIII. The results were found there was no difference in enzymatic activities between the control and the irradiated samples. The haemostatic effectiveness of fibrinogen(F)+ thrombin(T)+Ca was compared to fibrinogen+thrombin+Factor XIII(Ta).The result revealed the clotting time of the control(w/o coagulants) was 1.6X of F+T+Ca and 2.6X of F+T+Ta+Ca.(table 3). Thus, it was found factor XIII had an additory effect on the haemostatic function of fibrinogen and thrombin and Ca. The picture 1 was a clotting test of rabbit.

Conclusions

Cold sterilization could be used to kill the organisms presented in/on the coagulating factors, and this process did not affect the enzymatic activities of thrombin and factor XIII. It was also found the factor XIII had an additory effect on the haemostatic function of fibrinogen+thrombin added with Ca.

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Tables and Figures

Table 1 Effect of gamma-irradiation on the total microbial counts of fibrinogen, thrombin and factorXIII extracted from porcine blood

group	Fibrinogen	Thrombin	FactorXIII
Control (log CFU/g)	2.16	1.56	0.47
irradiation (log CFU/g)	0	0	0

Table 2 Effect of gamma-irradiation on enzymatic activities of thrombin and factor XIII

group	Thrombin	FactorXIII
Control	110 unit	0.016
irradiation	110 unit	0.017

Table 3

Clotting test of haemostat applied on New Zealand white rabbit

Group	Control	F+T+Ca	F+T+TG+Ca
Clotting time(second)	156	92	60

1. Control=without applying clotting agents.
2. F=fibrinogen, T=thrombin, TG=factorXIII and Ca=calcium.

Picture 1. Clotting Test on Animal

Control:



F+T+Ca:



F+T+TG+Ca:



- 1. Control=without applying clotting agents.
- 2. F+T+Ca=Fibrinogen+Thrombin+Calcium
- 3. F+T+TG+Ca=Fibrinogen+Thrombin+Factor XIII+Ca

COLOUR STABILITY AND OXIDATION IN RELATION TO TOCOPHEROL LEVELS IN RED DEER (CERVUS ELAPHUS) MEAT

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Key Words: Red deer, meat, venison, colour stability, vitamin E, TBARS

Introduction

Meat colour is an important quality attribute in consumer purchase decisions for fresh meat. The browning of meat, which determines the colour display life, is caused by oxidation of myoglobin and oxymyoglobin to metmyoglobin (Ledward, 1992). The reaction rate of the pigment oxidation process depends upon numerous factors including fatty acid (FA) composition and antioxidant content of the meat.

Differences in FA composition can influence colour stability (Liu *et al.*, 1996) since more unsaturated FA are more prone to oxidation (Cosgrove *et al.*, 1987), and the relationship between higher content of polyunsaturated FA (PUFA) and poor colour stability have been discussed. Levels of antioxidants, such as vitamin E, influence the rate and intensity of the oxidation process (Gray *et al.*, 1996), and thus lipid and colour oxidation in meat and meat products can be decreased by dietary supplementation with antioxidants (Buckley *et al.*, 1995; Liu *et al.*, 1996).

Meat from grazing ruminants contains in general a higher proportion of PUFA and especially n-3 FA compared to meat from pellet fed animals. Grass and leaves have a high content of 18:3 n-3 (Elgersma *et al.*, 2003) whereas pellets are often based on grains rich in n-6 FA and oils instead of seeds which leads to hydrogenation of unsaturated FA in the rumen by microorganisms, if they are not naturally protected by cell walls as in grass and seeds, or by synthetic coatings (Wood & Enser, 1997). However, naturally growing plants in general contain sufficient amounts of antioxidant substances, which might even be sufficient to protect the meat against oxidation.

Objectives

The purpose of the present study was to investigate the influence of feeding regimen on colour stability, vitamin content and levels of oxidation products in red deer meat, and to investigate effects of long-term storage on these parameters.

Methodology

A total of 16 male red deer (age 1 year) were included in the study. Eight animals had grazed pasture and eight had been fed a pelleted feed mixture (Standard Deer Nuts, Reliance Stockfoods Ltd, Dunedin, New Zealand) for 10 weeks prior to slaughter. The animals were exposed to normal pre-slaughter handling, including yarding at the farm, a short transport and subsequent overnight lairage at a deer slaughter premises. At slaughter, all animals were stunned with a captive bolt. The slaughter procedure included electrical stimulation of the carcasses using a MIRINZ low voltage stimulator. Samples from the left side *M. longissimus dorsi* were taken at 30 min *post mortem* and frozen in liquid nitrogen (-196°C). One day *post mortem*, *M. longissimus dorsi* from the left side were excised and cut in five pieces that were randomly allocated to sampling at 1 day *post mortem*, 1, 3, 6 or 12 weeks of refrigerated storage at -1.5°C . Finally all samples were stored at -80°C until further analyses.

Triplicate colour measurements were made on each freshly cut steak 2 hours after opening the vacuum bag, then twice daily using a Minolta Chroma meter (CR-300, Japan), as found appropriate for venison (Stevenson *et al.*, 1989). Days of acceptable colour (display life) were calculated as the time taken to reach an a^* value of 12 using linear interpolation between consecutive samples, as has been used previously for venison (Stevenson *et al.*, 1989; Wiklund *et al.*, 2001).

For the analysis of α -tocopherol, an HPLC method described by Högberg *et al.* (2002) was used. The HPLC column was a 4.0x250 mm RP-18 LiChroCART (Merck KGaA, Darmstadt, Germany). Mobile phase was pumped at a flow rate of 1.2 ml/min. Identification and quantification were done by external standards.

For the analysis of lipid oxidation products (thiobarbituric acid reactive substances (TBARS)) in red deer meat, a slightly modified method described by Miller (1998) was used to prepare samples before analysis by spectrophotometer at a wavelength of 530 nm. Quantification was made by using malondialdehyde (MDA) as an external standard, and a blank was measured to detect background absorbance. Amounts of TBARS were calculated as MDA equivalents by subtracting the blank from all standards and samples and by subtracting the sample blank from each sample.

Results & Discussion

Meat from the grazing deer had significantly longer colour display life at day 1 and after 1, 3 and 6 weeks of refrigerated storage (Fig. 1). TBARS increased during storage, but no significant differences between the treatment groups were found (Table 1). However, when the meat had been stored for 12 weeks, samples from the pellet fed animals had a tendency towards higher amounts of TBARS ($P = 0.067$) compared with samples from the grazing group (Table 1). Meat from grazing animals had a significantly higher content of α -tocopherol compared with meat from pellet fed red deer (Table 1). Storage did not decrease levels of α -tocopherol.

It is suggested that the difference in α -tocopherol content and not FA composition was mainly responsible for the shorter colour shelf life (faster browning) and for the slightly higher formation of TBARS in the meat from pellet fed deer during storage and display. Since oxidation prone n-3 FA (Cosgrove *et al.*, 1987) are present in high

proportions in pasture, opposite results for colour and lipid oxidation would be expected if meat FA composition was the main factor. It has been shown earlier that α -tocopherol has a protecting effect against pigment oxidation (Monahan *et al.*, 1994; Faustman *et al.*, 1998). Gatellier *et al.* (2004) and Gatellier *et al.* (2005) showed similar effects in beef, where grazing led to higher amounts of PUFA but also higher vitamin E content and better oxidation stability. In the same studies a slightly better colour stability of the meat from grazing animals was also found, indicating that negative effects of FA composition on colour stability can be reduced by efficient supplementation with antioxidants. Because significant differences in colour stability were already apparent in fresh red deer meat but differences in lipid oxidation were not, our results suggest that meat pigment oxidation occurs faster than lipid oxidation, and can reveal effects of diet and storage earlier than measurements of lipid oxidation. A similar conclusion was reached by Monahan *et al.* (1994), who found that myoglobin oxidation in pork stored at +4 °C preceded lipid oxidation.

Conclusions

Meat from grazing red deer (with higher vitamin E content) had superior colour stability. Differences in vitamin E intake had larger influence on meat colour than did FA composition. Shelf life or colour stability was closely related to feeding regimen. Meat pigment oxidation occurs faster than lipid oxidation and thereby reveals effects of diet and storage earlier than measurements of lipid oxidation.

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Tables and Figures

Table 1. Content of TBARS and α -tocopherol ($\mu\text{g/g}$) in *M. longissimus* from red deer grazing or fed pellets. Fresh meat and meat after storage in vacuum packages at $-1.5\text{ }^{\circ}\text{C}$.

	Fresh meat		6 weeks storage		12 weeks storage	
	grazing	pellet fed	grazing	pellet fed	grazing	pellet fed
α -tocopherol	1.94 ^a	0.39 ^b	n.m.	n.m.	2.08 ^a	0.34 ^b
TBARS	0.22 ^a	0.23 ^a	0.45 ^b	0.45 ^b	0.43 ^b	0.58 ^b

Means with different superscripts within a row differ significantly ($P < 0.05$)

n.m.= not measured

Display life (hours)

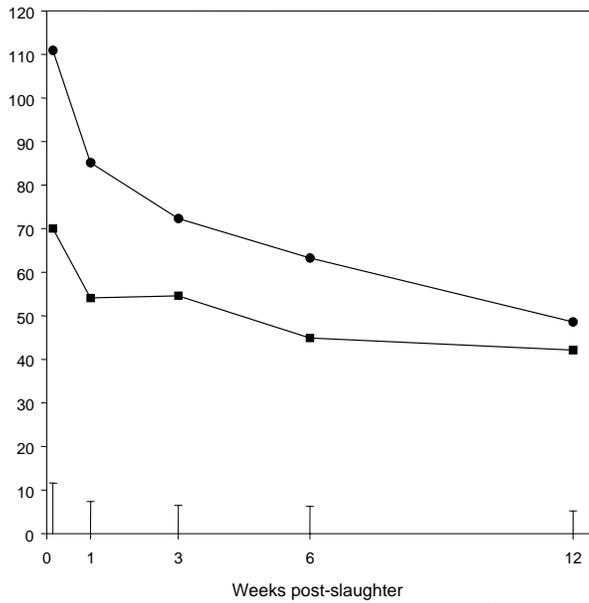


Figure 1. Mean display life (hours of Minolta a^* value ≥ 12) in *M. longissimus* from the red deer from two treatments (● pasture grazing and ■ pellet fed), measured at 1 day, 1, 3, 6 and 12 weeks of refrigerated storage (-1.5°C) in vacuum bags, with error bars indicating standard error of difference (S.E.D).

ANTIOXIDANT ACTIVITY OF *ILEX PARAGUARIENSIS* IN GROUNDED CHICKEN MEAT SUBMITTED TO THERMAL TREATMENT

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Key Words: antioxidant, “mate”, *Ilex paraguariensis*, chicken meat.

Introduction

Lipid oxidation is an important factor for meat and meat product quality since it can cause deterioration by changing the sensorial and nutritional value of meat products (TOKUSOGLU e BASMACIOGLU, 2004). Another factor to be considered is that the consumption of products that have suffered oxidative alterations cause countless health problems, being toxic to the cells, the liver, the kidneys, the cardiovascular system and also causing cancer and arteriosclerosis (FERRARI, 1999).

The use of synthetic antioxidants or controlling lipid oxidation can preserve meat quality. Studies have shown that synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) have been implicated into toxic effects. Thus these findings together with consumer interests in natural food additives have reinforced the need for effective antioxidants from natural sources (MELO & GUERRA, 2002).

“Mate” is a plant that is cultivated in Argentina, Uruguay, Paraguay and Brazil. The aerial parts of this plant are used to prepare a drink called “mate” (FILIP et al., 2000). Experiments have investigated that diseases caused by oxidative reactions in biological systems can be decelerated by the ingestion of natural antioxidants found in diets, especially phenolic compounds. In this sense, there are reports of antioxidant effects of *Ilex paraguariensis* (GUGLIUCCI & STAHL, 1995; SCHINELLA et al. 2000).

The results found in some studies have shown that “mate” extracts present antioxidant properties in boneless poultry meat (MILANI et al. 2001; MILANI et al. 2002) and some meat products (TERRA et al. 2002; FURTADO et al. 2004).

The NaCl is added in foods for a variety of purposes, including flavor and inhibition of microorganisms. However, it has been shown that NaCl accelerates lipid oxidation in a variety of meats, such as beef, pork, chicken and fish (CHEN et al. 1984, SAKAI et al., 2004).

Objectives

The objective of this study was to investigate the antioxidant activity of hydro-ethanollic extract of “mate” in grounded chicken meat submitted to thermal treatment as well as to test its antioxidant activity in presence of salt.

Methodology

Hydro-ethanollic “mate” extract: The hydro-ethanollic “mate” extract was donated by GERMINAL (Company of SP-International Speciality Products Group).

Chicken meat samples: Thigh and leg boneless, skinless chicken meat were used by grinding in a 5 mm disc. Four equal portions were separated to correspond to four treatments as: control (without salt or antioxidant), treatment 1 (addition of 0,5% of hydro-ethanollic “mate” (*Ilex paraguariensis*) extract, treatment 2 (addition of 2% of sodium chloride) and treatment 3 (addition of 2% of sodium chloride plus 0.5% of hydro-ethanollic “mate” extract). All portions, after homogenization, were molded as hamburger patties and thermically treated at 75 C/20minutes. Later, the samples were packaged in plastic bags and stored at 4C for 10 days.

Microbiological analysis: mesophyllic microorganism counts were done using plate count agar (PCA) and incubated at 36C/48 hours (BRASIL, 2003).

Thiobarbituric acid-reactive substances (TBARS) assay: The general extension of lipidic oxidation was determined by the TBARS content (RAHARJO et al., 1992) and expressed as mg of malonaldehyde per kg of sample.

pH assay: To the pH determination, 10 grams of sample were homogenized with 100 mL of distilled water (TERRA e BRUM, 1998).

Sensorial analysis: Hedonic scale with five points was used to evaluate the cooked samples in a conventional oven (ANSALDUA-MORALES, 1994).

Statistical analysis: The mean values were subjected to analysis of variance (ANOVA) and Tukey’s test was applied when appropriated at statistical significance of 5% level (COSTA NETO, 1977).

Results & Discussion

Figure 1 shows the variation of the TBARS values during storage period of the grounded chicken meat samples submitted to 75°C/20 minutes. The addition of 2% of salt accelerated the lipid oxidation of the samples showing TBARS values significantly higher than the control samples during the analyzed period. At the end of this period, it was observed that the addition of 2% of NaCl presented TBA value higher than 3.0 while the control samples obtained approximately a value of 0.8 mg of malonaldehyde/kg sample.

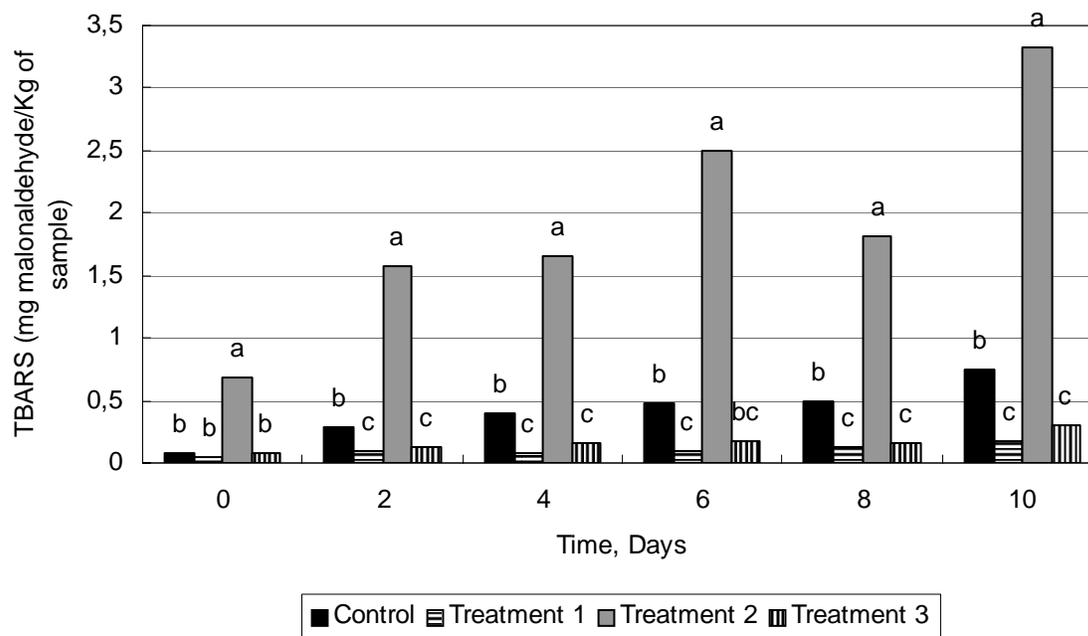


FIGURE 1: Antioxidant activity of grounded and cooked chicken meat control treated with hydro-ethanolic extracts, 2% of salt or a combination of both during days of storage at 4°C.

The pro-oxidant activity of salt is well known (CHEN et al. 1984; SAKAI et al., 2004). According to BELTRAN et al. (2004) sodium chloride is an important meat pro-oxidant for several reasons such as: ionic iron release, damaging of the cell membranes and decrease in reduction of antioxidant enzymes activity. Also cooking has been found to be a pro-oxidant treatment and if NaCl is present it releases the ionic iron from the heme pigment facilitating lipid oxidation (HALLIWELL e GUTTERIDGE, 1984).

AGUIRREZÁBAL et al. (2000) investigated that salt modified lipid peroxydation in sausage, especially in the absence of paprika. It was also noted that the values of reactive substances with the tiobarbituric acid (TBA) did not increase during the ripening of the sausage samples that contained paprika.

CHEN et al (1984) studied the effects of salt alone and with a few antioxidants on raw and cooked beef. These researchers also observed that NaCl accelerates lipid oxidation. In using both Tenox 4-coated salt and the mixture of BHT and the BHA with salt completely inhibited lipid oxidation in cooked meat during cooking and storage, although the tocopherol-coated salt did not control lipid oxidation during the storage after cooking.

BELTRAN et al. (2004) used 5% of sodium chloride and rosemary and acerola extracts to minimize rancidity in pressurized and cooked chicken slurries. Rosemary extracts showed as an antioxidant for pressure-treated samples, although had little effect on cooked ones while the acerola extract had none antioxidant effect.

In this experiment, the hydro-ethanolic “mate” extract showed antioxidant activity since the samples showed TBA values significantly lower than the control after 2 days of

storage (Figure 1), maintaining TBA values under 0.5 until the end of the analyzed period. In the presence of NaCl the hydro-ethanollic extract of “mate” (treatment 3) maintained its antioxidant activity, obtaining TBA values very close to the ones obtained in the samples treated with only the hydro-ethanollic extract of “mate” (Figure 1). Thus it was observed that even after the samples have received thermal treatment and in the presence of sodium chloride the hydro-ethanollic “mate” extract preserved its antioxidant activity showing its great antioxidant potential.

Others authors also observed the effect of different “mate” extracts. GUCLIUCCI and STAHL (1995) observed that aqueous and alcoholic “mate” extracts showed antioxidant capacity *in vivo*, protecting low density lipoprotein against oxidation. SCHINELLA et al. (2000) confirmed the antioxidant properties of “mate” extracts as inhibitor of lipid peroxydation when rat liver microsomes were used.

MILANI et al. (2002) studied the antioxidant effect of hydro-ethanollic and methyllic extracts of apple peel, artichoke leaves and methyllic “mate” extracts in mechanically deboned chicken meat (MDCM) maintained under refrigeration and freezing. It was observed that the methyllic extract of “mate” presented higher antioxidant effect when compared to the other extracts tested. After a month of storage the samples under refrigeration presented a 7.95 TBA level while the sample treated with methyllic extract showed a 1.68 value.

Antioxidant activity of hydro-ethanollic of “mate” extract is most likely related to the presence of phenolic compounds. FILIP et al. (2000) tested the presence of antioxidants in “mate” extracts of six natural and two commercial species. All the species presented higher antioxidant activity. TERRA et al. (2002) added BHA and the hydro-ethanollic “mate” extract to 0.5% and 1% in Italian type sausage to inhibited rancidity. It was observed that in the treatments with the addition of “mate” extract there was a protection against lipid oxidation comparable to the BHA. The treatments with 0.5% “mate” extract and BHA obtained the best sensorial results when compared to the treatments with the 1% “mate” extracts and control. The analysts considered the typical aroma of “mate” and rated the 1% “mate” extract samples as the worst result.

Table 1 shows that the hydro-ethanollic 0.5% “mate” extract did not alter significantly the sensorial characteristics in the analyzed samples. The NaCl treated samples showed higher values to flavor, color, odor, texture and for the acceptability.

Salt addition not only adds a salty taste in the final product but also favors the solubilization of muscle proteins that in turn allows gel formation and development of an optimum texture (ANDRÉS, 2004), which could explain the higher acceptability of the salt-treated samples comparing to the others.

The pH affects chemical and biochemical reactions and microorganisms development. The sample pH varied from 6.4 to 7.0 (Table 2). The samples began with pH levels superior to 6.4, which most likely favored microbial development. According to PRICE and SCHWEIGERT (1971) meats that have pH 6.5 are modified bacteriologically faster than meats with pH 5.3.

TABLE 1 - Sensorial analysis of grounded and cooked chicken meat control and treated samples with hydro-ethanollic extracts, 2% of salt or a combination of both during 10 days of storage at 4°C

Treatments	Storage Period (days)		
	0	5	10
COLOR			
Control	6.72 ^b	8.15 ^a	6.47 ^a
Treatment 1	6.77 ^b	7.45 ^a	4.77 ^a
Treatment 2	8.52 ^a	8.05 ^a	7.28 ^a
Treatment 3	7.92 ^{ab}	4.84 ^b	6.21 ^a
ODOR			
Control	7.65 ^a	5.22 ^a	4.38 ^a
Treatment 1	7.58 ^a	4.31 ^a	3.88 ^a
Treatment 2	8.75 ^a	6.07 ^a	5.43 ^a
Treatment 3	8.41 ^a	6.56 ^a	4.82 ^a
TASTE			
Control	6.89 ^b	nd	nd
Treatment 1	5.88 ^b	nd	nd
Treatment 2	8.78 ^a	nd	nd
Treatment 3	8.15 ^{ab}	nd	nd
TEXTURE			
Control	6.80 ^b	nd	nd
Treatment 1	6.99 ^b	nd	nd
Treatment 2	8.80 ^a	nd	nd
Treatment 3	8.50 ^{ab}	nd	nd
ACCEPTABILITY			
Control	6.15 ^b	nd	nd
Treatment 1	6.18 ^b	nd	nd
Treatment 2	8.18 ^a	nd	nd
Treatment 3	7.84 ^{ab}	nd	nd

Mean score in the same column which are not followed by the same lower case letter are significantly different ($p < 0,05$).

Control: did not contain any salt or antioxidant, treatment 1: addition of 0,5% of hydro-ethanollic extract of “mate”; treatment 2: addition of 2% of sodium chloride and treatment 3: addition of 2% of sodium chloride and 0,5% of hydro-ethanollic extract of “mate”.

Figure 2 shows the mesophyllic aerobic microorganism counts of samples during the storage period at 4°C. It can be seen that the control samples and the ones that received “mate” extracts treatment obtained a very close microbial development, increasing in both samples during the storage period. The observed results agree with antioxidant and antimicrobial effects of methanollic and ethanollic green tea, black tea and “mate” extracts in MDCM studies of MILANI et al. (2001).

TABLE 2. pH values of grounded and cooked chicken meat control and treated samples with hydro-ethanollic extracts, 2% of salt or a combination of both during 10 days of storage at 4°C

Treatments	Storage Period (days)					
	0	2	4	6	8	10
pH						
Control	6.6 ^a	6.6 ^a	6.7 ^a	6.7 ^a	6.7 ^a	6.8 ^b
Treatment 1	6.6 ^a	6.5 ^a	6.7 ^a	6.6 ^a	6.6 ^b	6.6 ^d
Treatment 2	6.4 ^b	6.5 ^a	6.6 ^b	6.6 ^a	6.6 ^b	7.0 ^a
Treatment 3	6.5 ^{ab}	6.5 ^a	6.6 ^b	6.6 ^a	6.6 ^b	6.8 ^b

Means scores in the same column which are not followed by the same lower case letter are significantly different ($P < 0.05$).

Control: did not contain any salt or antioxidant, treatment 1: addition of 0,5% of hydro-ethanolic extract of “mate”; treatment 2: addition of 2% of sodium chloride and treatment 3: addition of 2% of sodium chloride and 0,5% of hydro-ethanolic extract of “mate”.

FURTADO et al. (2004) while studying antimicrobial activity of *Achyrocline satureioides* extract in sausage also utilized hydro-ethanolic extract of “mate”. It was observed that this extract did not present antimicrobial activity on the total aerobic microorganisms.

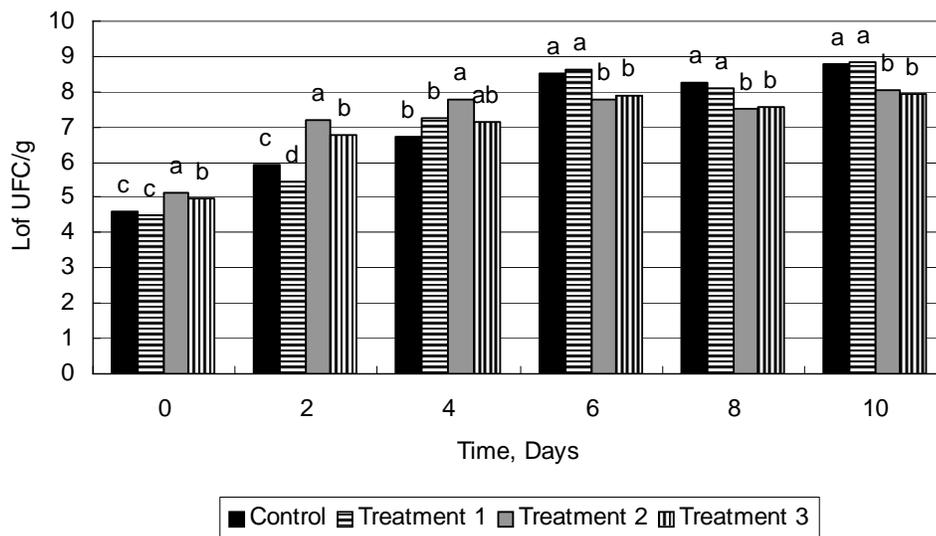


Figure 2. Mesophyllic aerobic microorganisms of grounded and cooked chicken meat control and treated samples with hydro-ethanolic extracts, 2% of salt or a combination of both during 10 days of storage at 4°C.

Conclusions

The hydro-ethanolic “mate” extract presented antioxidant activity in grounded chicken meat submitted to cooking at 75C/20 minutes and in the presence of salt. The 0.5% of hydro-ethanolic “mate” extract did not show antimicrobial activity and did not affect sensorial characteristics of the samples.

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STUDIES OF THE EFFECTS OF CATECHIN ISOMERS ON OXYMYOGLOBIN OXIDATION AND LIPID OXIDATION IN MUSCLE MODEL SYSTEMS

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Key Words: Tea catechins, oxymyoglobin, lipid oxidation

Introduction

Catechins are the major group of polyphenolic flavonoids found in tea. In addition to reported health benefits, tea catechins are potent antioxidants and activity has been reported in a variety of test systems (He and Shahidi, 1997; Huang and Frankel, 1997). Polyphenolic compounds have also been shown to react with proteins for example, the muscle pigment myoglobin (Kroll and Rawel, 2001). The principle catechins found in green tea (*Camellia sinensis*) are (-)-epicatechin (-EC), (-)-epigallocatechin (-EGC), (-)-epicatechin gallate (-ECG) and (-)-epigallocatechin gallate (-EGCG). Structural differences between tea catechin isomers result in varying degrees of antioxidant potency. -EC has an ortho-dihydroxyl group on the B-ring at carbons 3 and 4 and a hydroxyl group at carbon 3 on the C ring. -EGC differs from -EC in that it has a tri-hydroxyl group at carbons 3, 4, and 5 on the B ring. -ECG has a gallate moiety esterified at carbon 3 on the C ring. -EGCG has both a tri-hydroxyl group at carbons 3, 4, and 5 on the B ring and a gallate moiety esterified at carbon 3 on the C ring (Higdon and Frei, 2003). Studies within our research group have focused on the enhancement of fresh beef quality through supplementation of animal diets with green tea catechins. Direct addition of the tea catechin supplement to minced beef resulted in greater colour and lipid stability (Maher et al., 2002). Following HPLC separation (Tsuchiya et al., 1998), the tea catechin supplement, added to animal diets or directly to minced beef, contained approximately 8% -EC, 10% -EGC, 18% -ECG, and 44% EGCG. The influence of individual catechin isomers on oxymyoglobin (oxyMb) oxidation and lipid oxidation in muscle based model systems, at a temperature (4°C) and pH (5.5) relevant to fresh beef in storage, merits investigation.

Objectives

The objective of this study was to examine the influence of individual catechin isomers (+C, -EC, -EGC, -ECG, and -EGCG) on oxyMb (horse heart) oxidation over time. The influence of catechin isomers on oxyMb and lipid oxidation in 25% *M. longissimus dorsi* (LD) homogenates was also investigated.

Methodology

Horse heart oxyMb was prepared according to a modification of the method of Brown and Mebine (1969). Incubates (3 ml) containing oxyMb (~ 1 mg/ml) and catechin isomers (+C, –EC, –EGC, –ECG and –EGCG) at concentrations of 50, 25, 12.5, 6.25 and 3.125 μ M in 150 mM KH₂PO₄-KOH, pH 5.5 were prepared. Methanol was used to dissolve catechin isomers and the methanol content of incubates was 5%. Incubates without catechin isomers containing methanol were run simultaneously as controls. Incubates were stored at 4°C and oxyMb oxidation was monitored over a 5 day storage period.

M. Longissimus dorsi (LD) homogenates (25%) were prepared in 0.12 M KCl 5mM histidine buffer using an Ultra Turrax tissue homogenizer. Lipid oxidation in 30 ml LD homogenate samples, held at 4°C, was initiated by the addition of 45 μ M FeCl₃/sodium ascorbate (1:1). Catechin isomers (+C, –EC, –EGC, –ECG and –EGCG) were added to LD homogenates at concentrations of 250, 100 and 50 μ M. Catechin isomers were dissolved in methanol and the final methanol content of LD homogenates was 5%. LD homogenates with methanol and without FeCl₃/ascorbate were run simultaneously as controls. Lipid oxidation and oxyMb oxidation was measured in samples held at 4°C for up to 24 hours.

Lipid oxidation in LD homogenates was measured following a modification of the 2-thiobarbituric acid-reactive substances (TBARS) procedure of Siu and Draper (1978). The protein content of the LD homogenates was determined according to the method of Markwell et al. (1978). Lipid oxidation was expressed as TBARS in nmoles malondialdehyde (MDA)/mg protein.

OxyMb oxidation in incubates containing horse heart oxyMb was measured by directly recording the absorbance at selected wavelengths. Spectral scans were recorded by scanning from 730 to 500 nm.

OxyMb oxidation in 25% LD homogenates was measured in the supernatant obtained after centrifuging 5 ml of LD homogenate at 14,000 g for 15 min at 4°C. The supernatant was subsequently filtered through Whatman 541 filter paper and re-centrifuged at 14,000 g for 15 min at 4°C.

The relative proportion of oxyMb (% of total myoglobin) in incubates containing horse heart oxyMb and in LD homogenates was calculated using absorbance measurements at selected wavelengths (572, 565, 545 and 525 nm) as described by Krzywicki (1982).

Each experiment was performed three times and all analysis was carried out in duplicate. Data was analysed using the SPSS (version 11.0) statistical package.

Results & Discussion

Catechin isomers exhibited varying degrees of pro-oxidant activity on horse heart oxyMb oxidation (Table 1). Catechins +C and its epimer –EC did not enhance oxyMb oxidation over the 5 day storage period. –EGC exhibited greater pro-oxidant activity, compared to +C and –EC, on oxyMb oxidation and, in general, oxyMb oxidation increased with increasing catechin concentration. Catechins containing gallate moieties (–ECG and –EGCG) exhibited the greatest pro-oxidant effects on oxyMb oxidation

(Table 1). The reactivity of plant phenolic compounds with myoglobin is dependent on the number and position of phenolic hydroxyl groups (Kroll and Rawel, 2001). In the present study, the pro-oxidant activity of catechin isomers on oxyMb oxidation increased proportionally with the number of hydroxyl groups. The exact mechanism by which catechin isomers promote oxyMb oxidation is unclear. Flavonoids are known to bind with proteins (Arts et al., 2002) and as such, it is possible that the different catechin isomers bind or interact with oxyMb in ways which render the pigment susceptible to oxidation. No oxyMb spectral shift was observed in the presence of 50 μM –EGCG indicating lack of an interaction between catechin molecules and oxyMb (Figure 1). Nakayama et al. (2002) reported that hydrogen peroxide (H_2O_2) is formed in aqueous solutions of tea catechins where –EGC and –EGCG contribute mainly to H_2O_2 formation. Since hydrogen peroxide is a potent catalyst of oxyMb oxidation, this may, in part explain the pro-oxidative nature of –EGC and –EGCG on oxyMb.

Addition of FeCl_3 /ascorbate (45 μM) resulted in increased lipid oxidation in LD homogenates over the storage period (Table 2). Graded addition of each catechin isomer ($p < 0.05$) reduced lipid oxidation compared to controls after 24 hours storage at 4°C. Lipid oxidation decreased with increasing concentrations of +C and –EC. Catechins, –EGC, –ECG and –EGCG exhibited similar antioxidant activity irrespective of catechin concentration. In addition, catechins containing gallate groups (–ECG and –EGCG) exerted the greatest antioxidant activity compared to other catechin isomers. Guo et al. (1999) suggested that the presence of the gallate group played the most important role in free radical scavenging ability of catechins, and that the additional hydroxyl group on –EGC also contributed to radical scavenging activity. OxyMb oxidation was reduced ($p < 0.05$) in the presence of each catechin isomer (Table 2). Catechin isomers did not enhance oxyMb oxidation in LD homogenates in contrast to the pro-oxidant activity of catechins on horse heart oxyMb reported above. This may be attributed to catechins having greater affinity for other components in LD homogenates such as other proteins or lipids.

Conclusions

Catechins did not delay horse heart oxyMb oxidation and isomers containing gallate groups exerted the greatest pro-oxidant activity. Catechins reduced lipid oxidation in LD homogenates and isomers containing gallate groups had the greatest antioxidant activity. Results indicate differing reactivity of catechin isomers with muscle lipids and oxyMb depending on the test system employed.

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Tables and Figures

Table 1. OxyMb oxidation following the addition (50, 25, 12.5, 6.25 and 3.125 μM) of catechin isomers (+C, -EC, -EGC, -ECG and -EGCG) and storage at 4°C.

Incubate	μM	Time, days					
		0	1	2	3	4	5
OxyMb ¹		96.0 \pm 4.0	83.4 \pm 2.1	69.2 \pm 4.0	58.2 \pm 3.4	48.5 \pm 4.5	41.8 \pm 5.1
OxyMb +							
+C	50	96.6 \pm 2.5 ^a	79.3 \pm 5.3 ^a	61.5 \pm 4.5 ^a	49.7 \pm 4.6 ^a	39.1 \pm 3.5 ^a	31.1 \pm 2.0 ^a
	25	96.4 \pm 2.3 ^a	78.9 \pm 3.2 ^a	60.9 \pm 3.9 ^a	49.2 \pm 3.4 ^a	38.4 \pm 2.8 ^a	31.1 \pm 3.2 ^a
	12.5	96.2 \pm 2.6 ^a	81.0 \pm 5.9 ^a	65.0 \pm 5.4 ^a	53.1 \pm 5.4 ^a	42.6 \pm 4.4 ^a	36.0 \pm 4.7 ^a
	6.25	95.9 \pm 3.0 ^a	81.2 \pm 5.7 ^a	65.4 \pm 4.8 ^a	53.7 \pm 5.2 ^a	43.6 \pm 4.4 ^a	36.3 \pm 4.1 ^a
	3.125	95.4 \pm 2.9 ^a	79.0 \pm 8.9 ^a	62.4 \pm 9.4 ^a	51.0 \pm 10.0 ^a	41.2 \pm 9.0 ^a	34.3 \pm 8.9 ^a
-EC	50	94.8 \pm 3.9 ^a	78.7 \pm 6.7 ^a	61.6 \pm 7.0 ^a	49.0 \pm 8.5 ^a	39.1 \pm 8.8 ^a	31.8 \pm 8.5 ^a
	25	94.8 \pm 3.2 ^a	78.5 \pm 7.4 ^a	62.7 \pm 9.4 ^a	50.7 \pm 10.0 ^a	40.8 \pm 9.2 ^a	33.3 \pm 10.0 ^a
	12.5	93.5 \pm 3.6 ^a	79.6 \pm 5.6 ^a	64.4 \pm 6.5 ^a	53.6 \pm 7.7 ^a	43.6 \pm 7.4 ^a	36.7 \pm 7.1 ^a
	6.25	95.2 \pm 3.3 ^a	82.1 \pm 2.4 ^a	67.4 \pm 3.0 ^a	57.1 \pm 3.5 ^a	47.2 \pm 3.8 ^a	39.9 \pm 3.4 ^a
	3.125	94.8 \pm 3.7 ^a	80.7 \pm 2.6 ^a	65.2 \pm 3.3 ^a	54.7 \pm 2.4 ^a	44.6 \pm 1.7 ^a	37.3 \pm 1.7 ^a
-EGC	50	91.9 \pm 5.8 ^a	70.8 \pm 6.7 ^a	51.2 \pm 6.2 ^a	38.4 \pm 6.2 ^a	28.7 \pm 4.8 ^{ab}	22.6 \pm 3.8 ^{ab}
	25	91.3 \pm 6.5 ^a	67.9 \pm 9.4 ^a	46.1 \pm 9.9 ^a	33.1 \pm 9.3 ^a	24.0 \pm 7.6 ^a	18.5 \pm 6.4 ^a
	12.5	91.6 \pm 5.4 ^a	72.7 \pm 7.9 ^a	55.4 \pm 8.7 ^a	43.6 \pm 9.2 ^a	34.5 \pm 8.0 ^{ab}	28.2 \pm 7.1 ^{ab}
	6.25	91.9 \pm 5.8 ^a	75.7 \pm 9.5 ^a	60.5 \pm 10.0 ^a	50.1 \pm 9.9 ^a	41.1 \pm 9.5 ^{ab}	34.8 \pm 9.7 ^{ab}
	3.125	93.5 \pm 5.3 ^a	79.0 \pm 4.8 ^a	64.7 \pm 5.1 ^a	54.7 \pm 5.9 ^a	45.7 \pm 5.7 ^b	39.5 \pm 4.9 ^b
-ECG	50	86.0 \pm 8.2 ^a	50.7 \pm 5.7 ^a	33.3 \pm 3.9 ^a	23.4 \pm 2.9 ^a	16.7 \pm 2.1 ^a	12.4 \pm 1.4 ^a
	25	87.7 \pm 7.8 ^a	55.6 \pm 3.0 ^a	38.2 \pm 3.0 ^{ac}	27.8 \pm 2.3 ^{ac}	19.6 \pm 1.9 ^a	14.2 \pm 1.7 ^{ac}
	12.5	90.2 \pm 5.6 ^a	61.8 \pm 6.3 ^{ab}	44.1 \pm 4.7 ^{ad}	33.3 \pm 4.6 ^{ad}	24.4 \pm 3.1 ^{ac}	18.0 \pm 2.5 ^{ad}
	6.25	88.8 \pm 5.6 ^a	66.4 \pm 9.0 ^{ac}	50.3 \pm 7.7 ^{bcd}	39.2 \pm 6.9 ^{bcd}	29.8 \pm 5.1 ^{bc}	22.0 \pm 3.7 ^{bcd}
	3.125	91.9 \pm 5.7 ^a	73.9 \pm 4.5 ^{bc}	57.9 \pm 5.1 ^b	46.6 \pm 5.4 ^b	35.6 \pm 5.3 ^b	27.0 \pm 4.5 ^b
-EGCG	50	78.8 \pm 9.4 ^a	38.0 \pm 8.8 ^a	22.7 \pm 5.7 ^a	15.4 \pm 4.6 ^a	11.4 \pm 2.7 ^a	8.4 \pm 2.0 ^a
	25	84.9 \pm 7.8 ^a	44.3 \pm 8.4 ^{ac}	26.1 \pm 5.3 ^a	17.9 \pm 3.7 ^a	12.5 \pm 2.4 ^a	9.3 \pm 1.8 ^a
	12.5	88.7 \pm 7.7 ^a	51.4 \pm 6.4 ^{ad}	30.1 \pm 2.5 ^a	22.4 \pm 2.5 ^a	16.6 \pm 1.7 ^a	12.4 \pm 0.8 ^a
	6.25	90.7 \pm 6.0 ^a	62.8 \pm 7.6 ^{bcd}	45.4 \pm 6.0 ^b	33.0 \pm 9.8 ^{ac}	28.7 \pm 5.0 ^b	23.5 \pm 3.9 ^b
	3.125	91.2 \pm 6.0 ^a	69.5 \pm 7.4 ^{bd}	54.3 \pm 7.7 ^b	44.8 \pm 7.9 ^{bc}	35.9 \pm 6.1 ^b	31.0 \pm 7.2 ^b

¹Oxymyoglobin, % of total, control containing 5% methanol. abcdMean values for the same catechin group within each day bearing different superscripts are significantly different, P < 0.05.

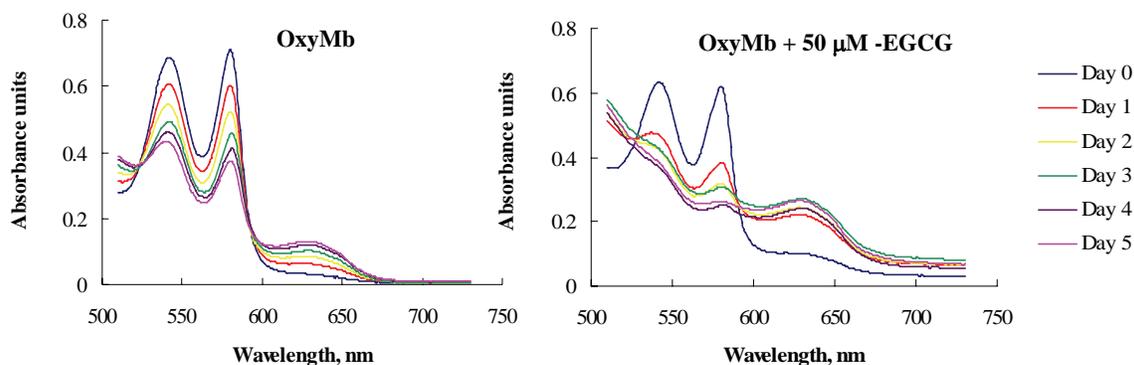


Figure 1. Absorbance spectra of oxyMb alone or oxyMb in the presence of 50 μM -EGCG.

Table 2. OxyMb oxidation and lipid oxidation in 25% *M. longissimus dorsi* homogenates following the addition (250, 100 and 50 μM) of catechin isomers (+C, -EC, -EGC, -ECG and -EGCG) and storage at 4°C.

Incubate	μM	Time, hours			
		0		24	
		¹ OxyMb, %	² TBARS	OxyMb, %	TBARS
H ³		87.6 \pm 10.8 ^a	0.008 \pm 0.005 ^a	83.8 \pm 5.7 ^a	0.021 \pm 0.012 ^a
H + P ⁴		84.6 \pm 12.4 ^a	0.010 \pm 0.003 ^a	51.2 \pm 6.8 ^b	0.264 \pm 0.043 ^b
H + P +					
+C	250	86.4 \pm 10.9 ^a	0.009 \pm 0.005 ^a	78.2 \pm 5.5 ^a	0.014 \pm 0.008 ^a
	100	86.7 \pm 10.5 ^a	0.010 \pm 0.006 ^a	77.4 \pm 5.0 ^a	0.053 \pm 0.036 ^{ad}
	50	87.5 \pm 9.7 ^a	0.009 \pm 0.004 ^a	75.2 \pm 3.7 ^a	0.115 \pm 0.049 ^{cd}
-EC	250	85.9 \pm 10.7 ^a	0.008 \pm 0.004 ^a	76.4 \pm 2.3 ^a	0.012 \pm 0.011 ^a
	100	86.6 \pm 10.9 ^a	0.011 \pm 0.004 ^a	78.3 \pm 5.1 ^a	0.025 \pm 0.016 ^{ad}
	50	86.9 \pm 11.0 ^a	0.015 \pm 0.011 ^a	75.4 \pm 4.1 ^a	0.085 \pm 0.038 ^{cd}
-EGC	250	86.3 \pm 10.7 ^a	0.009 \pm 0.002 ^a	78.7 \pm 5.4 ^a	0.009 \pm 0.007 ^a
	100	86.8 \pm 9.6 ^a	0.010 \pm 0.002 ^a	78.9 \pm 5.6 ^a	0.014 \pm 0.009 ^a
	50	86.4 \pm 10.6 ^a	0.008 \pm 0.005 ^a	76.2 \pm 3.0 ^a	0.024 \pm 0.009 ^a
-ECG	250	85.7 \pm 10.5 ^a	0.008 \pm 0.005 ^a	79.3 \pm 5.9 ^a	0.010 \pm 0.008 ^a
	100	85.8 \pm 10.1 ^a	0.010 \pm 0.003 ^a	79.5 \pm 6.2 ^a	0.008 \pm 0.007 ^a
	50	84.8 \pm 11.2 ^a	0.009 \pm 0.002 ^a	78.4 \pm 5.9 ^a	0.017 \pm 0.011 ^a
-EGCG	250	86.4 \pm 10.0 ^a	0.010 \pm 0.003 ^a	80.1 \pm 5.7 ^a	0.007 \pm 0.006 ^a
	100	87.1 \pm 10.4 ^a	0.009 \pm 0.003 ^a	80.3 \pm 5.7 ^a	0.006 \pm 0.005 ^a
	50	86.5 \pm 9.4 ^a	0.010 \pm 0.002 ^a	80.1 \pm 5.6 ^a	0.011 \pm 0.008 ^a

¹Oxymyoglobin, % of total. ²nmoles malondialdehyde/mg protein. 325% LD homogenate + 5% methanol. 4FeCl₃/ascorbate. abcdMean values within the same catechin group (and compared to controls H, H + P) bearing different superscripts are significantly different, P < 0.05.

LIPID OXIDATION IN DUTCH STYLE FERMENTED SAUSAGES WITH INCREASED LEVELS OF LINOLENIC ACID

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Key Words: Linolenic acid; Lipid oxidation; Fermented sausages; Flaxseed oil; Canola oil; PUFA/SFA ratio; *n-6/n-3* ratio

Introduction

In the 1970s researchers, struck by the low incidence of coronary artery disease among Greenland Inuit, associated this with the high content of *n-3* polyunsaturated fatty acids (PUFAs) in their diet (Bjerregaard et al., 2000). *n-3* PUFAs may also have a protective effect against breast and colon cancer (Rose and Connolly, 1999), rheumatoid arthritis and inflammatory bowel diseases (Alexander, 1998).

As meat (products), are some of the most important sources of dietary fat, modification of their lipid profiles by enhancing *n-3* PUFAs, can help to improve the nutritional quality of the occidental diet (Ansorena and Astiasaran, 2004). Nutritional authorities recommend that a dietary *n-6/n-3* PUFA ratio of less than 4 should be achieved and a polyunsaturated fatty acids/saturated fatty acids (P/S) ratio above 0.4 (Wood et al., 2004). Feeding animals PUFAs (basically *n-3*) had a positive effect on the fatty acid profile, but a negative effect on the sensory quality of the meat when added in high concentrations (D'Arrigo et al., 2002; Fontanillas et al., 1998; Hoffman et al., 2005; Hoz et al., 2004; Hoz et al., 2003; Matthews et al., 2000; Rey et al., 2001; Romans et al., 1995; SpechtOverholt et al., 1997).

The inclusion of vegetable oils in fermented sausages has been variously tested. Olive oil (Muguerza et al., 2003b; Muguerza et al., 2002; Muguerza et al., 2001; Severini et al., 2003), soybean oil (Muguerza et al., 2003a) and flaxseed oil (Ansorena and Astiasaran, 2004) were used as a source of monounsaturated fatty acids, to modify the P/S ratio, and to change both P/S and *n-6/n-3* ratios, respectively. The main problem of increasing *n-3* PUFAs in fermented sausages may arise from their susceptibility to oxidation.

Objective

The objective of this work was to evaluate the lipid modifications in dry-fermented sausages, when part of the animal fat was replaced by pre-emulsified flaxseed and canola

oil. Our work focuses on the changes in the P/S and *n-6/n-3* ratios and on the development of lipid oxidation in the final product during storage.

Methodology

Sausage Preparation

Dutch style cervelat, a semi-dry fermented sausage, were manufactured at Wageningen University. Lean beef, pork backfat, flaxseed and canola oil were used as raw materials. The flaxseed and canola oil (both obtained from a local supermarket) were pre-emulsified with soy protein isolate (Hoogenkamp, 1989a,b). Seven formulations of sausages, about 1.5 kg each, were prepared. The control contained 70% beef and 30% pork backfat. The other formulations were produced with 27, 25.5 and 24% backfat and 3, 4.5 and 6% flaxseed or canola oil, respectively. This corresponds to a substitution of backfat with 10, 15 and 20% flaxseed or canola oil, respectively. The experimental design and the formulations are given in Table 1. The relative amounts of the other ingredients, added (g) per kg meat mixture, were: nitrite-curing salt (25); starter sausage (10); glucose (7); glutamate (2); white pepper (1.2); paprika (1); crushed pepper (1); ascorbic acid (0.5); mace (0.25); clove (0.16); and garlic powder (0.15).

Product doughs were manufactured according to a strictly standardized procedure and stuffed into 52 mm diameter cellulose-based casings. The sausages were fermented for three days at 25°C and dried at 15°C and 80–65% R.H. for 12 days at Meester-Stegeman C.V. (Deventer). To accelerate the rate of oxidation the sausages were next sliced (thickness 6 mm) and stored in a modified atmosphere containing 55% oxygen, 24% carbon dioxide and 21% nitrogen. The slices were packaged at Hanskamp Vers Vlees B.V. (Deventer). Samples from all formulations were taken for analysis at day 0 (= packaging day), 18, 30, 41, 55, 69 and 83 of storage in the dark at 5°C.

Chemical Analyses

Determinations were done: of moisture (AOCS., 1997a); of total fat (AOAC., 2002); of peroxide values (AOCS., 1997b); of thiobarbituric acid reactive substances (TBARS) (Juncher et al., 2000) and of protein (according to the Dumas method with a NA 2100 Protein analyser). The fatty acid compositions of the lipid fractions were determined as fatty acid methylesters (AOCS, 1997c). The hexanal content was determined by a GC static head space method (Shahidi and Pegg, 1994).

Statistical Analyses

Analysis of variance (ANOVA) and the Tukey test were used to determine significant differences ($p \leq 0.05$). Software used was SPSS version 10.0 (© 1999, SPSS inc., Chicago).

Results & Discussion

The formulations of the sausages, with a total added backfat plus oil of 30% in the meat mix, are presented in Table 1. The flaxseed or canola oil added in the modified products increased from 10 to 20% of the total added backfat.

Table 2 shows the percentages of moisture, fat and protein of the different products. There was no exudation of oil/fat in the treatments with substitution of pork backfat by pre-emulsified flaxseed and canola oil during the manufacturing process. Bloukas et al. (1997), Muguerza et al. (2001) and Vural (2003) found that the partial replacement of pork backfat with oil pre-emulsified with soy protein isolate resulted in significant differences in moisture content. In contrast, we did not find differences in the moisture content for the different treatments.

The fatty acid profiles of the various sausages are shown in Table 3. SFA content showed a progressive decrease from control by substitution of 0 to 20% of backfat by flaxseed or canola oil. The low amount of palmitic and stearic acid in flaxseed and canola oil are mainly responsible for these changes. The Σ MUFA showed a progressive decrease in the sausages with flaxseed oil (low content of oleic acid), whereas Σ MUFA in the sausages with canola oil (high oleic acid content) showed a progressive increase. All the modified products showed a progressive increase in the Σ PUFA, especially the sausages with flaxseed oil. The P/S ratio increased from 0.32 in the control to 0.41–0.48 and to 0.49–0.70 in the sausages with canola and flaxseed oil, respectively. The increase in this ratio in relation to control is basically due to the increase in linolenic acid. Effectively, the *n*-6/*n*-3 ratio decreased from 11.29 in the control to 6.95–5.12 and to 1.93–1.05 in the sausages with canola and flaxseed oil, respectively.

Fig. 1 shows the peroxide values (P.V.) as a function of storage time of the different modified products. The P.V. of all the formulations were until day 55 quite similar, after that day the P.V. of the different products increased, especially for the sausages with flaxseed oil. After 83 days, the sausages with canola oil showed the same P.V. as the control. The sausages with flaxseed oil showed a higher increase of P.V. than the other formulations after that period of storage. Higher replacement of pork backfat by flaxseed oil, resulted in higher P.V. The high amount of linolenic acid in flaxseed oil is probably mainly responsible for these increments, because *n*-3 PUFAs are very susceptible to oxidation.

Secondary oxidation products (TBARS) were also determined (Fig. 2). After 45 days there was an increase in TBARS values of the sausages with flaxseed oil. All formulations with flaxseed oil had TBARS values around 7 μ g malonaldehyde/g sausage after 83 days of storage. These values were higher than for the other formulations; the higher unsaturated character of flaxseed oil is probably mainly responsible for this effect. The TBARS values of the sausages with canola oil were comparable to the values of the control.

The measurable formation of hexanal, a specific secondary lipid oxidation product, in sausages with flaxseed oil starts around day 30 compared to day 60 for the sausages with canola oil (Fig. 3). The hexanal end-values of the flaxseed oil containing sausages were much higher than the values of the canola oil containing products. The formation of hexanal was in the control even higher than in the sausages with canola oil.

Part of an explanation for the reduced lipid oxidation in the canola oil containing products may be an antioxidant effect of soy protein isolate (Bloukas et al., 1997) in combination with the relatively high content of vitamin E in this oil.

Conclusions

In the manufacture of Dutch style fermented sausages, up to 20% of pork backfat can be substituted with flaxseed or canola oil, pre-emulsified with soy protein isolate. The addition of flaxseed and canola oil progressively increased the PUFA/SFA ratio and decreased the *n-6/n-3* ratio leading to values closer to those considered optimal. The addition of canola oil, did not reduce the shelf life in terms of lipid oxidation, however, the addition of flaxseed oil showed an increased lipid oxidation during storage. Further research is needed to get more insight in the sensory and physical characteristics of the products.

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Tables and Figures

Table 1. Experimental design and raw materials used

Treatment s ^a	Backfat level ^b (%)	Oil replacing level (%)	Materials in g/kg of meat mixture					
			Beef	Pork backfat	Flaxseed oil	Canola oil	ISP ^c	Water ^c
Control	30	0	700	300	–	–	–	–
F10	30	10	700	270	30	–	3.0	24.0
F15	30	20	700	255	45	–	4.5	36.0
F20	30	30	700	240	60	–	6.0	48.0
C10	30	10	700	270	–	30	3.0	24.0
C15	30	20	700	255	–	45	4.5	36.0
C20	30	30	700	240	–	60	6.0	48.0

^aF10, F15 and F20, substitution of pork backfat with 10, 15 and 20% flaxseed oil, respectively; C10, C15 and C20, substitution of pork backfat with 10, 15 and 20% canola oil, respectively.

^bOn the day of preparation.

^cISP, isolated soy protein. ISP and Water added on top.

Table 2. Mean percentages of moisture, fat and protein of the different final products (N=2)

	Control	F10 ^a	F15	F20	C10	C15	C20
Moisture	33.3	32.3	33.3	30.8	33.5	32.4	32.8
Fat	40.4	38.6	40.5	40.8	40.4	38.4	39.3
Protein	23.0	24.0	24.1	25.5	24.3	25.6	26.1

^aSee corresponding subscript Table 1.

Table 3. Fatty acid content at the time of packaging for seven types of sausages (g/100 g fat)

Fatty acid		Control	F10 ^a	F15	F20	C10	C15	C20
Caproic	10:0	0.08 ^b a	0.04b	0b	0b	0b	0b	0b
Lauric	12:0	0.40a	0.37a	0.37a	0.32a	0.28a	0.37a	0.32a
Myristic	14:0	2.91a	2.63ab	2.49bd	2.37cd	2.60bc	2.41bd	2.26d
Palmitic	16:0	24.46a	22.92b	22.08bc	20.96de	22.92b	21.90cd	20.79e
Palmitoleic	16:1	3.05a	2.69bc	2.53cd	2.49de	2.73b	2.52ce	2.39de
Margaric	17:0	0.59a	0.55ab	0.51bc	0.50c	0.53ac	0.51bc	0.49c
Stearic	18:0	12.85a	12.06ab	11.86ab	11.21bc	11.76ab	11.35b	10.94c
Oleic	18:1	42.04c	39.65d	38.48de	37.31e	43.76b	44.63ab	45.97a
Linoleic	18:2	12.37e	12.53de	13.27c	12.72d	13.49bc	13.94ab	13.99a
Linolenic	18:3	1.10d	6.49c	8.42b	12.12a	1.94d	2.32d	2.73d
Arachidic	20:0	0.16a	0.08a	0a	0a	0a	0.05a	0.11a
Behenic	22:0	0a	0.01a	0a	0a	0a	0a	0a
Σ SFA ^c		41.45	38.64	37.30	35.36	38.08	36.59	34.92
Σ MUFA		45.09	42.34	41.00	39.80	46.48	47.14	48.36
Σ PUFA		13.46	19.02	21.69	24.84	15.43	16.26	16.73
P/S		0.32	0.49	0.58	0.70	0.41	0.44	0.48
n-6/n-3		11.29	1.93	1.58	1.05	6.95	6.01	5.12

^aSee corresponding subscript Table 1.

^bValues with different letters within a row are significantly different ($P < 0.05$; $N = 4$).

^cSFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; P/S, polyunsaturated fatty acids/saturated fatty acids.

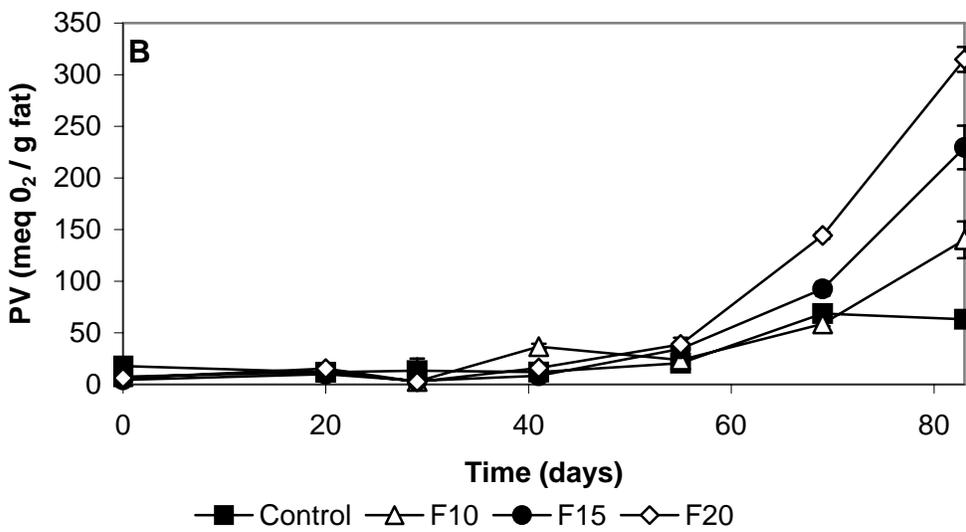
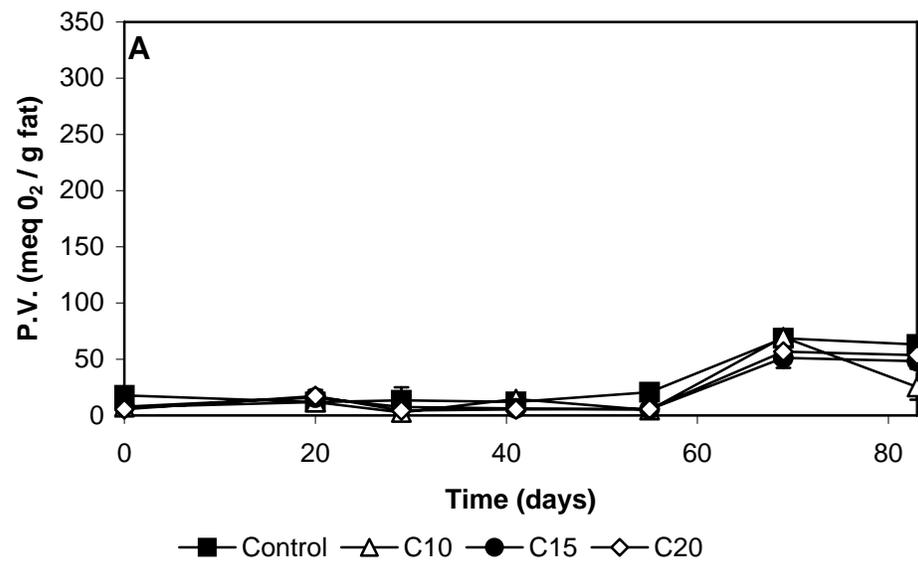


Fig. 1. Peroxide values of the different formulations during storage ($N=4$). **A:** Products with canola oil. **B:** Products with flaxseed oil. See further first subscript Table 1.

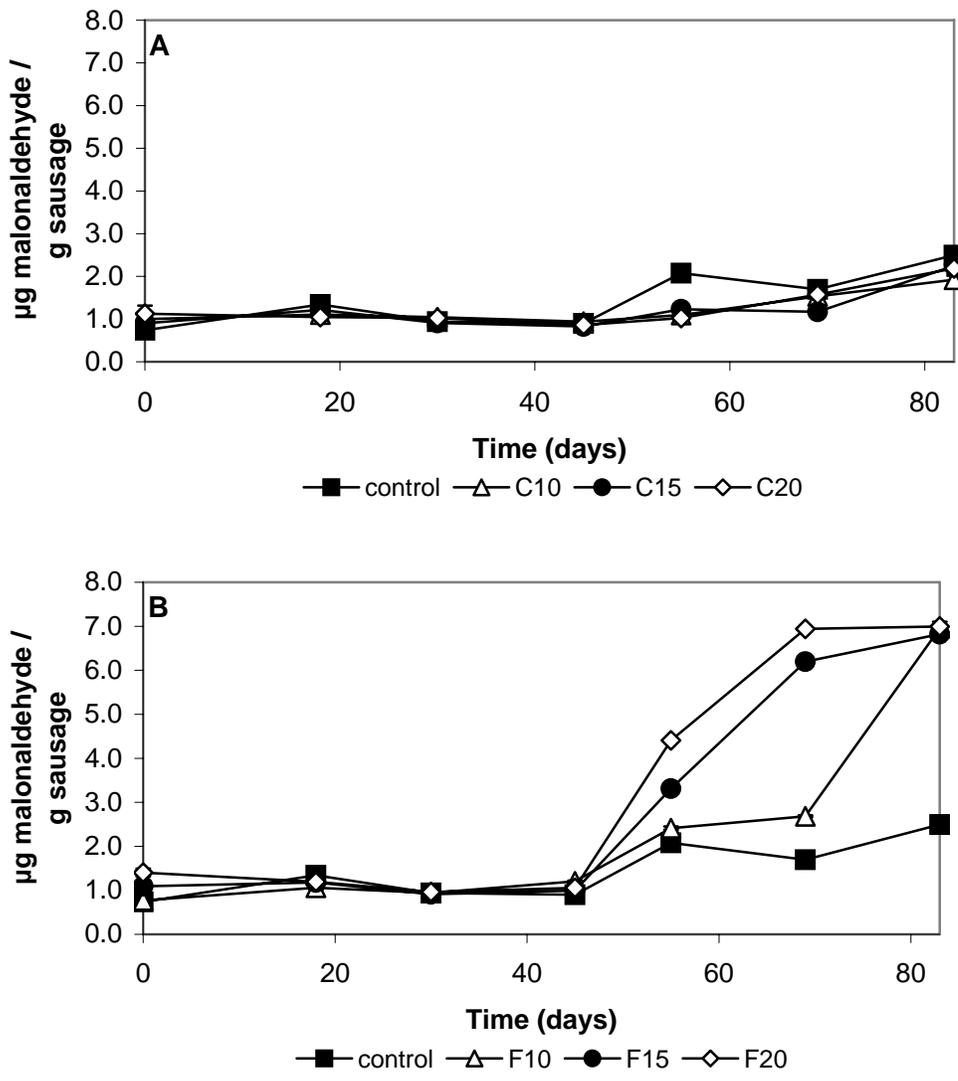


Fig. 2. TBARS values of the different formulations during storage (N=2). See further subscript Fig. 1.

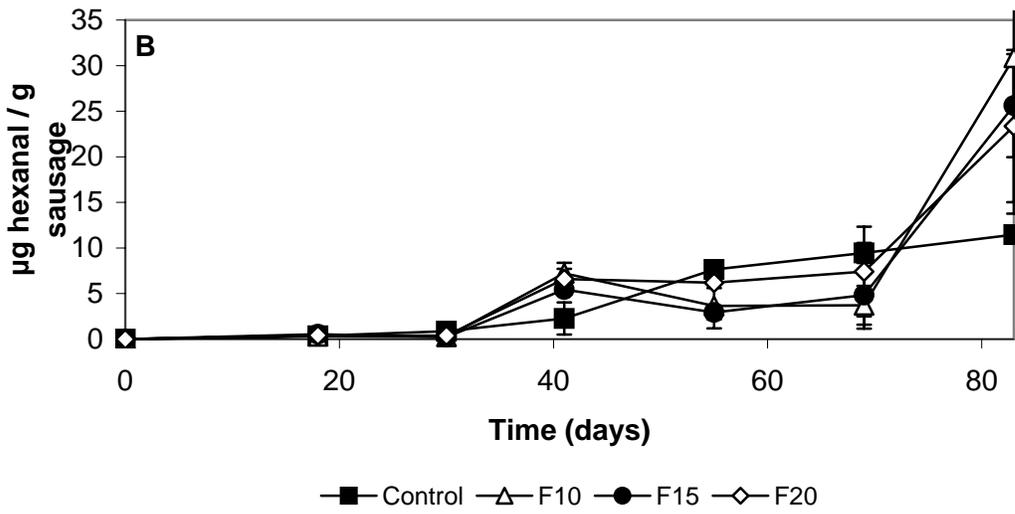
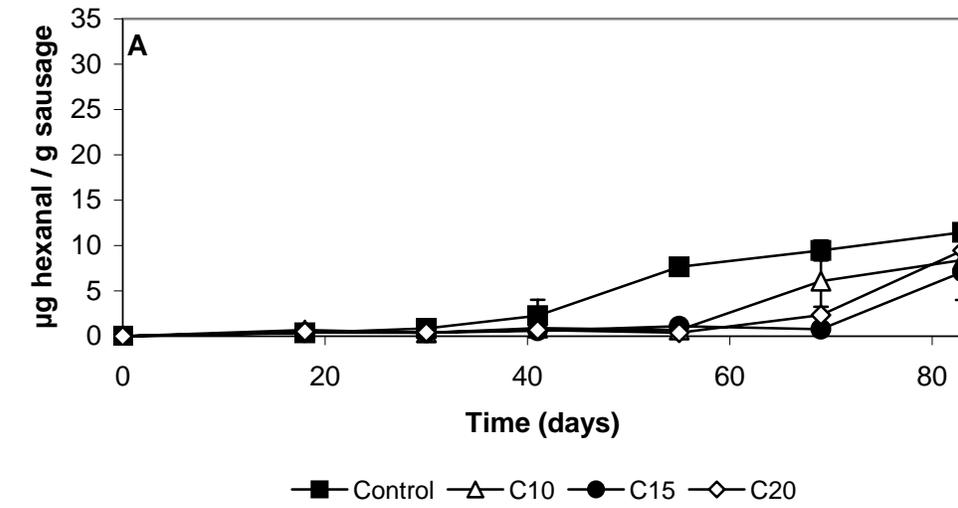


Fig. 3. Hexanal values of the different formulations during storage ($N=2$). See further subscript Fig. 1.

INHIBITION OF HEMOGLOBIN-MEDIATED LIPID OXIDATION BY THE COMBINATION OF ASCORBATE AND EDTA

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Key Words: washed cod, ascorbate, EDTA, reducing agent, metal chelator

Abstract

A model system consisting of washed muscle tissue and added hemoglobin can be used to rapidly test the potential efficacy of various antioxidants in meat products during cold storage. Ascorbate alone or EDTA alone did not inhibit Hb-mediated lipid oxidation. The combination of ascorbate and EDTA was highly inhibitory. The combination of urate and EDTA was not inhibitory. One possible mechanism by which EDTA and ascorbate act synergistically to inhibit Hb-mediated lipid oxidation is that the combination has a better ability to inhibit oxidation of hemoglobin compared to ascorbate alone. However, the combination of EDTA and ascorbate did not decrease the rate of met-hemoglobin formation compared to ascorbate alone. Thus, other mechanisms by which the combination of EDTA and ascorbate inhibit lipid oxidation should be considered. These studies indicate that the combination of EDTA and ascorbate may be a useful antioxidant system to control oxidative rancidity in meat products.

Introduction

Lipid oxidation can lead to rancidity, color defects, loss of nutritional value and toxic compound formation in muscle foods (1). Inhibiting oxidative processes is one of the major tasks for the meat industry. Improved knowledge of the oxidative mechanism in meat can lead to premium protection of the commercial products.

Hemoglobin, myoglobin and cytochrome c are the three major heme containing proteins in muscle foods. As an abundant and potent catalyst of lipid oxidation (2), hemoglobin is of our research interest. The potential mechanisms involve heme and iron displacement (3;4) and ferryl-radical formation (5). Ferryl-radical can abstract a hydrogen atom from polyunsaturated fatty acid and initiate lipid oxidation. Metals such as copper have been shown to accelerate hemoglobin oxidation (6). Hemoglobin oxidation is relevant since met heme proteins are more susceptible to heme release and formation of the ferryl radical than reduced hemoglobin (7). Among neutral lipid and phospholipids, phospholipids are thought to be the primary lipid class that is susceptible to lipid oxidation in muscle foods (8). Our study utilized washed cod muscle as the oxidation substrate. Washing removes most of the aqueous antioxidants and pro-oxidants of cod tissue. Myofibrillar proteins and membrane phospholipids remain in washed cod.

The term low molecular weight (LMW) iron represents iron bound to low molecular weight compounds (e.g. ADP). Iron is hence soluble and can have reactivity (9). Iron ions in aqueous solution exist either in the ferrous (Fe^{+2}) or the ferric (Fe^{+3}) state. When iron is tightly bound to a chelator molecule, be it a protein or a small chemical, the reactivity of the iron can be greatly dampened. The metal chelators, ethylenediamine tetraacetic acid (EDTA), tripolyphosphate and phytate, can be used to investigate the role of LMW iron in lipid oxidation processes. Physiologically, ferritin is the key iron storage protein. Ferrozine was often utilized by researchers for iron level measurement. It forms a stable complex with ferrous ion. Iron has been studied extensively as a catalyst of lipid oxidation (10) (11) and reported to be responsible for oxidation of fish sarcoplasmic reticulum (12) and can cause numerous disease processes biologically (13).

It should be kept in mind that the reactivity of iron is highly dependent upon its ligand environment. Any change in the ligand environment of iron, such as pH, buffer, chelator concentration and chelator type, will affect its reactivity (e.g. increase or decrease its ability to oxidize lipids). Harel and Kanner demonstrated that EDTA combined with ascorbate inhibited the activity of hydrogen peroxide-activated hemoproteins (14). The finding was based on lipid oxidation in microsomes.

Objectives

1. Test if the combination of urate and EDTA is as effective as ascorbate and EDTA in inhibiting Hb-mediated lipid oxidation.
2. Investigate the mechanism by which ascorbate and EDTA synergistically inhibit
3. Hb-mediated lipid oxidation.

Methodology

Washed cod muscle preparation. Cod fish (*Gadus morhua*) were obtained from a local seafood store or delivered overnight from Gloucester, MA. Cod muscle was ground (0.5 cm diameter) after removing dark muscle manually. It was then washed twice in distilled deionized water at 1:3 mince to water ratio (w:w) for 2 minutes and allowed to stand for 15 minutes before dewatering with fiberglass screen. Mince was subsequently mixed with 50 mM NaCl (pH 5.5) at the same weight ratio and homogenized thoroughly. It was finally centrifuged (15,000 g for 25 minutes at 4C) using a Beckman L8-70M ultracentrifuge (Beckman Instruments Inc.) and stored in -80C freezer until use.

Washed cod muscle/hemoglobin model system. Frozen washed cod was partially thawed and blended with pulsing (Waring blender Model 33BL79) to obtain a uniformed consistency. Streptomycin sulfate (200 ppm) was added to inhibit microbial growth during storage. Chemicals were added at this stage. pH of samples was then adjusted to ~6.3 with 1 M NaOH or 1 N HCl when necessary. Final moisture content was adjusted to 90% by adding distilled deionized water. Trout Hb (12 μM) was added at the end after all other materials were mixed thoroughly. Samples were stored at 2C for 8–10 days.

Thiobarbituric acid reactive substances (TBARS). TBARS were determined according to a modified procedure of Buege and Aust (15). Fifty percent trichloroacetic acid (TCA) containing 1.3% TBA was heated to 65C on the day of use to dissolve the

TBA. TCA-TBA mixture was added to samples at 1:10 weight:volume ratio and incubated for 1 hour at 65°C. After cooling in cold room for 1 hour and centrifugation (10,000 g for 3.5 minutes), the absorbance of the supernatant at 532 nm was measured. A standard curve was constructed using tetrathoxypropane. Lag phase was defined as the time it took for TBARS values to reach 20 µmol/kg washed cod during ice storage.

Methemoglobin formation. Hb solutions were diluted into 50 mM sodium phosphate buffer (pH 6.3). Spectra were obtained at regular time intervals between 700 and 500 nm using the UV-2401 spectrophotometer. The percentage of methemoglobin was calculated according to the equations of Benesch et al. (16). The slopes generated from linear regression plots were used to express the relative rates of methemoglobin formation.

Statistics. Each study was replicated at least once. Analysis of variance with a MIXED procedure of the SAS system was used to evaluate data from storage studies (17). Means were separated using differences of least squares.

Results & Discussion

Addition of trout hemoglobin to washed cod muscle caused formation of lipid oxidation products to occur rapidly during 2°C storage based on formation of thiobarbiturate reactive substances (TBARS) (Table 1). EDTA at a concentration of 111 µM did not inhibit nor accelerate Hb-mediated lipid oxidation (Table 1). Ascorbate at a concentration of 222 µM also did not inhibit Hb-mediated lipid oxidation (Table 1). In the presence of ascorbate (222 µM) and EDTA (111 µM), the TBARS formation was effectively inhibited for the entire 8 days storage period (Table 1). Urate (111 µM) combined with EDTA at 111 µM did not significantly inhibit Hb-mediated lipid oxidation compared to the combination of ascorbate (222 µM) and EDTA (111 µM) (Table 2). Ascorbate has a less positive reduction potential than urate which indicates that it is a stronger reductant than urate (18).

A possible mechanism by which EDTA and ascorbate in combination inhibited Hb-mediated lipid oxidation involves the metal inactivating ability of EDTA and the favorable reduction potential of ascorbate. Ascorbate also has the ability to regenerate tocopherol that is present in washed cod tissue. Around 30 mmol tocopherols per kg washed cod lipids were determined based on HPLC analysis (data not shown).

Table 1. Effect of ascorbate (222 µM), EDTA (111 µM), and ascorbate in combination with EDTA (222 µM/111 µM) on Hb-mediated lipid oxidation in washed cod muscle.

Sample ^a	Lag phase ^b prior to onset of lipid oxidation during 2°C storage (days)
WCM + Hb	0.3
WCM + Hb + ascorbate	0.4
WCM + Hb + EDTA	0.4
WCM + Hb + ascorbate + EDTA	> 8.0

^aThiobarbiturate reactive substances (TBARS) were used as the indicator of lipid oxidation. Hb level was 12 µmol/kg washed cod and the moisture content was adjusted to 90% in all samples. Final pH of the model system was 6.2 ± 0.1.

^bLag phase was defined as the extrapolated time until TBARS reached the level of 20 µmol/kg washed cod.

Table 2. Effect of urate (111 µM) and ascorbate (222 µM) in combination with EDTA (111 µM) on Hb-mediated lipid oxidation in washed cod muscle.

Sample ^a	Lag phase ^b prior to onset of lipid oxidation during 2 °C storage (days)
WCM + Hb + ascorbate	1.5
WCM + Hb + ascorbate + EDTA	>8
WCM + Hb + urate	0.7
WCM + Hb + urate + EDTA	1.1

^aThiobarbiturate reactive substances (TBARS) were used as the indicator of lipid oxidation. Hb level was 12 µmol/kg washed cod and the moisture content was adjusted to 90% in all samples. Final pH of the model system was 6.2 ± 0.1.

^bLag phase was defined as the extrapolated time until TBARS reached the level of 20 µmol/kg washed cod.

Met-Hb formation rate was considered one of the major mechanisms for hemoglobin to initiate lipid oxidation in muscle foods. Low molecular weight (LMW) metals have the capacity to accelerate met-Hb formation (6). Thus it was of our interest to analyze whether EDTA would enhance the ability of ascorbate to prevent met-Hb formation by tying up (LMW) metals that may be present. The trout met-Hb formation rate at pH 6.3 was not decreased significantly by the addition of EDTA (111 µM) in combination with ascorbate (222 µM), or EDTA in combination with urate (111 µM) (Table 3). Neither was it decreased by the addition of urate at 111 µM or EDTA at 111 µM. Only ascorbate at 222 µM decreased the hemoglobin autoxidation rate substantially (Table 3).

Table 3. Effect of EDTA, ascorbate and urate on formation of methemoglobin (pH 6.3, 50 mM sodium phosphate) during 2 °C storage.

Sample ^a	Met-Hb formation rate ^b
Hb	11.0
Hb + EDTA	9.5
Hb + ascorbate	3.8
Hb + ascorbate + EDTA	9.2
Hb + urate	13.0
Hb + urate + EDTA	10.4

^aFormation of methemoglobin from trout Hb (pH 6.3, 50 mM sodium phosphate). Samples were stored at 2 °C. Urate was added at the level of 111 µM. Ascorbate was added at the level of 222 µM. EDTA was added at 111 µM. Hb concentration was 12 µM.

^bRate is derived from the slopes obtained when plotting time vs % methemoglobin.

Conclusions

The fact that a combination of EDTA (111 μM) and ascorbate (222 μM) was required to inhibit Hb-mediated oxidation suggested that both inactivation of metals and a potent reducing system are needed to limit lipid oxidation processes. The specificity of the effect of these two components warrants further analysis.

Since the combination of EDTA and ascorbate did not decrease the met-hemoglobin formation rate, other potential mechanisms by which lipid oxidation was inhibited should be considered. Possible mechanisms include the ability of EDTA and ascorbate in combination to synergistically inhibit heme dissociation from hemoglobin.

The strong synergistic inhibition by EDTA and ascorbate was observed in washed cod containing trout hemoglobin. It is not known if the same synergistic inhibition will occur in consumable muscle foods.

FDA recognizes EDTA as a food additive that is generally recognized as safe (See the US Code of Federal Regulations-21 CFR 172.135 and 21 CFR 173.315). The combined usage of EDTA and ascorbate in muscle food product as antioxidants has potential application values.

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EVALUATION OF LIPID OXIDATION AND OXIDATIVE PRODUCTS AS AFFECTED BY MEAT CUT, PACKAGING METHOD AND STORAGE TIME DURING REFRIGERATED STORAGE

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Key Words: shelf-life, meat cut, packaging method, storage time, lipid oxidation

Introduction

To extend the shelf-life, meat and meat products are refrigerated during storage. Although meat and meat products are stored under the refrigerated temperatures to reduce the microbial growth, lipid oxidation is one of the most important chemical deteriorations that affect the quality of meat and meat products. There are several methods to determine the degree of lipid oxidation. Peroxide values (POV) and thiobarbituric acid reactive substance (TBARS) values have been used as indices to assess the degree of lipid oxidation. However, these methods were not appropriate to measure the degree of lipid oxidation in meat and meat products because TBARS values only correlated with POV when fat contained three or more double-bond fatty acids (Gray, 1978). In addition, it has been reported that TBARS values couldn't measure the degree of oxidation of lipids containing mono- or di-unsaturated fatty acids (Decker et al., 1998). Due to decreases after maximum level of POV, long-period stored meat and meat products couldn't be measured by POV. Hexanal values in cooked chicken tended to rise with increasing storage time (Beltran et al., 2003). The relationship between current methods of lipid oxidation and volatile compounds during storage time should be explained to assess the degree of lipid oxidation more accurately.

Objectives

The objectives of this study were to measure the degree of lipid oxidation as affected by pork meat cut, packing method and storage time on lipid oxidation, and to investigate the correlation between current methods of lipid oxidation and oxidative products produced by degradation of fatty acids during storage.

Methodology

Fresh pork loins and bellies were purchased from a wholesale meat markets in Gwangju, and analyzed pH, proximate composition, TBARS, FFA, and volatile oxidation products during refrigerated storage at 8°C. Chemical compositions were measured by the AOAC (1990) and pH was measured by pH meter. Lipid extraction was performed by the method of Folch et al. (1957). After extraction, extracts were evaporated and stored at

4°C until analyzed. TBARS and FFA values were conducted by the method of Witte et al. (1970) and AOCS (1987), respectively. Simultaneous distillation and extraction (SDE) was performed to extract the volatile oxidation products, with modified procedure of Heath and Reineccius (1986). Quantification and identification of volatile oxidation products were conducted by a gas chromatograph (GC) and mass spectrometer (MS). Total plate count (TPC) and violet red bile agar (VRB) have been used to determine total bacterial counts and coliform bacteria, respectively. Then, they were incubated at 37°C for 2 days and expressed as log cfu/g. The experiment was replicated triplicates, and the data were analyzed using two-way analysis of variance (ANOVA) in SPSS program, as factors for pork meat cut, packaging method and storage time. Means were separated by the Duncan's multiple range test.

Results & Discussion

Moisture, fat and protein contents of belly were 48.4, 38.5 and 10.2%, whereas those of loin were 73.5, 3.60 and 19.8%, respectively (Table 1). Belly fat content was higher ($p<0.05$) than loin, whereas moisture and protein contents of belly were lower ($p<0.05$) than those of loin. Since interactions between treatments (vacuum belly, aerobic belly, vacuum loin, aerobic loin) and storage time in all parameters were found ($p<0.05$), data were separated out by treatment and storage time (Table 2). Storage time did not affect ($p>0.05$) pH, and belly pH was higher than loin pH ($p<0.05$) due to high amount of fat in the belly cut. TBARS values increased ($p<0.05$) with increased storage time and belly had higher TBARS values ($p<0.05$) than loin in the latter period of refrigerated storage at 8°C. FFA values were also increased ($p<0.05$) as storage time increased, and loin had higher FFA values ($p<0.05$) than belly. But no differences ($p>0.05$) in pH, TBARS and FFA between vacuum and aerobic packaging were observed. After quantification of volatile compounds in belly and loin, approximately 23 compounds were identified and aldehydes (6), ketones (3), alcohols (3) and fatty acids (2) were the predominant compounds which were known to secondary by-products of lipid oxidation. Among aldehydes compounds, it was reported that hexanal was as an index to assess the degree of lipid oxidation due to increased hexanal contents with increased storage time (Beltran et al., 2003). However, hexanal values of this study were opposite trend. These results were partially due to the different extraction methods. In Table 2, hexadecanoic acid content extracted from belly were not affected ($p>0.05$) by storage time, whereas that of loin was increased ($p<0.05$) with increased storage time. Loin contained higher hexadecanoic acid content than belly in the latter period of storage. It was considered that hexadecanoic acid was to use as an index of lipid oxidation because it had high correlation coefficient with FFA ($p<0.01$) and TBARS ($p<0.01$). The correlation equations were as followed; Y (hexadecanoic acid content) = $0.12X$ (FFA) + 7.457, Y (hexadecanoic acid content) = $2.654X$ (TBARS) + 7.459 were developed.

Microbial counts are shown in Figure 1. It took 7 to 14 days to reach the total plate counts of vacuum and aerobic packed belly of 107 log cfu/g, respectively, and 14 and 21 days for vacuum and aerobic packed loins, respectively. It was indicated that belly could be spoiled faster than loin due to the high amounts of fat, and vacuum packaging extended the shelf-life, as compared to aerobic packaging. These results were in agreement with results of previous studies (Sachindra et al., 2005; Duffy et al., 2000).

Conclusions

pH and TBARS values were higher in belly than loin in the latter period of storage, whereas FFA values were higher in loin than belly. Among the volatile compounds produced by fresh pork meats during refrigerated storage, aldehydes, ketones, alcohols and fatty acids were predominant. The amount of hexadecanoic (palmitic) acid in loin was increased with increased storage time. Thus, this oxidative compound might be an indicator to determine the degree of lipid oxidation in fresh pork loins. In addition, belly was spoiled faster than loin, and vacuum packaging extended shelf-life of fresh pork meats, as compared to aerobic packaging.

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Tables and Figures

Table 1. Proximate composition(%) of belly and loin from pork

	Belly	Loin
Moisture	48.4 ± 6.02	73.5 ± 0.68
Crude fat	38.5 ± 9.89	3.60 ± 0.65
Crude protein	10.2 ± 3.05	19.8 ± 0.66
Total	97.1	96.9

Table 2. Change of pH, thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA) and hexadecanoic acid (HA) as affected by meat cut, packaging method and storage time at 8°C.

Parameters	Parts	Packaging	Storage time (days)					
			0	3	7	14	21	28
pH	Belly	Vacuum	6.09 ^A	6.11 ^A	5.97 ^{AB}	5.91 ^{AB}	5.96 ^{AB}	6.11 ^A
		Aerobic	6.05 ^{bA}	6.18 ^{abA}	6.22 ^{abA}	6.10 ^{bA}	6.24 ^{abA}	6.39 ^{aA}
	Loin	Vacuum	5.57 ^B	5.56 ^B	5.52 ^C	5.48 ^C	5.45 ^C	5.44 ^B
		Aerobic	5.63 ^B	5.63 ^B	5.76 ^{BC}	5.62 ^{BC}	5.58 ^{BC}	5.74 ^B
TBARS ^a (MDA mg/kg)	Belly	Vacuum	0.17 ^b	0.37 ^{ab}	0.49 ^{ab}	0.56 ^{ab}	0.39 ^{abAB}	0.84 ^{aAB}
		Aerobic	0.13 ^b	0.53 ^{ab}	0.84 ^{ab}	0.72 ^{ab}	0.83 ^{abA}	1.23 ^{aA}
	Loin	Vacuum	0.07	0.16	0.15	0.24	0.18 ^B	0.23 ^B
		Aerobic	0.07 ^b	0.16 ^{ab}	0.24 ^{ab}	0.31 ^a	0.26 ^{abB}	0.36 ^{ab}
FFA ^b (%)	Belly	Vacuum	1.18 ^{abB}	1.03 ^{abB}	0.93 ^{bB}	1.18 ^{abC}	1.57 ^{abB}	1.82 ^{ab}
		Aerobic	1.09 ^{cB}	1.09 ^{cB}	1.22 ^{cB}	1.57 ^{bcBC}	2.15 ^{bB}	3.17 ^{aAB}
	Loin	Vacuum	4.40 ^{bA}	3.57 ^{bA}	4.67 ^{bA}	3.87 ^{bB}	7.25 ^{aA}	7.57 ^{aAB}
		Aerobic	3.56 ^{cA}	4.33 ^{abA}	5.12 ^{abA}	6.92 ^{bA}	7.48 ^{bA}	13.0 ^{aA}
HA ^c (/g)	Belly	Vacuum	0.22	0.58	0.35	0.72 ^{AB}	0.41 ^C	0.36 ^B
		Aerobic	0.05	0.16	0.28	0.31 ^B	0.74 ^{BC}	0.48 ^B
	Loin	Vacuum	0.31 ^c	0.47 ^c	0.77 ^{bc}	1.21 ^{AB}	2.27 ^{aA}	1.71 ^{abA}
		Aerobic	0.18 ^c	0.44 ^c	0.63 ^{bc}	1.60 ^A	2.00 ^{abAB}	3.07 ^{aA}

aTBARS – thiobarbituric acid reactive substance; bFFA – free fatty acid; cHA hexadecanoic acid.

a–cMeans with a same superscript within a row are not significantly different (p>0.05).

A–CMeans with a same superscript within a column are not significantly different (p>0.05).

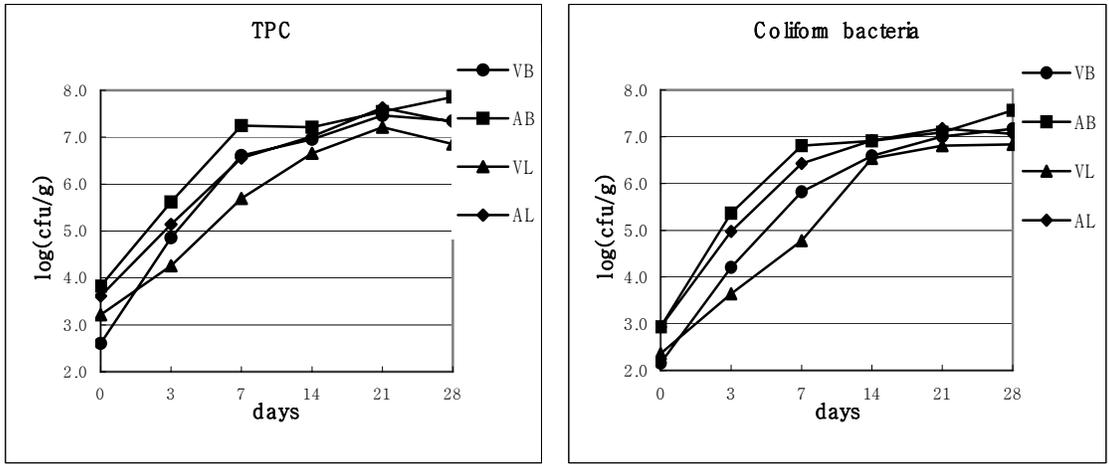


Fig. 1. Microbiological changes of pork belly and loin cuts as affected by meat cut, packaging method and storage time at 8°C.

VB – vacuum belly; AB – aerobic belly; VL – vacuum loin; AL – aerobic loin.

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF GRAPE SEED EXTRACT IN LARD

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Key Words: Grape seed extract, Lard, Malondialdehyde (MDA), Microbiology

Introduction

Oxidation of lipids in meat and meat products causes change of the functional and sensory characteristics, and decrease of shelf life. Synthetic antioxidants, such as BHT, BHA, and propyl gallate, have been utilised to extend the shelf life of product by retarding the development of rancidity. However, their use in food products is under great consideration for toxicological reasons, and thus interest in the natural ones steadily increases. The antioxidant properties of many herbs and spices are reported to be effective in retarding the process of lipid peroxidation in food (1, 2). In recent years, the use of grape seed extract has begun to become popular as a nutritional supplement that also has antioxidant activity. These extracts contain a heterogeneous mixture of monomers, oligomers, and polymers formed by subunits of flavan-3-ol (3). With regard to their pharmacological properties, these phenols have shown themselves active, against the oxidation of the low-density lipoproteins, at the same time as they appear to demonstrate anticarcinogenic, antimutagenic, and antimicrobial activity (4, 5). Inhibition of development of thiobarbituric acid reactive substances in dark poultry meat with pre- and post-mortem use of grape seed extract has been published (6).

Objectives

The aim of this study was to evaluate and compare the antioxidant and antimicrobial effectiveness of the grape seed extract and BHT added to lard.

Methodology

The lard used came from crossbreed pigs, live weight about 110 kg. After slaughtering the animal and sectioning of the meat, the lard, still attached to the skin, was sectioned off in blocks and stored at -24°C until further processed in the laboratory. Lard was allowed to taw overnight at 4°C . The skin was removed and the lard was cut into cubes before being ground in a food processor. After the addition of grape seed extract or BHT, lard was mixed with a hand-held utensil.

Samples were prepared without any addition (control), with addition of 0.01 % BHT, 0.01 and 0.7 % of grape seed extract (w/w). The samples were stored in stoppered glass jars at 4°C . Experiment lasted for 35 days. MDA quantities were determined on 0, 3, 6, 9,

12, 15, 25, 30 and 35 day; peroxide values on 0, 12, 30 and 35 day, while the microbiological analyses were done on 0, 6, 11, 20 and 33 day.

Malondyaldehyde (MDA), a major degradation product of lipid peroxides, was used as a marker for assessing the extent of lipid peroxidation. The determination of MDA was performed according to the method of Botsoglou et al. (7). Also, for the evaluation of the ability of antioxidants to inhibit lipid oxidation the peroxide value was determined, according to the A.O.C.S. Official Method Cd 8-53.

For the microbiological analysis, the sample was homogenized in a 2 % sodium citrate solution. Dilutions were prepared in a physiological solutions and the appropriate culture media were employed for the determination of *Salmonelae sp*, *Staphylococcus aureus*, *Clostridium*, *Proteus sp*, *E. coli*, molds, total count, and lipolytic microorganisms. The methods used, were according to the Regulation (8).

Experiment was conducted twice, with all measurements made in triplicate.

Results & Discussion

Maximum permitted level of BHT in lard is 0.01 % according to Regulations of Serbia and Montenegro, as well as of USDA. Grape seed extract on that level was checked for its activity, as well as in significantly higher concentration.

The results of MDA content of the lard samples are shown in Fig.1. During the first 9 days of experiment MDA content of all samples was low and unchanged. On the 15. day of experiment MDA content of control sample achieved 0.951 ppb, while MDA content of all spiked samples maintained initial and constant values within 35 days. The significance of these findings is that the grape seed extract, like BHT added to the lard was capable of maintaining MDA values below 0.1 ppb throughout the 30 – day study, whereas the MDA value of lard control sample exceeded 0.2 ppb by 6 days. Abilities of grape seed extract at both concentrations to retard lipid oxidation were not different from that of BHT. Furthermore, no difference between the activities of grape seed extract at both concentrations was noticed. MDA test is an indicator of late oxidative changes of lipids, and it pointed out only the difference between the control and spiked samples. The main products of lipid autooxidation during the initial stage of the process are hydroperoxides. Thus, the peroxide value indicates the early oxidative changes. Peroxide values are given in meqO₂/kg of lard (Fig.2). At 12. day peroxide value was 6.2 for the lard control sample, and reached 23.5 on 35. day. Peroxide value for the lard sample containing 0.01 % grape seed extract was 0.5 and 1.7, at 12 and 35. day, respectively. In the samples containing BHT and grape seed extract at 0.7 %, the value was 0. It is obvious that the BHT and the grape seed extract at 0.7 % inhibited the formation of hydroperoxides comparing to the lard control sample.

Within 35 days, there is no growth of any pathogen bacteria and molds. Total count (Fig.3) and lipolytic bacteria count (Fig.4) are given in log₁₀ CFU/g. Total count and lipolytic bacteria count rapidly increased in control sample at 11. day. Total bacteria count increased in lard sample containing BHT at 33. day of experiment. Lard samples containing grape seed extract, maintained low values of total and lipolytic bacteria count throughout the experiment. The grape seed extract has high content of total phenolics, which are active against bacteria (5, 9).

Conclusions

Results of this study show that grape seed extract added to lard, like BHT can retard lipid oxidation in lard, as well as it may be exploitable as antibacterial agents to prevent the deterioration of lard by bacteria.

Acknowledgment

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Tables and Figures

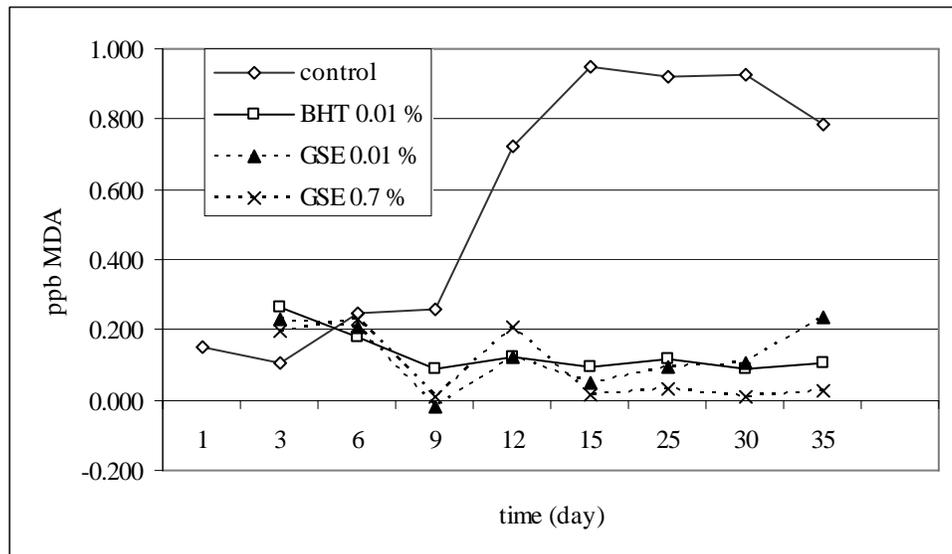


Figure 1. Evolution of the MDA value in lard samples. GSE- grape seed extract.

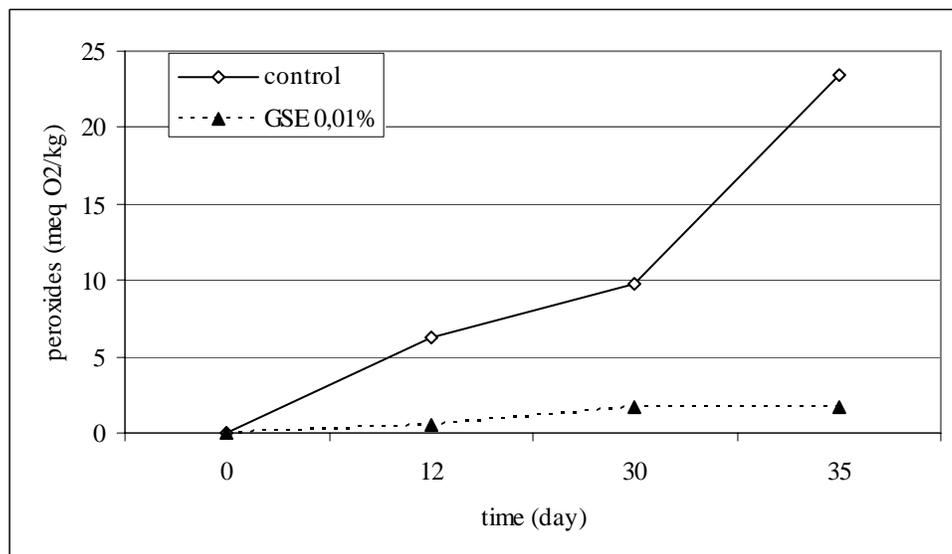


Figure 2. Evolution of the peroxide value in control sample and in lard sample containing 0.01 % of grape seed extract (GSE).

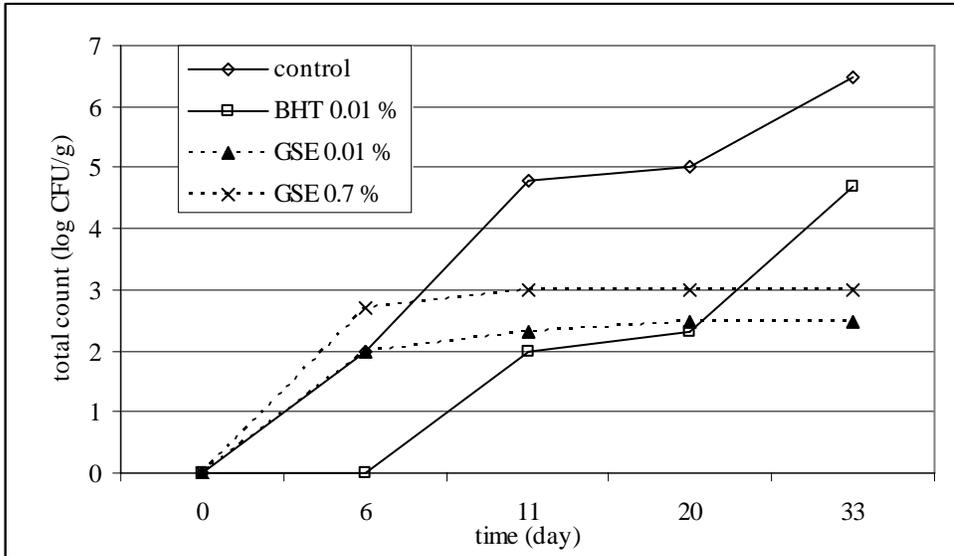


Figure 3. Total count of bacteria in the lard samples.

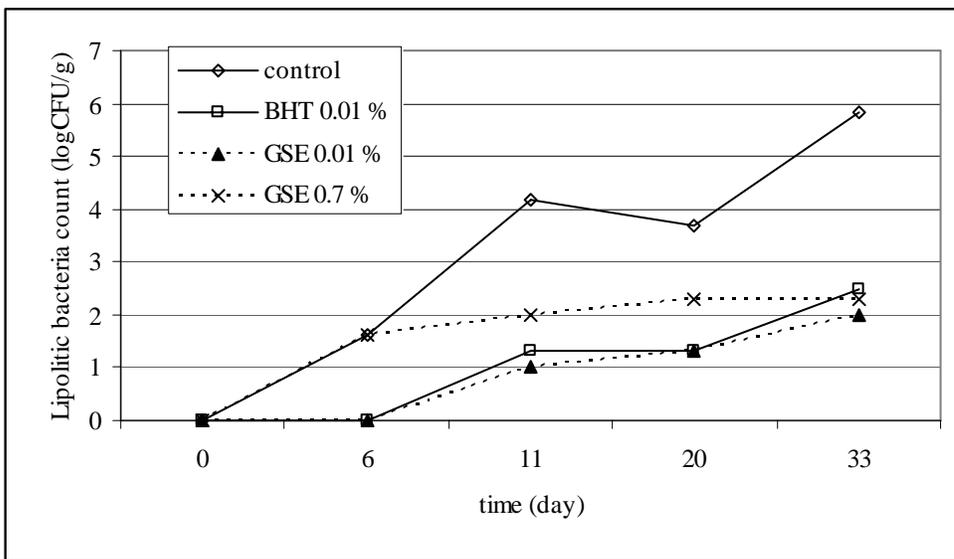


Figure 4. Lipolytic bacteria count in lard samples.

RELATIVE FRESHNESS OF TURKEY SAUSAGE WITH ROSEMARY EXTRACT

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Key Words: Turkey sausage, rosemary extract, oxidative rancidity

Introduction

Meat products typically spoil due to one of two major causes: microbial growth or chemical deterioration. The most common form of chemical deterioration is oxidative rancidity (Kanner, 1994). Oxidative rancidity in meat can vary greatly, ranging from extensive flavor changes, color losses and structural damage to proteins (Xiong, 1996) to a more subtle “loss of freshness” that discourages repeat purchases by consumers. The latter is probably the most important to food processors because it is not obvious, yet results in consumer dissatisfaction.

Several processed meat products are particularly susceptible to oxidative rancidity because of exposure to oxygen and/or elevated temperatures during processing. Meat sources with a high proportion of unsaturated fats, such as pork and poultry are particularly susceptible. Processed meats utilize antioxidants to control oxidative changes. Uncured meats typically depend on the synthetic phenolic antioxidants, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). BHA and BHT are very effective, but are a source of concern to some human health professionals and some consumers (Decker and Mei, 1996). The FDA regulations specify the GRAS limit for direct addition of total phenolic antioxidants to food to be 0.02% (200ppm), based on the fat content of the food. The Food Safety and Inspection Service of the USDA further restricts this for sausage to a total of 0.02% (based on fat content) of BHA/BHT specifically (FSIS Directive 7120.1, 2002)

Because of concerns about synthetic antioxidants, many consumers and, consequently, meat processors have been seeking natural alternatives to promote freshness. Rosemary (*Rosmarinus officinalis*) extracts have been used in meat systems (Lai et al., 1991, Offord et al., 1997, Güntensperger, et al., 1998, Yu, et al., 2002) but are generally considered to be less effective than BHA and BHT. However, rosemary extract is a natural compound and enjoys a highly positive consumer image. Consequently, rosemary extracts are of very significant interest to the meat industry and expanded applications are very likely if effectiveness in meat systems can be demonstrated.

Objectives

The overall objective of the experiments reported here was to establish the ability of a liquid rosemary extract (RE) to maintain the natural freshness of turkey sausage. Turkey

sausage is a lower-fat alternative to pork sausage; however, it is more susceptible to deterioration due to the higher proportion of unsaturated fats. The effectiveness of RE was assessed relative to the synthetic phenolics, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The results indicate that rosemary extracts offer a commercially viable alternative to the synthetic additives (BHA, BHT) currently used by the meat industry.

Methodology

Meat Processing. The shelf life of the sausage was tested under two conditions: (1) raw-frozen and (2) precooked-frozen. Five treatments were formulated as follows: (1) control; (2) 200 ppm BHA/BHT (BHA/BHT); (3) RE at 500 ppm (RE 500); (4) RE at 1000 ppm (RE 1000); (5) RE at 1500 ppm (RE 1500). Rosemary extract (Fortium™ natural rosemary extract, Kemin Food Ingredients, Des Moines, IA) inclusion was calculated on the basis of the total product weight; however, in compliance with USDA regulations, BHA/BHT (Danisco, Aubervilliers Cedex, France; Merisol, Houston, TX) inclusion was limited to 0.2 g/kg fat, with a fat content of 11.6%. The batch formulations were as described in Table 1.

Ground turkey (containing light meat, dark meat, and skin) and seasoning components were purchased from a local grocery store. The additives were mixed with spices to insure proper dispersion. Next, the ground turkey and seasonings were mixed for 2 minutes using a stand mixer (KitchenAid, St. Joseph, MI), with a paddle attachment. Each treatment was formulated and prepared as one batch. Two distinct batches of each treatment were prepared (n=2). Patties, 5.5 cm in diameter, were formed using a plastic petri dish. After preparation, patties were subdivided into groups according to their intended storage method. For cooked-frozen storage, patties were cooked to 71 °C (160 °F) internal temperature, and then set on wire racks to cool before packaging. All patties were wrapped individually in plastic film, placed into resealable plastic freezer storage bags and stored at -20 °C.

Table 1. Recipes for each of the treatments

Ingredient (g)	Treatment				
	Control	BHA/BHT	500 ppm RE ¹	1000 ppm RE	1500 ppm RE
Ground Turkey	454	454	454	454	454
Sucrose	6.9	6.9	6.9	6.9	6.9
Black Pepper	1.05	1.05	1.05	1.05	1.05
Salt	6.0	6.0	6.0	6.0	6.0
Sage	0.7	0.7	0.7	0.7	0.7
Test Additive	0	0.00526 each	0.227	0.454	0.681

¹RE: rosemary extract

Chemical Analyses. Malonaldehyde (MA) level and alkenal level were measured at each time interval using the SafTest™ System from SafTest, Inc. (Tempe, AZ).

Statistical Analysis. Statistical analysis of the data used the GLM procedures of STATGRAPHICS® Plus software (V5.1) to assess significance of treatment and time effects in two-way Analysis of Variance (ANOVA). When treatment or time effects

were significant ($P < 0.05$), the means were separated using Duncan's Multiple Range Test. In addition, one-way ANOVA was used to assess significant difference between the negative control, positive control (BHA/BHT) and RE (using the three levels as replicates ($n=6$)) for the RE treatment). Finally, multiple linear regression analysis was used to assess differences in rate of change of malonaldehyde and alkenal levels over time between the respective controls and RE via comparison of slopes. Data are presented graphically as outputs from the regression analyses.

Results & Discussion

Frozen, Raw Turkey Sausage. The results of the SafTest analyses are shown in Figures 1 and 2. Malonaldehyde concentration increased ($P < 0.001$) over the 16-week sampling period. However, the respective treatments impacted ($P < 0.001$) MA levels as well as the change in MA over time (time x treatment interaction, $P = 0.051$). SafTest suggests that MA greater than 0.4 mg/kg represent a cause for concern, whereas MA above 1.0 mg/kg indicates that substantial quality loss has occurred.

Turkey sausage patties containing RE had lower ($P < 0.05$) levels of MA when compared to the control and BHA/BHT sausage from 10 weeks onward. No dose response was observed for RE above 500 ppm. MA levels for untreated and BHA/BHT patties were above the level of concern from weeks 5–7 and 10–16. These results suggest that RE is more effective than BHA/BHT in raw-frozen turkey sausage patties. This is also confirmed by the regression analysis results displayed in Figure 1. BHA/BHT appeared to merely reduce the initial concentration of MA, but not the rate of formation of additional MA over time. A previous study in raw-frozen pork sausage patties also indicated that RE was more effective than BHA/BHT (Sebranek et al., 2005).

After 16 weeks, the control and BHA/BHT MA had increased substantially, however, turkey sausage containing RE remained virtually unchanged. The data also indicates the lack of a dose response beyond 500 ppm, thus offering meat processors flexibility in determining the appropriate inclusion rates for their required shelf life.

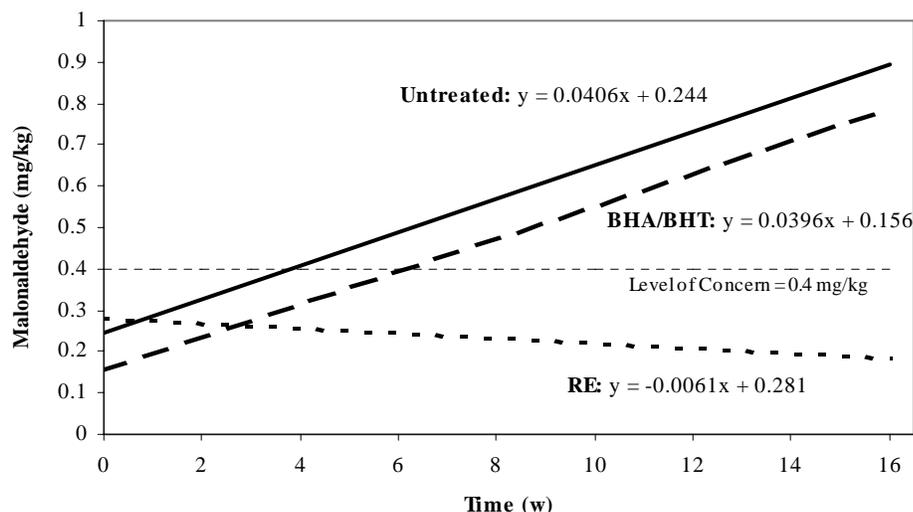


Figure 1. Regression lines representing change in malonaldehyde levels during frozen storage of raw turkey sausage as affected by synthetic (BHA/BHT) and natural (RE) additives. The RE regression line represents the average of all treatment levels (500, 1000, 1500 ppm). The slope (increase in MA over time) for both controls was steeper ($P<0.001$) than that of RE.

Alkenals increased ($P<0.0001$) in raw-frozen turkey sausage over the 16-week sampling period. Again, the respective treatments impacted ($P<0.001$) alkenal levels as well as the change in alkenals over time (time x treatment interaction, $P<0.001$). SafTest suggests that alkenals greater than 100 nmol/ml indicate that substantial quality loss has occurred. Alkenal levels for both the untreated and BHA/BHT patties were above the level of concern from weeks 12–16. From week 12 onwards, the turkey sausage with RE was lower in alkenals than sausage containing BHA/BHT ($P<0.05$). These results suggest that RE is more effective than BHA/BHT in raw-frozen turkey sausage patties, a conclusion that was also supported by multiple regression analysis (difference in slopes, $P<0.001$; see Figure 2).

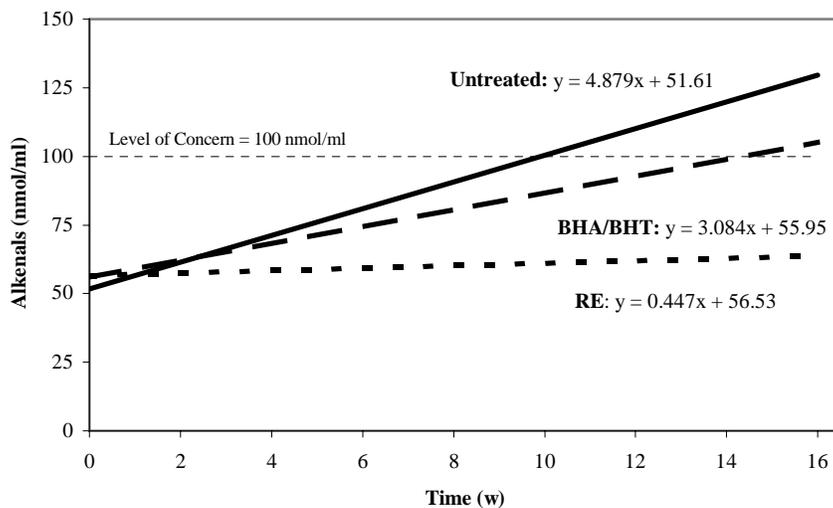


Figure 2. Regression lines representing change in alkenal levels during frozen storage of raw turkey sausage as affected by synthetic (BHA/BHT) and natural (RE) additives. The RE regression line represents the average of all treatment levels (500, 1000, 1500 ppm). The slope (increase in MA over time) for both controls was steeper ($P<0.001$) than that of RE.

Frozen, Cooked Turkey Sausage. The results of the SafTest analyses are shown in Figures 3 and 4. Similar to the raw-frozen sausage, both MA and alkenals increased ($P<0.001$) over the 16-week sampling period, and the treatments had a significant ($P<0.001$) impact on the malonaldehyde and alkenal levels. However, only a weak trend for treatment x time interaction was detected ($P=0.18$). The MA and alkenal levels for turkey sausage containing RE were significantly ($P<0.05$) lower than the untreated control and were similar to sausage containing BHA/BHT. However, by week 12, 14, and 16, turkey sausage containing RE tended to have lower MA levels than sausage with BHA/BHT ($P<0.10$). The high week-to-week variation in the MA data (not shown) made it challenging to distinguish trends among treatments; however, the untreated control was above the level of concern from 4 weeks onwards. Multiple regression analysis of time

on MA was able to more clearly differentiate RE from positive and negative controls, each one of which had steeper slopes than RE treated sausage ($P < 0.01$ and $P < 0.001$, respectively, see Figure 3). In addition, contrasting the RE MA against the untreated control revealed significantly ($P < 0.05$) lower MA on weeks 4, 5, and 8 to 16.

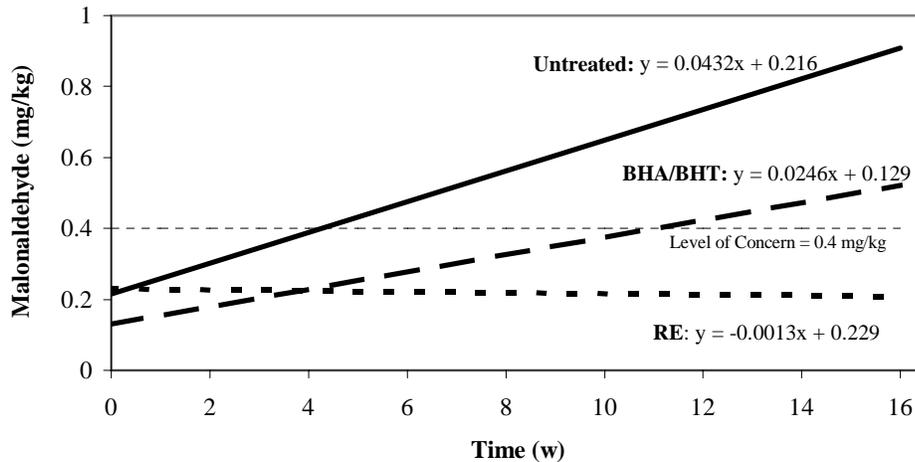


Figure 3. Regression lines representing change in malonaldehyde levels during frozen storage of cooked turkey sausage as affected by synthetic (BHA/BHT) and natural (RE) additives. The RE regression line represents the average of all treatment levels (500, 1000, 1500 ppm). The slope (increase in MA over time) for both controls was steeper ($P < 0.01$) than that of RE.

After 10 weeks of frozen storage, MA of the untreated control was well above the level of concern, but the turkey sausage with either BHA/BHT or RE had MA levels within the acceptable limit. By week 16, however, sausage containing BHA/BHT was not improved over the untreated control, yet the sausage containing RE was still within the acceptable limit. This suggests that RE is more effective than BHA/BHT in cooked sausage intended for long-term (greater than 3 months) frozen storage.

Alkenals showed little increase until week 10 when especially the negative control increased, but they remained below the level of concern until week 14. Although turkey sausage with either BHA/BHT or RE tended to maintain lower alkenal levels than the negative control ($P < 0.001$), neither analysis of variance nor regression analysis (Figure 4) detected a difference between RE and the positive control. Contrasting alkenals for the untreated control against the RE treatments confirmed that the RE treated sausage maintained lower alkenals ($P < 0.05$, weeks 8–16) than the negative control during long-term frozen storage.

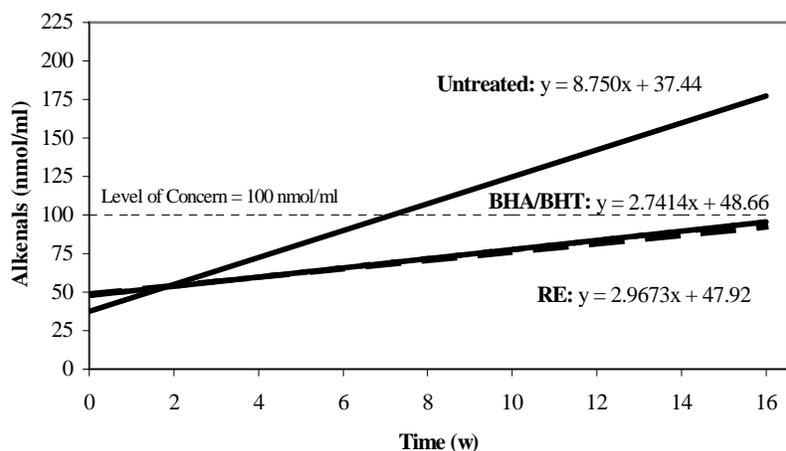


Figure 4. Regression lines representing change in alkenal levels during frozen storage of cooked turkey sausage as affected by synthetic (BHA/BHT) and natural (RE) additives. The RE regression line represents the average of all treatment levels (500, 1000, 1500 ppm). The slope (increase in MA over time) for the negative control was steeper ($P < 0.001$) than that of RE.

Conclusions

The addition of natural rosemary extract clearly provided significant protection of freshness for turkey sausage products. RE was superior to BHA/BHT in maintaining low malonaldehyde and alkenal levels in raw-frozen turkey sausage and in delaying the formation of malonaldehyde during prolonged storage of cooked-frozen turkey sausage. The results of these experiments are in agreement with the previously demonstrated capacity of rosemary extract to suppress oxidative rancidity in pork sausage (Sebranek et al., 2005).

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ANTIOXIDANT EFFECTS OF RAISIN PASTE IN COOKED GROUND BEEF AND PORK

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Key Words: Raisin, antioxidant, cooked, beef, pork

Introduction

Raisins are recognized as a good source of dietary antioxidants. Grapes and raisins contain various antioxidant compounds, including bioflavonoids (Shalashvili et al., 2002) and proanthocyanidins (Murga et al., 2000). Lipid oxidation (warmed over flavor) limits the shelf life and acceptability of cooked meat items (Jayasingh and Cornforth, 2002). Although raisins contain antioxidant compounds, their possible antioxidant effectiveness in cooked meat systems has not been previously studied.

Objectives

To study the possible antioxidant effects of raisin paste in cooked ground beef or pork.

Methodology

Sample Preparation: Ground beef (15% fat), lean ground pork shoulder (20% fat) and whole dark raisins were purchased from local supermarkets. Raisin paste was prepared by blending 60 g raisin with 20 ml distilled water for 1 min. in an Osterizer blender (Sunbeam Products, Inc. Boca Raton, FL). The raisin paste was manually mixed with ground beef (400 g) at 0.5, 1.0, 1.5 and 2.0% of meat weight respectively. The raisin paste was manually mixed with ground pork (400 g) at 1.0, 2.0, 3.0 and 4.0% of meat weight respectively. The ground meats were thoroughly cooked to well done state on a grill at a temperature setting of 163°C. A small amount of water was added during cooking to prevent sticking and charring. After cooking, the ground meats were divided into 4 equal portions and were placed in sealable (Ziploc) bags and cooled for 10 min. at room temperature. Bags were then sealed and stored at 2°C for 1, 4, 7 or 14 days.

TBA Test: Thiobarbituric acid reactive substances (TBARS) values were measured on duplicate 10 g samples by the distillation method of Tarladgis et al. (1960) at each storage period (1, 4, 7 or 14 days).

Sensory Evaluation: A trained panel (n=6) evaluated cooked samples at 1, 4, 7 and 14 days of refrigerated storage. Panelists were selected based on their sensitivity and reproducibility for detection of rancid samples (TBA Value > 1.5) in preliminary tests. The panelists evaluated cooked samples for cooked beef or pork flavor intensity, rancid flavor intensity and raisin flavor intensity on a scale of 1 to 5 where 1 = no detectable

flavor, 2 = slightly intense flavor, 3 = moderately intense flavor, 4 = very intense flavor and 5 = extremely intense flavor respectively.

Experimental Design: For beef and pork the experiment was done in 3 or 2 separate replicates, respectively (3 or 2 separate two-week test intervals, respectively). Analysis of variance (ANOVA) was done on the data sets. Treatment means were compared by the least significance difference (LSD) test. Significance was accepted at $p < 0.05$.

Results & Discussion

Cooked Ground Beef

TBA values were significantly ($p < 0.05$) reduced by addition of raisin paste to ground beef before cooking (Table 1). The mean TBA values (pooled over storage times) for the various treatments are shown in Table 2. TBA values increased significantly ($p < 0.05$) with storage time after cooking (Table 2).

The interaction of raisin content X storage time also significantly ($p < 0.05$) affected TBA values (Table 3). The control samples without raisins increased to a TBA value of 6.81 after 14 days storage at 2°C. In general TBA values > 1.4 are associated with detectable rancid odor and flavor (Jayasingh and Cornforth, 2003).

The trained panel sensory scores for rancid odor / flavor were in good agreement with the TBA values. Panelists ($n=6$) unanimously rated control samples (without added raisin paste) with scores > 3.0 , where 3.0 = moderately intense rancid flavor. The panelists did not detect raisin flavor even at the highest level (2.0% of added raisin). 2.0% raisin was sufficient to prevent detection of rancid flavor for 14 days, in cooked ground beef.

Cooked Ground Pork

Preliminary experiments established that cooked ground pork was more susceptible to lipid oxidation, with development of higher TBA values than observed in cooked ground beef samples. Thus, higher levels (up to 4%) of raisin paste were evaluated for possible inhibition of rancidity. The main effects of treatment and storage time affected TBA values of cooked ground pork (Tables 4,5). The interaction effects of treatment X storage time on cooked ground pork samples are shown in table 6.

Similar to cooked ground beef, the panelists noted an increase in rancid odor and flavor of control samples stored for 14 days at 2°C. Raisin paste at 2–4% levels significantly ($p < 0.05$) reduced the perception of rancidity.

Conclusions

The addition of raisin paste was highly effective in reducing lipid oxidation and the perception of rancidity in cooked ground beef and pork. 2.0% raisin paste was adequate for prevention of rancidity in cooked ground beef. 2–4% raisin paste was found to be adequate for significant ($p < 0.05$) reduction of TBA values and rancid flavor in cooked ground pork.

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Tables and Figures

Table 1: Mean TBA values for the treatment main effects pooled over storage time (n=24). Means with the same letter are not different ($p < 0.05$).

Treatment	TBA value
Control	4.63 a
0.5% raisin	2.47 b
1.0% raisin	1.48 c
1.5% raisin	1.21 cd
2.0% raisin	0.84 d

Table 2: Mean TBA values for the storage time main effects pooled among treatments (n=30). Means with the same letter are not different ($p < 0.05$).

Storage Days	TBA value
1	1.29 c
4	1.93 bc
7	2.36 ab
14	2.93 a

Table 3. Interaction effects of treatment X storage time on TBA values (n=6) of cooked ground beef formulated with raisin paste (0, 0.5, 1.0, 1.5, 2.0% of meat weight).

Treatment	Storage Days @ 2°C	Mean TBA Value
Control	1	2.43 ef
Control	4	4.13 c
Control	7	5.16 b
Control	14	6.81 a
0.5% Raisin	1	1.45 hi
0.5% Raisin	4	2.34 ef
0.5% Raisin	7	2.77 e
0.5% Raisin	14	3.34 d
1.0% Raisin	1	1.00 ik
1.0% Raisin	4	1.21 hij
1.0% Raisin	7	1.66 gh
1.0% Raisin	14	2.05 fg
1.5% Raisin	1	0.88 jk
1.5% Raisin	4	1.16 ijk
1.5% Raisin	7	1.32 hij
1.5% Raisin	14	1.48 hi
2.0% Raisin	1	0.70 k
2.0% Raisin	4	0.81 jk
2.0% Raisin	7	0.88 jk
2.0% Raisin	14	0.97 ijk

a – k means with the same letter are not different ($p < 0.05$).

Least significant difference among means ($LSD_{0.05}$) = 0.50.

Table 4: Mean TBA values for the treatment main effects pooled over storage time (n=16). Means with the same letter are not different ($p < 0.05$).

Treatment	TBA value
Control	12.79 a
1.0% raisin	6.48 b
2.0% raisin	3.59 c
3.0% raisin	2.63 c
4.0% raisin	2.51 c

Table 5: Mean TBA values for the storage time main effects pooled among treatments (n=20). Means with the same letter are not different ($p < 0.05$).

Storage Days	TBA value
1	3.05 b
4	5.19 ab
7	6.87 a
14	7.28 a

Table 6. Interaction effects of treatment X storage time on TBA values (n=4) of cooked ground pork formulated with raisin paste (0, 1, 2, 3, 4 % of meat weight).

Treatment	Storage Days @ 2°C	Mean TBA Value
Control	1	8.37 c
Control	4	11.84 b
Control	7	15.17 a
Control	14	15.77 a
1.0% Raisin	1	2.64 ef
1.0% Raisin	4	6.53 cd
1.0% Raisin	7	7.84 c
1.0% Raisin	14	8.90 c
2.0% Raisin	1	1.63 fg
2.0% Raisin	4	3.32 efg
2.0% Raisin	7	4.47 def
2.0% Raisin	14	4.93 de
3.0% Raisin	1	1.03 g
3.0% Raisin	4	2.08 fg
3.0% Raisin	7	3.80 defg
3.0% Raisin	14	3.60 efg
4.0% Raisin	1	1.61 fg
4.0% Raisin	4	2.16 efg
4.0% Raisin	7	3.04 efg
4.0% Raisin	14	3.22 efg

Means with the same letter are not different ($p < 0.05$).

Least significant difference among means (LSD0.05) = 2.79

MODE OF ACTION OF MILK MINERAL IN PREVENTING OXIDATIVE RANCIDITY IN COOKED MEAT SYSTEMS

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Key Words: Milk, meat, antioxidant

Introduction

Milk mineral (MM) is a by-product of the production of whey protein concentrates. It is a fine powder (particle size $<7 \mu\text{m}$), obtained by purifying and drying the ultra-filtration permeate of whey. MM has been shown to significantly reduce the rate of oxidative rancidity in cooked meat systems (Cornforth and West, 2002, Jayasingh and Cornforth, 2003). It has been suggested that the calcium phosphate fraction of MM may function as a Type II antioxidant, as does sodium tripolyphosphate (STP), by binding iron and effectively preventing it from catalyzing lipid oxidation (Cornforth and West, 2002).

Objectives

To study the mode of action by which MM functions to prevent lipid oxidation.

Methodology

Iron Binding Column Preparation: Columns were prepared using small (14.5 cm length) disposable borosilicate Pasteur-type pipettes (Scientific Products, McGraw Park, IL). Columns were plugged with glass wool, then filled with test material to a depth of 2.5 cm. The amount of test material added to each column was determined by weight difference. MM (Glanbia, Monroe, WI), STP (Fisher, Fairlawn, NJ), calcium phosphate monobasic (CM; JT Baker, Phillipsburg, NJ), and calcium pyrophosphate (CP; Aldrich, St. Louis, MO) were used as test materials. Columns were pre-wetted with 1 ml of distilled water (DI), then 0.5 ml of 1 mg/ml iron standard (ferrous chloride, JT Baker, Phillipsburg, NJ) and 0.5 ml DI were added. Eight additional 1 ml DI rinses were added, for a total wash volume of 10 ml. At least 9.75 ml of filtrate was recovered in all cases. Ten replicates ($n=10$) were performed for each test material.

Percent Packing Loss: Spent iron binding columns were dried overnight at 90°C , then cooled in a dessicator. Columns were weighed to determine the amount of packing solubilized. Results were expressed as a percent of the original packing weight for each column.

Iron Retention: Total iron content of the filtrates was determined using the Ferrozine assay (Carter, 1971). Samples were read at 562 nm using a Shimadzu UV2100U spectrophotometer (Columbia, MD) as a measure of total iron concentration. Each filtrate was assayed in duplicate. Iron retention, in mg iron / g packing compound, was

calculated based on a target value of 0.05 mg iron / ml filtrate, the expected concentration where no iron is retained by the column.

Microscopy: Lean ground beef (90%) was obtained from the USU Meat Lab. Samples were prepared by adding MM or STP at 0.75% and 1.5% levels to 50 g of meat. Samples were mixed thoroughly (kneading 25 times), wrapped in plastic film, then placed in resealable sandwich bags and held under refrigeration for three days. Samples were prepared for light microscopy by dehydrating, embedding in paraffin, sectioning, and re-hydrating. Von Kossa staining (Sheehan and Hrapchak, 1980) was performed on the re-hydrated sections to test for the presence of undissolved calcium. Slides were viewed at 40x magnification.

Ascorbate Test: To test the catalytic activity of iron and MM or STP solutions, the ascorbate test of Buettner (1988) was used. Briefly, 0.3 g MM or STP was spiked with 25 μ l of 0.1 mg/ml iron standard (ferrous chloride), then 3.25 ml distilled deionized water (DDI) was added (3.5 ml for unspiked MM and STP controls). Samples were vortexed for 3 seconds every 10 minutes for one hour, then allowed to sit overnight. To 3 ml aliquots of samples and controls, 3.5 μ l of 0.1 M ascorbic acid solution (Fisher, Fair Lawn, NJ) was added, and loss of ascorbate was monitored at 265 nm for 15 minutes. Controls (DDI, DDI + iron, and STP or MM only) were assayed once. Samples (STP or MM + iron) were prepared and assayed in triplicate and graphed as an average.

Experimental Design: Statistical analysis was performed for Iron Retention and Percent Packing Loss values. Analysis of variance (ANOVA) was used to identify significant differences at the 95% confidence level. Least significant difference (LSD) tests were used to separate means of treatments (packing compound type).

Results & Discussion

Iron Binding and Packing Loss: The type of packing compound used significantly affected the amount of iron bound by the columns ($p < 0.0001$) and the percent of the packing that was solubilized ($p < 0.0001$). MM was found to bind more iron per gram than any of the other three compounds (Table 1), and was less soluble than STP and CM (Table 2). On the average, 20% of the MM column packing was lost. MM contains approximately 80% mineral, 10% lactose, and 4 – 5% protein and moisture; it is likely the portion lost from the column was solubilized lactose and protein, though some small fraction of the finest mineral particles may have been lost as well. This is in contrast to STP, which lost 70% of its original weight. Some STP columns drained more slowly than others, allowing the packing to be in contact with DI for a longer period of time. In these cases, almost 100% of the packing was lost, suggesting that over time, STP will completely dissolve in aqueous systems (such as meat). Neither of the calcium phosphate forms (CP and CM) bound as much iron as did MM. However, once corrected for mineral content, the solubility of MM was closer to that of CP, suggesting that the form of calcium phosphate present in MM is more similar to CP than CM.

Microscopic Examination for Undissolved Calcium: The presence of insoluble calcium in ground beef + MM samples is verified by the black spots seen in Fig. 1. The absence of such spots in the ground beef + STP samples is not surprising, as no calcium was added. However, evidence supporting the solubility of STP can also be seen. Compared to the control, the 0.75% STP sample shows much less muscle fiber shrinkage

(white gaps between red muscle fibers). In the 1.5% STP sample, this difference is even more pronounced. In addition to iron, soluble phosphates will bind water. This series of slides shows the effect of increasing levels of STP; as the percentage increases, so does the water binding capacity, and less cell shrinkage is observed.

Catalytic Activity of Chelated Iron: In systems without added iron (Fig. 2), ascorbate loss was minimal, and did not vary greatly between treatments. In systems with added ferrous iron (Fig. 3), greater differences were observed. Ascorbate loss was greater with STP-iron complexes than with those of MM-iron. In sample preparation for this assay, STP was observed to be highly water soluble, while MM formed a suspension that settled over time. This observation may partially explain the results seen in Fig. 3.

Although STP is recognized as an effective iron chelator and inhibitor of lipid oxidation, it allowed measurable oxidation of ascorbate in this study. As free iron is not highly soluble in water, it must be bound to some water-soluble chelator to become catalytically active in an aqueous system (Kanner, Hazan, and Doll, 1988). Polyphosphate-type iron chelators are believed to promote the autoxidation of bound ferrous iron, stabilizing the ferric state (Aust, Morehouse, and Thomas, 1985). STP-iron mixtures may be forming soluble ferric iron-phosphate complexes with the ability to interact with and oxidize ascorbate. STP would not render the iron completely catalytically inert. Iron-MM complexes were not seen to dissolve, and in fact they settled out relatively quickly. Thus, MM could act to remove iron from solution, reducing the loss of ascorbate by limiting the catalytic activity of iron.

Conclusions

MM is an effective Type II, iron-chelating antioxidant. It was found to bind significantly more iron per gram than STP, despite containing only 80% mineral. The ability of MM to bind iron and remain insoluble may account for its antioxidant effect.

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Tables and Figures

Table 1. Iron Binding Capacity (mg iron bound / g column packing). Means sharing a letter are not different at $p > 0.05$.

	Mean \pm SD	
Milk mineral	1.71 \pm 0.24	a
Calcium pyrophosphate	1.16 \pm 0.15	b
Sodium tripolyphosphate	1.11 \pm 0.08	b
Calcium phosphate monobasic	0.44 \pm 0.45	c

Table 2. Column Packing Loss (% initial weight). Means sharing a letter are not different at $p > 0.05$.

	Mean \pm SD	
Calcium phosphate monobasic	75.2 \pm 9.4	a
Sodium tripolyphosphate	69.4 \pm 25.3	a
Milk mineral	20.7 \pm 0.6	b
Calcium pyrophosphate	0.9 \pm 0.6	c

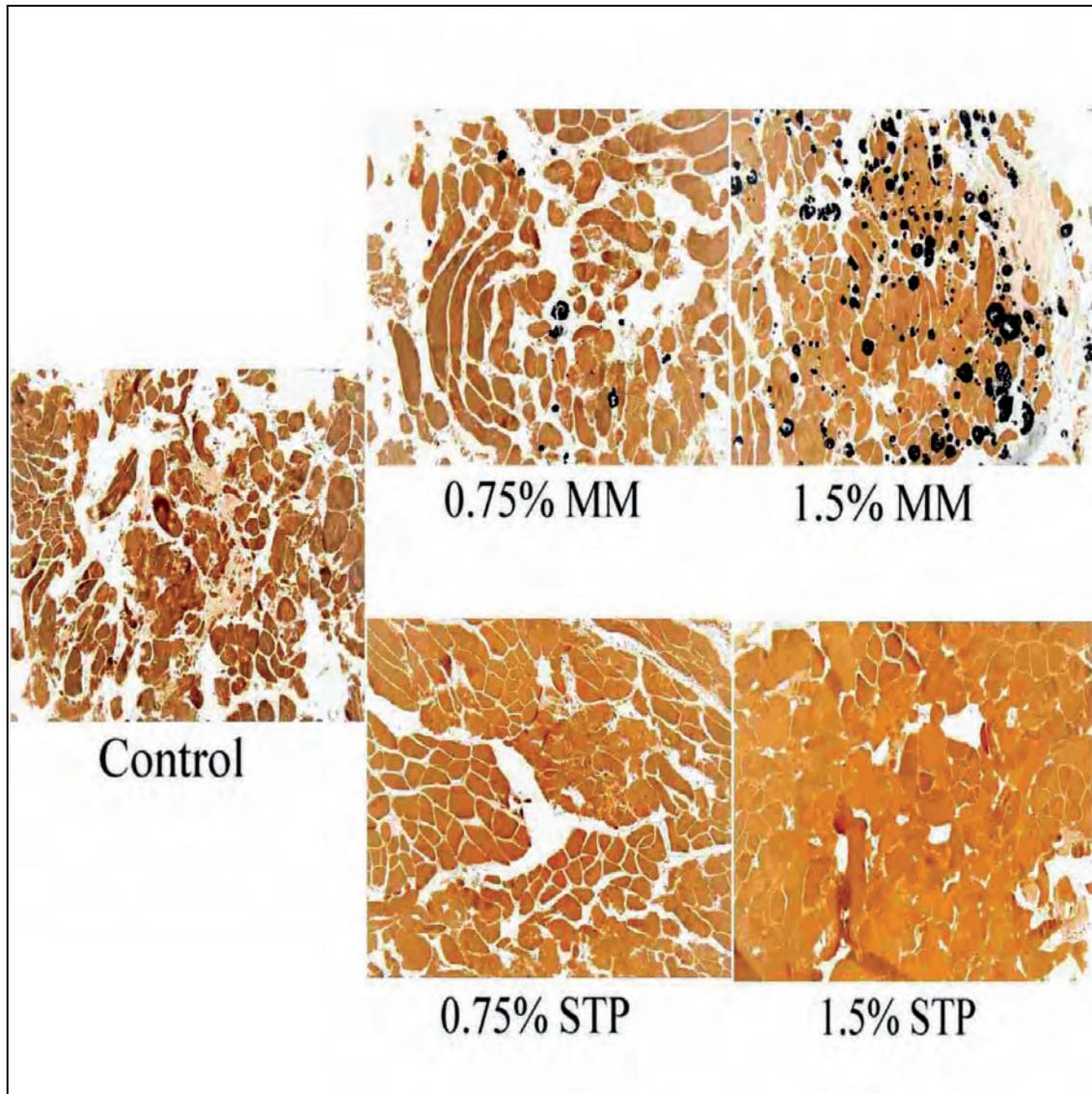


Fig. 1. Light microscopy of lean ground beef samples containing milk mineral (MM) or sodium tripolyphosphate (STP). Samples were treated with the Von Kossa stain; calcium-containing particles are black. Cell shrinkage during fixation is indicated by white gaps between muscle fibers. (40x magnification)

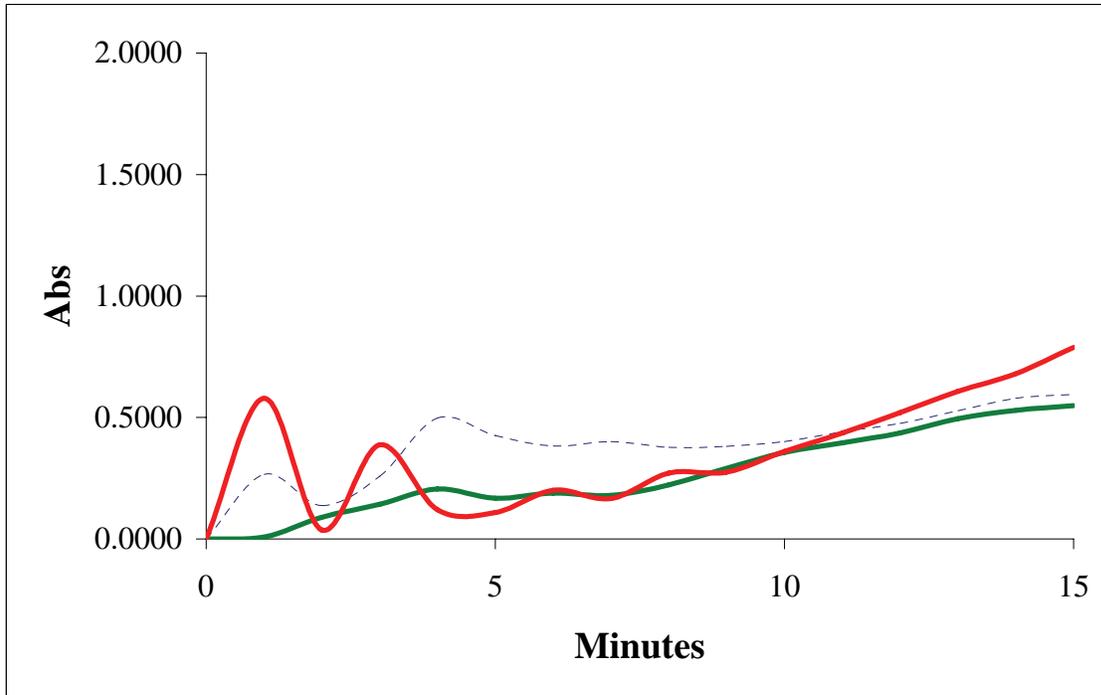


Fig. 2. Ascorbate degradation (increase @ A_{265}) in iron-free systems (- -, distilled deionized water; —, milk mineral; —, sodium tripolyphosphate).

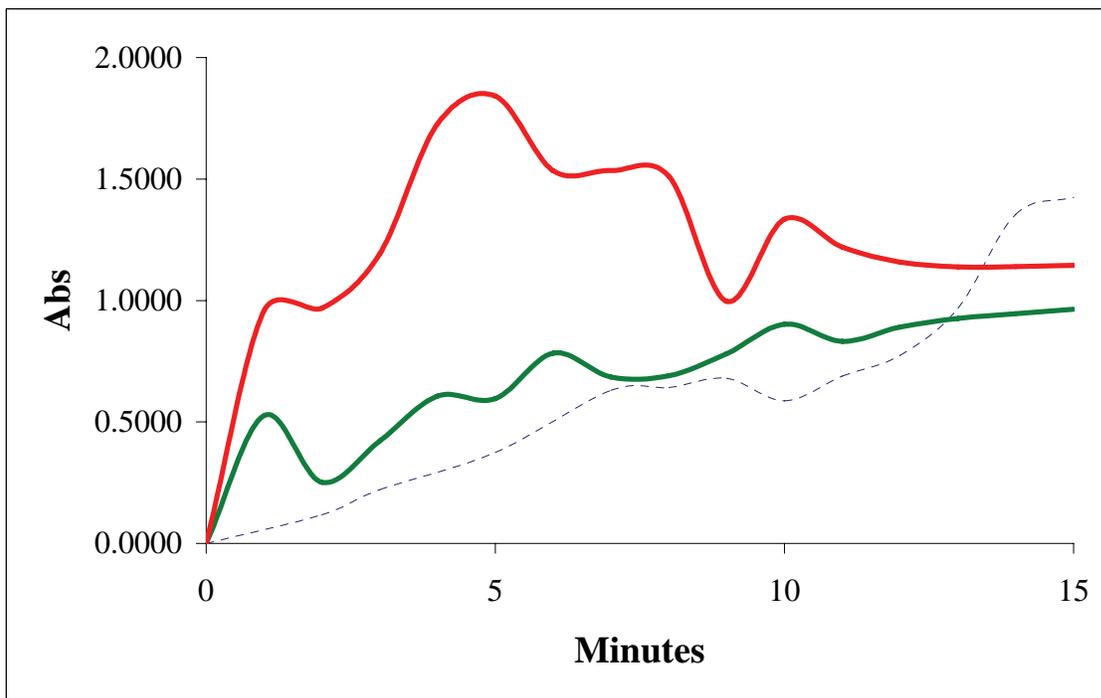


Fig. 3 Ascorbate degradation (increase @ A_{265}) in iron-added systems (- -, distilled deionized water + Fe; —, milk mineral + Fe; —, sodium tripolyphosphate + Fe).

EVALUATION OF ANTIOXIDATIVE PROPERTIES OF HOLY BASIL AND GALANGAL AND THEIR APPLICATION TO INCREASE THE OXIDATIVE STABILITY OF COOKED GROUND PORK

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Key Words: Antioxidative properties, Galangal, Holy basil, Oxidative stability, Cooked ground Pork

Introduction

Lipid oxidation is one of great concern to the meat during the storage and processing of foods, because it can lead to the development of unpleasant tastes and off-flavors as well as changes in color, rheological properties, and solubility, and potential formation of toxic compounds such as 4-hydroxy-nonenal (Addis, 1989). Ground meat tends to become brown and rancid more rapidly than whole muscle retail cuts (Ho, McMillin, & Huang, 1996). Because of its relatively high content of unsaturated fatty acid, pork oxidizes more rapidly than either beef or lamb (Pearson, Love, & Shorland, 1977). Additionally, thermal processes can promote lipid oxidation by disrupting cell membranes and releasing prooxidants, thereby inducing “warm-over flavor (WOF)”, which rapidly develops in cooked meat products during refrigerated storage and subsequent reheating (Sato & Hegarty, 1971). Therefore, it is essential to control lipid oxidation to delay the development of those WOF, because this is one of the major reasons for spoilage of meat-based products.

The most common synthetic antioxidants used in raw and precooked ground poultry, beef and pork products during storage to retard lipid oxidation are butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate (Shahidi, Rubin, & Wood, 1987; St. Angelo, 1996; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001a, 2001b). However, the demand for natural antioxidants has recently increased because of the toxicity and carcinogenicity of synthetic antioxidants (Branen, 1975; Ito, Fukushima, Hasegawa, Shibata, & Ogiso, 1983). Thus, there is an increasing interest in finding natural herbal plants that show high antioxidant activity.

Several research studies have documented the effectiveness of antioxidative components in herbal plants such as flavonoids, phenolic or polyphenolic and related compounds for the prevention of lipid oxidation in meat and meat products (Cuvelier, Richard, & Berset, 1996; Botsoglou, Christaki, Fletouris, Florou-Paneri, & Spais, 2002; Ahn, Grün, & Fernando, 2002; Fernández-López, Sevilla, Sayas-Barberá, Navarro, Marín, & Pérez-Alvarez, 2003). The antioxidant activity of phenolic compounds in herbs and spices is mainly due to their redox properties and chemical structure, which can act as reducing agent, free radical scavenger, Fe²⁺-chelator or quenchers of the formation of

singlet oxygen (Zheng & Wang, 2001; Pizzale, Bortolomeazzi, Vichi, Uberegger, & Conte, 2002).

The culinary herbs and spices such as lemon grass, ginger, galangal and holy basil are widely used in Thai cooking (Cousminer & Hartman, 1996; Uhl, 1996). Galangal (*Alpinia galanga*), a rhizome closely related to the ginger family, has been one of the most important ingredients in Thai curry paste and commonly used as flavoring (Uhl, 1996). Basil is generally used in Italian, Southeast Asian, and Mediterranean food for its clean flavor and sweet scented herbal bouquet. There are many types of basil, which vary in size, color and flavor. The most commonly used types in Thai cooking are holy basil (*Ocimum sanctum* Linn), which has spicy and lemony notes (Uhl, 1996). Several researchers have reported that galangal and basil extracts showed antioxidant activity in a model system (Barik, Kunda, & Dey, 1987; Wang, Chen, Liu, & Guo, 1997; Cheah & Abu-Hasim, 2000; Javanmardi, Stushnoff, Locke, & Vivanco, 2003). However, a few studies are available about its application in meat systems.

Objectives

1. To study the total phenolic content, antioxidant activity against a β -carotene-linoleic acid emulsion system, DPPH scavenging activity, superoxide anion scavenging activity, Fe^{2+} chelating activity and reducing power of ethanolic extracts from Holy basil and Galangal.
2. To study the effectiveness of natural antioxidant including dried Holy basil and dried Galangal powder and ethanolic extracts from Galangal and Holy basil as inhibitor of lipid oxidation in cooked ground pork.
3. To compare the effectiveness of a commercial antioxidant mixture of citric acid, ascorbic acid and α -tocopherol and natural antioxidants from Holy basil and Galangal.

Methodology

Materials

Fresh Holy basil (*Ocimum sanctum* Linn) leaves and Galangal (*Alpinia galanga*) rhizomes, imported from various locations in Thailand, were purchased from Asian supermarkets in Vienna, Austria. Samples were cleaned, washed with water, cut into small pieces, dried overnight in an air dryer (Memmert-GmbH+Co.KG, type UM 200-800, Germany) at 40°C, ground to a particle size of 25 mesh by using a grinder (Moulinex, Type MCU 1A, France), and stored at -20°C in an airtight container until use. Pork meat was obtained from a local market in Vienna, Austria.

Preparation of herbal extracts

In the preliminary study, antioxidative properties such as total phenolic content, reducing power, and antioxidant activity of Holy basil and Galangal were influenced by

extraction conditions. Optimum conditions for extraction of antioxidants from Holy basil and Galangal in this study are followings:

Dried Holy basil powder (4.50 ± 0.05 g dry basis) were extracted by stirring with 50 ml of ethanol and water (3:1, v/v) at 75°C and 300 rpm for 30 min, whereas, dried Galangal powder (4.5 g dry basis) was extracted with 50 ml of ethanol and water (1:1, v/v) at 50°C for 1 h. Each extract was then filtrated through filter paper (595 ½ folded filters, Ø125 mm, Ref. No. 10311644, Schleicher&Schuell GmbH, Germany); the filtrates were collected and dried using a rotary evaporator (Büchi rotavapor (R), Switzerland) at 40°C for 15 min, filled in a plastic bottle and stored at -20°C until use.

Total phenolic content

The total phenolic content was measured by the method described by Weurman and Swain (1955). The phenolic extracts (0.5 ml) was added to 5 ml of distilled water and vortexed for 1 min, then 1 ml of Folin and Ciocalteu's Phenolic Reagent was added and mixed well by using vortex mixture (Bender & Bobein AG, Model K-550-GE, Switzerland). After 5 min, 1 ml of saturated sodium carbonate solution was added and the mixture was vortexed again. The sample was allowed to develop color for 1 hr. The absorbance was measured at 640 nm by using a spectrophotometer (Hitachi U-1500 spectrophotometer). A standard curve was prepared at the same time with chlorogenic acid at concentration ranging from 0–100 µg/ml. The quantity of total phenolic content in the sample was calculated as chlorogenic acid equivalent by using the standard curve.

Antioxidant activity

Antioxidant activity based on coupled oxidation of β -carotene and linoleic acid emulsion system was evaluated by some modification of the method described by Taga, Miller, and Pratt (1984). The β -carotene (2 mg) was dissolved in 20 ml of chloroform. A 3 ml aliquot of the solution was put into a 50 ml beaker and 40 mg linoleic acid and 400 mg Tween 20 were added. Chloroform was removed by purging with nitrogen. Oxygenated distilled water (100 ml), which was generated by aerating air bubble into distilled water for 1 h, was added into the β -carotene emulsion and mixed well by using a vortex mixer (Bender & Bobein AG, Model K-550-GE, Switzerland). Aliquots (3 ml) of the oxygenated β -carotene emulsion and 0.12 ml of ethanolic extracts at concentration 1 mg/ml were placed in capped culture tubes and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50°C . Oxidation of β -carotene emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm in a Hitachi U-1500 spectrophotometer. Absorbance was measured at 0, 10, 20, 30 and 40 min. A control was prepared by using 0.12 ml of ethanol instead of the ethanolic extracts. Degradation rate of the extracts was calculated according to first order kinetics using equ. 1 (Al-Saikhan, Howard, & Miller, 1995).

$$\ln (a/b) \times 1/t = \text{sample degradation rate} \quad (\text{equ. 1})$$

ln = natural log

a = initial absorbance (470 nm) at time zero

b = absorbance (470 nm) at time 40 min

t = time (min)

The antioxidant activity (AA) was expressed as % inhibition relative to the control using equ. 2:

$$AA = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample} \times 100}{\text{Degradation rate of control}} \quad (\text{equ. 2})$$

DPPH scavenging activity

The effect of the ethanolic extracts on the content of 2,2-diphenyl-2-picrylhydrazyl radical (DPPH[•]) was estimated according to the modified method of Hatano, Kagawa, Yasuhara, and Okuda (1988). An aliquot (0.5 ml) of the DPPH[•] solution (50 mg/ml) was diluted in 4.5 ml of methanol, and 0.1 ml of the ethanolic extracts at various concentrations was added. The mixture was shaken vigorously and allowed to stand for 45 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (without extract) in a Hitachi U-1500 spectrophotometer. The decrease in absorbance depends on the antioxidant and radical concentration, the molecular structure of the antioxidant and its kinetic behavior (Brand-Williams, Cuvelier, & Berset, 1995).

From a calibration curve obtained with different amounts of ethanolic extracts, the ED₅₀ was calculated. The ED₅₀ was defined as the concentration of an antioxidant extracts which was required to quench 50% of the initial DPPH[•] under the experimental conditions given.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of the ethanolic extracts was based on the method described by Liu, Ooi, and Chang (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT).

In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 μM) solution, 1 ml of NADH (78 μM) solution and ethanolic extracts at concentration 1.0 mg/ml. The reaction started by adding 1 ml of phenazine methosulphate (PMS) solution (10 μM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and its absorbance at 560 nm was recorded against blank samples in a Hitachi U-1500 spectrophotometer. A lower absorbance of the reaction mixture indicated a higher superoxide anion scavenging activity. Superoxide anion scavenging activity (SASA) was calculated using equ. 3.

$$\text{SASA [\%]} = \left[1 - \frac{\text{Absorbance of sample at 560 nm}}{\text{Absorbance of control at 560 nm}} \right] \times 100 \quad (\text{equ. 3})$$

Chelating activity on Fe²⁺

The chelating activity of the ethanolic extracts on ferrous ions Fe²⁺ was measured according to the method of Decker and Welch (1990). A 1 ml of ethanolic extracts at concentrations 1.0 mg/ml was mixed with 3.7 ml of deionized water. The mixture was left for reaction with FeCl₂ (2 mM, 0.1 ml) and ferrozine (5 mM, 0.2 ml) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a Hitachi U-1500 spectrophotometer. A lower absorbance indicates a higher chelating power. Chelating activity was calculated according to equ. 4.

$$\text{Chelating activity [\%]} = \left[1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}} \right] \times 100 \quad (\text{equ. 4})$$

Reducing power

The reducing power of the ethanolic extracts was measured according to the method of Oyaizu (1986). A 0.5 ml of ethanolic extracts at concentration 1 mg/ml was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %). The mixture was incubated at 50°C for 20 min. TCA (10 %: 2.5 ml) was added. The mixture was centrifuged at 650x g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride and the absorbance was measured at 700 nm in a Hitachi U-1500 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Preparation of pork meat samples

The pork was trimmed to remove bone, skin and visible fat, cut into small pieces and then divided into six portions for each experiment prior to the addition of the test compounds. The pork of each portions was mixed with salt (2%) and the antioxidants according to the following formulation: (1) control (no antioxidant); (2) commercial antioxidant mixture of 0.3 % citric acid + 0.5 % ascorbic acid + 0.02% α-tocopherol; (3) 0.1 % ethanolic extracts of Holy basil; (4) 0.345 % dried Holy basil powder; (5) 0.1 % ethanolic extract of Galangal; (6) 0.350 % dried Galangal powder. Each portion was mixed for 2 min in a cutter (Dianawerk Model 65020), Austria. The concentration of dried Holy basil and dried Galangal powder and their ethanolic extracts were set according to the results obtained from preliminary experiments. The concentration of dried herb powder is based on the concentration of ethanolic extracts. Each portion of

ground pork was placed in polyethylene and then evenly spread to a thickness of 1 cm. All samples were packed bag under vacuum and heated on an open electric water bath until the final internal temperature of 80°C (measure with a thermocouple). After cooling down to room temperature, cooked ground pork of each portion was divided into smaller portion (about 100 g). Each sample was individually overwrapped tightly with oxygen-permeable cling film. The samples were displayed in a refrigerator (5°C) for 14 days. The samples in each experiment were evaluated at 0, 3, 7, 10, and 14 days of storage. Six replications at day 0 and 14 and duplication at day 3, 7 and 10 of storage from each treatment were sampled and then separately chopped in a microblender to obtain homogeneous samples. The samples in each treatment were analyzed TBARS value, conjugated diene, hexanal content and peroxide value.

Conjugated dienes

The formation of conjugated dienes was determined according to the procedure described by Sirinivasan, Xiong, and Decker (1996) with some modifications. Meat samples (0.5 g) were suspended in 5 ml of distilled water and homogenized to form a smooth slurry. A 0.5 ml of aliquot of this suspension was mixed with 5 ml of extracting solution (3:1 hexane:isopropanol) for 1 min. After centrifugation at 2000 g for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of conjugated diene was calculated using the molar extinction coefficient of 25,200 M⁻¹cm⁻¹ and the results was expressed as µmole per mg of meat sample.

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Witte, Krause, and Bailey (1970). In brief, ten grams of sample were homogenized in 50 ml of 10% (w/w) trichloroacetic acid. After homogenization the mixture was transferred to measuring flask and adjusted to 50 ml with distilled water. The dispersion was filtered through a folded filter paper (MN 615 ¼, Ø150 mm, Cat. No. 531015, Macherey-Nagel GmbH & Co. KG., Germany). The supernatant (5 ml) was mixed with 5 ml 2-thiobarbituric acid (2.88 g l⁻¹ H₂O) and heated in a boiling water bath for 10 min to develop the rose-pink color by reaction between malondialdehyde and 2-thiobarbituric acid (C₄H₄N₂O₂S) and cooled to room temperature. The absorbance was measured at 532 nm, against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a UV-VIS spectrophotometer (Hitachi U-1100). Thiobarbituric acid reactive substances (TBARS) were calculated from a standard curve (8-50 nmol) of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane). The TBARS value was calculated as mg of malondialdehyde per kg of sample.

Analysis of peroxide value

Peroxide value was determined according to AOAC method 965.33 (Association of official Analytical Chemists, 1995) and expressed as meq O₂/ kg meat.

Hexanal content

A Fisons GC 8000 gas chromatograph and HS-6 headspace sampler (Fisons Instruments SpA, Milan, Italy) were used. The volatiles in cooked ground pork samples were separated on a high polarity DB-5MS fused silica capillary column (15 m x 0.248 mm internal diameter, 0.25 μm film, J & W Scientific Inc., California, U.S.A.) was used. Operating conditions for GC were: helium flow 2 ml min^{-1} , initial oven temperature 40 $^{\circ}\text{C}$ for 3 min, raised to 220 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C}/\text{min}$, and held at 220 $^{\circ}\text{C}$ for 2 min. The injector and flame ionization detector (FID) temperature were adjusted to 250 $^{\circ}\text{C}$ and held at this temperature throughout the analysis. Total run time was 15 min.

For head space (HS) analysis, 3 g portion of minced cooked ground pork sample were transferred to 10 ml glass vials, capped with Teflon-lined septa, crimped and then placed in HS-6 magazine assembly to preheat at 60 $^{\circ}\text{C}$ for a 30 min equilibration. Chromatogram peak areas were expressed as integrator count units. Comparing relative retention time of GC peaks with those of commercially available standards tentatively identified the volatile compounds. Quantitative determination of hexanal was accomplished using 2-heptanone as an internal standard.

Statistical analysis

The statistical assessment was carried out with the program system of SPSS for Windows (Version 9). The results of TBARS value, peroxide value, conjugated diene and hexanal content were analyzed using one-way analysis of variance (ANOVA). Differences were considered significant at the $P < 0.05$ level. Comparison of treatment mean was based on Duncan's multiple range test (Montgomery, 1991). Furthermore, a correlation procedure (Pearson's correlation coefficient) was performed to evaluate any relationship between the TBARS value and hexanal content.

Results & Discussion

Antioxidant properties of ethanolic extracts from Holy basil and Galangal

Table 1 shows the total phenolic content, antioxidant activity against a β -carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power of ethanolic extracts from Holy basil and Galangal. So far as plant phenolic constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. From the result, ethanolic extracts of Galangal contained less total phenolic content than ethanolic extracts of Holy basil. The antioxidant activity, which reflected the ability of both extracts to inhibit the bleaching of β -carotene, was measured. The results indicated that ethanolic extracts from Holy basil exhibited greater antioxidant activity compared to ethanolic extracts from Galangal. Radical scavengers were evaluated by their reactivity toward a stable free radical, DPPH. DPPH radical scavenging activity (EC_{50}) of ethanolic extracts from Holy basil was better than that of ethanolic extracts from Galangal. Superoxide radical is

known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases (Halliwell & Gutteridge, 1999). The results showed that ethanolic extracts from Holy basil had higher superoxide radical-scavenging activity than ethanolic extracts from Galangal. Metal chelating capacity was significant since it reduce the concentration of the catalyzing transition metal in lipid oxidation (Duh, Tu, & Yen, 1999). Fe^{2+} chelating activity of ethanolic extracts from Holy basil was better than that of ethanolic extracts from Galangal. Additionally, the power of certain antioxidant is associated with their reducing power (Jayaprakasha, Singh, & Sakariah, 2001), which is associated with the presence of reductones (Duh, 1998). The results revealed that ethanolic extract from Holy basil had higher reducing power than ethanolic extracts from Galangal.

The results of the present work indicated that ethanolic extracts from Holy basil and Galangal possess high antioxidant activity, reducing power, free radical scavenging activity, Fe^{2+} chelating activity and superoxide anion scavenging activity. This was postulated to be due to the diversity and complexity of natural mixtures of antioxidant phenolic compounds in both extracts. Plant phenols and polyphenolic compounds are widely distributed in natural herb and spice extracts, and they have been found to possess significant antioxidant activity (van Acker et al., 1996; Zheng & Wang, 2001). However, it is rather difficult to characterize every compound and assess or compare their antioxidant activities. Generally, each herb contained different phenolic compounds, and each of these compounds differing amounts of antioxidant activity.

Changes of TBARS values during storage of cooked ground pork

The 2-thiobarbituric acid reactive substances values (TBARS) represent the content of secondary lipid oxidation products, mainly aldehydes (or carbonyls), which contribute to off-flavors in oxidized in meat and meat products. The effect of the antioxidants on TBARS value of cooked ground pork over 14 days of refrigerated storage is shown in Fig 1. The analysis of variance for the TBARS data indicates that the TBARS values were significantly affected ($P < 0.05$) between the control sample and those treated with the various antioxidants throughout storage. The overall lipid oxidation was drastically accelerated as storage progressed due to the denatured structure of the muscles by cooking and aerobic storage conditions. The TBARS value of commercial antioxidant mixture dried Galangal and dried Holy basil powder and ethanolic extracts from Galangal and Holy basil were considerably lower ($P < 0.05$) than the control, thus indicating high protection of meat against lipid oxidation. The ability of dried Galangal and dried Holy basil powder and their ethanolic extracts to inhibit lipid oxidation probably maybe related to the ability of their polyphenol content and structure. Hettiarachchy, Glenn, Gnanasambandam, and Johnson (1996) and Akamittath, Brekke, and Schanus (1990) found that the effectiveness of synthetic and natural antioxidants in controlling lipid oxidation in meat products. Cheah and Abu Hasim (2000) reported that 10 % Galangal extracts were as affective as 0.10 % α -tocopherol and 0.02 % BHT inhibiting/minimising lipid oxidation in raw beef during storage at 4 °C.

Initial (day 0) TBARS vaules for all antioxidant-containing samples were significantly ($P < 0.05$) lower than those for the control. This result suggests that these antioxidants retarded lipid oxidation during and immediately after cooking. The results agree with that reported by Ahn, Grün, and Fernando (2002); Fernández-López, Sevilla,

Sayas-Barberá, Navarro, Marín, and Pérez-Alvarez (2003). Sato and Hegarty (1971) reported that non-heme iron was the active catalyst in cooked meats. Chen, Pearson, Gray, Fooladi, and Ku (1984) exhibited that iron was released from heme pigments during cooking and proposed that the resultant increase in non-heme iron was responsible for lipid oxidation. Moreover, phospholipids are the primary substrates of lipid oxidation and are membrane components in close contact with the catalysts of lipid oxidation, which are located in the aqueous phase of the muscle cell (Gandemer, 1998). Gray and Pearson (1987) demonstrated that the membrane phospholipids, which are high in polyunsaturated fatty acid are responsible for the initial development of oxidation in cooked meat products during storage.

The results of this study indicated that the dried herb powder were much more effective on inhibition of lipid oxidation than ethanolic extracts from herbs. This is postulated to be due to decomposition of some active compounds, which had efficiency in oxidation prevention during the ethanolic extraction. It also could be because all antioxidative compounds in herbs could not be extracted by extracting solvent. This disagrees with the results of Abd El-Alim, Lugasi, Hóvári, and Dworschák (1999) who found that the ethanolic extracts of sage, basil, thyme and ginger at concentration 200 mg/ml were much more effective on inhibition of lipid oxidation than the dried spices at concentration 10 g/kg.

In addition, it was observed that the addition of Galangal was more effective than addition of Holy basil in retarding lipid oxidation throughout the storage period. Using the dried herbs, it was observed that Galangal was more effective than Holy basil in inhibiting the lipid oxidation. Thus, TBARS values did not correlate with total phenolic content, antioxidant activity against a β -carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power of both herbs. It could be due to the pro-oxidant effect of Holy basil, by the presence of chlorophyll. Endo, Usuki, and Kaneda (1985) have also reported that chlorophylls and their derivatives promote oxidation of lipids during storage. He and Shahidi (1997) have shown that antioxidant effect of green tea extracts in white muscles of mackerel might be markedly influenced by the presence of chlorophyll and other impurities.

It was found that TBARS value in cooked ground pork treated with dried Galangal powder was less than 1.63 mg MDA kg^{-1} sample on day 7. The threshold of TBARS values of oxidized flavor in cooked beef was between 0.5-1.0 and perceived by trained panelists (Tarladgis, Watts, & Younathan, 1960) and between 0.6-2.0 by inexperienced panelists (Greene & Cummuze, 1982). With this threshold as indicator dried Galangal powder added at a concentration of 0.35 % (w/w) almost controlled the development of lipid oxidation in cooked ground pork.

The efficiency of the sample treated with the various antioxidants in inhibiting lipid oxidation throughout refrigerated storage is in the following order: commercial antioxidant mixture (0.3 % citric acid + 0.5 % ascorbic acid + 0.02% α -tocopherol) > dried Galangal powder > dried Holy basil powder > Galangal extracts > Holy basil extracts > control. At the end of storage time (14 days) treatments added with dried Galangal and dried Holy basil powder and ethanolic extracts from Holy basil and Galangal resulted in significantly lower ($P < 0.05$) TBARS values compared to the control, which indicates that addition of Holy basil and Galangal exhibited antioxidant

properties. Beyond that, extension of shelf life without further oxidative changes would be possible with addition of dried Galangal and dried Holy basil powder and ethanolic extracts from Holy basil and Galangal.

Changes of peroxide value during storage of cooked ground pork

Peroxide values were used as indices to assess the level of lipid oxidation in cooked ground pork during storage at 5 °C (Fig. 2). The peroxide value of the control sample and those treated with the various antioxidants throughout storage increased during 14 days refrigerated storage. The increase of POV in cooked ground pork during storage may result from catalysis of intracellular compounds, the destruction of the cell structure by NaCl and processing.

On the other hand, the peroxidation of lipid also may be facilitated by oxygen during storage. Nevertheless, all treated samples had significantly ($P < 0.05$) effected with lower peroxide value compared to the control. The addition of both dried powders and ethanolic extracts from Holy basil and Galangal in cooked ground pork markedly inhibited the lipid peroxidation by decreasing POV, probably due to the phenolic constituents in Galangal and Holy basil function as antioxidants by terminating free radical chain-type reaction.

At the end of the storage (14 days), the effectiveness of the cook ground pork treated with the various antioxidants was in the following decreasing order: commercial antioxidant mixture > dried Galangal powder > dried Holy basil powder > Galangal extracts > Holy basil extracts > control. However, no significant differences ($P > 0.05$) were found between cooked ground pork treated with dried Galangal and dried Holy basil powder and also between cooked ground pork treated with ethanolic extracts from Galangal and Holy basil extracts on day 14. However, samples treated with Holy basil had higher POV than those treated with Galangal.

Changes of conjugated diene during storage of cooked ground pork

The development in conjugated diene was assessed on the basis of the hydroperoxides formed in extracted lipid of the cooked ground pork. All treated sample was able to decrease the formation of conjugated diene in cooked ground pork (Fig. 3). The concentration of conjugated diene increased significantly ($P < 0.05$) for all treatment with exception of the sample treated with ethanolic extracts from Holy basil during the first 3 days of storage and then decreased with a further storage time increased beyond 14 days.

This result was in agreement with Peña-Ramos and Xiong (2003) who report that the concentration of conjugated diene significantly increased on the first day, followed by decrease thereafter for cooked pork patties treated with whey and soy protein hydrolysates. Frankel (1998) noted that the formation of conjugated diene, which parallels the production of hydroperoxides, occurs in the early stages of lipid oxidation. Conjugated hydroperoxides are expected to decompose to the secondary products, and from the results, it is noteworthy that the decrease in conjugated dienes was accompanied by an increase in TBARS in cooked ground pork. Additionally, cooked ground pork treated with dried Galangal and dried Holy basil powder had lower concentration of conjugated diene compared to cooked ground pork containing ethanolic extracts from Galangal and Holy basil. No significant difference ($P > 0.05$) in conjugated diene was found between all treatments and control on day 3 and 7.

At the end of the storage period (14 days), cooked ground pork treated with synthetic antioxidants mixture was the most susceptible treatment to inhibit lipid oxidation, evidenced by the lowest conjugated diene, followed by cooked ground pork treated with dried Galangal powder, dried Holy basil powder, Galangal extracts and Holy basil extracts. However, no significant ($P > 0.05$) differences in conjugated diene were detected between cooked ground pork treated with commercial antioxidants mixture, dried Galangal and dried Holy basil powder on day 14.

Changes of hexanal content during storage of cooked ground pork

Hexanal has been used to follow the course of lipid oxidation and off-flavor development in cooked food (Dupuy, Bailey, St Angelo, Legendre, & Verceletti, 1987). The analysis of variance for the hexanal content data indicates that hexanal content for all treatment was significantly ($P < 0.05$) lower after cooking on day 0 than that of the control (Fig. 4). While significant increases in hexanal content were observed for all samples treatments throughout the storage period, the hexanal content for all antioxidant-containing treatments was consistently lower than that for the control.

At the end of the storage (14 days), the effectiveness of the cooked ground pork treated with various antioxidants was in the following decreasing order: commercial antioxidant mixture > dried Galangal powder > dried Holy basil powder > Galangal extracts > Holy basil extracts > control. Increasing in hexanal content in all treatments during storage may indicate persistent formation of aldehyde in the meat. Oxidation of linoleic acid and further oxidation of preformed volatiles have been considered to be responsible for the abundant occurrence of hexanal in food systems (Barbut, Josephson, & Maurer, 1985). Matthews (1971) noted that large amounts of hexanal were formed from the further oxidation of 2,4, -decadienal.

On the other hand, hexanal has been previously tested as qualitatively comparable to the TBARS test (Brunton, Cronin, Monahan, & Durcan, 2000; Brunton, Cronin, & Monahan, 2001; Beltran, Pla, Yuste, & Mor-Mur, 2003), and its determination is faster and easier than conventional methods for evaluating lipid oxidation. In the present study, changes in hexanal content were similar to changes in TBARS value. TBARS values and hexanal contents correlated well ($r^2 = 0.87$; $P < 0.05$) during storage period. This results was in agreement with findings by Ahn, Grün, and Fernando (2002) who report that hexanal contents in cooked ground beef treated with ActiVin™, BHA/BHT, rosemary and α -tocopherol increased in the same manner as the TBARS values.

Conclusions

The findings of this study demonstrated that ethanolic extracts of Holy basil showed better total phenolic content, antioxidant activity against a β -carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power than ethanolic extracts of Galangal. Additionally, the addition of commercial antioxidant mixture, dried Holy basil and Galangal powder or ethanolic extracts of Holy basil and Galangal were all inhibitory of lipid oxidation in cooked ground pork by reducing TBARS value, conjugated diene, POV and hexanal content compared to control during refrigerated at 5 °C for 14 days. Commercial

antioxidant mixture of 0.3 % citric acid + 0.5 % ascorbic acid + 0.02% α -tocopherol was identified as being the most effective antioxidant in retarding lipid oxidation in cooked ground pork. Dried powder of Galangal and Holy basil was more potent than ethanolic extracts of Galangal and Holy basil in suppressing lipid oxidation. Furthermore, addition of Galangal in cooked ground pork was more effective with elimination of lipid oxidation compared to addition of Holy basil. TBARS value and hexanal contents were well correlated.

Galangal and holy basil are culinary herbs and spices without any known toxic effect and has increasing use in preparation of ethnic food. This study brings attention to the antioxidant potential of dried powder and ethanolic extracts of Galangal and Holy basil as natural antioxidants for improving oxidative stability in meat products during storage. However, dechlorophyllization of ethanolic extracts from Holy basil may be necessary to avail them for application to meat products in which the original color of the ethanolic extracts might be of concern or when chlorophyll might act as pro-oxidant. Moreover, application of herbs antioxidant might be limited if the sensory quality of the meat products were affected, which needs further investigation.

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Tables and Figures

Table 1. Total phenolic content, antioxidant activity against a β -carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power of ethanolic extracts from Holy basil and Galangal

	Holy Basil	Galangal
Total phenolic content [mg (100g)^{-1}]	4712.45 \pm 10.87	2288.39 \pm 8.76
Antioxidant activity ^b (%)	97.35 \pm 0.65	96.94 \pm 0.31
DPPH scavenging activity (ED_{50}) ^c (mg/ml)	0.34 \pm 0.02	0.41 \pm 0.01
Fe^{2+} chelating activity ^b (%)	51.75 \pm 0.19	24.17 \pm 0.35
Superoxide anion scavenging activity ^b (%)	87.36 \pm 0.23	65.84 \pm 0.37
Reducing power ^b (absorbance 700 nm)	2.344 \pm 0.009	0.564 \pm 0.007

^aValues are means \pm standard deviation of three replicate analyses.

^bThe data concerning the antioxidant activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power were obtained with solution containing 1 mg of extracts/ml of extracting solvent.

^c ED_{50} is the concentration of Holy basil and Galangal extracts to quench 50% DPPH[•] under the chosen experimental conditions.

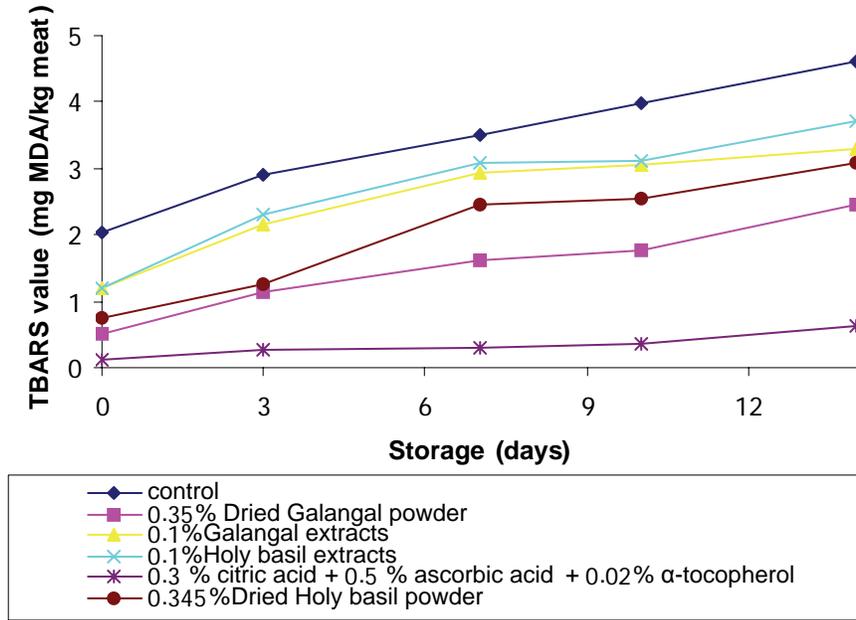


Fig. 1. Changes in TBARS value of cooked ground pork treated with different antioxidants during storage at 5 °C

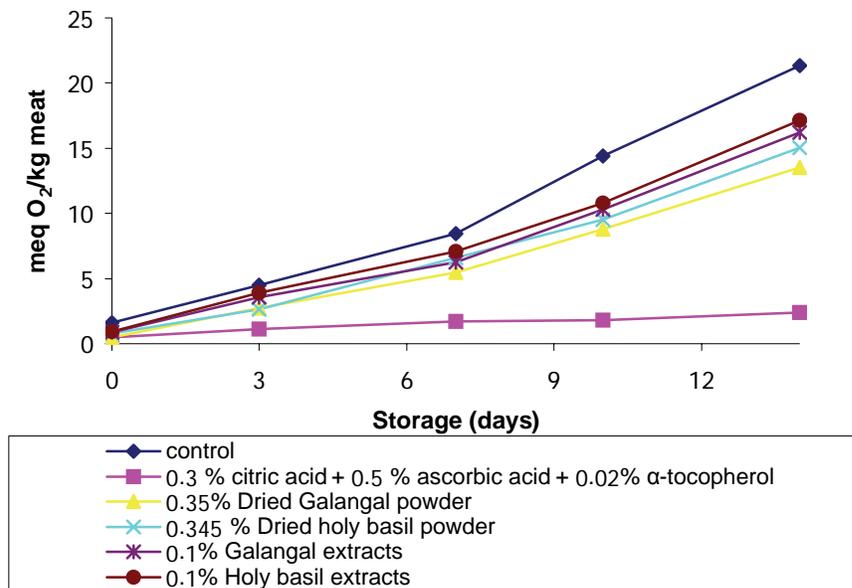


Fig. 2. Changes in peroxide value of cooked ground pork treated with different antioxidants during storage at 5 °C

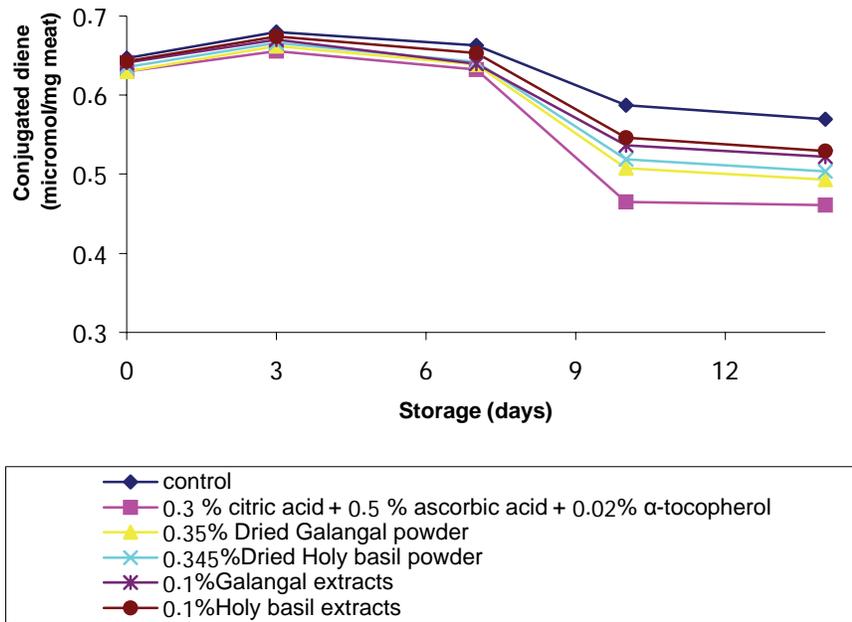


Fig. 3. Changes in conjugated diene of cooked ground pork treated with different antioxidants during storage at 5 °C

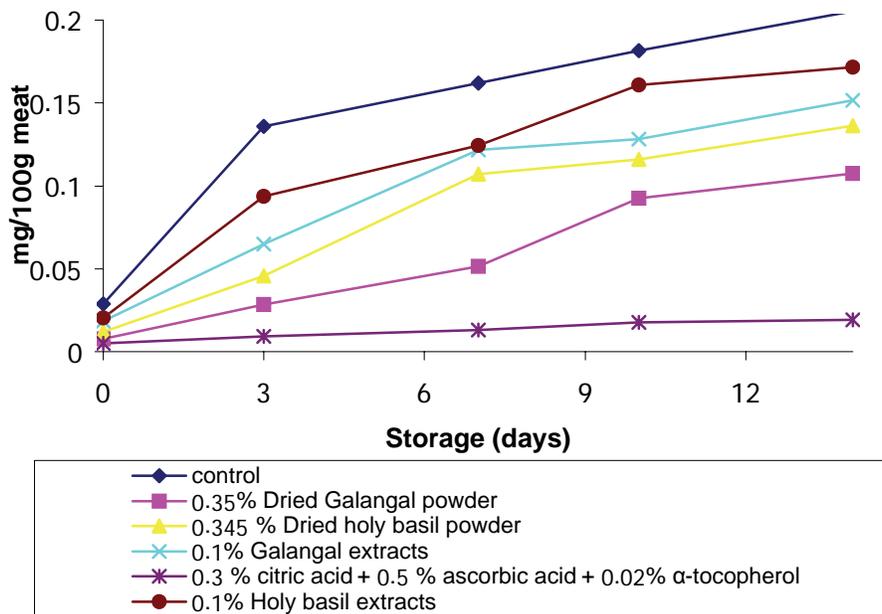


Fig. 4. Changes in hexanal content of cooked ground pork treated with different antioxidants during storage at 5 °C

**COMPARISON AND SEASONAL VARIATION OF LIPID-SOLUBLE
ANTIOXIDANT VITAMINS IN TWO PORTUGUESE BOVINE MEATS:
CARNALENTEJANA-PDO BEEF AND BARROSA-PDO VEAL**

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Key Words: meat; antioxidants; tocopherols; beta-carotene; autochthonous cattle.

Introduction

Barrosa, from the Northwest Atlantic territory of Portugal, and Alentejana, from the South Mediterranean territory of Portugal, are two of the most important Portuguese autochthonous cattle breeds (DGDR, 2002). The meat of these two breeds is commercialized under the Protected Origin Designation (PDO) certification and producers are obliged to fulfill the traditional rearing methods. The nutrition of Barrosa calves is essentially based on dam's milk until weaning at the age of 6 months and natural forage, receiving some supplementation based on agricultural resources such as potato, corn and rye (Commission Regulation n°1263/96 of 1/7, EC). Carnalentejana bullocks, which meat is commercialized as Carnalentejana-PDO, are raised on extensive pasturage, feeding on natural forage and corn, in a low stocking rate (less than 1.4 heads/hectare), receiving a 3 to 6 month finishing period based on pellet feeding (Commission Regulation n°1107/96 of 12/06, EC).

These beef production systems based on pasture feeding and traditional practices respect the physiological and behavioral needs of cattle and provide them good welfare, without the risk of metabolic and feed-related disorders commonly associated with the intensive feeding systems (Nielsen and Thamsborg, 2005).

Meat sensory attributes from PDO production systems do not represent, by itself, a marketing advantageous towards the meat produced in intensive production systems, inclusively the darker meat color, associated with grazing and exercise (Nielsen and Thamsborg, 2005), and the yellow fat due to high levels of carotene in the feedstuff that is not totally degraded to vitamin A (Nielsen and Thamsborg, 2005), represents disadvantageous characteristics in consumers perception of quality. Nevertheless, meat obtained from pasture based systems offer nutritional advantages to the consumers, because of their higher contents in polyunsaturated fatty acids (PUFAs; Yang *et al.*, 2002a) and conjugated linoleic acid (CLA; Enser, 2000), but these health promoting compounds may also act as pro-oxidant components promoting lipid oxidation, that is apart from microbiological spoilage, the primary cause of quality loss in meat products (Monahan, 2000). On the other hand, alpha- and gamma-tocopherol, the major vitamin E isomers present in meat, together with beta-carotene, a pro-vitamin A compound, are the

major lipid-soluble antioxidants present in meat (Kerry *et al.*, 2000), playing an important role in controlling lipid oxidation. However, their contents in grazing ruminants are expected to reflect the variability of pasture biomass and respective quality, which is highly dependent on cultural practices, season and geographical factors (Moloney *et al.*, 2001).

Objectives

The aim of this study was to compare and assess seasonal variations of major lipid-soluble antioxidant vitamins (alpha-tocopherol, gamma-tocopherol and beta-carotene) in Carnalentejana-PDO beef and Barrosa-PDO veal and assess their seasonal variations.

Methodology

Carnalentejana bullocks (n=31; 366±37 kg of carcass weight and 19±2 months of age), and Barrosa calves (n= 27; 223±37 kg of carcass weight and 8±0.9 months of age) were raised according the PDO specifications. Meat samples from the ribeye (LT; T1-T3 of *longissimus thoracis* muscle), the loin (LL; L4-L6 of *longissimus lumborum*) and from the distal portion of *semitendinosus* muscle (ST) were collected 2–3 days after slaughter (+1°C). All meat samples were ground using a food processor (3 × 5 s), vacuum packed and stored at –80° C until analysis.

Vitamin E homologues and β-carotene were simultaneously quantified by normal-phase HPLC, using fluorescence (tocopherols) and UV-visible photodiode array beta-carotene) detections in tandem, as described by Prates *et al.* (2005; Food Chemistry, *in press*).

Statistical treatment of data was conducted by ANOVA at a significance level of 5% (H_0 : $p < 0.05$), using the one-way ANOVA procedure of Statistix for Windows. When the F-test was significant, the comparison of means was assessed by the LSD method also at a significance level of 5%.

Results & Discussion

Barrosa-PDO veal showed no seasonal variation ($p > 0.05$) in the major lipid soluble antioxidants analyzed (alpha-tocopherol, gamma-tocopherol and beta-carotene). This stability in the major antioxidants during the year may outcome from the good climatic and geographical conditions that provide abundance of water and permanent pasture in the high-lands of Minho, where the breed is raised. Carnalentejana-PDO beef also showed no seasonal variation of alpha-tocopherol contents, what may result from the finishing period, when the animals receive pellet feeding with alpha-tocopherol supplementation. Evidence for seasonal variation in Carnalentejana bullocks' meat occurs in gamma-tocopherol and beta-carotene contents, which are not supplemented during the finishing period (Table 1). Carnalentejana-PDO beef exhibited higher gamma-tocopherol and lower beta-carotene contents during spring, while autumn beef had higher contents of beta-carotene and lower contents of gamma-tocopherol ($p > 0.05$). This seasonal fluctuation on Carnalentejana-PDO beef antioxidants must be related with seasonal

changes in the breed's nutritional management that results from the loss of pasture biomass, due to high summer temperatures and lack of water.

The contents of alpha-tocopherol Barrosa-PDO veal are significantly higher than those in Carnalentejana beef for LT and ST muscles, but not in LL. These contents in Barrosa-PDO meat are in conformity with the alpha-tocopherol contents quantified in meat from pasture raised cattle (Yang et al., 2002a; Realini et al., 2004; Descalzo et al., 2005). The contents of Carnalentejana beef alpha-tocopherol are, on another hand, in between the grain fed and the pasture raised animals (Yang et al., 2002a; Descalzo et al., 2005), which is in agreement with the feeding management, based on pasture raised with a final period of 3–6 month finishing.

The average beta-carotene contents in Carnalentejana beef was 0.17 $\mu\text{g/g}$ during the autumn season and 0.07 $\mu\text{g/g}$ of meat in the spring season, while the Barrosa veal had 0.09 $\mu\text{g/g}$ during the spring season and 0.08 $\mu\text{g/g}$ in the autumn season. All these meat beta-carotene contents were higher than those associated with grain fed cattle (Yang et al., 2002a; Descalzo et al., 2005). Nevertheless, all beta-carotene meat contents, exception for Carnalentejana autumn bullocks, are in the lower limit of the range described for beta-carotene in meat from cattle grazed on good green pasture (0.9–0.22 $\mu\text{g/g}$ of meat (Yang et al., 2002b).

The gamma-tocopherol, despite being the most common vitamin E homologue in plant foods (Decker et al., 2000), is present in meat in small amounts due to the lower preference of TTP (alpha-tocopherol Transfer Protein) for this isomer. The gamma-tocopherol contents in meat are low and no information in the literature was available for comparison. However, the values reported for reindeer's meat (Sampels et al., 2004) raised on pasture with a 2 month finishing period (0.09 $\mu\text{g/g}$ meat) are similar to the values obtained for Barrosa veal.

The differences in the contents of major lipid-soluble antioxidant vitamins present in PDO meats can be explained by the different natural forage availability and finishing period between the two production systems (extensive and semi-extensive production systems of Barrosa and Carnalentejana, respectively).

Conclusions

The contents of lipid-soluble antioxidant vitamins in Barrosa-PDO veal are in agreement with a pasture raised product while the figures obtained for Carnalentejana-PDO beef are in between the grain-fed and pasture raised animals, with no important seasonal variations for both bovine meats. The results suggest that Barrosa-PDO veal is more stable against lipid oxidation than Carnalentejana-PDO beef, which is of major importance for the preservation of sensorial and nutritional meat proprieties.

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Tables and Figures

Table 1. Contents of major lipid-soluble antioxidant vitamins (alpha-tocopherol, gamma-tocopherol and beta-carotene) in Carnalentejana-PDO beef and Barrosa-PDO veal sampled in early autumn and late spring.

	Carnalentejana-PDO beef		Barrosa-PDO veal	
	Autumn	Spring	Autumn	Spring
	<i>Longissimus thoracis</i> (µg/g meat)			
α -Tocopherol	2.26±1.43 ^a	2.09±0.69 ^a	3.92±1.10 ^b	3.76±0.70 ^b
γ -Tocopherol	0.04±0.05 ^a	0.17±0.03 ^b	0.15±0.08 ^b	0.16±0.08 ^b
β -Carotene	0.19±0.01 ^a	0.09±0.05 ^b	0.09±0.04 ^b	0.11±0.07 ^b
	<i>Longissimus lumborum</i> (µg/g meat)			
α -Tocopherol	2.18±1.57 ^{a,b}	1.69±0.49 ^a	3.30±1.28 ^c	2.78±0.70 ^{b,c}
γ -Tocopherol	0.04±0.04 ^a	0.16±0.06 ^b	0.13±0.05 ^b	0.12±0.05 ^b
β -Carotene	0.0.16±0.02 ^a	0.07±0.03 ^b	0.07±0.03 ^b	0.10±0.06 ^b
	<i>Semitendinosus</i> (µg/g meat)			
α -Tocopherol	1.94±1.36 ^a	1.40±0.34 ^a	3.56±1.32 ^b	2.79±0.45 ^b
γ -Tocopherol	0.02±0.05 ^a	0.13±0.02 ^b	0.14±0.06 ^b	0.13±0.06 ^b
β -Carotene	0.16±0.01 ^a	0.05±0.02 ^b	0.08±0.04 ^c	0.06±0.03 ^{b,c}

Means with different supra-scripts in the same row are significantly different (p<0.05).

THE MECHANISM OF BEEF MARROW DISCOLORATION: A SUMMARY OF CAUSES, EFFECTS, AND PREVENTION

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Key Words: Bone Marrow, Color, Packaging, Hemoglobin, Myoglobin, Discoloration

Introduction

The appearance of muscle-based food products is the primary determinant of consumer-purchasing decisions. Although there is an abundance of peer-reviewed literature focused on the mechanism of muscle discoloration, published research evaluating the discoloration of bone marrow is lacking. This is somewhat surprising because industry has expressed an interest in techniques that limit marrow discoloration during display in case-ready packaging. To study the mechanism of bone discoloration, we assessed the roles of bone marrow's primary components (hemoglobin, lipid, and iron) in color and discoloration.

Marrow from carcasses is found in two forms, red and yellow, both of which are a direct result of bone functionality and location. Vertebrae and ribs are abundant in "red, erythropoietic marrow" that is composed of hematopoietic cells, whereas long bones are composed of "yellow, fatty marrow" with little or no hematopoietic potential. As the name implies, this marrow is abundant in fat, yet lacks pigment because of a relatively small amount of hemoglobin and iron. Coinciding with the lack of pigment, Grobbel (2004) reported that humeri packaged in high-oxygen and PVC did not discolor during display. Long bones containing fatty marrow also mature earlier than vertebrae and thus, are designed more for lipid storage (85% lipid in beef femurs compared with 26 to 56% in vertebrae; Kunsman et al., 1981). It is estimated that approximately 34% of the weight of beef cervical vertebrae is marrow (Field, 1999).

Beef hematopoietic marrow contains significantly more total pigment than muscle (28.2 compared to 3.7 mg/g fresh tissue; Field et al., 1980). Essentially all of the pigmentation in beef erythropoietic marrow is due to hemoglobin (99.7% of total pigment content) as opposed to myoglobin, which accounts for a negligible amount of total marrow pigment (0.33% of total; Field et al., 1980). Conversely, muscle color is primarily due to myoglobin. Similar results were reported by Demos and Mandigo (1995), who concluded that the total pigment content of marrow was relatively high (42 mg/g), especially when compared to 85% lean ground beef (6 mg/g). Grobbel (2004) reported that ribs and vertebrae contained more hemoglobin than humeri. Field et al. (1980) suggested that the total pigment values (mg/g) reported in their work also were representative of hemoglobin content because hemoglobin was the only pigment found in cervical and lumbar marrow.

Total iron content was significantly higher in cervical marrow than ground beef (13 compared to 1.6 mg/100g; Demos and Mandigo, 1995). These researchers reported a

strong correlation ($r = 0.85$) between pigment concentration and iron content. However, Field et al. (1980) noted that iron in bone marrow is not exclusive to hemoglobin, as it can also be stored as free iron and non-heme compounds. Nevertheless, marrow that is susceptible to discoloration (marrow from ribs and vertebrae) contains more total iron than marrow that lacks pigmentation (humeri marrow; Grobbel, 2004).

Numerous characteristics of bone marrow such as its pigment, lipid, and iron content make it susceptible to oxidation and discoloration. However, little work in meat science has focused on determining the mechanism of marrow discoloration.

Objectives

Figure 1 summarizes potential factors involved in erythropoietic bone marrow discoloration. From this, we developed four research questions to better understand the etiology of bone marrow discoloration.

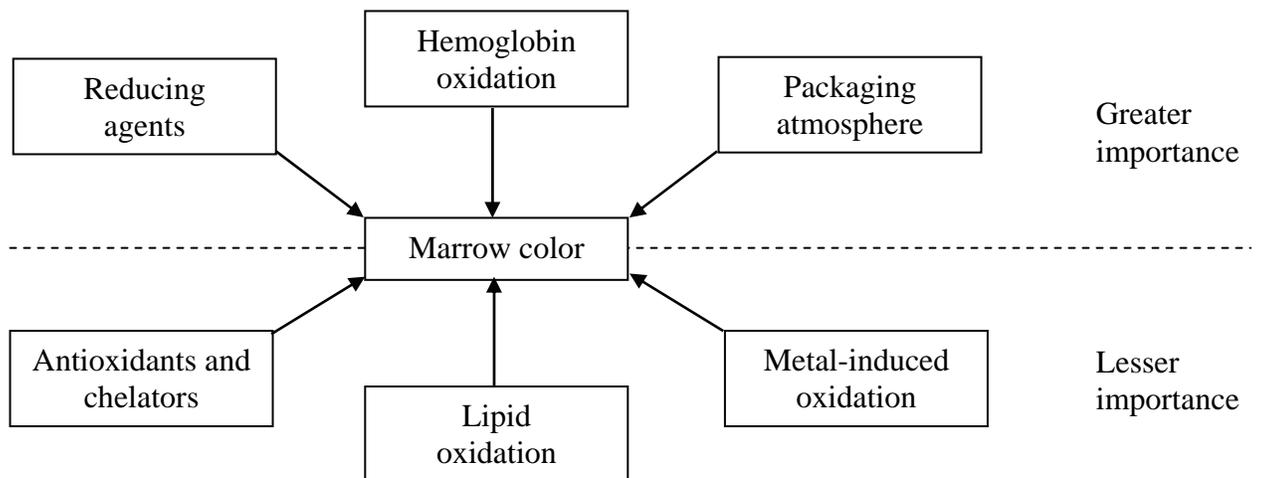


Figure 1: Potential factors involved in the discoloration of erythropoietic bone marrow.

Results & Discussion

Research question 1: What is the role of packaging atmosphere in beef erythropoietic marrow discoloration?

Mancini et al. (2005) and Grobbel et al. (2005a) suggested that excluding oxygen from modified atmosphere packages will improve the color stability of erythropoietic marrow from beef bones. Mancini et al. (2005) also reported that 0.4% carbon monoxide (30%CO₂ and 69.6%N₂) significantly improved vertebrae (red marrow) color stability during storage at 4°C. On the other hand, high-oxygen packaging (80% O₂) had a detrimental effect on vertebrae color during display (Mancini et al., 2004; Lanari et al., 1999). Other work has suggested that ribs and vertebrae will discolor in traditional aerobic packaging (polyvinyl chloride overwrap; Grobbel, 2005a). This implicates

hemoglobin's redox state in the color development and stability of red marrow. Hemoglobin's affinity for carbon monoxide suggests that the use of low levels of carbon monoxide in packages will maintain a bright-red marrow color during storage and display because of the formation of carboxyhemoglobin.

Research question 2: Can erythropoietic marrow discoloration be slowed by reducing agents such as ascorbic acid and sodium erythorbate?

Ascorbic acid or sodium erythorbate applied to the surface of ribs and vertebrae inhibited discoloration during display (Mancini et al., 2004). Although these reducing agents were most effective at concentrations between 0.5 and 10%, applications greater than 3% resulted in no significant color stabilizing advantage during 7-days of display. The effectiveness of ascorbic acid was improved by glutathione (Mancini, 2004), which plays a role in the recycling of water-soluble antioxidants. Grobbel et al. (2005b) also reported that ascorbic acid (2.5%) was useful for stabilizing marrow color. Grobbel (2004) noted that vertebrae marrow stability during display decreases with increased postmortem age.

Research question 3: Will minimizing lipid oxidation have a beneficial effect on bone marrow color?

The contribution of pigment and lipid oxidation to the surface color of cut bones is unknown. We hypothesized that hemoglobin oxidation, rather than lipid oxidation, was primarily responsible for the discoloration of erythropoietic marrow. Thus, a series of experiments (Mancini, 2004) were designed to determine whether the mechanism and sources of erythropoietic bone marrow discoloration are more water-soluble or lipid-soluble. Increasing ascorbic acid's lipophilicity diminished its ability to stabilize marrow color. Compared with ascorbate-6-palmitate (amphipathic), ascorbic acid better stabilized the surface color of ribs and vertebrae displayed in high-oxygen MAP (80% oxygen). Other lipid soluble antioxidants such as vitamin E, propyl-gallate, and dodecyl-gallate had no positive effects on vertebrae marrow color. Water-soluble glutathione tended to minimize discoloration more than lipoic acid. During display, TBARS values for untreated vertebrae in high-oxygen slightly increased from 0.6 to 0.8 (Grobbel, 2004).

Research question 4: Can marrow discoloration be minimized by chelation of metal catalyzing oxidation?

The contribution of iron to the oxidative instability of muscle foods is well documented. However, the role of iron in the oxidation of bone marrow pigments is unknown although marrow from erythropoietic bones contains more hemoglobin and iron than muscle. Vertebrae treated with 2.5% EDTA (topical application) will significantly discolor within 1 day after packaging in high-oxygen (Mancini, 2004). The "moderately gray" to "all gray" color of EDTA-treated vertebrae remained relatively stable throughout display. The inability of EDTA to minimize vertebrae surface discoloration suggests that free iron has a minor role in both marrow pigment oxidation and surface discoloration. Citric acid oxidized hemoglobin on the surface of vertebrae, resulting in discoloration that was reversed by ascorbic acid. Combining EDTA or citric acid with ascorbic acid provided no additional benefits compared to treatment with only ascorbic acid; thus,

inactivation of ascorbic acid via metals in erythropoietic marrow likely has little effect on the reducing agent's ability to stabilize vertebrae color.

The aqueous phase of erythropoietic beef marrow is the primary candidate for discoloration whereas the lipid portion had no significant role in marrow color (Mancini, 2004). Within the water-soluble phase, hemoglobin's redox state was the principal determinant of marrow color; thus, manipulating pigment redox status will have a dramatic effect on the color life of erythropoietic marrow. This could be accomplished by promoting ferrous hemoglobin with the use of either water-soluble reducing agents or packaging atmosphere, both of which were effective at maximizing color stability during storage and display. Reducing lipid oxidation and chelating metals appeared to have no impact on the oxidative stability of hemoglobin within erythropoietic marrow.

Conclusions

To improve the color stability of erythropoietic marrow, the beef industry can utilize technologies that minimize hemoglobin oxidation such as ascorbic acid, sodium erythorbate, or ultra-low oxygen packaging combined with low levels of carbon monoxide. Focusing on the water-soluble fraction of marrow, rather than lipid oxidation and other more non-polar fractions, should be the most effective way to improve marrow color stability.

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EFFECT OF LACTOPEROXIDASE SYSTEM ON QUALITY OF GROUND PORK

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Key Words: Lactoperoxidase; Total plate counts; Lipid oxidation; Metmyoglobin; Ground pork

Introduction

Lactoperoxidase (LP) is one of the most prominent enzymes in bovine milk and catalyzes the inactivation of microorganism in a lactoperoxidase system (LPS). Its activity is based on the oxidation of sulphhydryl groups of enzymes and other proteins by the products of reaction of LP with thiocyanate (SCN^-) and/or iodide (I^-) and hydrogen peroxide. (de Wit, 1995). LPS has been shown to be effective at reducing and inhibiting microbial populations on meats (Kennedy et al. 2000; Elliot, et al. 2004).

Objectives

Our objectives were to determine the effect of LPS with NaSCN or KI on total plate counts, lactic acid bacteria, coliforms, TBARS values, percent metmyoglobin, and color (a^* value) of ground pork during storage at 4 °C for 0, 2, 4 and 6 days.

Methodology

Frozen and thawed ground pork loins were divided into three batches (1.7 kg each) and each one was randomly assigned to one of the following treatments: (1) no additive (control), (2) LPS-NaSCN treatment (lactoperoxidase system consisting 2 ppm lactoperoxidase, 20 ppm NaSCN and 75 ppm hydrogen peroxide), and (3) LPS-KI treatment (consisting 2 ppm lactoperoxidase, 20 ppm KI and 75 ppm hydrogen peroxide). Meat samples were placed on white polystyrene foam meat tray, wrapped with polyvinylchloride film and stored at 4 °C for 0, 2, 4 and 6 days. Total plate counts and lactic acid bacteria were enumerated in plate count agar (Difco) and de Man Rogosa Sarpe (MRS) agar (Difco), respectively, after inoculation at 35 °C for 48hr. Coliforms were enumerated in violet red bile agar (Difco) after inoculation at 35 °C for 24 hr. All bacterial population was expressed as the \log_{10} colony forming units (cfu/g). Thiobarbituric acid reactive substances (TBARS) values of ground pork were determined according to Ockerman (1985). TBARS values were expressed as mg malonaldehyde/kg meat. Metmyoglobin (%) of ground pork was determined by the methods described by Chu et al. (1987). The color (CIE a^*) of ground pork was determined using a color meter (ZE, 2000, Nippon, Denshoku, Tokyo, Japan). All evaluations were replicated four times.

Data were analyzed as a factorial design, using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Differences between treatments means were separated using least squares mean procedures. Means were considered different at $P < 0.05$.

Results & Discussion

From the results of the analysis of variance, it was found that total plate counts (TPC) of meat samples were not significantly affected ($P > 0.05$) by LPS. No differences in TPC between the LPS-KI treatment and the control were observed during storage (Fig. 1). However, at days 4 and 6, the LPS-NaSCN treatment had lower ($P < 0.05$) TPC than the control. This indicated that the growth of TPC probably could be inhibited by the LPS-NaSCN treatment at later storage period. Kennedy et al. (2000) reported that the LPS-NaSCN treatment could inhibit the growth of naturally present microbial populations in ground beef. Elliot et al. (2004) indicated that the treatment with LPS-NaSCN was more effective at storage temperatures not for rapid bacterial growth.

Lactic acid bacteria of meat samples were not affected ($P > 0.05$) by LPS. No significant ($P > 0.05$) differences were found in lactic acid bacteria between treatments during storage (Fig. 2). The growth of lactic acid bacteria was not inhibited by the LPS-NaSCN or by the LPS-KI treatment. Elliot et al. (2004) also found that the LPS-NaSCN did not prevent the development of native lactic acid bacteria in beef cubes stored at chilling temperatures. In general, Gram-positive bacteria (i.e. lactic acid bacteria) are more resistant to the LPS than Gram-negative bacteria (de Wit, 1995).

The growth of coliforms was significantly affected ($P < 0.05$) by LPS. The LPS-NaSCN treatment had lower ($P < 0.05$) coliforms than the other two treatments during storage (Fig. 3). This indicated that the LPS-NaSCN treatment could effectively inhibit the growth of coliforms. However, no significant differences were found in coliforms between the LPS-KI treatment and the control, suggesting that the LPS-KI treatment did not depress the growth of coliforms. Kennedy et al. (2000) reported that the LPS-NaSCN could inhibit the growth of *E. coli* O157:H7 in ground beef. Zajac et al. (1983) also reported that coliforms in LP-activated milk remained unchanged for a longer period than those in the controls.

The TBARS values of meat samples were significantly affected ($P < 0.05$) by LPS. At day 0, the TBARS values among all treatments were similar (Fig. 4); but at days 2 and 4, the LPS-NaSCN treatment had higher ($P < 0.05$) TBARS values than the LPS-KI treatment and the control; and at days 6, the TBARS values of all treatments were not different. In general, the TBARS values of the LPS-NaSCN and the LPS-KI treatments were higher than the control during storage. This indicated that the LPS treatments consisting of H_2O_2 and SCN^- or I^- anions could probably accelerate lipid oxidation in ground pork, and increase TBARS values. The LPS-NaSCN treatment had greater effect on TBARS values than the LPS-KI treatment. The TBARS values of all samples were low initially and remained low (less than 0.4 mg/kg) throughout the entire storage time.

The values for a^* of meat samples were significantly affected ($P < 0.05$) by LPS. During storage, the LPS-KI treatment had lower a^* values than the control (Fig. 5), suggesting that the LPS-KI treatment could accelerate meat discoloration in ground pork; however, a^* values for the LPS-NaSCN treatment and the control were similar. As storage time increased, the values for a^* of all treatments decreased. These color changes

were expected, since extended exposure of fresh meat, resulted in myoglobin oxidation and meat discoloration.

The percent metmyoglobin of ground pork was significantly affected ($P < 0.05$) by LPS. The LPS-KI treatment had higher percent metmyoglobin, but the LPS-NaSCN treatment had lower percent metmyoglobin than the control during storage (Fig. 6). It was found that the higher percent metmyoglobin, the lower a^* values (Fig. 5) for all meat samples. The percent metmyoglobin was negatively correlated ($r = -0.512$) with a^* values ($P < 0.05$). The percent metmyoglobin of all meat samples increased with storage time.

Conclusions

The growth of coliforms could be inhibited by the LPS-NaSCN treatment. Both the LPS-NaSCN and the LPS-KI treatments would accelerate lipid oxidation and increase TBARS values. Ground pork treated with LPS-NaSCN had lower percent metmyoglobin and higher a^* values. The LPS-KI treatment had less effect on microbial growth, and it would increase percent metmyoglobin and decrease a^* values of ground pork.

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Tables and Figures

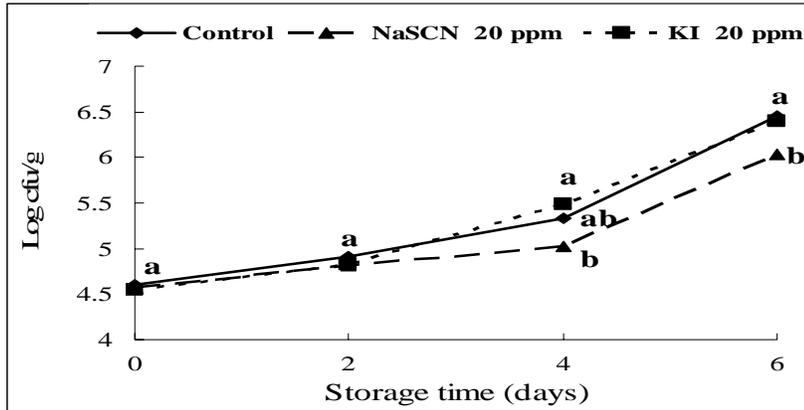


Fig. 1. Total plate counts in ground pork as affected by LPS with NaSCN or KI. ^{ab} Means within a storage period having the same superscripts are not significantly different ($P<0.05$).

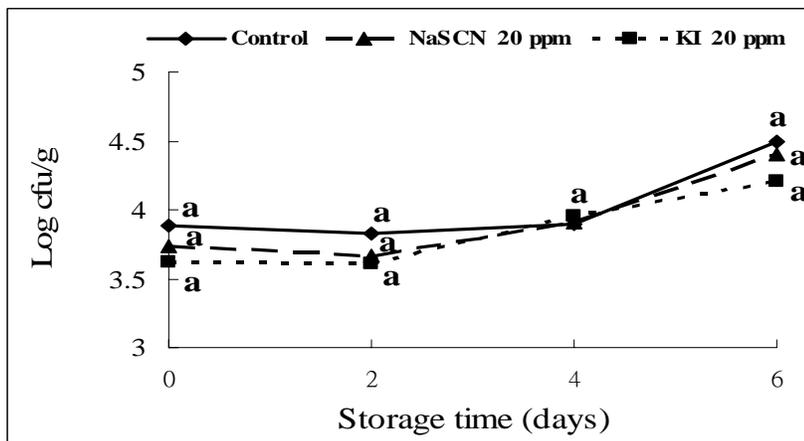


Fig. 2. Lactic acid bacteria in ground pork as affected by LPS with NaSCN or KI. ^a Means within a storage period having the same superscripts are not significantly different ($P<0.05$).

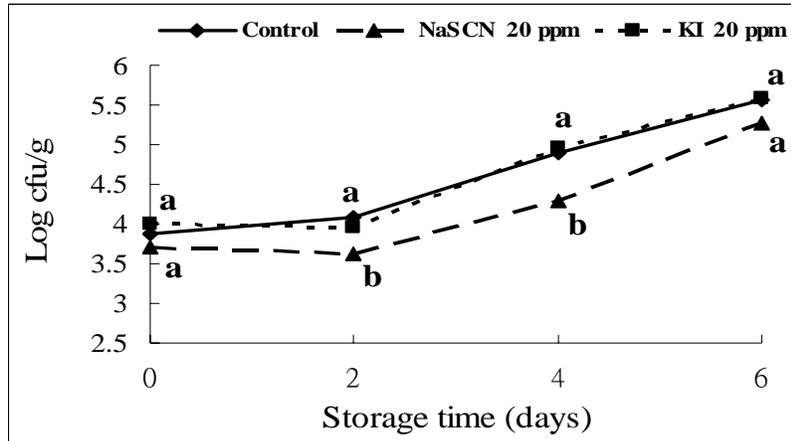


Fig. 3. Coliforms in ground pork as affected by LPS with NaSCN or KI. ^{ab} Means within a storage period having the same superscripts are not significantly different ($P<0.05$).

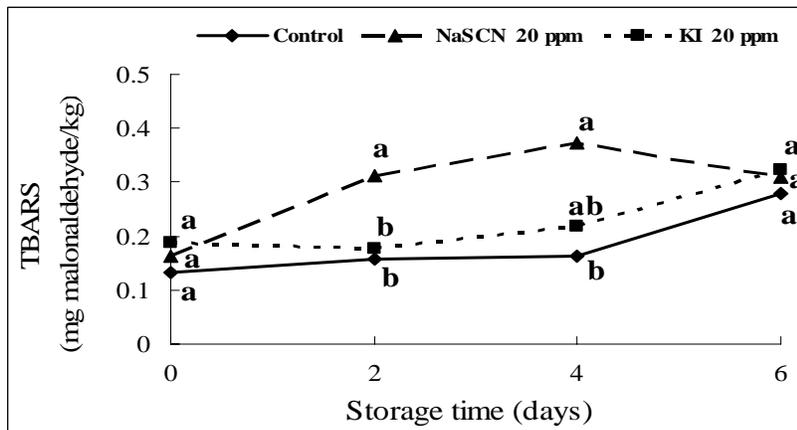


Fig. 4. TBARS values of ground pork as affected by LPS with NaSCN or KI. ^{ab} Means within a storage period having the same superscripts are not significantly different ($P<0.05$).

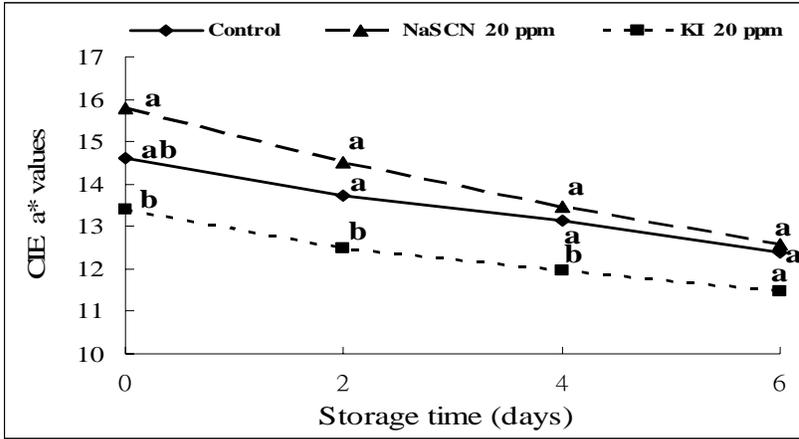


Fig. 5. The a* values of ground pork as affected by LPS with NaSCN or KI. ^{ab} Means within a storage period having the same superscripts are not significantly different ($P < 0.05$).

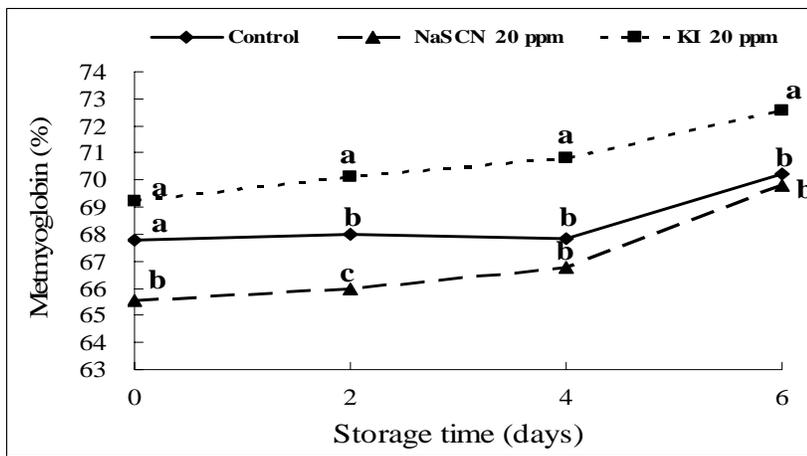


Fig. 6. Percent metmyoglobin of ground pork as affected by LPS with NaSCN or KI. ^{abc} Means within a storage period having the same superscripts are not significantly different ($P < 0.05$).

ROSEMARY OLEORESIN EXTENDS THE SHELF LIFE OF GROUND BEEF STORED AT ABUSIVE TEMPERATURES

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Key Words: Beef, Rosemary oleoresin, Lactic acid bacteria, Packaging

Introduction

While pathogens are of large concern to the meat industry, other factors including cost and quality are important as well. Spoilage characteristics that are typically associated with meats include color, odor, and texture changes. These characteristics can be affected by many different factors including: storage temperature, atmospheric oxygen, light, meat constituents, microorganisms, and endogenous enzymes (Lambert et al., 1991). Ground beef is affected not only by microbial growth, but also by chemical reactions such as lipid oxidation. Rosemary oleoresin has been found to have comparable antioxidant properties to that of a BHT/BHA/citric acid mix (Barbut et al., 1985). However, numerous health benefits associated with lactic acid bacteria (LAB) have been studied, including their role as biopreservatives in foods. They are found in many foods, because of their ability to grow under a wide variety of environmental conditions. The LAB also play an important role in food fermentations, having a preservative effect on the product (Stiles, 1996). Additionally, LAB have GRAS (generally recognized as safe) status (Brashears et al., 2005).

Objectives

The objective of this study was to determine color stability, odor characteristics, and oxidative rancidity susceptibility when rosemary oleoresin and LAB were added to ground beef packaged in modified atmosphere packaging and displayed at refrigeration and abusive temperatures.

Methodology

The spoilage characteristics of ground beef were evaluated by inoculating ground beef with a combination of LAB or sterile distilled water (control). Samples were stored in retail display cases with a light intensity of approximately 1900 lux and collected at the following times during display: 0, 24, 48, 72 and 84 h for patties displayed at 0°C, and 0, 12, 24, and 36 h for those displayed at 10°C. Coarse-ground beef was obtained from a commercial beef-packing facility. A cocktail of 4 strains of lactic acid bacteria consisting of *Lactobacillus acidophilus* NP 51, *Lactobacillus crispatus* NP 35, *Pediococcus*

acidilactici, and *Lactococcus lactis* spp. *Lactis* was provided by Culture Systems, Inc. (Mishawaka, IN) for use in this study at an inoculation level of 10^9 cfu/g meat.

For each replication (n = 3), 34.85 kg of ground beef was divided into four, 8.71 kg treatments: (1) control; (2) rosemary oleoresin; (3) LAB only; and (4) LAB + rosemary oleoresin. For LAB treatment groups, 17.42 kg ground beef was mixed thoroughly for 1 min, then 250 mL of a 10^9 cfu LAB/g solution suspended in sterile distilled water was added to the ground beef, the mixer direction was reversed, and the ground beef was mixed for 1 min. Using a 0.32 cm fine grind plate, the meat was passed through a three-phase meat grinder. Patties weighing 145.2 g were formed using a patty machine. The remaining 17.42 kg of ground beef was processed as above, except 250 mL of sterile distilled water only was added to serve as the control. Herbalox Type HT – rosemary oleoresin (Kalsec, Inc; Kalamazoo, MI) was added at a level of 0.01% (w/w; 1000 ppm) to the ground beef. Equipment was cleaned thoroughly between treatments and rinsed with 180°C water.

Once the patties were formed, 2 patties were placed in a plastic lidded tray. A gas flush, tray sealing machine was used to seal the film onto the trays. Packages were flushed with a targeted of 80% O₂ and 20% CO₂. Packages in each treatment group were randomly identified and assigned to either a 0°C or 10°C coffin-style retail display case for display. The display cases were kept under fluorescent lighting at an intensity of approximately 1900 lux. Headspace was analyzed on random packages during processing. A total of 40 packages per replication were prepared (n = 10 packages per treatment group).

Trained and consumer panelists were utilized to detect differences in color and odor of the treated ground beef. Trained panelists scored the patties for ground meat color (1 = very bright red; 5 = very dark red or brown), as well as surface discoloration (1 = no discoloration; 2 = slight, 1–10%; 3 = small, 11–20%; 4 = moderate, 21–60%; 5 = severe discoloration, 61–100%) as outlined by the AMSA color guidelines (AMSA, 1991). Untrained panelists served as the consumer panel and were asked to determine if the meat had good color (1 = very strongly agree; 7 = very strongly disagree) and how likely they were to purchase (1 = definitely would purchase; 5 = definitely would not purchase) the package (AMSA, 1991).

Odor panels were also conducted on packages removed from the cases at each sampling interval. The packages were opened in a random order and the panelists were allowed to smell the patties, using verbal descriptors adapted from Payne et al., 2002. Briefly, panelists were asked to determine if an immediate off-odor was present (1 = no off-odor; 5 = extreme off-odor) and to characterize the off odor if present (1 = rancid; 2 = arid; 3 = sweet; 4 = sour; 5 = acid; and 6 = putrid). Consumer panelists were asked if the meat in the package smelled fresh (1 = very strongly agree; 7 = very strongly disagree) and how likely they were to consume the meat (1 = definitely would consume; 5 = definitely would not consume) based upon the odor.

Commission Internationale de l'Eclairage (CIE) L* (luminance or muscle lightness), a* (redness or muscle redness), and b* (yellowness or muscle yellowness) were taken on one patty from each package opened at the sampling interval. Each patty was evaluated using the MiniScan XE Plus (Hunter Association Laboratory, Incorporated; Reston, VA). Two readings from randomly selected locations were taken from each patty and averaged to determine the L*, a*, and b* values of the patty.

TBA assays were performed to estimate oxidative rancidity on the remaining one-half of the patties in the Texas Tech University Animal and Food Sciences Nutrition Laboratory according to modified procedures outlined by Buege and Aust (1978).

Results & Discussion

Temperature Display at 0°C

Trained panelist responses for lean color and surface percentage brown discoloration did not significantly differ between treatments for ground beef patties displayed at 0°C. Also, trained panelist responses for immediate off-odors of ground beef patties did not significantly differ between treatments. Trained panelists also characterized off-odors at each sampling interval. At 0 h, 100% of the patties for each treatment were characterized as having no off-odor. Later in the display period, a large portion of the LAB-inoculated ground beef patties containing resin were characterized as not having an off-odor present, compared to patties without resin where a majority of the patties were either characterized as rancid or sour.

No significant differences were found between treatments for consumer responses for lean color of ground beef and purchase intent based upon ground beef patty color. Consumer panelist responses to questions concerning the freshness of odor of the ground beef as well as the consumer's likelihood to consume the product did not significantly differ between treatments. Hunter L*, a*, and b* values did not significantly differ between treatment groups. These results agree with the trained and consumer panelists responses.

Significant ($P < 0.05$) differences did occur between treatments for TBA values. Treatment groups with resin had significantly ($P < 0.05$) lower values than treatment groups without resin, despite the addition of lactic acid bacteria. Therefore, lipid oxidation would be slowed with the addition of rosemary to ground beef.

Temperature Display at 10°C

Trained panelists analyzed lean color and no significant differences were found in responses for this trait between treatments. No significant differences were found between treatments for panelist responses for percent discoloration. At time 0 h, panelist scores for immediate off-odor were not significantly different for treatments applied to the ground beef. After 24 h of display, uninoculated and inoculated ground beef patties with added rosemary oleoresin had significantly ($P < 0.05$) lower scores than the uninoculated and LAB-inoculated ground beef without added resin. This trend continued throughout the remainder of the display period. Rosemary oleoresin slows lipid oxidation, which produces unpleasant odors. Therefore, the treatment groups with added oleoresin would be expected to have lower immediate off-odor scores as compared to the treatment groups without added oleoresin. Also, trained panelists were asked to characterize the off-odor. At time 0 h, 100% of the uninoculated ground beef patties with or without resin were characterized as having no off-odor. As display progressed, the percent of uninoculated ground beef patties without resin characterized as rancid and sour increased. Also, a larger percentage of patties with added resin had no off-odor. LAB-

inoculated ground beef patties with and without added rosemary oleoresin were classified as having no off-odor at time 0 h. As compared to the LAB-inoculated ground beef patties without added rosemary oleoresin, a larger percentage of the LAB-inoculated ground beef patties with rosemary oleoresin were characterized as not having an off-odor at the end of the display period. This indicated that the addition of the oleoresin slowed the on-set of undesirable odors through the inhibition of lipid oxidation.

Significant ($P < 0.05$) differences were found between treatments in consumer panelist response scores for lean ground beef color. LAB-inoculated ground beef without added oleoresin had significantly ($P < 0.05$) higher scores than the treatment groups with added oleoresin, indicating a darker, less desirable color. Based on lean ground beef color, the consumers indicated how likely they were to purchase the package of ground beef if it had been available in retail stores. The consumer scores were not significantly different between treatments. Significant ($P < 0.05$) differences between treatments were also found in consumer panelists responses for odor freshness. The treatment groups with added oleoresin had significantly ($P < 0.05$) fresher odor scores than the uninoculated ground beef patties without added resin. No significant differences were found between treatments in response scores for the likelihood of consumption based on the odor of the meat.

Hunter L^* , a^* , and b^* values were taken at each sampling interval. No significant differences were found in L^* values for any treatment group displayed at abusive temperatures. The treatment groups with added oleoresin had significantly ($P < 0.05$) higher a^* values than the uninoculated ground beef patties without added resin. The uninoculated ground beef patties without resin had significantly ($P < 0.05$) lower b^* scores than the treatment groups with added resin. By adding rosemary oleoresin, the deterioration of ground beef color was slowed.

Initially, TBA values did not significantly differ between treatments. After 12 h of display, the treatment groups with added oleoresin had significantly ($P < 0.05$) lower TBA values than the treatment groups without resin. The treatment groups without added oleoresin had significantly ($P < 0.05$) higher TBA values than those treatment groups with added resin after 24 and 36 h of display. These results indicate that the addition of rosemary oleoresin slowed lipid oxidation as the display period progressed despite the addition of LAB.

Conclusions

The results of this study showed that rosemary oleoresin was effective in extending the shelf life of ground beef patties stored in MAP packaging by decreasing lipid oxidation at both 0 and 10°C. Also, consumer color and odor scores revealed that the addition of resin produced brighter red, longer-lasting fresher beef patties when stored at 10°C, and more desirable odor scores at 0°C as well. However, analysis of both trained and consumer color and odor panels revealed no significant differences between samples with added LAB and those without. Additionally at 0 and 10°C, trained color and odor panels of beef patties containing resin were not significantly different compared to those without added resin. At 0 and 10°C, Hunter a^* values were significantly higher, and b^* values were significantly lower, indicating rosemary oleoresin helped to maintain beef patty color during retail display. TBA assays showed that the addition of rosemary

slowed the lipid oxidation process at both 0 and 10°C. This is in agreement with several researchers who also found that lipid oxidation was inhibited by the addition of the natural antioxidant (Sanchez-Escalante et al., 2001; Ho et al., 1995; Barbut et al., 1985). Therefore, this natural antioxidant can maintain color and freshness for extended periods of time, even at abusive temperatures.

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ANTIOXIDANT EFFICACY OF CRANBERRY PRESS CAKE EXTRACTS ON THE OXIDATION OF MECHANICALLY SEPARATED TURKEY

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Key Words: Mechanically Separated Turkey, Cranberry press cake, Cranberry powder, Microwave Assisted Extraction, Solvent extraction.

Introduction

Lipid oxidation is a major cause of quality deterioration in muscle foods (1). Oxidation could be retarded by the addition of antioxidants exogenously to food systems (2). Due to the safety and toxicity concerns related to synthetic food antioxidants (3), consumers prefer the use of natural antioxidants in food products. Cranberry press cake is an under utilized by-product of cranberry processing industry. The press cake contains a number of phenolic compounds which could be used as potential food antioxidants(4).

Objectives

The objective of our research was to prepare extracts from powdered cranberry press cake and test their antioxidant efficacy in controlling the oxidation of mechanically separated turkey (MST). The antioxidant efficacy of cranberry press cake extracts were also compared with a chloroform extract of cranberry powder.

Methodology

Antioxidant extracts from powdered cranberry press cake was prepared using two extraction methods, solvent extraction (5) and Microwave Assisted Solvent Extraction (MASE) (6). Five different extraction solvents, 100% acetone, 50% acetone, 100% ethanol, 50% ethanol and water were used. The antioxidant extracts were evaporated, freeze dried and tested for their efficacy on MST at 0.15% based on the muscle weight. Antioxidant extract from cranberry powder (90MX from Ocean Spray Cranberries) was prepared by extraction using (1:1) chloroform and methanol. The chloroform phase was separated, evaporated, freeze dried and tested for its antioxidant efficacy on MST at 0.01%, 0.05%, 0.1% and 0.2% of the muscle weight. Ethanol was used as the antioxidant carrier solvent. The total phenolic content of the antioxidant extracts were determined as quercetin equivalents. Oxidation of MST was monitored by the measurement of thiobarbituric acid reactive substances (TBARS) and lipid peroxides.

Results & Discussion

Irrespective of the method of extraction, the press cake extracts prepared using, 100% ethanol, 100% acetone, 50% ethanol or 50% acetone, significantly ($p < 0.01$) increased the oxidative stability of MST compared to the control when oxidation was measured in terms of TBARS (**Table 3, 5**) and lipid peroxides (**Table 4, 6**). The water extracts of cranberry press cake prepared by MASE or solvent extraction did not have any significant ($p > 0.01$) effect in controlling the oxidation of MST. Among all the press cake extracts, the microwave extract (MASE) prepared using 100% ethanol and the solvent extract prepared using 100% acetone, were most effective in inhibiting lipid oxidation. When compared to the control which oxidized in 5 days, the MASE extract prepared using 100% ethanol and added at 0.15% of muscle weight could inhibit TBARS formation for up to 12 days and a solvent extract prepared using 100% acetone could inhibit oxidation for around 11 days. In comparison, a chloroform extract prepared from cranberry powder could inhibit TBARS formation for up to 20 days at 0.05% level and 35 days at 0.1% level while the control was completely oxidized in 15 days (**Table 7**). Although, at similar levels of usage, the cranberry powder extract was more effective than the cranberry press cake extracts in inhibiting the oxidation of MST, the yield of the press cake extracts was much higher compared to the powder extracts (**Table 2**). Among the different press cake extracts, the overall effectiveness in controlling the oxidation of MST in terms of TBARS measurements were: 100% acetone, MASE 100% ethanol > 100% ethanol > 50% ethanol, 50% acetone, MASE 50% ethanol, MAE 100% acetone, MASE 50% acetone > water, MASE water, where water, ethanol and acetone indicate the solvents used for extraction. However, when the total phenolic content of the press cake extracts were measured as quercetin equivalents (QE), the decreasing order of quercetin equivalents were 100% acetone > MASE 100% acetone > 100% ethanol, 50% ethanol, MASE 100% ethanol, MASE 50% acetone > 50% acetone > water and MASE water (**Table 1**). The water extracts had the lowest phenolic content of around 0.022 QE/g of extract while the acetone extract had the maximum phenolic content of 0.53 QE/g of extract. The difference in the quercetin equivalent of the extracts and their corresponding antioxidative efficacy indicate that the total phenolic content may not be a good parameter to estimate the antioxidant efficacy of the press cake extracts.

Between the two extraction methods, MAE and solvent extraction, the addition of water to the organic solvents increased the yield of the MAE extracts (**Table 2**). Hence, for the same amount of starting material i.e. powdered cranberry press cake, the extracts obtained using MAE could protect more amount of MST compared to the extracts obtained using simple solvent extraction.

Conclusions

Among all the cranberry press cake extracts obtained using solvent extraction and MAE, the two extracts obtained using 100% acetone extraction and 100% ethanol MASE extraction, inhibited the oxidation of MST most in terms of TBARS measurement. When water was present in the organic extraction solvents, the antioxidative potential of the extracts were similar irrespective of the method of extraction. However, the yield of the press cake extracts using MASE was higher than that prepared using solvent extraction.

The water extracts were the least inhibitive of all the extracts in inhibiting the oxidation of MST. At a similar level of usage, extracts prepared from cranberry powder was more effective in inhibiting the oxidation of MST compared to press cake extracts. However, when the cost of the starting raw material is considered, cranberry press cake is much cheaper than cranberry powder (4). Moreover, the yield of the press cake extracts was much higher than that of powder extracts. Hence, on an economy of scale, cranberry press cake may be a useful raw material for the preparation of food grade antioxidant extracts.

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Tables and Figures

Table 1. Quercetin Equivalents of Cranberry Press Cake Powder Extracts: Microwave Assisted Solvent Extraction (MASE) vs. Simple Solvent Extraction^a

Solvents used for extraction	Quercetin equivalents (mmole/ g of extract)	
	MASE	Solvent extraction
Ethanol (100%)	0.30 ± 0.03	0.24 ± 0.09
Ethanol (50%)	0.12 ± 0.12	0.28 ± 0.01
Acetone (100%)	0.53 ± 0.08	0.40 ± 0.04
Acetone (50%)	0.27 ± 0.03	0.23 ± 0.10
Water	0.022 ± 0.01	0.024 ± 0.01
Chloroform:Methanol*	–	0.23 ± 0.00

^a n = 2

MASE: Microwave assisted solvent extraction at 125°C (10 min ramp, 10 min hold)

*Cranberry powder was extracted using 1:1 chloroform and methanol. The chloroform phase was used for determining the yield.

Table 2. Yields of Cranberry Press Cake Powder Extracts: Microwave Assisted Solvent

Extraction (MASE) vs. Simple Solvent Extraction

Solvent used for extraction	Yield in terms of dry powder weight (%)	
	MASE	Solvent extraction
Ethanol (100%)	2.13	2.17
Ethanol (50%)	7.75	2.35
Acetone (100%)	2.99	2.92
Acetone (50%)	3.70	2.51
Water	17.6	9.97
Chloroform:Methanol*	–	0.3

MASE: Microwave Assisted Extraction at 125°C (10 min ramp, 10 min hold)

*Cranberry powder was extracted using 1:1 chloroform and methanol. The chloroform phase was used for determining the yield.

Table 3. Effect of various extracts of powdered cranberry press cake obtained by MASE on the TBARS measurement of MST^a.

	TBARS (µmole/ kg of MST)					
		100%	50%	100%	50%	
Days	Control	EtOH	EtOH	Acetone	Acetone	Water
0	3.3 ± 0.8	2.9 ± 0.2	4.3 ± 0.4	3.2 ± 0.4	3.4 ± 0.5	2.4 ± 0.5
3	5.1 ± 0.8	3.6 ± 1.7	3.5 ± 2.1	4.0 ± 1.2	5.1 ± 0.8	4.3 ± 0.7
7	3.9 ± 0.1	3.5 ± 0.1	8.1 ± 1.1	6.47 ± 0.9	8.4 ± 3.2	19.8 ± 0.2
9	20.8 ± 0.2	6.5 ± 1.6	18.8 ± 2.1	11.9 ± 1.3	16.3 ± 0.3	41.5 ± 4.2
12	30.4 ± 0.0	9.0 ± 2.8	44.2 ± 11	37.9 ± 2.3	42.0 ± 2.8	–
14	–	25.2 ± 11	–	–	–	–
16	–	43.9 ± 6.0	–	–	–	–

^a n = 2

All extracts were added at 0.15% of the muscle weight. MST: Mechanically separated turkey; MASE: Microwave assisted solvent extraction; EtOH: Ethanol; Control: MST; Solvents used for extraction: 100% ethanol, 50% ethanol (1:1 ethanol and water), 100% acetone, 50% acetone (1:1 acetone and water) and water.

Table 4. Effect of various extracts of powdered cranberry press cake obtained by MASE on the lipid peroxide measurement of MST.

	Lipid peroxide (mmole/ kg of MST)					
		100%	50%	100%	50%	
Days	Control	EtOH	EtOH	Acetone	Acetone	Water
0	0.063	0.095	0.062	0.141	0.064	0.074
3	0.083	0.126	0.124	0.117	0.132	0.152
7	0.20	0.17	0.19	0.18	0.26	0.66
9	0.50	0.20	0.22	0.22	0.23	0.58
12	0.53	0.53	0.59	0.54	0.48	–
14	–	0.65	–	–	–	–

All extracts were added at 0.15% of the muscle weight. Abbreviations: See Table 3.

Table 5. Effect of various extracts of powdered cranberry press cake obtained by solvent extraction on the TBARS measurement of MSTa.

Days	TBARS ($\mu\text{mole/ kg of MST}$)					
	Control	100% EtOH	50% EtOH	100% Acetone	50% Acetone	Water
0	3.3 \pm 0.8	3.5 \pm 0.8	4.6 \pm 1.1	3.3 \pm 0.6	3.8 \pm 0.8	2.7 \pm 0.2
3	5.1 \pm 0.8	3.6 \pm 0.8	2.8 \pm 0.7	3.5 \pm 0.4	2.8 \pm 1.0	3.6 \pm 0.5
7	3.9 \pm 0.1	5.7 \pm 0.2	6.2 \pm 0.9	4.7 \pm 0.1	5.0 \pm 0.5	16.2 \pm 0.5
9	20.8 \pm 0.2	9.6 \pm 2.1	18.2 \pm 0.4	6.8 \pm 0.7	12.2 \pm 2.1	33.9 \pm 2.9
12	30.4 \pm 0.0	25.6 \pm 1.9	52.3 \pm 9.3	14.0 \pm 2.3	34.8 \pm 11	–
14	–	34.6 \pm 0.9	–	19.0 \pm 3.1	–	–
16	–	–	–	40.0 \pm 1.2	–	–

a n = 2

All extracts were added at 0.15% of the muscle weight. MST: Mechanically separated turkey; EtOH: Ethanol; Control: MST; Solvents used for extraction: 100% ethanol, 50% ethanol (1:1 ethanol and water), 100% acetone, 50% acetone (1:1 acetone and water) and water.

Table 6. Effect of various extracts of powdered cranberry press cake obtained by solvent extraction on the lipid peroxide measurement of MST.

Days	Lipid peroxide (mmole/ kg of MST)					
	Control	100% EtOH	50% EtOH	100% Acetone	50% Acetone	Water
0	0.063	0.077	0.059	0.097	0.056	0.058
3	0.083	0.123	0.097	0.136	0.088	0.134
7	0.20	0.20	0.21	0.21	0.25	0.60
9	0.50	0.21	0.21	0.21	0.22	0.59
12	0.53	0.56	0.47	0.61	0.57	–
14	–	0.66	–	0.58	–	–

All extracts were added at 0.15% of the muscle weight. Abbreviations: See Table 5.

Table 7. Effect of different concentration of cranberry powder extracts prepared using chloroform: methanol (1:1) solvent extraction on the TBARS measurement of MSTa.

Days	TBARS ($\mu\text{mole/ kg of MST}$)				
	Control	0.01%	0.05%	0.1%	0.2 %
14	29.61 \pm 5.12	8.3 \pm 1.8	1.7 \pm 0.8	1.2 \pm 0.1	1.5 \pm 0.1
18	–	24.5 \pm 7.1	1.9 \pm 0.1	1.8 \pm 0.6	2.0 \pm 0.3
21	–	39.2 \pm 15.3	8.9 \pm 2.7	2.1 \pm 0.3	1.8 \pm 0.0
27	–	–	44.0 \pm 3.4	4.6 \pm 1.7	3.6 \pm 2.0
34	–	–	90.8 \pm 6.8	5.2 \pm 1.6	2.3 \pm 0.0
42	–	–	–	86.5 \pm 7.3	6.2 \pm 3.0

a n = 2

MST: Mechanically separated turkey; Extracts were added to MST based on the muscle weight. Amount added = 0.01%, 0.05%, 0.1% and 0.2%.

EFFECT OF 2 YEARS OF STORAGE ON SENSORY QUALITY OF PORK LOIN WITH A HIGH CONTENT OF POLYUNSATURATED FAT

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Key Words: Frozen storage, rancidity, tenderness, polyunsaturated fat, shelf life, pork loin, oxidation

Introduction

Inclusion of polyunsaturated fatty acids (PUFA) in pigs' diets increases the risk of lipid oxidation in both the living organism and the carcass (Jakobsen, 1995). It means that the shelf life of meat and meat products during storage will normally become poorer and the storage time will be shorter compared to meat from pigs fed a 'normal' diet. In meat there are three different types of fat: lard (back fat) covering the meat, triglycerides in the inter- and intramuscular fat and phospholipids in the cell membranes. Phospholipids are mainly polyunsaturated, whereas triglycerides are mainly monounsaturated, making the phospholipids more oxidative labile. The diet will mainly influence the fatty acid composition in back fat and triglycerides whereas the fatty acid compositions in phospholipids are the same (Lauridsen *et al.*, 1999). Polyunsaturated fatty acids are desirable in meat due to their nutritive quality in the human diet, but large amounts will lead to softening of the products and make them susceptible to lipid oxidation (Warnants *et al.*, 1998). Oxidation occurs initially on the surface of the product when the meat/fat is exposed to oxygen. The oxygen or the primary or secondary oxidation products from the lipid oxidation might diffuse inside the meat and accelerate the oxidation in the rest of the product, so the entire product will get a rancid taste and off-flavour.

Objective

To investigate the shelf life and sensory quality in the back fat and middle of pork loins stored for two years at -20°C and compare them with the fatty acid composition in back fat, triglycerides and phospholipids in the loin.

Methodology

Thirty-six female pigs with an average slaughter weight of 75 kg were used for this experiment. The pigs were fed three different feeding stuffs, each resulting in a high level of unsaturated fat in the meat. After slaughtering at a commercial slaughterhouse (Danish Crown, Holstebro) both loins from each carcass were vacuum-packed, matured for four days and then stored at -20°C until thawing. Prior to the sensory analysis by a panel of 8 panelists, the loins were thawed for approximately 20 hours at 4°C . The loins were sliced in 20 mm thick chops. A cast iron frying pan was heated to 200°C before the chops were

placed on the pan, and the chops were turned every two minutes and fried to center temperature of 65–68°C. After frying the chops were cut in two and served to the panelists. Chops from the right loin were served after two years on frozen storage, and the following attributes of the meat were judged on a continuous scale from 0 (no intensity) to 15 (high intensity): tenderness, juiciness, sour taste, fried meat flavour, rancid flavour in back fat, rancid flavour in meat, faded flavour.

At the time of slaughter, the meat was analyzed for fatty acid composition of triglycerides and phospholipids, and the back fat for fatty acid composition of triglycerides using the GC-FID method. The fatty acid was divided into saturated fatty acid **SFA**, monounsaturated fatty acid **MUFA** and polyunsaturated fatty acid **PUFA**.

Results & Discussion

The fat content of the three types of feeding stuff all came from different vegetable fat sources and led to a higher level of PUFA in the lipids of the carcass compared with pigs fed a conventional diet. Lauridsen et al. (1999) found that PUFA in back fat and triglycerides from Danish pigs fed a basal (basic) diet was 11 and 5 compared with 27 and 10 in this experiment (Table 1).

There was no difference in the sensory analyzes in relation to the three types of feeding stuff after two years of storage, and the level of tenderness, juiciness and fried meat flavour in this experiment (table 2) was at the same level, that is normally found in fresh Danish pork shops (Magnussen, 2004).

After two years' frozen storage, the panelists were able to detect whether the meat had a rancid and faded flavour. The rancid flavour differs whether it is back fat or meat from the loin. The back fat was found more rancid, 50% of the loin had an intensity score of > 3, whereas 80% of the loins were <1 for rancid flavour in the meat, so the off-flavour product from the oxidation of the back fat will not be able to diffuse into the meat and result in a more rancid flavour.

Oxidation of the fat will commence in the back fat fraction and will led to a higher intensity of rancid flavour of the fat. The oxidation of the lipids inside the meat (triglycerides and phospholipids) will not produce sufficient off-flavour products for a trained person to be able to detect the flavour even after two years' frozen storage (Table 2 and Table 3).

The correlation between %PUFA in the back fat and the rancid flavour is significant, however, on a low level. While the level of rancid flavour is low and not detected in 80% of the meat samples, there will be no correlation between the % PUFA in the triglycerides and phospholipids and the rancid flavour of meat (Table 4). In this experiment the variation in PUFA was small (from 21 to 34). This could explain for the low correlation between PUFA and rancid flavour.

Conclusions

A storage time of up to two years will lead to a higher intensity of rancid flavour in the back fat, but not in the meat, because oxidation of PUFA will commence in the back fat. In this experiment the level of PUFA in the back fat was more than twice as high as in a basic pig feed, but it did not lead to an accelerated oxidation and a more rancid flavour of the meat. The amount of PUFA in back fat is significantly correlated to rancid flavour.

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Tables and Figures

Table 1. The fraction of the fatty acid analyzed in the three fat types.

	Loin		Back fat
	Triglycerides	Phospholipids	Total lipid
SFA %	39	39	33
MUFA %	52	18	40
PUFA %	10	43	27

Table 2. The sensory analysis of the chops after 2 years of storage

	Intensity (0 – 15)
Tenderness	9.5
Juiciness	9.1
Sour taste	6.5
Fried meat flavour	7.0
Rancid flavour, back fat	3.4
Rancid flavour, meat	0.6
Faded flavour	1.4

Table 3. Frequency of loins (%) in groups with an increasing intensity of rancid and faded flavour

	Rancid		Faded
	Back fat	Meat	
< 1	22	81	36
1 – 2	19	8	36
2 – 3	8	6	19
3 – 4	8	5	9
4 – 5	18	–	–
5 – 6	6	–	–
6 – 7	14	–	–
> 7	5	–	–

Table 4. The correlation (Pearson) between the rancid flavour and the PUFA % of the three different fat types.

	Rancid flavour, back fat	Rancid flavour, meat
PUFA, phospholipid	0.301 (p=0.07)	0.200 (ns)
PUFA, triglycerides	0.138 (ns)	0.006 (ns)
PUFA, Back fat	0.133 p<0.05	0.035 (ns)

INFLUENCE OF FEED ENRICHED WITH NATURAL ANTIOXIDANTS ON THE OXIDATIVE STABILITY OF FROZEN BROILER MEAT

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Key Words: oxidative stability; natural antioxidants; broiler meat

Introduction

Meat as well as precooked and restructured meat products have a low oxidative stability, which causes discoloration, drip losses, off-odor and off-flavor development, and the production of potentially toxic compounds (Gray et al., 1996; Morrissey et al., 1998). Freezing slows down oxidation, but it does not stop the process (Kanner, 1994).

To maximize the oxidative stability of meat, antioxidants, mostly α -tocopheryl acetate (α TAc), are added to PUFA rich feeds. The beneficial effect of dietary α TAc supplementation on the subsequent stability of lipids in foods from animal origin has been extensively reported for poultry, beef cattle, veal calves and pigs (see e.g. Gray et al., 1996; Jensen et al., 1998). More recently, an increasing number of studies report on the antioxidative properties of plant products and compounds (Halvorsen et al., 2002; Pellegrini et al., 2003; Schwarz et al., 2001). Several studies have already demonstrated that plant antioxidative compounds supplemented to meat post mortem are able to improve the lipid stability of the meat (Govaris et al., 2003; Lau et al., 2003; Nissen et al., 2000). However, only a limited number of studies have been performed investigating the effect of dietary administration of natural antioxidants, other than natural α -tocopherol (α TC), on the oxidative stability of meat or meat products (Botsoglou et al., 2002, 2003; Tang et al., 2000).

Objectives

The aim of this study was to investigate the effect of supplementation of a broilers' diet with several plant extracts, rich in natural antioxidants, on the oxidative stability of meat after 8 months of frozen storage. The effects of individual extracts were tested, as well as combinations of extracts to investigate possible additive or synergistic effects between antioxidants.

Methodology

Animals and experimental design

Two thousand and forty one-day old chickens (Ross 308, cocks) were divided over 60 pens and were fed for 6 weeks a diet containing 4% refined linseed oil (n-3 PUFA enrichment) and one of 20 antioxidants or antioxidant mixtures. Within each phase (3 phases), diets were formulated to an equal protein and energy content. In the premix no synthetic antioxidants were added, but a basal amount of 20 ppm α TAc was present to meet physiological requirements. The experimental antioxidants were mixed in the refined linseed oil prior to the feed manufacturing. Five extracts (natural α TC, rosemary, green tea, grape seed and tomato; Table 1) were supplemented separately at 100 ppm or 200 ppm, making 10 treatments. In addition, α TC, rosemary and green tea were supplemented in equal proportions in all combinations two by two and all three combined at a final concentration of 100 or 200 ppm, making a further 8 treatments. Two control treatments with a mixture of synthetic antioxidants (BHT, BHA, ethoxyquin) and with or without 200 ppm α TAc were also included. All antioxidant extracts were supplemented at 100 or 200 mg total product/kg feed and not as 100 or 200 mg active compounds/kg feed. These 20 antioxidant treatments were replicated in three pens (Table 2).

Sampling

At the moment of slaughter, 5 birds per pen were selected with a live weight close to the average pen weight. After slaughter, the right part of the breast muscle of the 5 selected animals was pooled, minced, vacuum-packed and stored at -18°C . After 8 months, three patties (approximately 100g) were prepared and over-wrapped in an oxygen permeable polyethylene film and placed in an illuminated chill cabinet (illuminance of 1000 lux) at 3°C for 11 days. Samples were analysed for lipid oxidation at day 1, 4 and 11 of display and for protein oxidation at day 11.

Methods

Lipid oxidation was assessed by the TBARS-method (thiobarbituric acid reactive substances) based on Tarladgis et al. (1960) and is expressed as μg malondialdehyde/g tissue.

Oxidative damage to proteins was determined by measuring the decrease in the amount of thiol groups. Thiol groups are expressed as nmol free SH-groups/mg protein (Batifoulier et al., 2002).

Statistical analysis

Data of lipid and protein oxidation were analysed by a linear model including the fixed effects of antioxidant treatment and days of storage. The interaction term was not significant and was therefore not included in the model. TBARS values were log

transformed to account for heterogeneity of variances. To compare single antioxidant treatments and to test for dose effects and possible synergistic action, specific contrasts were defined at a significance level of $P < 0.05$ (e.g. c1 vs. c2; for number of treatments see Table 2). The analyses were performed in S-Plus 6.1.

Results & Discussion

TBARS values significantly increased with time of display ($P < 0.05$). After thawing (day 1), the meat samples gave low TBARS values ($< 0.25 \mu\text{g malondialdehyde/g meat}$). These values increased considerably during storage in the illuminated chill cabinet (Table 2). TBARS values on fresh broiler meat samples from the same treatments (reported by Smet et al., 2005) similarly increased during display, but the increase was much more marked for the frozen/thawed samples in this study. So, freezing slows down but does not stop the oxidation process. As lipid-free radicals are soluble in the lipid fraction and more soluble at lower temperatures, it allows them to diffuse to longer distances and to spread the reaction (Kanner, 1994).

Compared to all other antioxidant treatments, the treatment with 200 ppm αTAc resulted in the lowest TBARS values ($< 0.6 \mu\text{g malondialdehyde/g meat}$). Even after 11 days of refrigerated storage, these meat samples still had acceptable values. Also in literature, it has been clearly demonstrated that αTAc supplementation results in good oxidative stability (e.g. Botsoglou et al., 2003; Coetzee & Hoffman, 2001; Lopez-Bote et al., 1998).

For the other antioxidant treatments, some dose effects were observed. Grape seed and tomato showed lower TBARS values at 200 vs. 100 ppm (c5 – c10; c7 – c12; $P < 0.05$), while αTC and rosemary showed no difference between the two doses (c3 – c8; c4 – c9; $P > 0.05$). In contrast, TBARS values were much higher for the 200 ppm compared to the 100 ppm green tea treatment (c6 – c11; $P < 0.05$). For the combined extracts, some trends for an antioxidative dose effect were observed ($\text{TBARS}_{100\text{ppm}} > \text{TBARS}_{200\text{ppm}}$), however this was not significant. These findings confirm the work of Lau et al. (2003) for grape seed. However for green tea, Tang et al. (2000) observed a clear antioxidative dose-response effect, which is in contrast with our results. The different effects between our study and the one of Tang et al. (2000) could be due to a different content of catechins present in the green tea extracts.

Possible synergistic effects were investigated by making combinations vs. single antioxidant additions. However, few synergistic effects were revealed because of the high variation between different pen repetitions. Nevertheless, the combination of αTC and green tea at 200 ppm yielded significantly ($P < 0.05$) lower TBARS values compared to 200 ppm αTC or 200 ppm green tea alone, which may suggest a synergistic effect between αTC and green tea at this dose. Further, the combination of rosemary and green tea at 100 and 200 ppm resulted in significantly higher TBARS values than 100 ppm rosemary or green tea alone. This suggests that the combination of both extracts gave a lower oxidative stability than the single addition. Few data are available about possible synergistic effects of natural extracts on the oxidative stability of meat. Basmacioglu et al. (2004) reported a synergistic effect for oregano and rosemary essential oils in broilers at a dose of 300 ppm. Also a synergistic action between oregano oil and αTAc (200 ppm)

resulted in a higher oxidative stability than 200 ppm α TAc alone in turkeys (Botsoglou et al., 2003; Papageorgiou et al., 2003).

As oxidation is a very general process, also proteins, DNA, pigments and carbohydrates are affected (Kanner, 1994). Therefore, oxidative stability should not only be evaluated by lipid oxidation (Dotan et al., 2004). In this experiment, protein oxidation was also measured by the amount of thiol groups disappearing during storage. However, the thiol content at day 11 (Table 2) did not differ much between dietary treatments and did also not differ from our results on the fresh broiler meat (Smet et al., 2005), suggesting no effect on protein oxidation.

Conclusions

During frozen storage, the oxidative stability of broiler meat decreased, which resulted in higher TBARS values compared to fresh meat evaluated in a previous study. Yet, for protein oxidation no differences were revealed compared to the fresh meat. The addition of different antioxidants to the feed influenced lipid stability. The highest oxidative stability of meat was obtained with 200 ppm α -tocopheryl acetate. Grape seed and tomato showed an antioxidative dose-response effect. In contrast, the addition of green tea resulted in a pro-oxidative dose-response effect. However, the combined use of α -tocopherol and green tea resulted in a net antioxidative effect.

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Tables and Figures

Table 1: Specifications of the antioxidant extracts (supplied by Nutri-AD International, Belgium)

Extract	Specification
Synth antioxidant	BHT/ethoxyquin/BHA
α -tocopheryl acetate	500 IU/g
α -tocopherol	700 mg/g mixed tocopherols with 80-150 mg α -tocopherol, 10-30 mg β -tocopherol, 350-450 mg γ -tocopherol, 140-200 mg δ -tocopherol
Rosemary	3% carnosic acid, 0.1% carnosol, 0.03% Me-carnosate
Green tea	Cafein 4% to 8%, EGCG > 14,5%, epicatechins > 2.4%, catechins (as epicatechins) > 60%, catechins (as EGCG) 23.25% to 26.75%
Grape seed	89% polyphenols, containing 3.1% catechins; 11.2% oligomeric procyanidins
Tomato	No information available

Table 2: Mean values for lipid oxidation (μg malondialdehyde/g meat) and protein oxidation (nmol free SH groups/mg protein) of the breast muscle stored in a chill cabinet during 1, 4 or 11 days (after 8 months of frozen storage)

Antioxidant treatment		Lipid oxidation				Protein oxidation	
		Day 1	Day 4	Day 11	SEM	Day 11	SEM
1	Synthetic	0.16	0.45	1.09	0.17	70.7	2.39
2	Synthetic + 200 ppm αTAc	0.04	0.31	0.51	0.16	76.6	3.67
3	100 ppm $\alpha\text{-tocopherol}$ (αTC)	0.19	0.38	1.42	0.34	71.7	2.07
8	200 ppm $\alpha\text{-tocopherol}$	0.20	0.53	1.65	0.39	85.5	11.1
4	100 ppm rosemary (ros)	0.12	0.80	2.44	0.50	76.9	1.77
9	200 ppm rosemary	0.20	0.45	1.14	0.17	70.9	3.00
5	100 ppm grape seed	0.20	2.02	4.32	0.81	77.0	5.38
10	200 ppm grape seed	0.11	0.49	1.06	0.16	79.7	2.38
6	100 ppm green tea	0.20	0.48	1.02	0.15	67.8	8.38
11	200 ppm green tea	0.23	1.39	4.51	0.66	68.5	0.80
7	100 ppm tomato	0.22	2.23	5.90	0.86	61.7	8.88
12	200 ppm tomato	0.19	0.61	2.96	0.47	75.3	0.84
13	αTC + ros (100 ppm)	0.17	0.33	2.24	0.37	70.6	3.35
16	αTC + ros (200 ppm)	0.18	0.30	1.31	0.24	76.0	2.77
14	αTC + tea (100 ppm)	0.18	0.30	1.23	0.28	77.6	2.39
17	αTC + tea (200 ppm)	0.14	0.34	1.01	0.15	73.0	4.32
15	Ros + tea (100 ppm)	0.22	1.54	4.32	0.89	67.4	9.19
18	Ros + tea (200 ppm)	0.22	0.78	3.06	0.67	72.8	0.20
19	αTC + ros + tea (100 ppm)	0.24	0.53	1.88	0.30	86.0	5.76
20	αTC + ros + tea (200 ppm)	0.23	0.47	2.31	0.46	69.3	0.29

INFLUENCE OF SOME COMBINED PRESERVATIVE FACTORS ON THE SHELF LIFE OF “MARANHOS”

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Key Words: meat products, sodium nitrite, ascorbic acid, sodium metabisulphite, potassium sorbate.

Introduction

“Maranhos” (Figure 1) is a goat meat sausage traditionally homemade in Beira Baixa region in the center of Portugal, where it is a very popular dish. It is a product with low price raw materials, easily perishable, with a reasonable composition, reasonable energetic value and low salt content (Salavessa & Barreto, 2003).

The ingredients hygienic quality is generally low therefore the raw product is heavily contaminated with microorganisms. Sometimes pathogens can be present. The shelf life of the raw product is very short and, therefore, it can be considered a dangerous product because it may be a source of cross contamination to other foods. The heat processing of the product improves its hygienic standards and eliminates vegetative forms of pathogens but not all spore forms. However, it cannot be overestimated because there is a risk of recontamination during assembly that may present a food safety hazard. The shelf life of this heat-processed product depends on the control of many factors. The growth of microbiological flora can be controlled by product formulation, packaging systems and chill storage conditions, so modifications based on technology and combined preservative factors should be researched in order to develop a hurdle technology able to prevent microbiological and chemical spoilage as well as the risk of food borne diseases to the consumer (Salavessa & Barreto, 2004).

Sodium nitrite (E250) is frequently used as antimicrobial and color preservative as well as flavor enhancer. Ascorbic acid (E300) enhances the action of nitrites on meat pigments, stabilizes the color of meat, and inhibits growth of microorganisms and formation of N-nitrosamines. Potassium sorbate (E202) acts like an antimicrobial preservative especially on Fungi broad spectrum. Sodium metabisulphite (E223) is generally used as an antimicrobial, antioxidant and bleaching agent (Smith & Hong-Shum, 2003).

Objectives

The combined preservative action of sodium nitrite (E250), ascorbic acid (E300), potassium sorbate (E202), and sodium metabisulphite (E223) has been tested in order to get a shelf life extension of “Maranhos”. The potential synergic effect of using these preservatives together against food borne pathogens and spoilage flora is evaluated. We

hope to improve the shelf life of this product, which is very important in order to expand the selling market.

Methodology

Two different batches of “Maranhos” were prepared and the experience was repeated four different times. “Maranhos” were prepared from raw ground adult goat meat mixed with rice and seasoned with salt, peppermint (*Mentha* sp.) and white wine. All the ingredients were then stuffed into natural casings, small bags especially made from the goat gastric compartments. Batch L1 was prepared according to the traditional recipe, 1% NaCl added, boiled for 75 minutes and then cooled in current water and vacuum packaged according the technological procedures used by a local manufacturer. In batch L2, salt was added with nitrite (1.5%) and ascorbic acid (1.5%). After the boiling process, the product was cooled inside a water solution containing sodium metabisulphite (1%) and potassium sorbate (2,5%). All the batches were brought to the laboratory under cool conditions and stored at 4°C, random samples were collected and analyzed at 0, 7, 14, 21 and 28 days of storage.

pH was measured with a pH-meter HI9023-HANNA INSTRUMENTS and Water activity (aw) was measured with the ROTRONIC HYGROSKOP DT, with the measure cell WA-14TH at 25°C of constant temperature as described by Martins & Patarata (1993).

For the microbiological analysis, 25 g sample of each sausage was aseptically transferred to a sterile plastic bag and pummeled in a stomacher LAB BLENDER-400 with 225 ml of buffered peptone water (DIFCO). Decimal dilutions of suspension were prepared using triptone salt solution (SCHARLAU) and plated in duplicate on different growth media. The following media and incubation conditions were used: total aerobic in Plate Count Agar (SCHARLAU) at 30°C for 2 days; total psychrophiles counts in Plate Count agar (SCHARLAU) at 7°C for 10 days; total anaerobes count in Anaerobic Agar acc. to Brewer (MERCK) inside an anaerobic jar at 7°C for 10 days; total thermophiles counts in Plate Count agar (SCHARLAU) at 42°C for 2 days; moulds and yeasts counts in Cook Rose Bengal agar with chlorophenicol (OXOID) at 25°C for 5 days; Enterobacteriaceae counts in Violet Red Bile agar (OXOID) at 37°C for 2 days; lactic acid bacteria counts on Man Rogosa Sharpe agar (OXOID) at 30°C for 3 days; *E. coli* detection with Kovacs reagent in Brilliant Green broth (SCHARLAU) and Peptone water (DIFCO) at 45°C for 2 days; Clostridium sulphite reducers spores detection in Sulfadiazine Polimyxine Sulphite agar (MERCK) at 45°C for 2 days; *S. aureus* detection by the coagulase test after isolation of suspicious colonies in Baird Parker agar (OXOID) and then Brain Heart Infusion (DIFCO) at 37°C for 1 day; *Salmonella* spp. identification by biochemical test API 20E (BIOMÉRIEUX) after isolation of suspicious colonies; and *Listeria monocytogenes* identification by biochemical test API Listeria (BIOMÉRIEUX) after isolation of suspicious colonies.

Means of results were compared using paired-samples T test to verify the significance of differences between treatments, L1 and L2, with SPSS 12.0.1 statistical package for Windows.

Results & Discussion

The evolution of pH, aw and the microbiological results during the storage time are shown in Table 1. It is possible to observe that the pH values drops more quickly in batch L1 than in L2, indicating a bigger incipient spoilage rate in L1, this difference was observed to be very significant at 28 days of storage. In relation to aw no significant differences were observed between batches, presenting values of 0.948 in L1 and 0.946 in L2 in day 0, remaining practically constant along the storage time. Due to the product characteristics and especially by the observation of the pH and aw values, both batches results as easily perishable meat products.

In this kind of meat product after the cooking step, usually it seems that only sporulating forms of bacteria can resist, generally came from some vegetable ingredients that can harbour sporulating forms of *Bacillus* that are used in products composition. The presence of microbial flora in product 24 hours after packaging is commonly due to the post-cooking contamination of the product that happens during the cooling and packaging steps (Borek et al., 2002).

In the aerobic plate counts very significant differences ($p < 0.01$) were observed since storage day 7, when L1 products already presents 6.68 log cfu/g. The combined preservatives tested in L2 had a positive effect decreasing the initial microbial load of the packed product, it was also verified an inhibition of spoilage flora, presenting only 1.89 log cfu/g at the end off the trail, without changes on pH parameter. It was possible to observe that psychrophiles and anaerobes total counts were always quite similar to the aerobic plate counts. These presences were higer in batch L1 being significantly ($p < 0.01$) different from batch L2. L1 started with 3.60 log cfu/g and 3.08 log cfu/g for psychrophiles and anaerobes counts respectively and finished the trail with 8.09 log cfu/g and 7.87 log cfu/g. In Moulds, no significant differences were observed between L1 and L2 without any increase in their counts during all the storage time. This is effect of the vacuum package action on moulds growth, once these kinds of microorganism are strictly aerobe. In relation to yeast counts, we observed very significant ($p < 0.01$) differences between batches L1 and L2 in days 0, 7 and 14. L1 reveled always a higher level of this kind of Fungi, which plays an important role on the shelf life of this meat product. In L2 it was noted the known inhibitory action of potassium sorbate on the growth of the Fungi population during all storage time. No growth of Enterobacteriaceae was verified in L2, the counts were significantly different ($p < 0.01$) from L1 with values of 1.56 log cfu/g and 7.23 log cfu/g respectively on day 0 and 28. Similar effect was observed in relation to lactic acid bacteria, an important dominating group of the spoilage population. The potential antimicrobial effect of sodium metabisulphite on the microbiological safety of the product is noted by no *Staphylococcus aureus* coagulase positive presence in 1g of L2 product as well as no detection of *E. coli* or *Clostridium* sulphite reducers' spours (absent in 1 g), *Salmonella* spp. and *Listeria monocytogenes* (absent in 25 g), something that didn't happen in L1 where sometimes were detected the presence of *Listeria monocytogenes* and *Staphylococcus aureus* coagulase positive.

Conclusions

According to the results found in this work, the combined effect of tested additives in L2 have a positive effect on extension of microbiological shelf life of “Maranhos”, that can go over 30 days. Besides, this combination does not seem to decrease the sensory attributes of the product, however further work must be done in order to prove that the use of these combined substances does not take risk to the consumer as well to the typical sensory properties of the final product.

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Tables and Figures

Table 1 – pH, a_w and microbiological counts (log cfu/g).

		Days				
		0	7	14	21	28
pH	L ₁	6.31	6.28	6.16	5.94	5.85**
	L ₂	6.24	6.26	6.24	6.13	6.11**
a_w	L ₁	0.948	0.950	0.948	0.944	0.943
	L ₂	0.946	0.945	0.945	0.947	0.944
<i>Aerobic</i>	L ₁	3.73	6.68**	7.59**	8.20**	8.22**
	L ₂	1.76	2.02**	1.72**	1.44**	1.89**
<i>Termophiles</i>	L ₁	1.87	3.08**	4.55	4.89*	4.51*
	L ₂	1.01	1.48**	0.76	1.09*	0.82*
<i>Psicrophiles</i>	L ₁	3.60	6.94**	7.64**	8.27**	8.09**
	L ₂	1.00	0.54**	0.75**	1.15**	1.62**
<i>Anaerobes</i>	L ₁	3.08	6.81**	7.65**	8.27**	7.87**
	L ₂	0.64	0.41**	0.25**	0.25**	0.65**
<i>Moulds</i>	L ₁	0.50	0.50	0.62	0.65	0.25
	L ₂	1.05	0.54	0.33	0.37	0,00
<i>Yeasts</i>	L ₁	1.95**	2.97**	3.47*	3.73	3.90
	L ₂	0.50**	0.00**	0.25*	1.79	0.58
<i>Enterobactereacea</i>	L ₁	1.56	4.69**	6.31**	6.96**	7.23**
	L ₂	0.00	0.00**	0.00**	0.00**	0.00**
<i>Lactic acid bacteria</i>	L ₁	0.99	4.21**	6.37**	6.90**	7.27**
	L ₂	0.00	0.00**	0.25**	0.25**	0.00**

Significant * $p < 0.05$, very significant ** $p < 0.01$



Figure 1 – “Maranhos”.

EFFECT OF ANTIOXIDANTS ON FRESH PORK SAUSAGE, AND FISH OIL FORTIFIED GROUND BEEF

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Key Words: Antioxidant, fish oil, fortification, color, lipid oxidation

Introduction

Muscle food products comprise a major portion of the average American's diet and have the potential to contribute a substantial quantity of long chain (LC) n-3 fatty acids on a daily basis. Ollis et al. (1999) reported that canola oil and fish were the primary sources of linolenic acid and LC n-3 fatty acids in the Australian diet, respectively. However, the authors specifically noted the unexpected finding that meat products were a major source of LC n-3 polyunsaturated fatty acids (PUFAs) (i.e. 29% of total dietary contribution). Although meat is not generally considered a rich source of LC n-3 PUFAs, meat products could be an efficacious delivery medium for LC n-3 PUFA.

One potential challenge presented by increasing the PUFA of meat products, whether by dietary supplementation of the live animal or process fortification of the meat raw material, is greater susceptibility to lipid oxidation. The degree to which PUFA alterations have affected flavor has ranged from no effect (Leskanich et al., 1997) to a significant sensory detection of off-flavors (Coxon et al., 1986; O'Keefe et al., 1995). Utilization of antioxidants to minimize lipid oxidation in meat products, with or without alteration of the fatty acid profile, has been an effective means to delay off-flavor development (Crawford et al., 1976; Faustman, 1993).

Increasing the LC n-3 PUFA concentration of fresh meat products is a logical approach for delivering this nutrient in a 'functional food'. However, it is critical that the increased oxidative susceptibility expected from this processing approach is anticipated and addressed.

Objectives

To determine the suitability of antioxidant systems for ground beef fortified with stabilized fish oil vs. non-stabilized (unstabilized) fish oil, and for non-fortified fresh pork sausages.

Methodology

Preparation of Meat Products

Fresh pork sausages

Fresh boneless pork shoulder butts were ground coarsely (19.2 mm), mixed with breakfast sausage seasoning containing salt, red pepper, sage, sugar and black pepper (No.25, A.C.Legg Packing Co., Inc., Birmingham, AL), mixed with antioxidants (DL α -tocopherol, 95% liquid mixed tocopherol, BHA/BHT, rosemary extract), ground fine, and formed into patties (5 cm dia, 1.5 cm thick, 25 g). Patties were wrapped with oxygen-permeable PVC film ($15,500$ - $16,275$ cm³ O₂/m²/24 h at 23 °C) and stored at 4°C or -18°C. Analyses were performed on patties stored fresh for 0, 2, 4 and 6 days, and frozen for 0, 3, and 6 weeks.

Controls were pork sausages only (CON) or pork sausages containing the amount of coconut oil used to deliver antioxidants (CON+COCONUT)(i.e. DL α -tocopherol, 95% liquid mixed tocopherol, BHA/BHT and rosemary extract). Treatments were DL- α -tocopherol, A-TOC (0.03%, w/w based on fat content); 95% liquid mixed tocopherol, 95MIX-TOC (0.03%, w/w based on fat content); butylated hydroxyanisole/butylated hydroxytoluene, BHA/BHT (0.02%, w/w based on fat content); rosemary extract, ROSE (0.2%, w/w).

Ground beef patties

Fresh coarse ground beef (85% lean, 15% fat), obtained locally, was mixed with fish oil and antioxidants (mixed tocopherol, ascorbyl palmitate, rosemary extract, and butylated hydroxyanisole/butylated hydroxytoluene), ground fine (5 mm) and formed into patties (5 cm dia, 1.5 cm thick, 25 g). Patties were packaged on trays with oxygen-permeable PVC film ($15,500$ - $16,275$ cm³ O₂/m²/24 h at 23 °C) and stored at 4°C or -18°C. Analyses were performed on patties stored fresh for 0, 2, 4 and 6 days, and frozen for 0, 3, and 6 weeks.

Unstabilized fish oil (U-OIL), a refined blend of fish oil triglycerides (ROPUFA '30' n-3 Food Oil) and the same product stabilized with mixed tocopherol and rosemary (S-OIL), were obtained from DSM Nutritional Products Inc. (Belvidere, NJ, USA). Controls were ground beef only (CON), ground beef containing unstabilized fish oil (U-OIL) or ground beef containing fish oil commercially stabilized with mixed tocopherol and rosemary (S-OIL). Treatments were stabilized oil with mixed tocopherol, S-OIL+TOC; stabilized oil with mixed tocopherol and rosemary extract, S-OIL+TOC+ROSE; stabilized oil with butylated hydroxyanisole/butylated hydroxytoluene (S-OIL+BHA/BHT); stabilized oil with ascorbyl palmitate (S-OIL+AP); stabilized oil with ascorbyl palmitate and mixed tocopherol (S-OIL+AP+TOC); stabilized oil with ascorbyl palmitate and rosemary extract (S-OIL+AP+ROSE); stabilized oil with ascorbyl palmitate, mixed tocopherol and rosemary extract (S-OIL+AP+TOC+ROSE). TOC was added at 0.03% (w/w based on fat content); ROSE was added at 0.2% (w/w based on fat

content); AP was added at 0.02% (w/w based on fat content); BHA/BHT was added at a maximum level of 0.02% (w/w based on fat content).

Measurement of Color and Lipid oxidation

Colorimetric values (CIE L*, a*, b*) were determined by measurement with a Minolta Chromameter (Model CR-200b, Minolta Co., Osaka, Japan) and hue angle was calculated as $\tan^{-1} (b^*/a^*) \times 360^\circ / 2$ (Chan et al., 1996). The thiobarbituric acid (TBA) procedure of Yin et al. (1993) was used to assess lipid oxidation and reported as TBA-Reactive Substances (TBARS).

Results & Discussion

Fresh pork sausages

A decrease in a* value and increase in hue angle are each consistent with discoloration of fresh meat. A-TOC was most effective for maintaining color stability (greater a* values, lower hue angle values) during storage at 4°C when compared to other treatments ($p < 0.05$) (results not shown). While increased muscle concentrations of tocopherol have proved effective at slowing meat discoloration via dietary supplementation of beef cattle with vitamin E, the effect has not been well documented for vitamin E as an exogenous ingredient (Faustman et al., 1998).

The results for the effect of antioxidant treatments on lipid oxidation (TBARS) in fresh and frozen pork sausage patties are presented in Figure 1. In general, all antioxidant treatments were effective at minimizing lipid oxidation relative to the respective controls (CON and CON+COCONUT). There was no difference between the different antioxidants during storage at 4°C ($p > 0.05$), whereas BHA/BHT was most effective at minimizing lipid oxidation during storage at -18°C when compared to A-TOC, 95MIX-TOC or ROSE ($p < 0.05$).

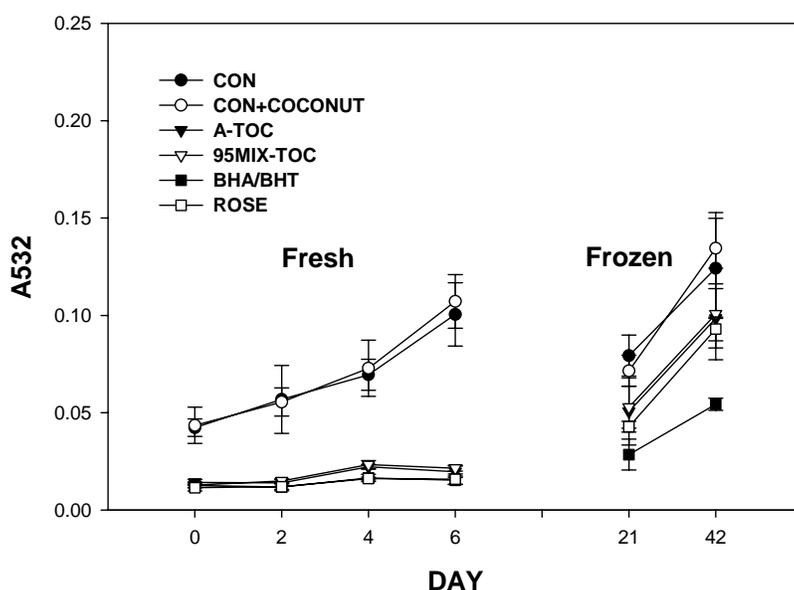


Figure 1. Changes in lipid oxidation (TBARS) of control (CON), control with coconut oil (CON+COCONUT), DL α -tocopherol (A-TOC), 95% mixed tocopherol (95MIX-TOC), BHA/BHT and rosemary extract (ROSE) treated fresh pork sausages stored at 4°C or -18°C. Standard deviation bars are indicated.

Ground beef patties

All antioxidant treatments except S-OIL+AP stabilized color when compared to controls (CON, CON+UOIL and CON+SOIL)($p < 0.05$)(results not shown). There were no consistent effects of antioxidant treatments on hue angle during storage ($p > 0.05$) (results not shown).

The effects of antioxidant treatments on lipid oxidation (TBARS) in fresh and frozen ground beef patties are presented in Figure 2. As expected, the lipid oxidation was considerably high in beef patties fortified with non-stabilized (unstabilized) fish oil. On the other hand, when fortified with S-OIL (ROPUFA 30 Food Oil, which is stabilized), the lipid oxidation was actually lower than the control at refrigerated conditions. The oxidation was further reduced by incorporating antioxidant systems. BHA/BHT followed by TOC + ROSE showed the greatest antioxidant effect during storage. There was no significant lipid oxidation and no effects of antioxidants were observed during frozen storage ($p > 0.05$). The combination of AP, TOC and ROSE delayed lipid oxidation more effectively relative to each of these antioxidants alone. Calvert and Decker (1992) suggested that antioxidant combinations would inhibit lipid oxidation more effectively than the use of any single antioxidant, and synergism might result from the action of mixed free radical scavengers.

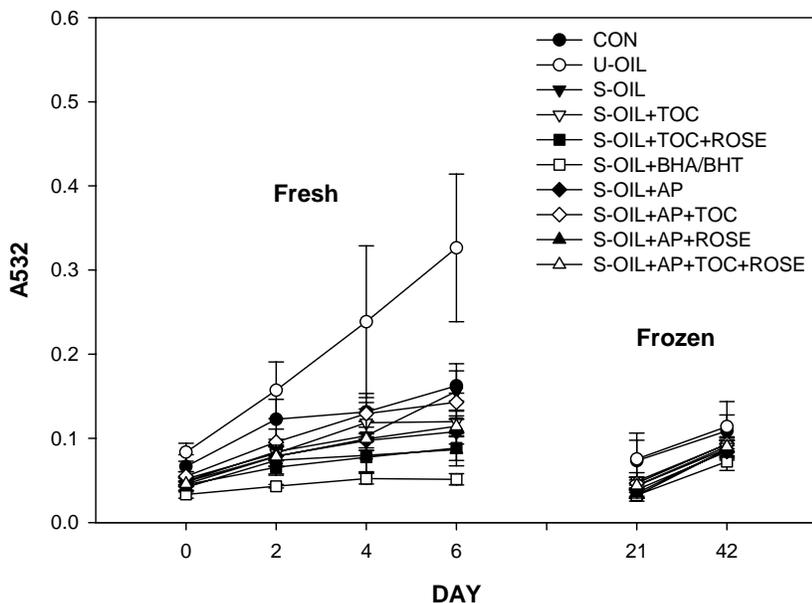


Figure 2. Lipid oxidation (TBARS) in control (CON), control with unstabilized fish oil (U-OIL), control with stabilized fish oil (S-OIL), stabilized fish oil + mixed tocopherol (S-OIL+TOC), stabilized fish oil + mixed tocopherol + rosemary (S-OIL+TOC+ROSE), stabilized fish oil + butylated hydroxyanisole/butylated hydroxytoluene (S-OIL+ BHA/BHT), stabilized fish oil + ascorbyl palmitate (S-OIL+AP), stabilized fish oil + ascorbyl palmitate + mixed tocopherol (S-OIL+AP+TOC), stabilized fish oil + ascorbyl palmitate + rosemary (S-OIL+AP+ROSE), and stabilized fish oil + ascorbyl palmitate + mixed tocopherol + rosemary (S-OIL+AP+TOC+ROSE) treated ground beef patties during storage at 4°C (fresh) or -18°C (frozen). Standard deviation bars are indicated.

Conclusions

Antioxidants of tocopherol product type were incorporated into fresh pork sausages and proved effective at minimizing lipid oxidation. A-TOC appeared most effective for stabilizing color in fresh pork sausages. Stabilized fish oil appeared to be more suitable for incorporation in ground beef patties when compared to unstabilized fish oil. An antioxidant combination containing mixed free radical scavengers (mixed tocopherols and rosemary) incorporated into LC n-3 PUFA fortified ground beef patties effectively minimized lipid oxidation.

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EFFECTS OF CARNOSINE AS ANTIOXIDANT IN TAIWANESE SILKY FOWL COMPARED WITH BROILER

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Key Words: Antioxidant · Carnosine · Taiwanese silky fowl · Broiler

Introduction

Carnosine is a naturally occurring skeletal muscle peptide possessing β-alanine and histidine. CHAN et al. (1994) noted the carnosine antioxidant to be present in endogenous skeletal muscle. DECKER and FARAJI (1990) observed carnosine to inhibit lipid oxidation and oxidative rancidity in salted and cooked ground pork (DECKER and CRUM, 1991;1993). The antioxidant activity of this compound is expressed by various mechanisms and metal chelation and free radical scavenging have also been noted as additional functions (DECKER et al., 1992).

Silky fowl (**Photo 1**), in addition to serving as pet, has been found ideal for medical research. The bird contains the ingredients for use in Chinese medicinal food and wine preparations, thus demonstrating its commercial value. A long time ago, a Chinese Dr. LEE (1578) has reported that certain constituents from this fowl may be used for treating dizziness, heartache and impotence. In Japan, blood powder, silky fowl egg yolk oil and vinegar pickled egg are among various products that can be prepared from this bird.

The authors previously noted in Taiwanese silky fowl (TSF) higher protein and lower fat content compared to broiler, with broiler collagen content being 7 times less than in the former (LIN and WU, 2000ab). In TSF, IMP is higher and meat flavor is superior. SDS-PAGE results indicate TSF and broiler muscle to be similar, though thigh muscle of the former has an additional band at the 45KD position. This band was shown due to predominant melanin content (WU, 1998). KAN and KAN (1992) noted silky fowl to be rich in Fe²⁺, Ca²⁺ and Zn²⁺.



Photo 1 Silky fowl ♂

Objectives

In the present study, carnosine content in TSF and broiler was compared with respect to antioxidant capacity and in muscle, Fe²⁺ content was also determined. The results were used to determine medical application.

Methodology

Chicken preparation

Fifty broilers (Aber Acre, twenty-five males, twenty-five females, average weight 1.6 kg) were reared for a period of 6 weeks. Fifty TSFs (twenty-five males, twenty-five females, average weight 1.4 kg) were maintained for 14 weeks on a corn-soybean diet procured commercially. Breast and thigh meat samples were taken and stored at -20°C until use.

Carnosine determination

This compound was taken from breast and thigh muscle of both species, as described by CHAN et al. (1993). Muscle specimens, after thawing, were homogenized (Waring Blender, Model 31BL91, USA), centrifuged $10,000\times g$ (Sigma, 2k15), 4°C , 30min heating at 100°C , 10 min then centrifuged $2000\times g$, 4°C , 30min (Sigma, 2K15), demineralized and freeze-dried.

Carnosine content was determined as follows:

Carnosine content (%): $\text{Weight of carnosine after freeze-dried} / \text{Weight of muscle} \times 100$

Antioxidant activity determination

1. Ferric thiocyanate method (MITSUDA et al., 1966)
2. **Take 0.2 ml (1 mg/ ml) sample extract, standard (BHA) or control (deionized water)**
3. 10 ml linoleic acid / methanol solution addition (0.13:10, v/v)
4. 10 ml 1/30M phosphate buffer (pH 7.0) addition
5. Distilled water addition to 25 ml (5) Incubation at 40°C
6. Antioxidant activity measured every 24 hrs as follows:
 - (1) Take 0.2 ml above solution
 - (2) Add 9.4 ml 75% methanol (Merck)
 - (3) Add 0.2 ml $\text{NH}_4(\text{SCN})$ liquid
 - (4) Add 0.2 ml NaCl liquid with FeCl_2
7. At 3 minutes reaction time, absorbance is measured at 500 nm (Spectrophotometer U-2000, Hitachi, Japan).

Reduction capacity

This parameter was determined according to OYAIKU (1986), using Prussian blue $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ (Sigma) as an index. Maximum absorbance was noted at 700 nm. Higher absorbance was indication of greater reduction capacity.

Effects of chelating on Fe^{2+}

These effects were examined in accordance with the method of DINIS et al. (1994), which involves a color reaction of the Fe^{2+} /ferrozine complex at 562 nm. Chelation was

found to reduce absorbance at 562 nm. Lower absorbance would thus be indication of the effect of this process.

Determination of total iron

This determination was made as indicated by SCHRICKER et al. (1982). Samples were ashed at 525°C over 12 hrs, dissolved in 5 ml 1N-NH₄Cl, maintained at 100°C in a hot-water bath for 20 min, filtrated through filter paper (Whatman 4) treated with sulfonated bathophenanthroline and incubated for 10 min. Absorbance was measured at 540 nm.

Statistical analysis

The General Linear Model Procedure of the SAS Institute (SAS, 1989) was carried out for this analysis. Significance was assessed by the Student's T-test (MULLEN and PUR, 1980).

Results & Discussion

Carnosine content and color

Broiler content values of carnosine in breast and thigh are shown to be 1.87% and 1.33% in **Fig. 1**, along with those for TSF as 1.9% and 1.53%. This content is clearly shown greater in breast meat than thigh ($p < 0.05$), though the amounts in the two species were essentially the same, as also noted by CHAN et al. (1993). The greater concentration of enzymes required for glycolysis in breast muscle may possibly be the reason for this. Owing to lack of oxygen, more lactic acid may be produced in the breast so as to decrease pH. The greater amount of carnosine would serve to buffer pH and reduce rancidity due to lipid oxidation (DAVEY, 1960).

TSF meat was deeper dark in color, compared to that from broilers and even more so in the case of thigh meat. Breast was whitish in color, which is peculiar to TSF. Hemoglobin and myoglobin content in the thigh may be the factors responsible for the darker meat color of this compound.

Comparison of antioxidant capacity

Ferric thiocyanate method

This method was used here for antioxidant activity assessment, in which the high energy of hydroperoxide produced by lipid oxidation to oxidize Fe²⁺ to Fe³⁺ is taken into consideration. Fe³⁺, by reaction with SCN, may become red Fe (SCN)²⁺ whose absorbance at 500 nm is maximum. At high lipid oxidation, hydroperoxide increases and takes on a darker color. Based on absorbance at 500 nm, antioxidation capacity may thus be determined (MITSUDA et al., 1966).

The conditions for measuring standard carnosine antioxidation are specified in **Fig. 2**. This process was noted to be enhanced with increase in carnosine concentration. With 1 mg carnosine addition on day 3 of storage, oxidation became significantly greater.

Subsequent to 5, 10, 20 and 50 mg additions, on the third day, antioxidation was still strong and significantly more compared to that noted for the 1 mg addition ($p < 0.05$). On the 6th day, antioxidation for different additions was basically the same. Antioxidation is thus shown to significantly increase with carnosine concentration. At more than 20 mg carnosine addition, antioxidation was strong but not sufficient to be of any economical significance. Carnosine antioxidation may possibly become less with storage, perhaps due to lipid oxidation.

Antioxidation by BHA (butylated hydroxyanisole) and carnosine for the two species is compared in **Table 1**. Control treatment antioxidation can be seen to have decreased significantly on the first day of storage ($p < 0.05$). BHA antioxidation was high throughout 7 days storage. Antioxidation by TSF and broiler carnosine had decreased at 4 days storage and was entirely absent on day 6. Antioxidation by carnosine is thus shown less than by BHA but more than by control treatment. The reason for lesser carnosine antioxidant activity may be that this compound was unable to eliminate hydroperoxide continuously in linoleic acid emulsion that caused the complete loss of antioxidation by 6 days storage. Breast meat antioxidation was greater than in thigh, possibly owing to greater Fe^{2+} content which would diminish antioxidant capacity. At 5 and 10 mg carnosine, antioxidation can be seen from Table 1 to increase with concentration. This was also noted for carnosine obtained from Sigma. With TSF carnosine, antioxidation was greater than in broilers during storage. At 1~3 days storage, the differences were significant ($p < 0.05$). Whether melanin or other constituents in TSF contribute to the greater carnosine antioxidation is a point that should be clarified through further study.

Comparison of reduction capacity

Reduction capacity was measured based on Prussian blue as an index. $\text{K}_3\text{Fe}(\text{CN})_6$ is reduced to $\text{K}_4\text{Fe}(\text{CN})_6$ and then reacts with Fe^{3+} to form Prussian blue. Changes in absorbance at 700 nm serve as basis for assessment of reduction capacity. At higher absorbance, reduction has been shown more extensive (OYAIZU, 1986).

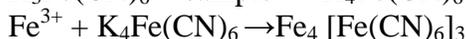


Fig. 3 shows the reduction capacity of standard carnosine at different concentrations. This capacity was noted to rise with increase in the concentration of this compound. Reduction was noted to differ significantly at different concentrations ($p < 0.05$). Reduction capacity in breast meat for the two species is compared in **Fig. 4**. This capacity was maximum with BHA treatment and significantly more than by any other treatment ($p < 0.05$). 10 mg carnosine exhibited considerably greater reduction capacity than 5 mg carnosine. This capacity in breast meat was greater than in thigh for either species. TSF carnosine showed greater reduction compared to broilers ($p < 0.05$). PITOTTI et al. (1995) maintains that antioxidation by Maillard reaction products (MRP) is significantly correlated with reduction capacity by reductone. The two amino acids that constitute carnosine may possibly provide electrons that inhibit fatty autoxidation (ANTHONY et al., 1980). It thus follows that carnosine also has antioxidant capacity. Antioxidation by carnosine would not derive solely from reduction capacity.

Fe²⁺ chelation

In the redox cycle, only a few metal ions efficiently produce free radicals so as to expedite lipid oxidation (BROWN, 1988; GORDON, 1990; DECKER et al., 1992). Fe²⁺ has been shown the greatest accelerator of lipid oxidation (YAMAGUCHI et al., 1988). Using the Fe²⁺ complex color reaction and ferrozine at 562 nm, the chelation of Fe²⁺ sample was measured.



Fig. 5 shows the chelation of Fe²⁺ by carnosine at different concentrations and the extent to increase with concentration. Comparison was made at 50 mg > 20 mg > 10 mg > 5 mg carnosine and the differences were significant. Carnosine chelating capacity may derive primarily from the imidazol ring that may form compounds with metal ions which function to inhibit active oxidation (BROWN, 1988; CHAN et al., 1993; LEE and HENDRICKS, 1977). This ring does not inhibit the oxidation of all ions, but in some cases, may actually promote this process. Carnosine has been shown to possibly inhibit lipid oxidation induced by Fe²⁺ via the elimination of free lipid radicals, but not by chelation. Carnosine was found in this study to actually chelate Fe²⁺.

Carnosine chelation of Fe²⁺ at different concentrations for breast and thigh meat of the two species is shown in **Fig. 6**. The extent was greater at 10 mg than 5 mg, though not significantly so, in breast meat than thigh (p<0.05). Greater Fe²⁺ content in the latter may possibly be the reason for this. With broiler carnosine, chelation was greater, possibly owing to Fe²⁺ content.

Total iron in TSF and broiler

Fig. 7 shows the values for total iron in TSF and broiler and these values to be higher in thigh than breast (p<0.05), this being due to higher content of hemoglobin and myoglobin in the former. Total iron of TSF was higher than in broilers (p<0.05), in agreement with the findings of KAN and KAN (1992). This greater content may contribute to greater carnosine chelation capacity in broilers compared to TSF, as shown in Fig. 6. The relation of total iron to melanin in TSF should be clarified in greater detail.

Conclusions

The antioxidant activity of carnosine in TSF and broiler was assessed and Fe²⁺ content in each species was determined. Carnosine was found more abundant in breast meat than thigh for either species, with over-all content greater in TSF. TSF displayed more intense dark color which, in both species, was more intense in the thigh than breast meat. The antioxidant effect of carnosine increased with its concentration. Antioxidant effect in all cases was greater for breast meat. During storage, TSF extract antioxidation was always greater. Carnosine reduction capacity varied significantly in proportion to concentration and was greater in TSF breast meat. Chelating capacity was in proportion to chelating iron in carnosine, being greater in breast meat from broiler, compared to thigh. Thigh total iron content was always higher than in breast meat, with that in TSF being significantly greater than in broilers.

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Tables and Figures

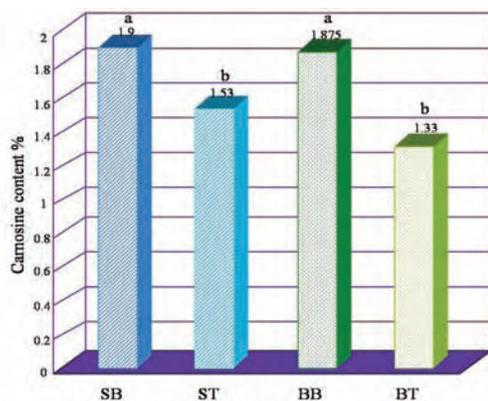


Fig. 1: Content of Taiwanese silky fowl (TSF) and broiler carnosine in breast and thigh
SB: Breast in TSF ST: Thigh in TSF
BB: Breast in broiler BT: Thigh in broiler
a,b Different subscripts on column tops indicate significantly different mean values (p<0.05)

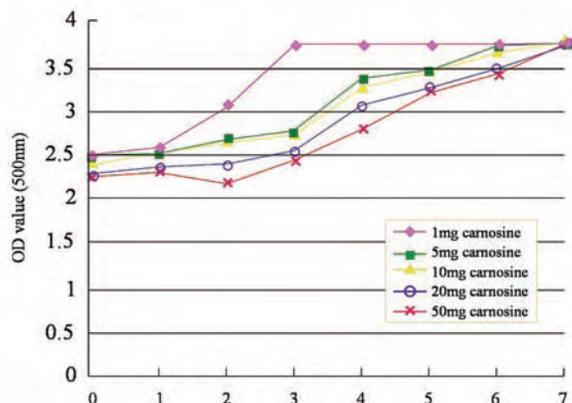


Fig. 2: Antioxidation of standard carnosine with different concentrations during storage (40°C)

Table 1: Antioxidation of carnosine in breast and thigh of TSF and broiler (40°C)
(OD value at 500 nm)

Day	Cont.	BHA	Broiler				TSF			
			Breast		Thigh		Breast		Thigh	
			1mg	5mg	10mg	5mg	10mg	5mg	10mg	5mg
0	2.38 ^{av}	2.33 ^{aw}	2.49 ^{ay}	2.49 ^{az}	2.54 ^{ay}	2.54 ^{az}	2.49 ^{az}	2.49 ^{az}	2.53 ^{az}	2.53 ^{ay}
1	2.95 ^{aw}	2.33 ^{cw}	2.64 ^{bcx}	2.53 ^{cz}	2.76 ^{bx}	2.71 ^{by}	2.58 ^{bcy}	2.44 ^{cz}	2.71 ^{byz}	2.58 ^{bcy}
2	3.51 ^{aw}	2.35 ^{cw}	2.68 ^{bcx}	2.64 ^{bcy}	2.85 ^{bx}	2.85 ^{by}	2.66 ^{bcy}	2.61 ^{bcy}	2.68 ^{bcyz}	2.64 ^{bcy}
3	3.70 ^{aw}	2.39 ^{cw}	2.88 ^{bx}	2.74 ^{by}	2.95 ^{bx}	2.90 ^{by}	2.61 ^{by}	2.69 ^{by}	2.93 ^{by}	2.75 ^{bx}
4	3.69 ^{aw}	2.43 ^{cw}	3.41 ^{bw}	3.24 ^{bx}	3.42 ^{bw}	3.37 ^{bx}	3.37 ^{bx}	3.27 ^{bx}	3.38 ^{bx}	3.34 ^{bx}
5	3.70 ^{aw}	2.45 ^{cw}	3.42 ^{bw}	3.42 ^{bw}	3.43 ^{bw}	3.38 ^{bx}	3.42 ^{bw}	3.38 ^{bx}	3.42 ^{bw}	3.42 ^{bw}
6	3.70 ^{aw}	2.52 ^{bw}	3.53 ^{aw}	3.71 ^{aw}	3.69 ^{aw}	3.71 ^{aw}	3.71 ^{aw}	3.62 ^{aw}	3.59 ^{aw}	3.54 ^{aw}
7	3.72 ^{aw}	2.54 ^{bw}	3.67 ^{aw}	3.71 ^{aw}	3.69 ^{aw}	3.71 ^{aw}	3.71 ^{aw}	3.71 ^{aw}	3.71 ^{aw}	3.71 ^{aw}

BHA : Butylated hydroxyanisole

^{a-c} Means within the same row without the same superscript are significantly different (p<0.05)

^{w-z} Means within the same column without the same superscript are significantly different (p<0.05)

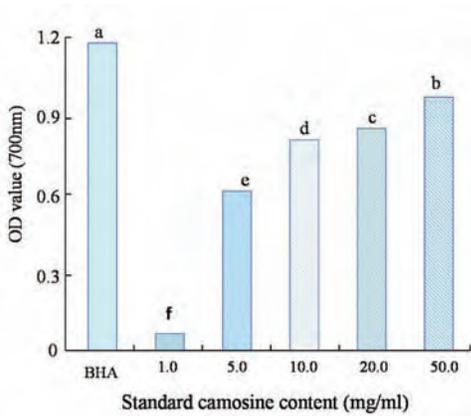


Fig. 3: Reduction capacity of standard carnosine with different concentrations
BHA : 1.0 mg/ml
a-f Different subscripts on column tops indicate significantly different mean values (p<0.05)

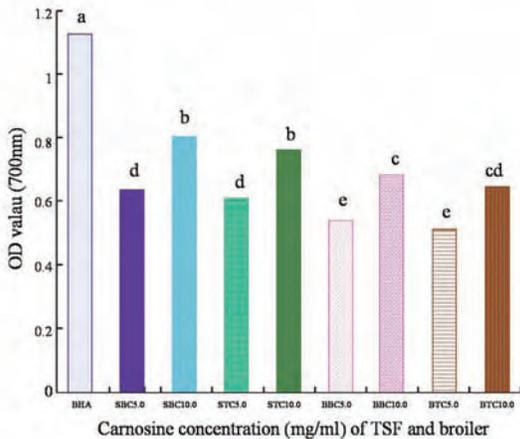


Fig. 4: Reduction capacity of TSF and broiler carnosine in breast and thigh
BHA : 1.0 mg/ml
SBC: Carnosine in breast of TSF
STC: Carnosine in thigh of TSF
BBC: Carnosine in breast of broiler
BTC: Carnosine in thigh of broiler
a-e Different subscripts on column tops indicate significantly different mean values (p<0.05)

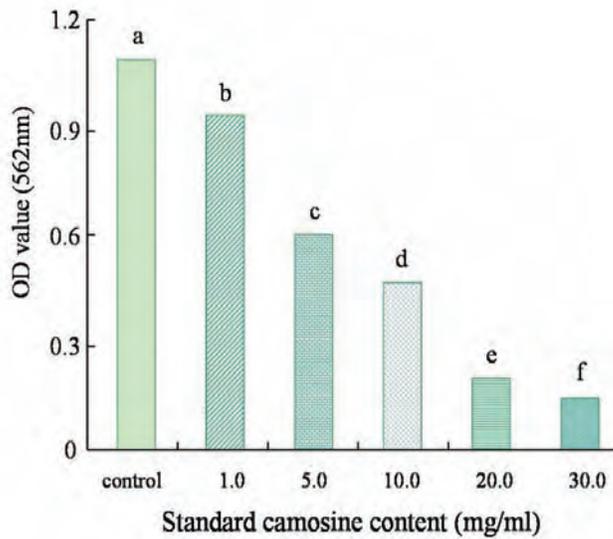


Fig. 5: Chelating effect of standard carnosine on Fe²⁺ ion with different concentrations
 a~fMeans a~fDifferent subscrips on column tops indicate significantly different mean values (p<0.05)

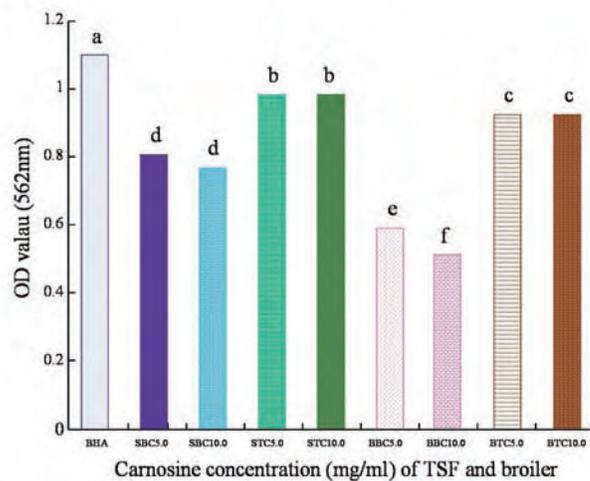


Fig. 6: Chelating effect of carnosine on Fe²⁺ ion in breast and thigh of TSF and broiler
 SBC: Carnosine in breast of TSF
 STC: Carnosine in thigh of TSF
 BBC: Carnosine in breast of broiler
 BTC: Carnosine in thigh of broiler
 a~fDifferent subscrips on column tops indicate significantly different mean values (p<0.05)

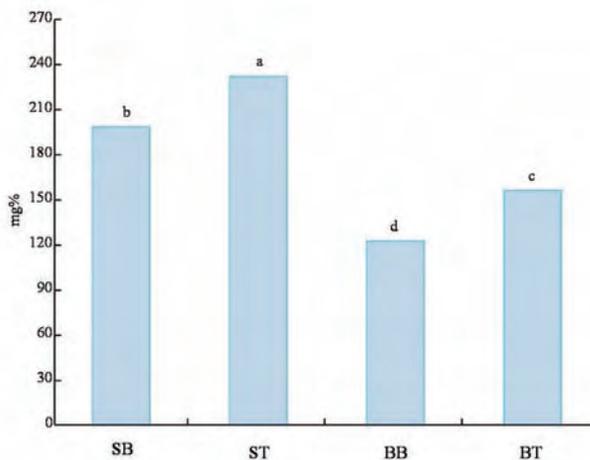


Fig. 7: The concentration of total iron in TSF and broiler
 SB: Breast in TSF ST: Thigh in TSF
 BB: Breast in broiler
 BT: Thigh in broiler
 a~dDifferent subscrips on column tops indicate significantly different mean values (p<0.05)

**ASSESSMENT OF HEALTH PROMOTING PLANT DERIVED
NEUTRACEUTICALS WITH POTENTIAL ANTIOXIDANT PROPERTIES FOR
USE IN MEAT SYSTEMS**

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Key Words: phytochemicals, plant extracts, oxidative stress, meat quality

Introduction

Phytochemicals and plant extracts, present in fruit, vegetables, plants, herbs and beverages exhibit a wide range of biological activities in vitro, attributed in part, to the presence of polyphenolic compounds with antioxidant and free radical scavenging properties (Beattie et al., 2005). In general, there is now a greater demand than ever by consumers for foods perceived as natural, fresh tasting, healthy and more nutritious. Concerns over the safety of synthetic compounds have resulted in renewed interest in the incorporation of natural compounds into foods as a means of improving product quality and shelf life. Lipid oxidation is one of the major causes of quality deterioration in muscle foods, resulting in adverse effects on flavour, colour, texture and nutritional value. The antioxidant activity of plant-based extracts has been reported in beef (Mansour and Khalil, 2000), turkey (Botsoglou et al., 2003) and pork (Rey et al., 2005; Nissen et al., 2004; McCarthy et al., 2001). In light of the current dietary recommendations for increased consumption of fruit and vegetables, the dramatic rise in the use of plant derived extracts and phytochemicals as supplements in the human diet, and consumer demands for quality enhanced meat products serving as nutraceutical sources for consumer health, studies on the biological effects of such compounds merits investigation.

Objectives

The objective of the present study was, to investigate the biological activity of two phytochemicals, resveratrol (RES) and citroflavan-3-ol (C3ol) and four plant derived extracts, grapeseed polyphenols (GSP), olive leaf extract (OLE), bearberry (BB) and echinacea purpurea (ECH) under conditions of oxidative stress in a human monocytic blood cell line, U937 cells. The effect of direct addition of selected plant extracts, GSP and BB, on colour, lipid stability, pH and microbial status of minced pork was also investigated.

Methodology

The compounds were solubilised in methanol, with the exception of echinacea purpurea in distilled water and added to U937 cells at incremental concentrations.

Samples were incubated for 24 h at 37°C and cell viability was assessed by the fluorescein diacetate-ethidium bromide (FDA/EtBr) assay, a microscopic staining assay (Ryan et al., 2004). The concentration of compound that inhibited cell growth by 50% (IC₅₀) was determined. To examine the antioxidant and genoprotective effects, cells were pretreated with each compound at levels below the IC₅₀. U937 cells were then exposed to oxidants: 0.5µM etoposide or 100µM hydrogen peroxide (H₂O₂) or 400µM *tert*-butylhydroperoxide (tBOOH). Cellular glutathione (GSH) levels were measured as an indicator of etoposide induced oxidative stress (O'Callaghan et al., 2002). H₂O₂ and tBOOH induced DNA damage was assessed by the alkaline single-cell gel electrophoresis (ASCGE) assay or comet assay (Woods et al., 1999) and results expressed as olive tail moment (OTM).

Pork loin (*M. longissimus dorsi* (LD)) was minced through a plate with 4mm holes. Following mincing, GSP and BB, solubilised in distilled water, were added at incremental levels of, 0, 50, 100, 200, 300 and 400µg/g muscle and 0, 10, 20, 40, 60 and 80µg/g muscle respectively and subsequently the minced LD was formed into pork patties. Trays containing LD patties were stored under modified atmosphere conditions (75% O₂ : 25% CO₂) for up to 12 days at 4°C.

Colour measurements were made at 3 day intervals using a Cr-300 Chromameter (Minolta Co.Ltd. Japan) set on the LAB colour scale and results were reported as the 'L' lightness, 'a' redness and 'b' yellowness values.

Lipid oxidation was measured by the distillation method of Taradgis et al. (1960) as modified by Ke et al., (1977) and results expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde/kg muscle.

The pH of minced LD homogenates (10%) were measured using a pHm 201 portable pH meter (Radiometer, Copenhagen) at 20°C. Total plate counts were determined at 3 day intervals, and samples were incubated at 30°C for 72 h. Results were expressed in log colony forming units/g (cfu/g).

All analysis was performed in triplicate. Data was analysed by ANOVA using the Prism statistical package followed by Dunnett's test (P<0.05).

Results & Discussion

RES was the most toxic compound in U937 cells demonstrating the highest IC₅₀ value of 13.7µg/ml. ECH was the least toxic plant extract with the lowest IC₅₀ at 9400µg/ml (Data not shown). Exposure of cells to etoposide alone resulted in a significant decrease in glutathione content to 52% of control value (Table 1). GSP, C3ol, ECH and RES increased glutathione levels to a range of 64% to 76% of the control value. OLE and BB resulted in a significant (P<0.05) increase in glutathione content to 94% and 112% of the control, respectively following the trend: BB > OLE > RES > GSP > C3ol > ECH.

None of the compounds alone increased DNA damage as measured by the olive tail moment (OTM). Exposure of U937 cells to H₂O₂ alone increased DNA damage, resulting in a 13-fold increase in DNA OTM versus that of the untreated sample (Table 1). C3ol, ECH and RES had a slight but nonsignificant effect on reducing H₂O₂ induced DNA damage. GSP and BB significantly (P<0.05) decreased the formation of DNA single strand breaks. The order of effectiveness in reducing H₂O₂ induced DNA damage was as follows: BB > GSP > C3ol > ECH > RES > OLE. Exposure of U937 cells to tBOOH

alone also increased DNA damage, resulting in a 4-fold increase in DNA OTM versus the untreated sample (Table 1). The two plant extracts, GSP and BB, lead to a reduction in DNA single strand breaks caused by tBOOH treatment following the trend: GSP > BB > C3ol > ECH > OLE > RES.

The antioxidant effectiveness of GSP is believed to be due to the presence of oligomer procyanidins within grapeseed, which possess a greater protective effect than monomer components (Llopiz et al., 2004). Furthermore, this protection is also related to the closeness of catechol moieties in monomer components and the resulting reduction in oxidation potential of grapeseed, found to be twenty times greater than vitamin E and fifty times that of vitamin C (Shi et al., 2003). The observed antioxidant effect of BB may be explained by the vast array of hydroquinone derivatives present in this extract, such as arbutin, tannins, flavonoids, triterpenes and phenolcarboxylic acids (Tziveleka et al., 2002).

From this screening process, the direct addition of the selected extracts, GSP (0, 50, 100, 200, 300, 400µg/g) and BB (0, 10, 20, 40, 60, 80µg/g), resulted in greater lipid stability, relative to controls, in LD patties stored under modified atmosphere conditions at 4°C (Table 2). After 6, 9 and 12 days of storage, lipid oxidation in LD patties decreased with increasing concentrations of GSP and BB. The antioxidant activity of GSP (200µg/g) (Nissen et al., 2004) and BB extract (100, 200 and 500µg/g) (Pegg et al., 2001) in cooked pork has been previously reported. The surface redness 'a' values of LD patties increased with GSP and BB concentration, relative to controls (Table 2). The lightness 'L' values (range=58.27-62.19) and yellowness 'b' values (range=8.88-10.23) were essentially unaffected by the incorporation of GSP and BB until 9 and 12 days of storage. The pH (5.6-5.78) and total plate count (log 3.3-3.6 cfu/g) of LD patties were unaffected by the presence of GSP and BB.

Conclusions

In conclusion, the overall data support the view that non-nutrient dietary constituents may act as significant bioactive compounds *in vitro* and that plant extracts may play a role in the modulation of oxidative processes *in vivo*. Our results also support the view that plant based extracts may act as nutraceuticals in "true terms", having the potential to improve oxidative stability, and thus eliminate the need for synthetic antioxidants. Further research is necessary to ascertain the effects of plant extracts under different packaging conditions. The influence of dietary supplementation with health promoting nutraceuticals such as GSP and BB as a means of enhancing pork quality also needs to be investigated.

Acknowledgements

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Table 1. Effect of resveratrol, citroflavan-3-ol and plant derived extracts on etoposide induced oxidative stress, H₂O₂ or tBOOH induced DNA damage in U937 human blood cells.

Treatment	Glutathione (% control)	DNA damage (OTM) ^x	
	0.5μM Etoposide	100μM H ₂ O ₂	400μm tBOOH
Control	100 ± 1.21	0.39 ± 0.09	0.39 ± 0.09
Etoposide	52 ± 1.18 ^a	—	—
H ₂ O ₂	—	5.36 ± 0.59 ^a	—
tBOOH	—	—	1.60 ± 0.44 ^a
GSP 50μg/ml	72 ± 0.29 ^a	1.70 ± 0.06 ^b	0.57 ± 0.07 ^b
BB 10μg/ml	112 ± 0.19 ^b	1.64 ± 0.46 ^b	0.66 ± 0.04 ^b
OLE 50μg/ml	94 ± 1.46 ^b	6.96 ± 0.36 ^a	2.01 ± 0.67 ^a
C3ol 100μg/ml	65 ± 0.94 ^a	3.82 ± 0.33 ^a	1.63 ± 0.36 ^a
ECH 1mg/ml	64 ± 0.67 ^a	4.12 ± 0.40 ^a	1.83 ± 0.47 ^a
RES 2μg/ml	76 ± 0.81 ^a	4.59 ± 0.28 ^a	3.44 ± 0.68 ^a

All six compounds were co-incubated with etoposide or H₂O₂ or tBOOH. ^{ab} Mean values (± SEM) in the same column bearing different superscripts are significantly different, P<0.05. ^xOTM= olive tail moment.

Table 2 Effect of the direct addition of GSP and BB on lipid oxidation and colour in minced *M. Longissimus dorsi* (LD) stored in modified atmosphere packs (75% O₂ : 25% CO₂) at 4°C

	Storage time, days									
	0		3		6		9		12	
	TBARS ^b	'a' value ^c								
Control	0.44	10.92	0.4	8.86	0.75	7.79	0.9	7.67	2.02	6.25
GSP 50µg/g	0.46	10.96	0.31	8.85	0.32	8.6	0.34	8.11	0.58	7.03
GSP 100µg/g	0.29	10.9	0.39	9.19	0.42	9.21	0.31	8.12	0.37	7.28
GSP 200µg/g	0.12	10.91	0.54	9.51	0.2	8.89	0.23	8.54	0.32	7.75
GSP 300µg/g	0.13	11.21	0.32	9.7	0.12	8.87	0.22	8.13	0.19	8.04
GSP 400µg/g	0.1	11.2	0.22	9.58	0.15	9.55	0.19	8.21	0.2	7.93
Control	0.44	10.92	0.4	8.86	0.75	7.79	0.9	7.67	2.02	6.25
BB 10µg/g	0.24	10	0.63	9.4	0.61	7.94	0.65	7.76	1.27	6.5
BB 20µg/g	0.09	10.87	0.6	9	0.4	8.37	0.41	7.79	0.46	7.05
BB 40µg/g	0.08	10.99	0.3	8.52	0.27	8.38	0.33	7.84	0.37	7.96
BB 60µg/g	0.08	10.62	0.16	9.13	0.16	8.04	0.18	7.79	0.27	7.45
BB 80µg/g	0.09	10.49	0.1	8.73	0.18	8.47	0.16	7.21	0.3	7.29

^b TBARS, mg MDA/kg muscle, ^c 'a' value denotes redness of meat

**COMBINED EFFECT OF MODIFIED ATMOSPHERE PACKAGING AND
ADDITION OF ROSEMARY, ASCORBIC ACID, RED BEET ROOT AND
SODIUM LACTATE AND THEIR MIXTURES ON THE STABILITY OF FRESH
PORK SAUSAGES**

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Key Words: Pork, fresh sausage, modified atmosphere packaging, antioxidants, rosemary, ascorbic acid, red beet root, sodium lactate.

Introduction

The colour of fresh meat products is of major importance from consumer viewpoint. The widespread of modified atmosphere packaging (MAP) for fresh meat products has generated problems regarding colour stability of fresh ground meat products stored under high concentrations of oxygen during retail display (Martínez et al., 2005). In addition, the use of natural colorants has been investigated with respect to colour stability of fresh meat products (Martínez et al., 2004). Lipid and myoglobin oxidation are closely related (Faustman and Cassens, 1989). Currently, the search of crude extracts of fruits, herbs, vegetables, cereal, and other plant material rich in phenolics are increasingly of interest in the food industry since they retard oxidative degradation of lipids and thereby improve the quality and nutritional values of food (Kahkonen et al., 1999). Rosemary (*Rosmarinus officinalis*) is a natural plant of the *Labiatae* family; its major and most active components are carnosol, carnosic acid and rosmarinic acid. Several authors had demonstrated that rosemary, alone or with ascorbic acid (AA), effectively contributes to oxidative stability of meat products (Djenane et al., 2003; Sánchez- Escalante et al., 2003). Red beet root (*Beta vulgaris*) is a rich source of the betalain pigments group betacyanins (red) and betaxanthins (yellow). Red beet root powder has been used to simulate cured meat colour in cooked, smoked, semidry and fermented sausages. Besides oxidative processes, growth of psychrotrophic microorganisms results in microbial spoilage and limited shelf life. Sodium or potassium salts of lactic acid have been shown to delay growth of meat spoilage microorganisms (Jensen et al., 2002).

Objectives

The objectives of the present research was to determine the effects of natural antioxidant rosemary in combination with ascorbic acid, natural colorant red beet root and sodium lactate, and their mixtures, on the inhibition of both lipid and pigment oxidations, and consequently, on the extension of quality characteristics of fresh pork sausages packaged in modified atmosphere.

Methodology

Preparation of samples. Four pork forelegs (initial pH 5.5-5.7) were excised from two pork carcasses 48 h post-slaughter from a local supplier (MARBE, Zaragoza, Spain), trimmed external fat off, and ground using an industrial grinder machine (Gesame, S. L., Barcelona, Spain) through a plate with 4 mm holes. Minced meat was divided in eight batches, which were mixed with NaCl (to a final concentration of 2%) and with either: 1) Control (no additions), 2) rosemary powder (1000 ppm) + ascorbic acid (500 ppm), 3) sodium lactate (3%), 4) rosemary powder (1000 ppm) + ascorbic acid (500 ppm) + sodium lactate (3%), 5) red beet root (1 ml kg⁻¹), 6) red beet root (1 ml kg⁻¹) + rosemary powder (1000 ppm) + ascorbic acid (500 ppm), 7) red beet root (1 ml kg⁻¹) + sodium lactate (3%), 8) red beet root (1 ml kg⁻¹) + rosemary powder (1000 ppm) + ascorbic acid (500 ppm) + sodium lactate (3%). The fresh sausages were stuffed into collagen casings, Colfan F (Viscofan S.A., Caseda, Spain), placed on polypropylene trays, introduced in a pouch made of a polyethylene and polyamide and filled with a 80% O₂ + 20% CO₂ gas mixture. Sausages were stored for 20 days at 2 ± 1°C in the dark.

Red beet juice preparation. Fresh red beet roots (*Beta vulgaris*) were purchased from a commercial store. Samples were washed, dried and cut into cubes of about 1cm x 1cm, which were boiled for 5 min for blanching (Han et al, 1998). After rapid cooling, beet juice was extracted with a standard kitchen food processor. The crude juice was boiled at 100°C for 1 min. After rapid cooling, it was filtered stepwise (MN 640w, Machinery Nagel GmbH & Co. KG, Düren, Alemania). The clear beet juice was stored at refrigeration until use.

Meat colour and metmyoglobin analysis. Meat colour was measured at the surface of fresh sausages using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan), 30 min after packaging opening. CIE L*, a*, b* parameters were recorded. The metmyoglobin percentage at the surface was estimated spectrophotometrically, according to Stewart et al. (1965), by measuring reflectance at 525 and 572 nm. The average value for each fresh sausage was the mean of 30 determinations.

Lipid oxidation analysis. Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method of Pfalzgraf (1995). TBARS values were expressed as mg malonaldehyde/kg sample.

Microbial analysis. Counts of aerobic psychrotrophic flora were determined in Plate Count Agar (PCA; Merck; Darmstadt, Germany) after incubation at 10°C for 7 days (Elliott et al., 1983). Counts were expressed as the log₁₀ of colony forming units (CFU)/g.

Sensory evaluation: Six-member trained panel evaluated fresh pork sausages for discolouration and off-odour according to procedures of Djenane et al. (2001). Discoloration; scores referred to percentage of discolored surface: 1 = none, 2 = 0-10%, 3 = 11-20%, 4 = 21-60%, and 5 = 61-100%; Odour scores referred to the intensity of odours associated to meat spoilage: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme. Results were expressed as the predominant score given by panellists.

Statistical analyses. The significance of differences among samples at each day of storage was determined by analysis of variance using the least square difference method of the General Linear Model procedure of SPSS (SPSS 11.5, 2002).

Results & Discussion

Colour (a* values; Fig. 1). Samples with red beet root (RBR) added had higher a* values ($p < 0.05$) than control sausages (Martínez et al., 2004). Later they were lower (about 7.5), similar to control samples at the 8th day. After that they suffered a loss of redness with the exception of rosemary + AA + NaL samples with or without RBR, which maintained its colour (8) until the 12th day ($p < 0.05$) and 16th day, respectively. Tan and Shelef (2002) found that red colour was enhanced by lactates immediately after their addition to the meat. On the other hand, Jensen et al. (2003) found in pork chops that the use of 2% NaL preserved red colour the first days of storage, after that meat suffered a pronounced decrease of their a* values.

Metmyoglobin percentage (Fig. 2). The amount of metmyoglobin was lower ($p < 0.05$) in samples containing sodium lactate and natural antioxidants which maintained a high level of acceptability (below 30%) after 12 days of storage. Djenane et al. (2002) reported that about 30-40% metmyoglobin was needed for causing relevant meat discoloration. Only control samples, RBR and RBR + NaL exceeded this level of acceptability after 12 days of storage ($p < 0.05$). The best effect was shown by RBR + rosemary + AA + NaL, these samples had values near 30% after 16 days ($p < 0.05$).

Lipid oxidation (Fig. 3). Lipid oxidation was low in sausages packaged with antioxidant alone or with NaL added throughout all the storage time ($p < 0.05$), TBARS values after 16 days of the storage were just below 1 mg/kg in all samples with rosemary + AA added, with the exception of RBR with antioxidants, that increased their values to near 1.5 larger than the other sausages packaged with rosemary + AA ($p < 0.05$). Results did not show an effective antioxidant activity of RBR on TBARS after packaging. A possible explanation is found by Kahkonen et al. (1999), because red colour of the beet root extract interfered with the determination of the pink TBA chromogen. While the antimicrobial effects of lactates are well documented (Brewer et al., 1995), there are limited reports on the effect of lactates on lipid oxidation and meat pigments. Nnanna et al. (1994) indicated a suppression of lipid oxidation by lactates in raw ground pork during storage for up to 7 days at 0 and 5 °C. At the last day of storage, all samples that remained with low TBARS increased their values to just above 1 mg/kg.

Microbial counts (Fig. 4). All samples started with very high microbial counts of near 5 log₁₀ (CFU /g), which are common in fresh meat products and should be referred to intense manipulation during meat preparation, grinding and stuffing. Fresh sausages did not show significant differences among different treatments after 4 days of storage ($p > 0.05$). Samples formulated without sodium lactate had rapidly growing counts, which reached 7 log₁₀ (CFU /g) at 12th day ($p < 0.05$). These samples had counts of about 8 log₁₀ (CFU /g) at the end of the storage period. The rest of the samples with sodium lactate showed no significant differences among them ($p > 0.05$) at 8th day, although they were significantly lower ($p < 0.05$) than the rest of sausages during all the storage period. Those results clearly demonstrated the inhibitory effect on microbial growth of sodium lactate by extending the lag phase, in accordance with previous reports regarding other authors (Brewer et al., 1995).

Sensory evaluation (Table 1). Discoloration of fresh sausages markedly increased since the 8th day and throughout storage following all treatments. Control, RBR with or without NaL were given a score of 4, corresponding to 21-60 % discoloration, until the 12th day. Rosemary + AA + NaL with or without RBR, were given the lowest scores,

either 2.5 or 3 after 16th of storage. They would be considered acceptable according to their colour, while the rest of samples would be considered unacceptable. These results appeared to be in close agreement with those of metmyoglobin formation and a* index. After 20 days of storage all samples scored 5 points. Regarding off-odour, scores increased throughout storage for all samples. Sensory results of odour had a very close relationship with those of lipid oxidation. After 12th days only control, RBR and RBR + NaL overpassed the limit of acceptability (3 points). At 16th day, all samples with rosemary + AA alone or with NaL added remained below 3 points, the lowest scores corresponded to RBR with antioxidants and NaL.

Conclusions

The use of a mixture containing red beet root juice, rosemary powder, ascorbic acid and sodium lactate extended the shelf-life of fresh pork sausages stored in modified atmosphere. The samples improved their red colour, metmyoglobin, lipid oxidation and microbial indices after 16 days of storage, showing better sensory scores than the rest of samples. If red colour is used by consumers as freshness indicator and as willingness of purchasing, the use of this natural mixture enhanced the shelf life of this product.

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Table 1. Influence of different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate on sensory panel scores (Predominant score) of fresh pork sausages packaged in modified atmosphere at 2 ± 1 °C.

Treatments		Days of storage					
		0	4	8	12	16	20
Discoloration (1)	Control	1	1	2	4	5	5
	Rosemary + AA	1	1	1	3	3,5	5
	NaL	1	1	1	3	3,5	5
	Rosemary + AA + NaL	1	1	1	3	3	5
	Red Beet Root	1	1	2	4	5	5
	Red Beet Root + Rosemary + AA	1	1	1	3	5	5
	Red Beet Root + NaL	1	1	1	4	5	5
	Red Beet Root + Rosemary + AA+ NaL	1	1	1	3	3,5	5
Off-odour (2)	Control	1	1	2	4	5	5
	Rosemary + AA	1	1	1	1	3	4
	NaL	1	1	1	3	4	5
	Rosemary + AA + NaL	1	1	1	1	1	4
	Red Beet Root	1	1	1	5	5	5
	Red Beet Root + Rosemary + AA	1	1	1	2	4	4
	Red Beet Root + NaL	1	1	2	4	5	5
	Red Beet Root + Rosemary + AA+ NaL	1	1	1	2	3	4

¹ Discoloration: 1 = none, 2 = 0 to 10%, 3 = 11 to 20%, 4 = 21 to 60%, and 5 = 61 to 100%.

² Off odor: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme.

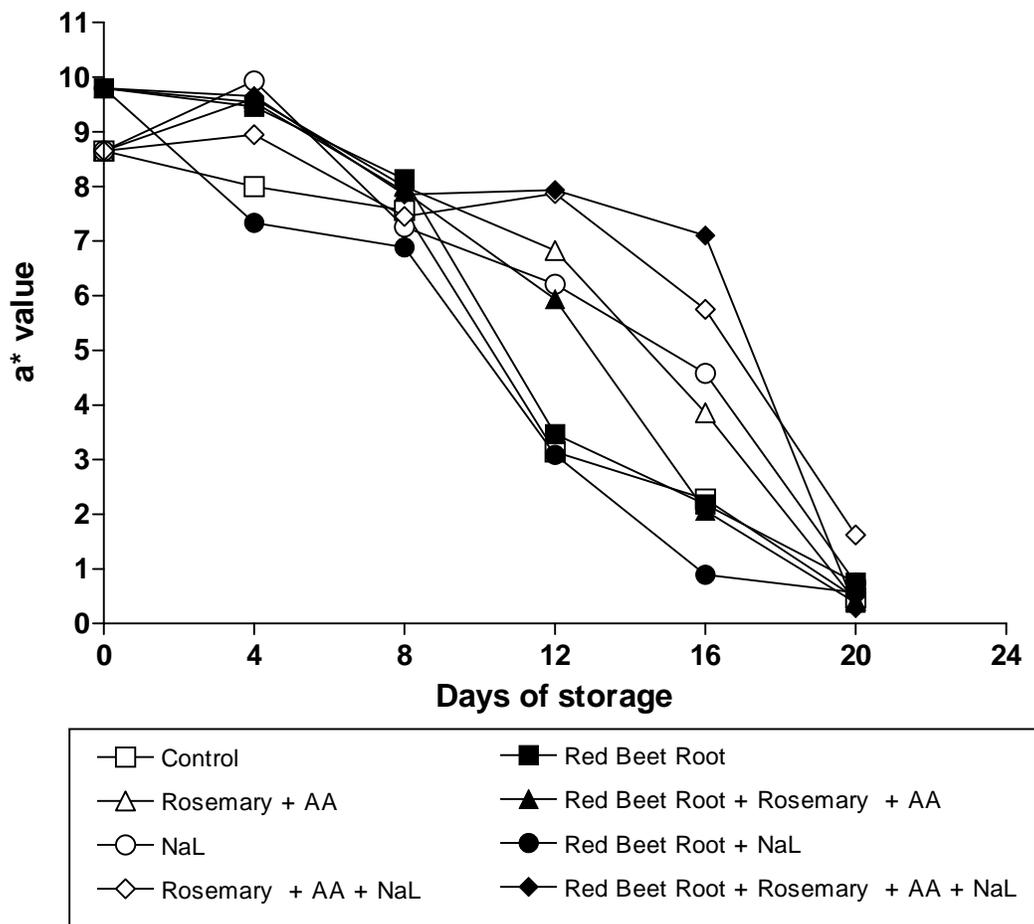


Figure 1. Evolution of a^* values in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at $2\pm 1^\circ\text{C}$.

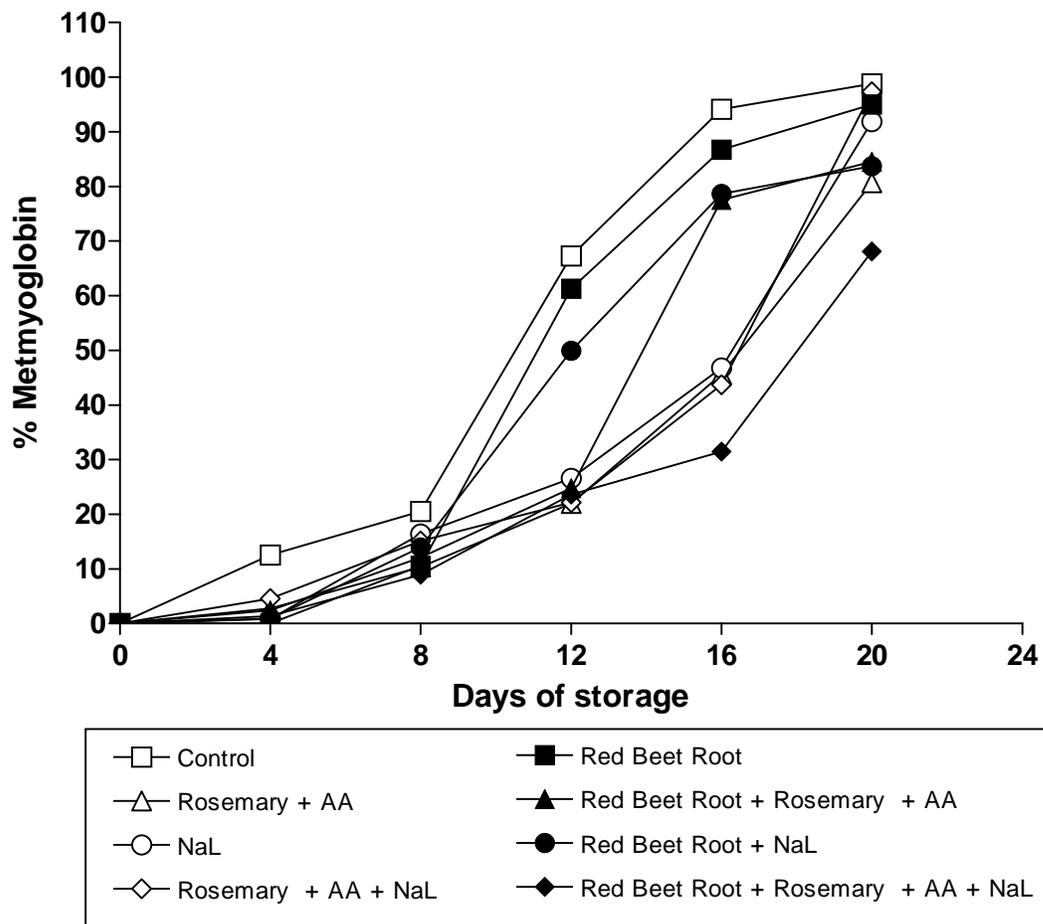


Figure 2. Evolution of metmyoglobin formation (%) in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at 2±1°C.

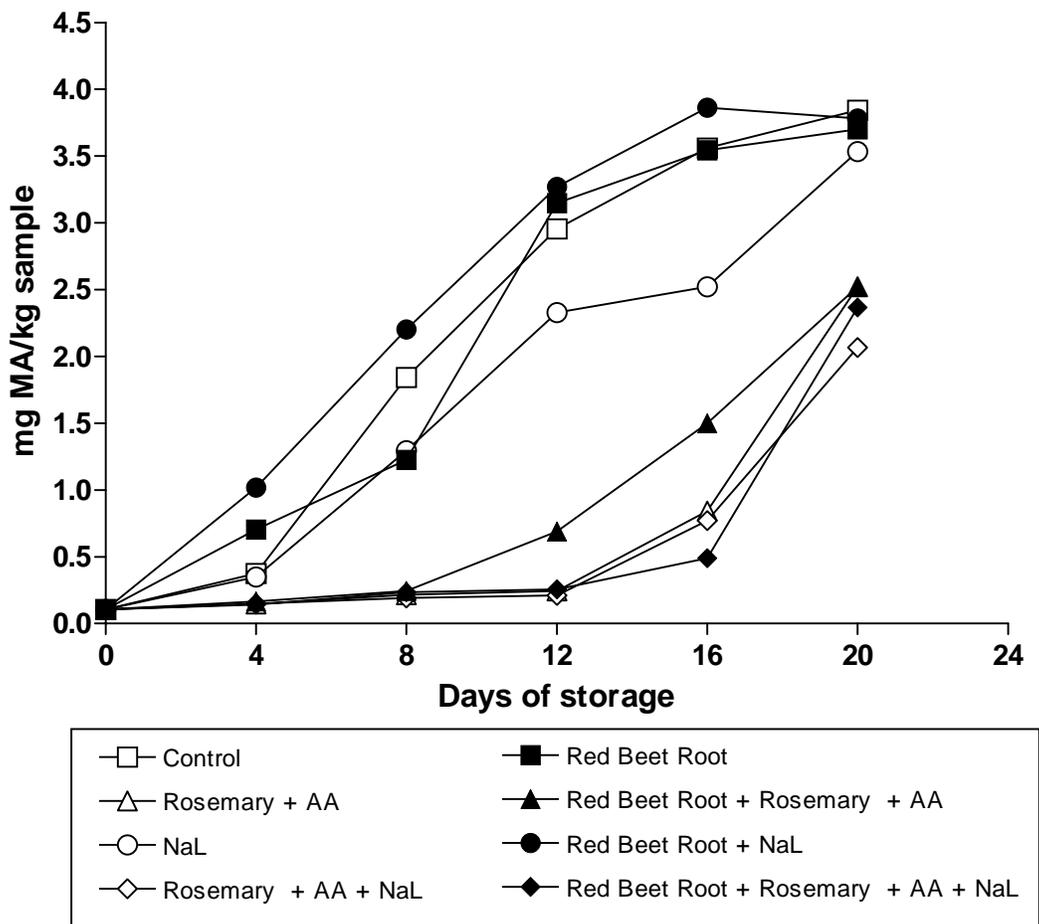


Figure 3. Evolution of TBARS values in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at $2\pm 1^{\circ}\text{C}$.

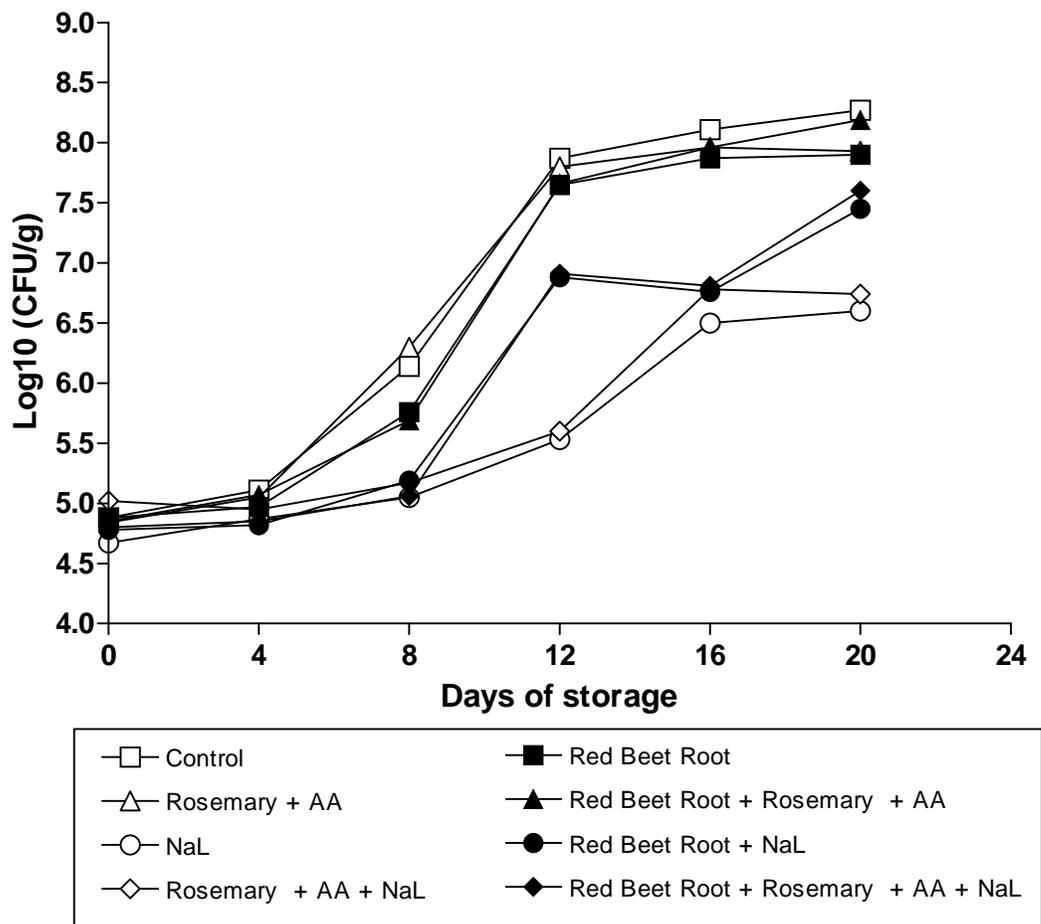


Figure 4. Evolution of psychrotrophic aerobes in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at $2\pm 1^\circ\text{C}$.

SENSORY CHARACTERIZATION OF SPANISH AND FRENCH DRY-CURED HAMS IN RELATION TO SPANISH TYPICALITY PROFILES

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Key Words: Dry-cured ham, sensory characterization, typicality

Introduction

Dry-curing of hams is a common practice in the South European countries. It is one of the most important ways of processing pork in Spain and Italy. The dry-curing process was originally used as a preservation method. However, this process has been improved to develop a more desirable flavour and a softer texture producing the characteristic Mediterranean Hams (González et al. 2000). In the Mediterranean regions of Europe, the term "cured" is used when they produce hams utilizing a long period of aging (usually between 6 to 12 months) where enzymatic action occurs and a distinctive flavour is developed. However, in the northern region, the term "cured" is limited to products that receive nitrite during processing (Flores 1997).

Spanish, Italian and French hams are representative of high-quality dry-cured hams of the Mediterranean areas. That is why, in 1992, the European Union created systems known as PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and TSG (Traditional Speciality Guaranteed) to promote and protect them. So among Spanish, Italian and French hams it is possible to find these brands which play a key role by leading consumer choice in a positive feeling of reassurance. Brands give a guarantee cue to consumers.

The quality of the dry-cured ham depends on multiple factors, such as animal breeding, animal age, feeding, environmental conditions previous to slaughtering (antemortem factors) and meat handling and ham manufacturing (postmortem factors). However, the most important factors that influence the sensory properties of hams are the raw materials and the ripening conditions (Toldrá et al. 1997; Cilla et al. 2005). In France, the production of dry-cured hams is lower than in Italy or Spain, but the consumption of this product is continuously increasing (Buscailhon et al. 1994). Dry-cured hams are made in France from white pigs (around 110-120 kg live weight). The curing mixture is made exclusively of dry salt, nitrite and/or nitrate and spices. They are not smoked. Generally, the total processing duration does not exceed nine months. The Spanish Iberian hams are produced from the Iberian breed from the Southwestern region of Spain fed in pastures with acorns, weighing 85-90 kg (the age of slaughter is 10-12 months). The product achieves a high degree of marbling, soft texture and typical dry cured-ham flavour. The common dry-cured hams are produced from crossbreed white pigs with a low marbling score, firmer texture and a typical dry-cured-ham flavour,

which depends on the length of ripening (Toldrá et al. 1997). In Spain the traditional dry-curing process consists of a mixture of curing adjuncts that are applied to hams without any added water or spices. In this process, the curing mixture penetrates by diffusion aided by the original moisture of the meat. Common ham is ripened for 9 to 15 months while the Iberian ham may be held for 18 to 24 months. The complexity and combination of biochemical reactions occurring during the ripening stage determine the sensory characteristics of the hams (Verplaetse 1994; Flores 1997).

Objectives

The objectives of this work were to find typicality parameters that allow to characterize and group objectively French (white) and Spanish (Iberian and white) hams, as well as to find relations among sensory traits and factors such as breed, maturation, origin and quality label which define each group of hams in order to establish differences.

Methodology

Twenty-one Spanish hams (8 Iberian hams and 13 white hams) and 20 French hams were processed by local manufacturers using the traditional method of each geographical origin (Flores et al., 1993; Sabio et al. 1998). Table 1 shows the codes of the samples, their geographical origin, breed, maturation time and quality label according to Regulations (EEC) n° 2081/92 and 2082/92 (DOCE, 1992). Hams were deboned and portions were taken in a transversal cut at 6 cm below the femur head which included Biceps femoris (BF), Semimembranosus (SM) and Semitendinosus (ST) muscles. The obtained portions of about 10 cm thick were stored at $4 \pm 2^{\circ}\text{C}$ in vacuum until they were required for sensory analysis and then cut in slices (1.5 mm thick) using a slicing machine. Typicality traits were defined by a group of Spanish experts on that field (scientist and producers) with a deep knowledge of the different dry-cured hams used in this work. Typicality attributes deal with several attributes of the given products which explained the ideal profile of traditional hams in order to fit with consumer expectations. This attributes were: marbling, aroma, hardness, softness, flavour, acorn flavour, saltiness and sheen. These parameters were included in the sensory test for trained panel and they were explained according to expert definitions. Dry-cured ham samples were individually assessed by a trained panel of 13 Spanish members (Universidad de Zaragoza). To acquaint panellists with product attributes and intensities, ten 1 hour training sessions took place over a 4 week period prior to sample testing. During this phase, panellists were presented dry-cured hams from a variety of manufacturers corresponding to maximum and minimum intensities that might be found for each attribute (1, very low, to 9, very high). The panel sessions were held at mid-morning, about 3 hours after breakfast. Slices were served on plates. A profile of 24 sensory attributes of dry-cured ham (croutage, colour of BF, SM, colour homogeneity, subcutaneous fat, fat colour, marbling, aroma intensity, rancidity, nut aroma, mould aroma, toughness, softness, crumbliness, fibrousness, pastiness, adhesiveness, ham flavour, saltiness, rancidity, nut flavour, sweet taste and sheen), as well as overall acceptability, was assessed. About 50 ml of water at room temperature and 20 g of unsalted bread were provided between successive hams. All sessions were done at 22°C in a sensory panel room equipped with white fluorescent

lighting (Philips TLD 86, 5600 °K, 800 lux). Four hams from different groups (Spanish and French) were successively evaluated in each session. The sample order was randomised.

Statistical analysis: The statistical study of the differences among the classes of samples (French vs. Iberian and Spanish white hams) was carried out by multivariate analyses by means of SPSS version 11.5 (2005). In order to verify typicality attributes as good criteria for classification of French vs. Iberian and Spanish white hams, a cluster analysis was carried out. Principal Components Analysis (PCA) was applied to check the results of the supervised procedure and to find the relations among sensory traits and sampling factors (breed, origin, country, maturation and quality label) which could explain the differences among the groups.

Results & Discussion

Sensory evaluation

Figure 1 is a plot of the PCA of sensory traits from trained panel loading for the first two partial least squares components. The first component was able to predict 37.38% of the total variation while the second component contributed a further 12.12%; thus an accumulative 49.5% of total variation was explained by the first two principal components. The first component might be interpreted as the main factors contributing to acceptability, since the factorial coefficients of acceptability and variables related to colour, flavour, aroma, odour, maturing time, origin and quality label showed the highest positive coefficients, whereas saltiness, pastiness and adhesiveness had negative. On component 2 positive loading had pastiness, adhesiveness, saltiness, fibrousness and hardness, whereas crumbliness and softness had negative coefficients. According to the plot of analyzed samples (Figure 2), the first component was able to discriminate most sharply samples in three different groups (French, Spanish white and Iberian hams). Iberian hams were located with positive loading on the first component 1 indicating the highest acceptance of this group according the Spanish trained panel. French were mainly located with negative loading on component 2 (less acceptance) probably due to shorter time of maturing and Spanish white hams located in a mid position. However, some samples were not classified as expected. S05, S17, S20 were located with negative loading on component 2. S05, S17, S20 were from Spanish white hams. Their location might be explained due to the detection of atypical sensory attributes. F13, F14 and F17 were classified into the Spanish white hams group probably due to similar sensory profile of Bayonne hams to this group.

PCA results were highly representative of the factors interactions. According to Pearson correlations, acceptability decreased with increasing pastiness (-0.390), adhesiveness (-0.309) and saltiness (-0.366) which were significant at $p < 0.05$ level. Similar results were found by Cilla et al. (2005). About sampling factors it is important to consider that breed, geographical origin, label and maturation time are representative of high-quality dry-cured hams due to the influence on acceptability, so these factors are good guarantees of quality.

Typicality parameters

Figure 3 shows the results from Cluster analysis. The samples were properly clustered according to typicality traits into expected groups (French, Spanish white and Iberian hams). Exceptions were F13 and F14 which were included with Spanish white hams. The results confirmed typicality attributes defined by experts as good criteria for classification.

Conclusions

Typicality parameters defined by experts constituted a good method for dry-cured ham classification depending on quality expectation. PCA results confirmed these grouping method, as well as to establish differences among groups, relations among sensory traits and sampling factors (breed, geographical origin and maturing time) on the Spanish trained panel's acceptability. Iberian hams were highly scored while French dry-cured hams registered lower scores. Spanish white hams and Bayonne hams had very similar sensory profile and maintained a mid position between Iberian and French hams. Among sensory traits, pastiness, adhesiveness and saltiness influenced negatively on acceptability whereas, aroma, flavour, odour and colour positively, indicating a suitable maturation time. Factors such as breed, geographical origin, and label constituted a guarantee for quality.

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Tables and Figures

Table 1. Geographical origin, kind of breed, maturing time and label of the coded samples

Code	Geographical origin (Region, Country)	Breed	Maturing time (months)	Label
S01	Teruel, Spain	NIB ⁽¹⁾	12-18	PDO ⁽²⁾ Teruel
S02	Teruel, Spain	NIB	12-18	PDO Teruel
S03	Teruel, Spain	NIB	≥18	PDO Teruel
S04	Teruel, Spain	NIB	8-11	-
S05	Miscellaneous, Spain	NIB	8-11	TSG
S06	Teruel, Spain	NIB	12-18	-
S07	Miscellaneous, Spain	NIB	8-11	-
S08	Miscellaneous, Spain	NIB	8-11	-
S09	Miscellaneous, Spain	NIB	12-18	-
I10	Southwest Spain	Iberian	≥18	PDO Guijuelo
I11	Southwest Spain	Iberian	≥18	PDO Huelva
I12	Southwest Spain	Iberian	≥18	PDO Los Pedroches
I13	Southwest Spain	Iberian	≥18	-
I14	Southwest Spain	Iberian	≥18	-
I15	Southwest Spain	Iberian	≥18	-
S16	Teruel, Spain	NIB	12-18	PDO Teruel
S17	Teruel, Spain	NIB	12-18	-
I18	Southwest Spain	Iberian	≥18	-
I19	Southwest Spain	Iberian	≥18	PDO Extremadura
S20	Miscellaneous, Spain	NIB	8-11	-
S21	Miscellaneous, Spain	NIB	12-18	TSG ⁽³⁾
F01	Auvergne, France	NIB	8-11	TSG
F02	Auvergne, France	NIB	8-11	-
F03	Auvergne, France	NIB	≥7	-
F04	Auvergne, France	NIB	≥7	-
F05	Auvergne, France	NIB	≥7	-
F06	Auvergne, France	NIB	12-18	-
F07	Auvergne, France	NIB	8-11	-
F08	Auvergne, France	NIB	8-11	-
F09	Aveyron, France	NIB	8-11	-
F10	Lacaune, France	NIB	8-11	-
F11	Lacaune, France	NIB	≥7	-
F12	Lacaune, France	NIB	8-11	TSG
F13	Bayonne, France	NIB	12-18	PDO Bayonne
F14	Bayonne, France	NIB	12-18	PDO Bayonne
F15	Auvergne, France	NIB	8-11	-
F16	Lacaune, France	NIB	8-11	-
F17	Bayonne, France	NIB	8-11	PDO Bayonne
F18	Miscellaneous, France	NIB	8-11	-
F19	Miscellaneous, France	NIB	≥7	-
F20	Miscellaneous, France	NIB	8-11	-

(1) NIB: Non Iberian Breed. Common European breeds: Large white, Landrace, Duroc and their crosses

- (2) PDO: Protected Designation of Origin
- (3) TSG: Traditional Speciality Guaranteed

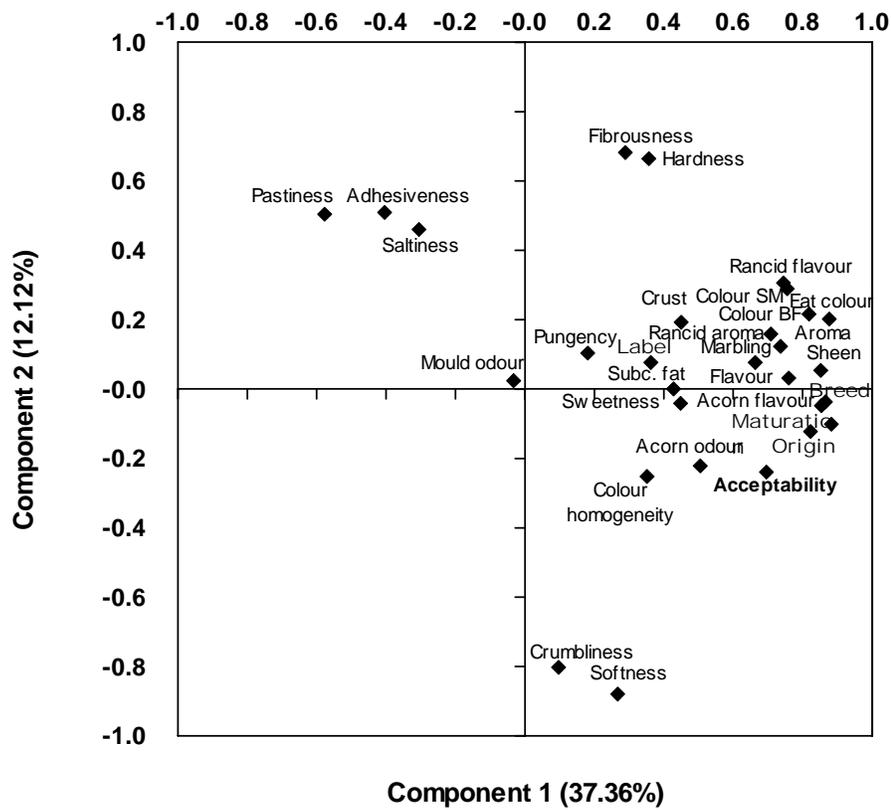


Fig. 1. Plot of the loadings obtained for the parameters included in the principal components analysis, (origin, breed, label, maturing time, sensory traits and overall acceptability).

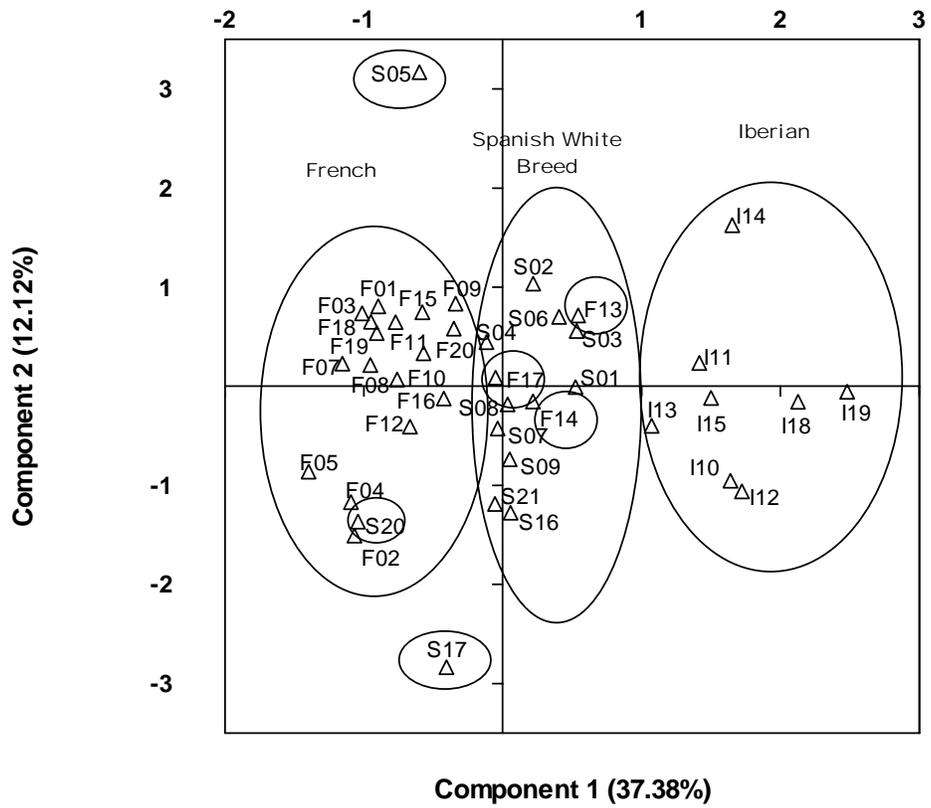


Fig. 2. Plot of analyzed samples (French, Spanish White and Iberian hams) in the principal component analysis test.

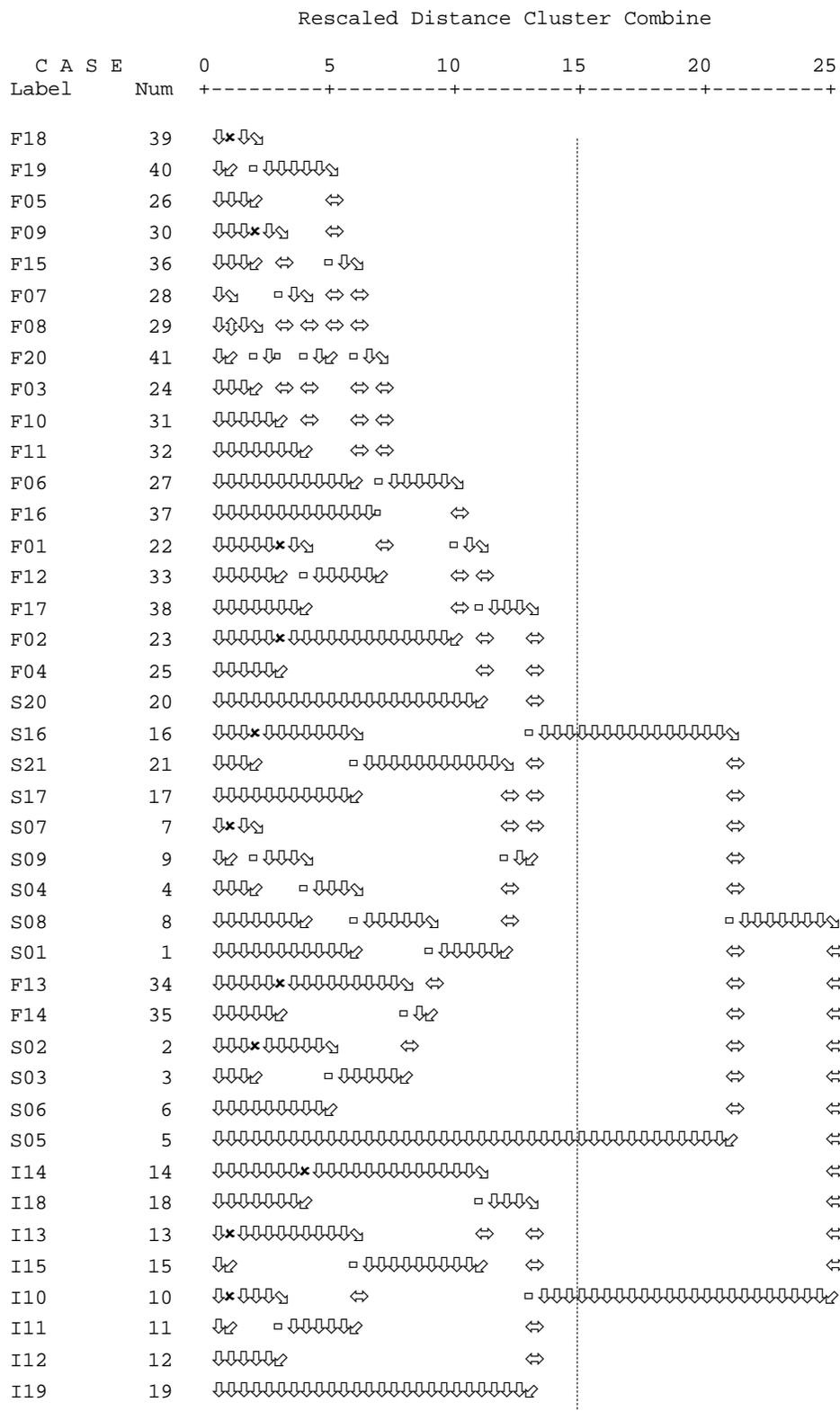


Fig. 3. Clustered display of data from typicality traits. Different cases were grouped at 13 points of rescaled distance.

INDONESIAN BAKSO MEATBALL COMPOSITION, TEXTURE, AND STRUCTURE WITH FROZEN MEAT AND PRODUCT STORAGE TIMES

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Key Words: bakso, frozen, texture, SEM structure, composition, processing

Introduction

Meatballs and fishballs are popular convenient meat products in Asia. Meatballs in Indonesia, known as bakso, are made from prerigor meat or with meat before completion of rigor mortis that has been emulsified and mixed with salt, tapioca starch, and garlic (Purnomo, 1990). The batter mixture is formed into balls and cooked before serving with boiled chicken stock or soup, commonly distributed from pushcarts at street corners (Pandisurya, 1983). Consumers prefer tougher, but more elastic, bakso (Yuliati, 1999). Commercial production of bakso has been difficult for Indonesian meat processors because sufficient quantities of prerigor or early postmortem meat materials are not readily available. Bakso from postrigor beef gave only slightly different textural properties than bakso using early postmortem meat with higher elasticity with 15% than 5 or 10% tapioca starch (Rahardiyani and McMillin, 2004). Use of frozen postmortem beef would provide more flexibility in raw material procurement and processing schedules.

Objectives

The objective of the study was to compare the texture, structure, and composition of bakso meatballs made from postrigor beef after different raw material frozen storage times and after different frozen storage times of the cooked bakso meatballs.

Methodology

Semimembranosus and *Semitendinosus* muscles were deboned within 3¾ hours postmortem from carcasses of commercially slaughtered (RPH Pegirian Slaughter House, Surabaya, East Java, Indonesia) local Ongole crossbred grass-fed cattle 2 to 4 years old. The beef was stored at 10°C during 1 hour transport to the meat plant (PT. Eloda Mitra, Sidoarjo, East Java, Indonesia) in a refrigerated truck. The meat was divided randomly into four equal portions for immediate usage (early postmortem), chilled overnight and used the following day (0 month postrigor), or stored frozen for usage after 2 or 4 months of storage (2 month postrigor and 4 month postrigor). The pH of early postmortem meat was 5.42-5.74 while the late postmortem meat had pH of 5.08-5.62.

Meat was weighed (20.51 kg, 81.4% of batch) and chopped (bowl chopper, K.G. Wetter, Germany) for 20 minutes with salt (1.6%), sodium tripolyphosphate (0.6% STPP, Na₅P₃O₁₀, Albert & Wilson Phosphate Groups, Indonesia), cane sugar (0.6%), monosodium glutamate (0.8% MSG, PT. Ajinomoto, Indonesia), and tapioca starch (15% National[®] 7, National Starch and Chemical, Singapore). Crushed ice was added (5%) to maintain batter temperature at 15°C. Balls of ~14g were formed (meatball former, Chuang Zong Baller, Taiwan) and then boiled at 100°C for 20 minutes in an open boiler (PT. Mastrada, Indonesia), drained on perforated aluminum trays until the surface was dry, and packed in polyethylene bags (limited low density 0.15 mm thickness, Top Printing Indonesia Co., Indonesia) at 40 balls/bag for vacuum packaging (Henkelman H-800 Double Chamber, Netherlands). Three packs each of early postmortem bakso and post rigor bakso were sampled for analysis time of 0 month. The remaining packs were frozen at -20°C and 3 packs each of early postmortem and post rigor bakso were thawed and tested after 2 and 4 months of frozen storage. After 2 and 4 months of frozen meat storage, 61.54 kg of meat were thawed and processed into cooked bakso balls using the previous procedures. Three packages of cooked bakso were tested initially and remaining bakso packs were stored at -20°C for an additional 2 or 4 months before testing.

Composition of bakso was determined as moisture (method 950.46) and fat (method 960.39, AOAC, 1990). Lipid oxidation was evaluated as TBARS values with the method of Wu et al. (2000). Texture analyses (Lloyd Universal Testing Model-1000s, England) according to Hidayati (2002) and Yuliati (1999) using 4 mm upper cycle limit, 3 mm lower cycle limit, compression mode, and 60 mm/min test speed on 10-mm sample cubes were reported as minutes/gram for elasticity and in newtons for gel strength (hardness) and shear force values. Samples for scanning electron microscope procedure were prepared according to Hidayati (2002) and Yuliati (1999) by slicing bakso 2 to 3-mm thick with a razor blade. Samples were fixed with 2% glutaraldehyde in a pH 7.3 phosphate buffer and critical point dried (Sumdri-780 Sample Drying, USA) for 72 hours. Each sample was coated with 24 carat gold with an ion sputter fine coater (JEOL-GLE4X, JEOL Technic Co. Ltd., Japan) for 1.5 minutes or 0.25-mm thickness for observation at 1500 times magnification (JEOL GSM-T100 Scanning Electron Microscope, JEOL Technic Co. Ltd., Japan).

Main effects of meat storage and bakso storage times, interactions, and replications were analyzed in two separate designs using analyses of variance and least squares mean separations at probability value of 0.05 (general linear model procedures for complete randomized design, 1998, SAS Inst., Inc., Cary, NC). The first design compared properties of bakso after post rigor meat frozen storage times of 0, 2, and 4 months and bakso frozen storage times of 0, 2, and 4 months. The second design compared bakso from early postmortem and post rigor meat after 0, 2, and 4 months of frozen bakso storage. Each batch of bakso was an experimental unit in the 6 experimental replications.

Results & Discussion

Replication in this study had no effects ($P > 0.05$) on any bakso properties. Of the composition, stability, and textural properties, only lipid stability, as indicated by TBARS, increased ($P < 0.05$) with increased frozen storage time of the frozen bakso meatballs (Table 1). The phospholipids in frozen meat are susceptible to fat oxidation

(McMillin, 1996). Bakso made of 0 month frozen stored post rigor meat appeared to have a more web-like protein strand network than in bakso made from meat stored frozen for 2 months (not shown) and 4 months (Figure 1).

Bakso composition was not different ($P>0.05$) with meat rigor condition or frozen storage times (Table 2). TBARS values of bakso from post rigor meat at 0 months of bakso frozen storage were higher ($P<0.05$) than bakso from early postmortem meat. Bakso from post rigor meat increased in TBARS value during the first 2 months of frozen storage, while bakso from early postmortem beef had a constant decrease ($P<0.05$) in lipid stability throughout the frozen storage period. Bakso from postrigor meat was less elastic and had less gel strength than bakso from early postmortem meat. Texture did not change with frozen storage time of bakso.

Conclusions

The substitution of post rigor meat for early postmortem meat in bakso production using 0.6% STPP, 1.6% NaCl, and 15% starch tapioca resulted in bakso with minimal composition and texture differences. The decrease in oxidative stability of bakso made from post rigor meat after 2 and 4 months of frozen storage compared with the use of early postmortem meat or bakso stored frozen for 0 months must be addressed by inclusion of commercial ingredients to minimize potential off-flavors of the precooked bakso products. Meat stored frozen for 2 and 4 months was still suitable as raw material for bakso production.

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Tables and Figures

Table 1. Proximate analysis, lipid stability, and texture of bakso from postmortem beef.

Meat storage (mo)	Bakso storage (mo)	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
0	0	70.75	0.22	1.524 ^c	0.552	38.884	22.272
0	2	70.73	0.21	3.061 ^a	0.537	38.804	22.232
0	4	70.57	0.19	3.036 ^a	0.537	38.778	22.210
2	0	70.77	0.22	1.612 ^{bc}	0.545	38.742	22.233
2	2	70.73	0.20	2.811 ^{ab}	0.531	38.532	22.218
2	4	70.62	0.19	2.989 ^a	0.523	38.519	22.208
4	0	70.75	0.21	1.519 ^c	0.536	38.537	22.160
4	2	70.71	0.20	2.706 ^{ac}	0.522	38.507	22.118
4	4	70.52	0.20	3.069 ^a	0.517	38.496	22.150
SEM		0.37 nd	0.01 nd	0.255	0.016 nd	0.202 nd	0.468 nd

Table 2. Properties of bakso from early postmortem and postrigor meat after 0, 2, and 4 months frozen storage.

Meat condition	Storage (mo)	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
Postrigor	0	70.75	0.22	1.524 ^{bc}	0.552 ^{bcd}	38.884 ^b	22.272
Postrigor	2	70.73	0.21	3.061 ^a	0.537 ^{cde}	38.805 ^b	22.232
Postrigor	4	70.57	0.19	3.036 ^a	0.537 ^c	38.778 ^b	22.210
Early	0	70.76	0.21	0.922 ^d	0.651 ^a	40.632 ^a	23.175
Early	2	70.75	0.20	1.438 ^{bcd}	0.624 ^{ab}	40.507 ^a	23.158
Early	4	70.61	0.18	1.655 ^b	0.613 ^{abc}	40.506 ^a	23.153
SEM		0.173 nd	0.01 nd	0.119	0.016	0.224	0.274 nd

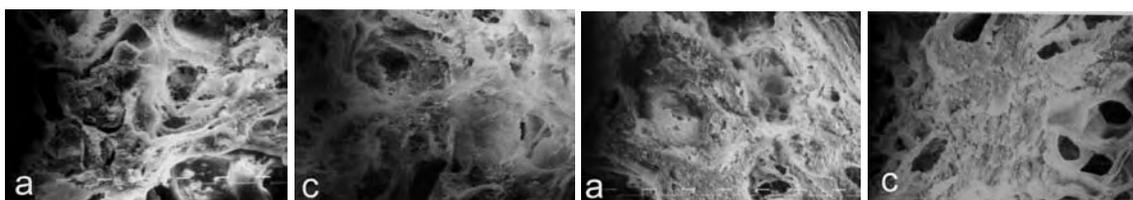


Figure 1. Scanning electron microscopy at 1500x magnification of bakso made of post rigor meat stored frozen 0 month (left micrographs) and 4 months (right micrographs) after 0 (a) and 4 (c) months of frozen bakso storage.

LOCALIZATION OF ZINC PROTOPORPHYRIN IX (ZPP) IN PARMA HAM

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Key Words: Parma ham, Color, Zn protoporphyrin IX, Localization, Autofluorescence

Introduction

The color of traditional dry-cured Parma ham is desirable red despite the fact that nitrite or nitrate is not added. It has been reported that an unidentified myoglobin derivative, which is extractable by 75% acetone, is present in Parma ham (Morita et al., 1996). We have recently shown that the red pigment is Zn protoporphyrin IX (ZPP), in which iron in heme is substituted by zinc (Wakamatsu et al., 2004a). It has been reported that the red pigment is formed by staphylococci (Morita et al., 1996), but the existence of microorganisms inside the products is suspected. We have established a model system in which ZPP is formed by anaerobic incubation of meat and myoglobin in the absence of microorganisms (Wakamatsu et al., 2004b). The results suggested that endogenous enzymes contribute to the formation of ZPP. However, the mechanism of formation and the localization of ZPP in Parma ham have not been elucidated.

Objectives

The objective of this study was to determine the localization of ZPP in Parma ham by observing autofluorescence by near-UV light irradiation in order to clarify the mechanism of ZPP formation.

Methodology

Materials. Whole Parma hams (deboned) were purchased from f.lli Galloni s.p.a..

Purple LED lighting. Five purple LEDs (Peak wavelength: 400 nm, OSSV5111A, OptoSupply) were connected in series at intervals of 10.16 mm, and nine LED series were connected in parallel. The current was regulated by two current regulative diodes (10 mA CRD) in parallel.

Measurement of autofluorescence spectra of Parma ham. Parma ham was cut by a slicer into slices of approximately 2 mm in thickness. The autofluorescence spectra of the lean meat and the subcutaneous fat tissue of Parma ham (approximately 1.5 cm square) were measured by using a spectrofluorophotometer (RF-5300C, Shimadzu Corp.). The excitation wavelength was 400 nm, and fluorescence spectra from 450 nm to 750 nm were analyzed.

Detection of ZPP in Parma ham by purple LED lighting. The slices of Parma hams (approximately 2 mm in thickness) were irradiated by purple LED lighting from two

directions and were photographed by using a digital camera (D70, Nikon Corp.) in a darkroom. The colors of the images were divided in RGB colors by using Adobe Photoshop 6.0 (Adobe Systems Inc.). Green (G) and blue (B) were deleted. The red emission was regarded as localization of ZPP.

Detection of ZPP in Parma ham by fluorescence microscopic observation. Parma ham was cut on a cryostat microtome (CM3000, Leica Microsystems) at -20°C. The frozen sections (10 µm in thickness) were mounted on slides and thawed at room temperature. The specimens were embedded in a mountant (Aqua-Poly/Mount, Plysciences, Inc.). The embedded specimens were viewed with a fluorescence microscope (BX50-FLA, Olympus Corp.). Using an excitation filter of 400-440 nm, a 565-nm dichroic mirror and a 580-nm barrier filter, the specimens were viewed at room temperature.

Results & Discussion

The autofluorescence spectra of Parma ham are shown in Fig. 1. Emission peaks of lean meat were detected at 473, 593 and 632 nm, and those of subcutaneous fat tissue were detected at 467, 584, 633 and 699 nm. Since the emissions at approximately 590 and 630 nm were consistent with that of ZPP and emission peaks of the autofluorescence spectra of the residue extracted from Parma ham by 75% acetone had disappeared (data not shown), the emission peaks were caused by ZPP. It was shown that ZPP existed in both lean meat and subcutaneous fat tissue. ZPP tended to be more abundant in subcutaneous fat tissue than in lean meat. As shown in Fig. 1, red emission other than that of ZPP was hardly detected. Therefore, it appears that the location of ZPP agreed with that of the red emission.

A sample of Parma ham was cut out from the portion shown by the arrow in Fig. 2A. A cross section of the sample is shown in Fig. 2B. The red emission of Parma ham irradiated by purple LED, that is, the location of ZPP, is shown in Fig. 2B. Although ZPP was distributed widely in Parma ham, it was more abundant in the intermuscular fat and subcutaneous fat than in the lean meat, in agreement with the results shown in Fig. 1. ZPP in the lean meat tended to be more abundant in the inner region than in the outer region, and ZPP in the subcutaneous fat also tended to be more abundant in the inner region than in the outer region. Since the Parma ham pigment, i.e., ZPP, is lipophilic (Møller *et al.*, 2003), ZPP might be transferred from lean meat to fat tissue during the processing, resulting in the small amount of ZPP in lean meat adjacent to subcutaneous fat. Further investigation on the transference of ZPP is needed. On the other hand, the intensity of red emission was weak in the superficial portions of semimembranosus (SM) and adductor (AD) muscles; these portions were the cutting plane and were exposed during the processing of ham. Since ZPP was formed under anaerobic conditions in a model system (Wakamatsu *et al.*, 2004b), oxygen might inhibit the formation of ZPP. The location of ZPP in other cross-sections was similar to the above-described results (data not shown).

ZPP was also detected by fluorescence microscopy as shown in Fig. 3. Although emission of ZPP was observed within muscle fibers, strong emission in the perimysium was not observed. Even in an enlarged image, ZPP was found to be localized within muscle fibers.

Conclusions

Red autofluorescence of Parma ham induced by near-UV light irradiation was found to be derived mainly from ZPP. Localization of ZPP in Parma ham was clarified by using purple LED lighting and the image processing. Although ZPP was distributed widely in the interior of Parma ham, it was more abundant in fat tissue than in lean meat. It was thought that ZPP transferred from lean meat to fat tissue during the processing. The cutting plane had only a small amount of ZPP. Exposure to oxygen might have inhibited the formation of ZPP in that portion. By microscopic examination, fluorescence of ZPP was observed within muscle fibers, whereas a strong positive reaction in the perimysium was not seen.

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Tables and Figures

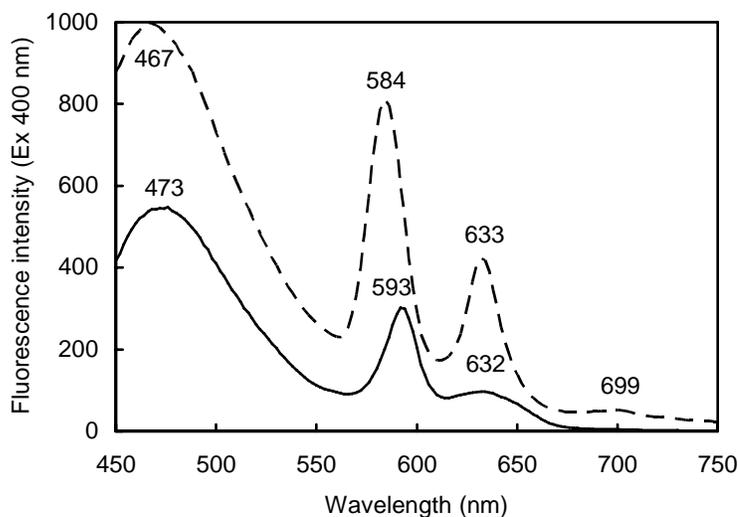


Fig. 1. Autofluorescence spectra (excitation: 400 nm) of lean meat (solid line) and subcutaneous fat tissue (broken line) in Parma ham.

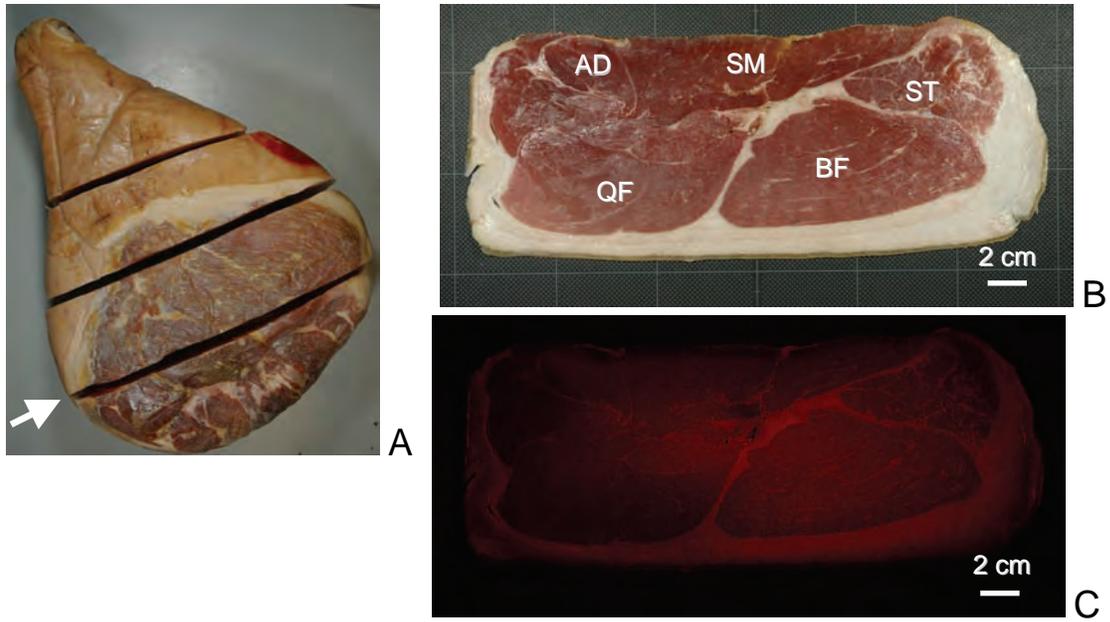


Fig. 2. Sampling from Parma ham (cutting line (A, arrow) and cross section (B)) and localization of ZPP detected by the irradiation using purple LED lighting (C). *Muscles:* AD, adductor; SM, semimembranosus; ST, semitendinosus; QF, quadriceps femoris; BF, biceps femoris.

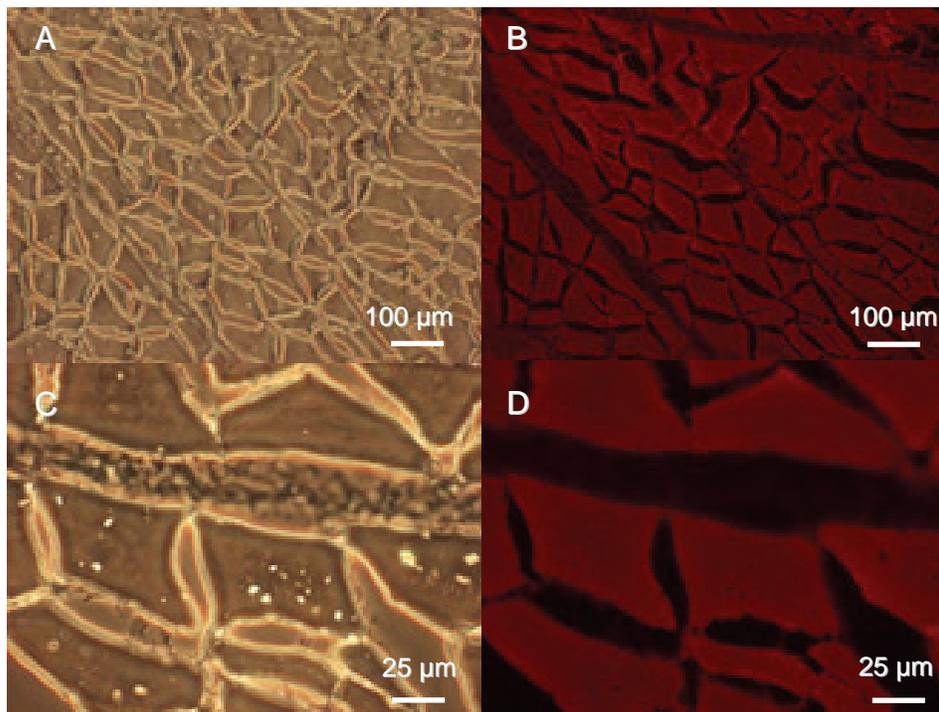


Fig. 3. Detection of ZPP by fluorescent microscopic observation. Phase-contrast views (A and C) and fluorescence views of emission by ZPP (B and D).

QUALITY CHARACTERISTICS OF BA-TSUNG FRESH PORK SAUSAGE AS AFFECTED BY ACID-INDUCED GELLED EGG WHITE POWDER AND SODIUM LACTATE

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Key Words: Ba-Tsung fresh sausage, Acid-induced gelled egg white powder, Sodium lactate, Antioxidant ability, Bacteriostatic ability

Introduction

Chen *et al.* (2004) reported that non-fermented acid Chinese-style semi-dry sausage formulated with acid-induced gelled egg white powder had the antioxidant ability and appetizing acidic traits. Sodium lactate was a natural salt of lactic acid to be extensively used in meat industry, which possessed the characteristics of increasing shelf-life and enhancing meat flavor, water-holding ability, antioxidant function, cooking yields on the meat products without affecting pH value of meat products. And it also had the function of reducing water activity and inhibiting the growth of aerobic, facultative and anaerobic microorganisms. The Chinese mixed herbs-Ba-Tsung powder had the antioxidant and appetizing effects on meat products due to containing the polyphenol functional groups.

The ground fresh pork sausage was an uncured and nitrite-non-added meat products which quality was easily affected by unsuitable raw meat handling processing procedures and storage time, and subsequently to cause the results of lipid oxidative rancidity flavor, color discoloration and microbial growth of products, and further, to reduce the economic value of products.

Objectives

The objective of this study was to investigate the characteristics of pH values, antioxidant ability, color stability and bacteriostatic ability of non-fermented and acidic fresh pork sausage formulated with Ba-Tsung powder as affected by differing ratios acid-induced gelled egg white powder and sodium lactate during 21 days refrigerated storage.

Methodology

Materials

1. The acid-induced gelled egg white powder were made as described by Chen *et al.* (2004).
2. The experimental design of non-fermented and acidic Ba-Tsung fresh pork sausage formulated with 1,2.5% of differing lactic acid concentration (6,7,8,9 and

10%) induced gelled egg white powder and 0,3% of sodium lactate(SL) was shown in table 1.

Methods

1. **Measurement of pH value, 2-thiobarbituric acid (TBA) value and metmyoglobin (MetMb) content** : a. The pH value was measured as described by Ockerman (1974). b. The TBA value was measured as described by Taladgis *et al.* (1960) and modified Zipser and Watts (1962) and Ockerman (1974). c. The MetMb was analyzed as described by Warriss (1979), Trout (1989) and calculated as described by Krzywicki (1982).
2. **Measurements of color difference and water-holding capacity (WHC)** : a. The color difference of Hunter *L, a, b* value was measured by color difference meter (Model TC-1, Nippon Denshoku Co., LTD, Tokyo, Japan). b. The WHC was measured as described by the methods of McCaw *et al.* (1994).
3. **Total plate counts (TPC) measurement** : The Total plate counts was obtained as described by FDA. (1996).
4. **Statistical analysis** : Triplicate measurements were conducted for each sample. The statistical analysis of the data was carried out by applying Duncan's new multiple range test using the statistical analysis system (SAS, 1996) by the General Linear Model.

Results & Discussion

The pH value, water-holding capacity, color difference(Hunter *L, a, b*), metmyoglobin content and total plate counts of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and sodium lactate across all storage days at 4 are shown in table 2, 3, 4, 5, 6, 7 and 8. Table 2 indicated that the pH value of treatment groups A1 and A2 were between 5.5~5.8, D1 and D2 to be lower than 5.4. Table 3 showed that the water holding capacity of all treatment groups were very stable, and A1 and A2 groups had significantly difference ($p < 0.05$) than the others. Table 4 and 5 indicated that the Hunter *L* and *a* value of all treatment groups were very stable, and contrasted with metmyoglobin content as shown in table 7, the A1, A2 and B2 had the high acceptability than the others. Table 6 showed that the TBA value of all treatment groups were increased with increasing storage days, and A1, A2, B1 and B2 groups had lower value than the others, and to be significantly difference ($p < 0.05$). It could be explained that the antioxidant ability of our experimental non-fermented and acidic Ba-Tseng pork sausage were stabilized by Chinese mixed herbs-Ba-Tseng powder. Table 8. indicated that the TPC of all treatment groups were very stable across storage days at 4 , and 2.5% of differing lactic acid concentration (6, 7, 8, 9 and 10%) induced gelled egg white powder and sodium lactate had significantly difference ($p < 0.05$) than the others. As we expected that the different ratio lactic acid induced egg white powder and sodium lactate had the synergistic effects on Ba-Tseng fresh pork sausage. Additionally, our experiments have indicated that the sensory evaluation group can not accept the pH value of non-fermented and acidic Ba-Tseng fresh pork sausage to be lower than 5.50.

Conclusions

According to the data of experimental items of pH value, antioxidant ability, color stability and bacteriostatic ability across all storage days at 4 °C, the Ba-Tseng pork sausage formulated with 1% of 6% lactic acid-induced gelled egg white powder and 3% sodium lactate had possessed the non-fermented and acidic appetizing traits.

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Tables

Table 1. Experimental design of non-fermented and acidic Ba-Tseng fresh pork sausage formulated with 1%, 2.5% of differing lactic acid concentration (6%,7%,8%,9% and 10%) induced gelled egg white powder and 0%,3% of sodium lactate

A1 :	1% of 6% lactic acid-induced gelled egg white powder+0%SL
A2 :	1% of 6% lactic acid-induced gelled egg white powder+3%SL
A3 :	2.5% of 6% lactic acid-induced gelled egg white powder+0%SL
A4 :	2.5% of 6% lactic acid-induced gelled egg white powder+3%SL
B1 :	1% of 7% lactic acid-induced gelled egg white powder+0%SL
B2 :	1% of 7% lactic acid-induced gelled egg white powder+3%SL
B3 :	2.5% of 7% lactic acid-induced gelled egg white powder+0%SL
B4 :	2.5% of 7% lactic acid-induced gelled egg white powder+3%SL
C1 :	1% of 8% lactic acid-induced gelled egg white powder+0%SL
C2 :	1% of 8% lactic acid-induced gelled egg white powder+3%SL
C3 :	2.5% of 8% lactic acid-induced gelled egg white powder+0%SL
C4 :	2.5% of 8% lactic acid-induced gelled egg white powder+3%SL
D1 :	1% of 9% lactic acid-induced gelled egg white powder+0%SL
D2 :	1% of 9% lactic acid-induced gelled egg white powder+3%SL
D3 :	2.5% of 9% lactic acid-induced gelled egg white powder+0%SL
D4 :	2.5% of 9% lactic acid-induced gelled egg white powder+3%SL
E1 :	1% of 10% lactic acid-induced gelled egg white powder+0%SL
E2 :	1% of 10% lactic acid-induced gelled egg white powder+3%SL
E3 :	2.5% of 10% lactic acid-induced gelled egg white powder+0%SL
E4 :	2.5% of 10% lactic acid-induced gelled egg white powder+3%SL

Table 2. The pH value of Ba-Iseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)						
	0	3	5	7	10	14	21
Control	5.83±0.01 ^a	6.24±0.11 ^a	6.05±0.03 ^a	6.23±0.06 ^a	6.36±0.03 ^a	6.25±0.03 ^a	6.15±0.04 ^a
A1	5.64±0.06 ^{abc}	5.78±0.07 ^{bc}	5.80±0.10 ^b	5.64±0.05 ^b	5.59±0.02 ^{bc}	5.78±0.07 ^{bc}	5.58±0.10 ^{bcd}
A2	5.57±0.05 ^{ab}	5.88±0.03 ^b	5.84±0.08 ^{ab}	5.64±0.06 ^b	5.64±0.02 ^b	5.90±0.05 ^b	5.65±0.02 ^{bc}
A3	4.76±0.03 ^{hi}	4.94±0.02 ^{ghi}	4.85±0.12 ^{de}	4.89±0.04 ^{fgh}	5.02±0.05 ^f	4.79±0.00 ^{hij}	4.85±0.05 ^{ef}
A4	5.02±0.005 ^g	5.04±0.03 ^g	4.94±0.16 ^d	5.12±0.04 ^e	5.04±0.03 ^f	5.00±0.00 ^{fg}	5.04±0.04 ^e
B1	5.63±0.10 ^{abcd}	5.76±0.09 ^{bc}	5.47±0.16 ^c	5.45±0.05 ^c	5.65±0.01 ^b	5.11±0.02 ^f	4.92±0.05 ^{ef}
B2	5.40±0.14 ^{def}	5.63±0.03 ^{cde}	5.43±0.02 ^c	5.44±0.02 ^c	5.54±0.04 ^{bcd}	5.50±0.00 ^d	5.62±0.04 ^{bc}
B3	4.75±0.01 ^{hi}	4.81±0.05 ^{ijk}	4.88±0.11 ^{de}	4.89±0.01 ^{hi}	4.78±0.01 ^{gh}	4.65±0.08 ^{lk}	4.86±0.07 ^{ef}
B4	4.92±0.02 ^{hgi}	4.97±0.03 ^{ghi}	4.83±0.08 ^{de}	5.07±0.00 ^{ef}	5.01±0.04 ^f	4.92±0.03 ^{gh}	4.95±0.01 ^{ef}
C1	5.30±0.24 ^f	5.52±0.05 ^{def}	5.44±0.03 ^c	5.46±0.02 ^b	5.33±0.03 ^e	5.50±0.00 ^d	5.55±0.02 ^{bcd}
C2	5.46±0.01 ^{cdef}	5.58±0.02 ^{de}	5.50±0.02 ^c	5.58±0.03 ^b	5.40±0.00 ^{de}	5.50±0.03 ^d	5.68±0.00 ^{bc}
C3	4.69±0.03 ⁱ	4.77±0.03 ^{kl}	4.69±0.02 ^e	4.91±0.00 ^{hi}	4.74±0.00 ^h	4.70±0.03 ^{jk}	4.75±0.03 ^f
C4	4.91±0.01 ^{hgi}	4.95±0.02 ^{ghi}	4.90±0.05 ^{de}	5.05±0.03 ^{efg}	5.02±0.03 ^f	4.90±0.02 ^{gh}	4.85±0.05 ^{ef}
D1	5.41±0.02 ^{cdef}	5.59±0.02 ^{de}	5.46±0.03 ^c	5.52±0.08 ^{bc}	5.35±0.05 ^e	5.69±0.11 ^c	5.41±0.06 ^d
D2	5.56±0.03 ^{bcd}	5.65±0.05 ^{cd}	5.53±0.01 ^c	5.53±0.01 ^{bc}	5.44±0.02 ^{cde}	5.75±0.01 ^c	5.51±0.02 ^{cd}
D3	4.72±0.00 ⁱ	4.76±0.01 ^{kl}	4.75±0.03 ^{de}	4.92±0.02 ^{gh}	4.77±0.01 ^{gh}	4.66±0.00 ^k	4.76±0.03 ^f
D4	4.96±0.02 ^{gh}	5.01±0.04 ^{gh}	4.91±0.00 ^{de}	5.06±0.01 ^{ef}	4.98±0.03 ^f	4.89±0.01 ^{ghi}	4.97±0.00 ^e
E1	5.39±0.08 ^{ef}	5.41±0.07 ^f	5.43±0.04 ^c	5.31±0.06 ^d	5.56±0.01 ^{bcd}	5.36±0.02 ^e	5.58±0.22 ^{bcd}
E2	5.56±0.07 ^{bcd}	5.48±0.04 ^{ef}	5.53±0.03 ^c	5.45±0.01 ^c	5.61±0.21 ^b	5.41±0.01 ^{de}	5.73±0.02 ^b
E3	4.76±0.04 ^{hi}	4.65±0.05 ^k	4.69±0.01 ^e	4.53±0.01 ^j	4.70±0.01 ^h	4.52±0.02 ^l	4.53±0.02 ^g
E4	4.98±0.02 ^{gh}	4.84±0.02 ^{hij}	4.91±0.01 ^{de}	4.78±0.03 ⁱ	4.92±0.01 ^{fg}	4.76±0.03 ^{ijk}	4.76±0.00 ^f

^{a-j} Mean within the same column with different superscripts are significantly different (p<0.05).

Table 3. The water holding capacity of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)						
	0	3	5	7	10	14	21
Control	70.66±1.14 ^{bcd}	74.22±1.02 ^a	74.23±0.79 ^a	72.13±1.51 ^{ab}	74.23±1.61 ^a	73.41±1.02 ^a	73.98±1.03 ^a
A1	68.11±1.86 ^{efgh}	70.16±1.31 ^{abcd}	70.69±1.28 ^{abcd}	71.16±0.35 ^{abcd}	69.15±0.10 ^{bcdef}	68.94±0.78 ^{cd}	70.85±0.32 ^{bc}
A2	67.95±0.62 ^{efgh}	69.46±0.30 ^{bcdef}	70.41±2.46 ^{abdef}	70.86±0.48 ^{abdef}	70.77±0.42 ^{bcde}	67.62±1.56 ^{de}	70.85±1.08 ^{bc}
A3	66.16±0.31 ^{gh}	66.84±0.52 ^{fghi}	67.52±0.53 ^{edf}	67.68±0.46 ^{fghi}	67.89±0.58 ^{ef}	66.97±0.38 ^{de}	67.06±0.35 ^e
A4	67.57±0.29 ^{efgh}	65.89±0.59 ^{hij}	67.16±0.28 ^{ef}	67.24±0.34 ^{ghi}	68.23±0.83 ^{def}	66.75±0.47 ^{de}	66.43±0.83 ^e
B1	73.72±1.18 ^a	72.15±0.92 ^{ab}	72.05±1.02 ^{abc}	72.85±0.37 ^a	72.34±0.75 ^{ab}	70.39±1.11 ^{bc}	68.22±1.21 ^{cde}
B2	72.55±1.12 ^{ab}	70.00±1.18 ^{bcde}	72.90±0.72 ^{ab}	71.89±0.68 ^{ab}	72.16±3.77 ^{abc}	72.75±0.04 ^{ab}	72.55±0.91 ^{ab}
B3	66.81±0.49 ^{fgh}	65.60±0.61 ^{ij}	67.18±0.72 ^{ef}	66.56±0.58 ^{hi}	67.76±0.16 ^{ef}	67.07±0.33 ^{de}	66.49±0.51 ^e
B4	66.63±0.54 ^{fgh}	66.16±0.45 ^{hij}	67.60±1.12 ^{edf}	66.17±0.15 ⁱ	67.30±0.46 ^f	67.30±0.71 ^{de}	66.42±0.97 ^e
C1	69.29±0.56 ^{cde}	71.47±1.29 ^{abc}	71.60±0.87 ^{abcd}	71.31±0.59 ^{abc}	67.17±1.76 ^f	68.32±0.92 ^{cde}	68.19±1.22 ^{cde}
C2	71.01±0.50 ^{bc}	69.83±0.78 ^{bcde}	69.62±0.84 ^{bcdef}	70.59±0.60 ^{bcde}	68.85±0.46 ^{cdef}	68.56±0.47 ^{cde}	70.13±2.12 ^{bcd}
C3	67.18±0.48 ^{efgh}	67.38±0.35 ^{defghi}	66.64±0.43 ^{ef}	68.03±0.10 ^{fghi}	68.52±0.33 ^{def}	67.24±0.51 ^{de}	66.81±0.39 ^e
C4	67.74±0.44 ^{efgh}	66.83±0.39 ^{fghij}	66.21±1.61 ^f	68.23±0.67 ^{fghi}	68.01±0.71 ^{def}	67.00±0.83 ^{de}	66.14±0.51 ^e
D1	68.83±0.89 ^{cdef}	68.05±0.57 ^{defghi}	68.20±1.93 ^{cdef}	68.98±0.91 ^{defg}	69.41±1.20 ^{bcdef}	68.64±1.90 ^{cde}	69.09±1.00 ^{cde}
D2	68.45±0.52 ^{defg}	69.57±1.14 ^{bcdef}	69.07±1.09 ^{bcdef}	68.00±0.67 ^{fghi}	71.35±0.41 ^{abcd}	68.27±1.06 ^{cde}	70.11±0.61 ^{bcd}
D3	67.88±0.24 ^{efgh}	66±510.40 ^{ghij}	66.72±0.97 ^{ef}	69.67±0.92 ^{cdef}	68.56±0.34 ^{def}	66.84±0.33 ^{de}	67.28±1.15 ^{de}
D4	68.17±0.54 ^{efgh}	65.76±0.29 ^{ij}	67.73±0.97 ^{edf}	68.38±0.25 ^{fgh}	68.98±0.18 ^{bcdef}	66.98±0.11 ^{de}	66.69±0.55 ^e
E1	68.49±0.75 ^{defg}	69.00±0.95 ^{cdefg}	68.41±0.47 ^{bcdef}	68.67±1.33 ^{efgh}	68.78±0.60 ^{bcdef}	68.19±0.53 ^{cde}	67.45±0.12 ^{de}
E2	68.57±0.35 ^{cdefg}	68.59±1.18 ^{defgh}	69.73±0.30 ^{bcdef}	68.04±0.62 ^{efg}	69.16±0.69 ^{bcdef}	68.94±0.79 ^{cd}	68.06±0.77 ^{cde}
E3	67.74±1.02 ^{efgh}	67.33±0.66 ^{efghi}	66.57±0.57 ^{ef}	67.29±0.73 ^{ghi}	67.09±0.29 ^f	66.21±0.31 ^e	66.31±0.43 ^e
E4	65.87±0.60 ^h	64.65±0.94 ^j	68.84±3.95 ^{bcdef}	66.13±0.62 ⁱ	67.55±0.01 ^{ef}	66.45±0.40 ^{de}	66.73±0.57 ^e

^{a-j} Mean within the same column with different superscripts are significantly different (p<0.05).

Table 4. The lightness (*L* value) of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)						
	0	3	5	7	10	14	21
Control	38.93±0.96 ^{ab}	43.35±1.87 ^a	42.64±0.44 ^a	40.33±0.36 ^a	38.64±1.05 ^{ab}	39.60±1.88 ^{ab}	41.10±1.46 ^{ab}
A1	33.83±1.07 ^{defg}	34.77±1.34 ^{def}	34.64±0.88 ^{defg}	33.66±1.65 ^{efgh}	35.56±0.44 ^{cde}	33.90±0.22 ^{efg}	34.75±1.11 ^{gh}
A2	33.16±0.89 ^{efg}	34.19±0.88 ^{def}	33.74±0.36 ^{efgh}	32.65±1.12 ^{efij}	33.86±0.32 ^{ef}	34.59±0.85 ^{def}	34.48±0.60 ^{gh}
A3	36.83±0.33 ^{bcd}	36.33±1.00 ^{cdef}	35.44±0.65 ^{def}	37.40±1.37 ^{bcddef}	35.82±0.58 ^{cde}	39.57±0.47 ^{ab}	37.28±0.14 ^{cdefg}
A4	34.29±0.84 ^{defg}	35.79±1.02 ^{def}	34.16±0.14 ^{defgh}	35.78±0.73 ^{defg}	36.90±0.59 ^{bc}	33.90±1.00 ^{efg}	34.86±0.92 ^{efgh}
B1	31.88±1.49 ^g	31.43±0.57 ^{gh}	30.79±0.84 ⁱ	29.89±0.51 ^{ij}	30.68±0.25 ^h	31.87±1.22 ^{fg}	35.82±1.09 ^{efgh}
B2	31.52±0.68 ^g	30.45±0.20 ^h	30.95±0.21 ⁱ	29.60±0.34 ^j	30.94±0.53 ^{gh}	31.55±0.49 ^g	34.27±1.66 ^{gh}
B3	36.20±0.44 ^{bcd}	37.32±0.84 ^{bcd}	38.13±0.41 ^{bc}	38.11±1.33 ^{abcd}	37.48±1.32 ^{abc}	40.15±1.70 ^{ab}	39.40±1.75 ^{abcd}
B4	34.30±0.79 ^{cdef}	35.82±1.05 ^{def}	36.23±0.71 ^{bcd}	36.22±0.16 ^{cdef}	35.85±0.96 ^{cd}	38.14±0.57 ^{abc}	38.03±0.72 ^{bcd}
C1	32.58±0.97 ^{fg}	33.46±0.24 ^{efgh}	31.93±0.55 ⁱ	32.93±0.23 ^{ghi}	33.38±0.54 ^f	33.28±0.50 ^{efg}	33.38±0.55 ^h
C2	31.99±1.18 ^g	33.10±0.49 ^{efgh}	32.55±0.25 ^{ghi}	33.25±0.84 ^{efgh}	33.57±0.61 ^f	32.95±0.74 ^{efg}	33.69±0.55 ^h
C3	38.79±1.40 ^{abc}	39.15±0.57 ^{bc}	38.16±0.44 ^{bc}	38.32±0.71 ^{abcd}	39.08±0.27 ^a	39.50±1.49 ^{ab}	39.97±0.60 ^{abc}
C4	37.55±0.92 ^{abc}	37.00±0.39 ^{bcd}	34.71±0.69 ^{defg}	37.07±0.10 ^{bcd}	35.66±0.62 ^{cde}	39.76±1.28 ^{ab}	38.20±1.70 ^{abcde}
D1	33.34±0.40 ^{efg}	34.71±1.00 ^{def}	35.47±0.06 ^{def}	35.25±0.96 ^{defg}	34.39±0.59 ^{def}	34.69±0.10 ^{def}	35.34±0.23 ^{efgh}
D2	33.47±1.39 ^{efg}	33.67±0.86 ^{efgh}	33.79±1.10 ^{efgh}	32.97±0.80 ^{ghi}	33.12±0.06 ^f	32.79±0.27 ^{efg}	35.34±0.88 ^{efgh}
D3	40.14±0.46 ^a	40.02±0.60 ^b	38.68±1.11 ^b	39.19±1.00 ^{abc}	39.25±1.01 ^a	40.87±1.18 ^a	40.83±1.33 ^{ab}
D4	35.67±0.59 ^{cdef}	36.28±1.99 ^{cdef}	36.17±0.69 ^{bcd}	34.92±1.16 ^{efgh}	36.30±0.73 ^{cd}	35.22±1.06 ^{cde}	38.06±0.87 ^{abcde}
E1	32.23±10.6 ^g	33.95±0.28 ^{efg}	33.52±0.53 ^{efgh}	33.97±0.79 ^{efgh}	32.81±0.38 ^{fg}	33.50±0.88 ^{efg}	34.50±0.80 ^{gh}
E2	31.25±1.52 ^g	33.32±1.56 ^{efgh}	32.46±0.90 ^{ghi}	32.48±0.49 ^{hij}	31.10±0.11 ^{gh}	33.90±0.53 ^{efg}	34.65±0.22 ^{gh}
E3	36.83±0.86 ^{bcd}	39.33±0.97 ^{bc}	36.64±0.59 ^{bcd}	39.59±1.51 ^{ab}	38.95±0.18 ^a	39.34±0.21 ^{ab}	41.28±1.43 ^a
E4	33.20±1.76 ^{efg}	36.96±1.12 ^{bcd}	35.99±2.34 ^{cdef}	36.39±0.78 ^{cdef}	36.81±0.17 ^{bc}	37.21±1.04 ^{bcd}	36.28±0.85 ^{bcd}

^{a-j} Mean within the same column with different superscripts are significantly different ($p < 0.05$).

Table 5. The redness (*a* value) of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)						
	0	3	5	7	10	14	21
Control	10.95±0.75 ^{bcdef}	7.35±0.26 ^{bcdef}	7.45±0.56 ^c	7.84±0.24 ^{bcd}	7.18±0.33 ^{bcd}	7.50±0.34 ^{abc}	6.63±0.51 ^{cde}
A1	10.83±0.38 ^{abc}	9.19±0.30 ^{ab}	9.00±0.56 ^{ab}	9.08±0.16 ^{ab}	7.74±0.36 ^{bcd}	7.61±1.21 ^{ab}	6.64±0.31 ^{cde}
A2	10.79±0.40 ^{abc}	9.90±0.52 ^a	9.10±0.37 ^{ab}	10.34±0.54 ^a	8.22±0.47 ^{bc}	7.89±0.63 ^{ab}	8.20±0.32 ^{ab}
A3	8.14±0.05 ^{efgh}	5.96±0.27 ^{defghi}	5.65±0.87 ^{ef}	4.64±0.29 ^{fg}	4.32±0.25 ^{gh}	5.84±0.59 ^{cdefg}	4.35±0.03 ^{hi}
A4	9.22±0.46 ^{bcd}	6.94±0.30 ^{cdefg}	5.89±0.05 ^{de}	5.40±0.48 ^{ef}	5.46±0.72 ^{fg}	5.36±0.01 ^{defghi}	6.95±0.58 ^{bcd}
B1	8.89±0.62 ^{cdefg}	8.18±0.36 ^{abc}	7.61±0.20 ^c	7.61±0.45 ^{cd}	10.00±0.46 ^a	9.10±0.64 ^a	7.51±0.19 ^{abc}
B2	9.49±0.70 ^{bcd}	8.38±0.54 ^{abc}	7.62±0.30 ^c	6.94±0.25 ^d	8.49±1.06 ^b	8.72±0.51 ^a	8.62±0.53 ^a
B3	8.45±0.61 ^{defgh}	5.85±0.74 ^{efghi}	4.16±0.26 ^{gh}	3.82±0.11 ^g	4.16±0.41 ^{gh}	4.22±0.17 ^{ghij}	4.92±0.32 ^{fghi}
B4	9.44±1.03 ^{bcd}	7.57±1.24 ^{bcd}	4.79±0.73 ^{efgh}	4.60±0.49 ^{fg}	4.56±0.40 ^{gh}	5.41±0.50 ^{defghi}	6.84±0.25 ^{bcd}
C1	10.30±0.10 ^{abcd}	8.28±0.71 ^{abc}	7.24±0.53 ^c	7.11±0.43 ^d	6.67±0.78 ^{def}	5.63±0.25 ^{defgh}	5.20±0.07 ^{fghi}
C2	10.10±1.21 ^{abcde}	8.62±1.15 ^{abc}	6.93±0.55 ^{cd}	7.52±1.11 ^{cd}	6.72±0.39 ^{cdef}	6.24±0.75 ^{bcd}	5.52±0.17 ^{efghi}
C3	7.18±0.59 ^{gh}	4.82±0.43 ^{hi}	3.87±0.19 ^{fgh}	3.94±0.36 ^g	4.11±0.59 ^{gh}	4.28±0.22 ^{ghij}	4.63±0.12 ^{ghi}
C4	8.31±0.26 ^{defgh}	6.25±0.64 ^{defg}	4.45±0.07 ^{gh}	4.13±0.11 ^{fg}	4.32±0.10 ^{gh}	4.59±0.13 ^{ghij}	5.67±0.34 ^{defgh}
D1	10.95±0.24 ^{ab}	9.80±0.24 ^a	8.98±0.24 ^{ab}	8.94±.78 ^{abc}	8.33±0.21 ^b	6.94±0.47 ^{bcd}	5.61±0.17 ^{defgh}
D2	11.75±0.62 ^a	9.69±0.07 ^a	9.58±0.15 ^a	8.78±0.45 ^{bc}	8.05±0.34 ^{bcd}	6.98±0.28 ^{bcd}	6.03±0.33 ^{defg}
D3	7.02±0.29 ^h	5.05±0.42 ^{hi}	3.87±0.36 ^h	3.79±0.46 ^g	4.17±0.07 ^{gh}	3.84±0.33 ^{ij}	4.98±0.20 ^{fghi}
D4	9.50±0.31 ^{bcd}	5.69±0.66 ^{hi}	5.01±0.20 ^{efgh}	4.36±0.41 ^{fg}	4.40±0.53 ^{gh}	5.20±0.10 ^{efghi}	6.04±0.23 ^{def}
E1	10.50±1.09 ^{abc}	7.73±0.36 ^{bcd}	6.90±0.21 ^{cd}	6.72±0.62 ^{de}	6.42±0.72 ^{ef}	6.57±1.08 ^{bcd}	5.01±0.13 ^{defgh}
E2	11.72±1.30 ^a	8.74±1.02 ^{abc}	7.91±0.09 ^{bc}	7.17±0.10 ^d	6.56±0.01 ^{def}	7.48±0.58 ^{abc}	6.28±0.07 ^{cdef}
E3	7.95±0.28 ^{efgh}	4.36±0.27 ^j	5.08±0.15 ^{efgh}	3.80±0.40 ^g	3.30±0.20 ^h	3.37±0.57 ⁱ	4.11±0.18 ^j
E4	9.08±0.19 ^{bcd}	5.11±0.13 ^{hi}	5.39±0.28 ^{efg}	4.09±0.07 ^{fg}	3.61±0.10 ^h	4.00±0.51 ^{hij}	5.07±0.42 ^{fghi}

^{a-j} Mean within the same column with different superscripts are significantly different ($p < 0.05$).

Table 6. The TBA value (mg MDA/g) of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)				
	0	3	5	7	10
Control	0.181±0.10 ^{cdef}	0.174±0.02 ^e	0.221±0.05 ^l	0.185±0.00 ^e	0.175±0.01 ^h
A1	0.188±0.16 ^{cdef}	0.200±0.04 ^e	0.319±0.11 ^f	0.759±0.09 ^e	2.882±0.83 ^{gh}
A2	0.078±0.01 ^f	0.235±0.05 ^e	0.234±0.05 ^f	0.530±0.03 ^e	0.933±0.08 ^h
A3	0.522±0.23 ^{ab}	1.887±0.84 ^{ab}	4.269±0.26 ^{bc}	6.701±1.70 ^{bc}	8.412±0.85 ^{bcde}
A4	0.468±0.09 ^{abcd}	1.53±0.20 ^{abc}	3.8714±0.50 ^c	9.259±0.61 ^{ab}	9.521±1.15 ^{abcd}
B1	0.157±0.01 ^{def}	0.232±0.02 ^f	0.276±0.04 ^f	1.316±0.44 ^{de}	0.751±0.17 ^h
B2	0.169±0.01 ^{cdef}	0.237±0.06 ^e	0.663±0.13 ^f	0.755±0.04 ^e	1.517±0.85 ^{gh}
B3	0.477±0.12 ^{abc}	1.574±0.43 ^{abc}	4.678±0.57 ^{bc}	8.084±1.52 ^{ab}	7.485±0.99 ^{cde}
B4	0.403±0.05 ^{bcde}	1.298±0.16 ^{bcd}	6.033±0.92 ^a	10.302±1.03 ^a	9.614±0.82 ^{abcd}
C1	0.224±0.02 ^{bcdef}	0.276±0.07 ^e	0.443±0.08 ^f	1.424±0.72 ^{de}	4.420±1.20 ^{fg}
C2	0.141±0.02 ^{ef}	0.235±0.01 ^e	0.496±0.08 ^f	1.288±0.57 ^{de}	6.13±1.29 ^{fe}
C3	0.726±0.193 ^a	2.130±0.23 ^a	4.066±0.71 ^{bc}	8.455±1.85 ^{ab}	7.714±0.29 ^{cde}
C4	0.743±0.18 ^a	1.550±0.28 ^{abc}	4.878±0.56 ^{abc}	9.92±0.33 ^a	11.074±1.60 ^{ab}
D1	0.161±0.08 ^{cdef}	0.296±0.00 ^e	0.686±0.13 ^f	1.610±0.20 ^e	1.610±0.202 ^{gh}
D2	0.141±0.00 ^{ef}	0.257±0.03 ^e	0.427±0.02 ^f	1.842±0.35 ^{de}	1.842±0.35 ^{gh}
D3	0.341±0.02 ^{bcdef}	1.476±0.17 ^{abc}	5.293±0.52 ^{ab}	9.179±1.44 ^{ab}	9.179±1.44 ^{abcd}
D4	0.296±0.02 ^{bcdef}	0.824±0.15 ^{cde}	4.466±0.36 ^{bc}	10.281±1.04 ^a	10.28±1.04 ^{abc}
E1	0.275±0.04 ^{bcdef}	0.295±0.04 ^e	0.747±0.21 ^{ef}	1.103±0.27 ^{de}	6.970±1.48 ^{bcde}
E2	0.280±0.06 ^{bcdef}	0.293±0.05 ^e	0.568±0.15 ^f	1.700±0.24 ^{de}	8.106±0.94 ^{bcde}
E3	0.340±0.07 ^{bcdef}	0.672±0.10 ^{de}	2.337±0.13 ^d	3.907±0.97 ^{cd}	9.926±0.45 ^{abcd}
E4	0.317±0.00 ^{bcdef}	0.452±0.09 ^e	1.981±0.83 ^{de}	3.872±0.35 ^{cd}	12.056±1.38 ^a

^{a-h} Mean within the same column with different superscripts are significantly different (p<0.05)

Table 7. The MetMb content (%) of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)						
	0	3	5	7	10	14	21
Control	34.87±3.04 ^{cd}	38.63±2.40 ^h	43.52±3.35 ^h	40.80±0.59 ^l	44.45±2.08 ^{fg}	48.68±1.41 ^h	30.28±2.96 ^e
A1	47.48±5.49 ^{ab}	48.19±2.69 ^{defg}	49.15±2.58 ^{gh}	52.83±6.00 ^{hg}	61.23±3.94 ^{cdh}	59.05±4.33 ^{fgh}	84.29±1.79 ^a
A2	46.79±0.03 ^{abc}	52.32±3.18 ^{bcdef}	55.68±2.13 ^{defgh}	54.13±2.08 ^{fgh}	56.23±0.76 ^{ef}	60.93±0.96 ^{efg}	66.48±1.66 ^{cd}
A3	37.80±4.92 ^{abcd}	58.81±1.93 ^{bcde}	65.99±6.72 ^{abcdef}	68.25±0.26 ^{abcd}	77.25±2.32 ^{ab}	72.89±1.26 ^{abcd}	79.15±2.43 ^{abc}
A4	37.36±0.80 ^{abcd}	61.24±3.81 ^{bc}	67.00±3.53 ^{abcdef}	72.02±2.21 ^{abcd}	73.62±2.02 ^{abcd}	71.57±0.10 ^{abcd}	75.48±1.20 ^{abc}
B1	33.16±2.23 ^{cd}	27.77±3.62 ^h	57.47±4.17 ^{defgh}	60.24±0.07 ^{efg}	39.22±10.02 ^e	77.42±3.32 ^{ab}	73.44±2.04 ^{abcd}
B2	43.93±1.17 ^{abcd}	48.76±0.66 ^{cdef}	53.23±9.79 ^{fgh}	63.36±4.41 ^{cdefg}	39.70±6.51 ^e	55.46±1.91 ^{fgh}	58.47±11.58 ^d
B3	32.19±3.26 ^d	59.61±3.48 ^{bcde}	69.35±1.28 ^{abcde}	74.92±0.40 ^{ab}	75.41±0.28 ^{abc}	76.43±0.80 ^{ab}	78.49±0.40 ^{abc}
B4	33.66±3.53 ^{cd}	61.59±1.15 ^{abc}	73.13±0.42 ^{ab}	73.96±1.57 ^{abc}	74.31±1.42 ^{abcd}	75.01±0.43 ^{abc}	72.11±2.08 ^{abcd}
C1	32.66±1.73 ^{cd}	46.20±5.66 ^{fg}	50.97±1.60 ^{gh}	55.55±3.31 ^{fgh}	58.55±0.16 ^{def}	64.08±1.14 ^{cdefg}	73.76±0.10 ^{abc}
C2	35.52±2.87 ^{abcd}	49.75±0.79 ^{cdefg}	54.38±2.51 ^{fgh}	56.13±0.81 ^{fgh}	63.33±0.87 ^{bcde}	66.09±3.66 ^{bcdef}	74.25±8.02 ^{abc}
C3	43.54±3.50 ^{abcd}	58.77±2.51 ^{bcde}	71.01±1.05 ^{abc}	73.96±2.69 ^{abc}	73.85±0.37 ^{abcd}	77.37±2.14 ^{ab}	82.00±3.53 ^{ab}
C4	39.40±2.38 ^{abcd}	73.50±2.12 ^a	75.12±1.04 ^{ab}	73.94±0.62 ^{abc}	75.46±0.57 ^{abcd}	72.98±3.15 ^{abcd}	74.50±2.33 ^{abc}
D1	42.59±5.49 ^{abcd}	46.99±4.16 ^{efg}	45.00±5.84 ^h	46.86±4.27 ^{hi}	54.34±3.95 ^{efg}	54.34±3.95 ^{gh}	81.73±0.18 ^{ab}
D2	42.97±1.34 ^{abcd}	49.02±0.07 ^{defg}	47.40±0.97 ^{gh}	56.46±4.54 ^{fgh}	54.81±0.31 ^{efg}	54.81±0.31 ^{fgh}	80.46±4.69 ^{ab}
D3	38.15±5.53 ^{abcd}	57.19±3.26 ^{bcdef}	67.00±4.32 ^{abcdef}	74.90±1.78 ^{ab}	76.58±0.58 ^{abc}	76.58±0.58 ^{ab}	80.28±2.93 ^{ab}
D4	37.80±2.39 ^{abcd}	62.06±5.60 ^{ab}	69.09±1.44 ^{abcde}	71.96±0.37 ^{abcd}	73.55±1.02 ^{abcd}	75.33±1.02 ^{abcd}	74.08±0.77 ^{abc}
E1	47.86±6.77 ^{ab}	48.56±2.07 ^{defg}	54.59±1.90 ^{defgh}	64.32±0.71 ^{bcdef}	80.33±0.25 ^a	76.41±1.43 ^{ab}	73.98±0.08 ^{abc}
E2	49.51±2.48 ^a	55.26±0.32 ^{bcdef}	60.48±0.74 ^{bcdef}	62.05±3.95 ^{defg}	69.66±11.21 ^{abcde}	77.50±0.46 ^{ab}	67.89±3.37 ^{bcd}
E3	49.23±3.90 ^a	64.12±1.54 ^{ab}	74.64±3.94 ^{ab}	75.69±0.67 ^a	76.25±0.55 ^{abc}	80.62±2.44 ^a	80.18±0.08 ^{ab}
E4	49.65±3.31 ^a	63.98±3.58 ^{ab}	75.77±0.79 ^a	75.64±1.92 ^a	78.17±0.30 ^{ab}	79.00±1.98 ^a	76.37±2.23 ^{abc}

^{a-i} Mean within the same column with different superscripts are significantly different (p<0.05).

Table 8. The TPC (log CFU/g) of Ba-Tseng fresh pork sausage as affected by different ratio lactic acid-induced gelled egg white powder and SL across all storage days at 4°C

Treatment groups	Storage time (days)						
	0	3	5	7	10	14	21
Control	3.96 ^{bc}	4.09 ^a	4.37 ^b	4.39 ^b	3.87 ^{ab}	3.4 ⁱ	5.06 ^e
A1	3.84 ^{cd}	3.60 ^f	3.38 ⁱ	3.94 ^c	5.15 ^{ab}	4.08 ^d	4.42 ^k
A2	3.49 ^{ef}	3.92 ^c	3.95 ^e	3.72 ^g	4.11 ^{ab}	3.5g ^{hi}	3.83 ^p
A3	3.87 ^{cd}	3.79 ^d	4.32 ^c	3.00 ^o	2.82 ^{ab}	4.06 ^d	4.83 ^f
A4	3.35 ^{fg}	3.54 ^f	3.31 ^j	3.33 ^l	4.26 ^{ab}	3.83 ^e	4.71 ^{gh}
B1	4.04 ^{abc}	4.06 ^{ab}	4.43 ^a	4.56 ^a	5.22 ^{ab}	5.39 ^a	3.76 ^q
B2	4.24 ^a	3.72 ^{de}	4.17 ^d	4.27 ^c	4.27 ^{ab}	4.95 ^b	4.52 ⁱ
B3	3.11 ^h	2.83 ^k	3.22 ^l	3.19 ^m	3.01 ^{ab}	3.56 ^{ghi}	4.74 ^g
B4	3.12 ^h	3.20 ^h	2.95 ⁿ	3.45 ^k	3.30 ^{ab}	3.99 ^d	5.21 ^c
C1	4.16 ^a	4.01 ^b	3.48 ^g	3.88 ^e	4.05 ^{ab}	4.08 ^d	4.02 ⁿ
C2	4.20 ^{ab}	3.73 ^{ed}	3.97 ^e	3.82 ^f	6.65 ^a	3.61 ^{gh}	4.11 ^m
C3	3.13 ^h	3.08 ^{ij}	2.80 ^o	2.90 ^p	2.99 ^{ab}	3.79 ^{ef}	4.69 ^{zh}
C4	3.68 ^{de}	3.00 ^j	2.66 ^p	3.08 ⁿ	3.59 ^{ab}	2.96 ^k	4.10 ^m
D1	3.18 ^{gh}	3.40 ^g	3.74 ^f	3.61 ⁱ	3.61 ^{ab}	3.23 ^j	5.15 ^d
D2	3.86 ^{cd}	3.01 ^j	3.25 ^k	3.69 ^h	3.99 ^{ab}	3.67 ^{fg}	3.88 ^o
D3	2.89 ⁱ	3.08 ^{ij}	2.66 ^p	3.19 ^m	3.16 ^{ab}	3.63 ^{gh}	4.86 ^f
D4	3.74 ^d	3.12 ^{hi}	2.47 ^q	2.81 ^r	2.72 ^b	3.54 ^{hi}	4.38 ^k
E1	3.47 ^{ef}	3.91 ^c	2.93 ⁿ	3.19 ^m	3.40 ^{ab}	4.46 ^c	5.43 ^a
E2	3.35 ^{fg}	3.70 ^e	3.39 ^j	2.84 ^q	3.14 ^{ab}	4.46 ^c	5.33 ^b
E3	3.01 ^{hi}	3.69 ^e	2.98 ^m	3.07 ⁿ	3.10 ^{ab}	3.04 ^k	4.46 ^j
E4	3.02 ^{hi}	3.43 ^g	3.43 ^h	3.51 ^j	3.09 ^{ab}	3.17 ^j	4.17 ^l

^{a-r} Mean within the same column with different superscripts are significantly different (p<0.05)

EFFECT OF RIPENING TEMPERATURE ON QUALITY CHARACTERISTICS OF CHINESE-STYLE SAUSAGES

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Introduction

A Chinese-style sausage is a popular traditional processed meat product in Taiwan and many other areas. Traditionally, after stuffing, it was common to hang sausages outdoors and drying under natural condition with sunshine. Without adding starter cultures, Chinese-style sausages would ferment with microorganisms naturally presented and produce specific flavors and texture for this type of product. However, due to sanitary concerns, possible lacking of good sanitation-control and also increasing numbers of modern meat processing facilities, more and more controls such as controlled temperatures and humidity have been applied to make sausage products. Limit research has been conducted to investigate the effect of keeping this traditional sausage in a ripening condition (12-18°C, 85% relative humidity) on the quality characteristics. Making quality traditional products with modern facilities is always a major interest for the meat industry.

Objectives

The objective of this study was to evaluate the quality characteristics of Chinese-style sausages made under various ripening conditions.

Methodology

Fresh pork ham was ground through a 10.0 mm plate. Fat was partially thawed in a cooler prior to manually dicing into size of 0.5×0.5×0.5 cm³. After trimming, raw materials including 80% pork ham and 20% cubed pork back fat, were mixed thoroughly with 7.5% sugar, 3.0% rice wine, 1.4% salt, 1.0% monosodium glutamate, 0.2% sodium sorbate, 0.15% polyphosphate, 0.1% white pepper powder, 0.1% sodium nitrate, and 100ppm sodium nitrite, and then curing at 2±2°C for 1 day. The meat mixture was then stuffed into natural casing. Raw sausages were linked and dried in a preheated oven at 50°C for 4hr. Following drying, sausages were randomly assigned into three groups, and stored in an incubator which had three separated chambers individually maintaining at 12, 15, or 18°C with 85% relative humidity. At days of 0, 5, and 10, samples were analyzed for moisture, water activity, pH, L*, a*, and b*, TPC and mold counts. Three

trials were conducted. Data were analyzed using SAS GLM with a 5% level of significance. Means were separated using Duncan's new multiple range test.

Results & Discussion

It shows that the moisture contents of the sausage samples for the 12°C ripening group increased significantly after 10 days of ripening, while the 15 and 18°C ripening group samples remained the same (Table 1). After 10 days of ripening, the water activity of the samples was 0.94, which was close to the report in other studies (Kuo and Chu, 2003; Lin and Lin, 2002). The data show that the pH values of the three group sausage samples significantly decreased with storage time, and agreed with other studies (Kuo and Chu, 2003; Wu, 2003). In this study, there was no significant difference of pH values of the sausages among the three treatments between days 0 and 5. It was observed that the samples ripened at 18°C had significantly lower pH of 5.70 and a higher pH reduction of 1.34 pH unit compared with the other two groups ripened at 12 and 15°C after ripening for 10 days. The possible reason is the higher temperature of the sausages ripened, the more the growth of microorganisms naturally presented in the sausages increased, thus results in a more fermentation, and finally a lower pH of products. The results indicated that L* values of the sausage samples remained stable during ripening (Table 2). No significant difference of L* and b* values of the sausages among three treatments was observed during 10 days of ripening. It was found that the a* values of the sausages decreased with storage time but were without significant difference among the different ripening temperature treatments. Also, it showed that the TPC of the 12°C group samples increased slightly with storage time but without significant difference, while the samples of 15°C and 18°C groups increased significantly after 5 days of ripening. After 10-day ripening, only TPC of the 12°C samples met a standard set by a local sanitary authority which was $\leq 3 \times 10^6$ CFU/g; both 15 and 18°C samples were considered as unqualified microbiologically based on the same standard. The mold counts of the samples increased with ripening for each treatment, and up to 4.4, 5.4, and 6.1 log CFU/g of mold for ripening at 12, 15, and 18°C samples respectively at day 10.

Conclusions

Based on the results obtained in this study, Chinese-style sausages should be ripened at 12°C based on the microbial evaluation in order to obtain products with less total plate counts and mold counts which qualified according to the local sanitary standard and with acceptable qualities.

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Tables and Figures

Table 1. Changes in moisture content and water activity of Chinese-style sausages during ripening

Parameter	Ripening temperature (°C)	Ripening time (day)		
		0	5	10
Moisture (%)	12	48.40 ^b	50.12 ^{ab}	51.15 ^a
	15	49.28	50.28	50.49
	18	50.26	50.89	49.97
Water activity	12	0.93 ^b	0.94 ^a	0.94 ^a
	15	0.93 ^b	0.94 ^a	0.94 ^a
	18	0.94 ^a	0.94 ^a	0.94 ^a

^{abc} Means within a row have different superscript are significantly different (p<0.05).

Table 2. Changes in pH value of Chinese-style sausages during ripening

Ripening temperature (°C)	Ripening time (day)		
	0	5	10
12	6.86 ^{ax}	6.54 ^{bx}	6.28 ^{cx}
15	6.89 ^{ax}	6.52 ^{bx}	6.12 ^{cx}
18	7.04 ^{ax}	6.56 ^{bx}	5.70 ^{cy}

^{abc} Means within a row have different superscript are significantly different (p<0.05).

^{xy} Means within a column have different superscript are significantly different (p<0.05).

Table 3. Changes in total plate counts and mold counts of Chinese-style sausages during ripening

Items	Ripening temperature (°C)	Ripening time (day)		
		0	5	10
Total plate count (CFU/g)	12	4.80 ^{ax}	5.26 ^{ax}	5.34 ^{ax}
	15	4.89 ^{bx}	5.74 ^{abx}	6.49 ^{ay}
	18	4.56 ^{bx}	6.95 ^{ay}	6.76 ^{ay}
Mold counts (CFU/g)	12	3.10 ^{bx}	3.74 ^{abx}	4.37 ^{ax}
	15	3.32 ^{bx}	4.35 ^{bx}	5.40 ^{ay}
	18	3.17 ^{bx}	5.49 ^{ay}	6.13 ^{ay}

^{ab} Means within a row have different superscript are significantly different (p<0.05).

^{xy} Means within a column have different superscript are significantly different (p<0.05).

Table 4. Changes in color values of Chinese-style sausages during ripening

Ripening temperature (°C)	Ripening time (day)		
	0	5	10
	L values		
12	37.45	38.50	37.51
15	37.28	39.16	38.22
18	37.14	37.37	38.23
	a values		
12	11.33 ^a	8.16 ^b	8.71 ^b
15	11.06 ^a	9.01 ^b	8.78 ^b
18	11.32 ^a	9.18 ^b	10.57 ^{ab}
	b values		
12	7.41	7.74	8.93
15	7.46	7.95	8.58
18	7.93	8.06	8.02

^{ab} Means within a row have different superscript are significantly different ($p < 0.05$).

QUALITY CHARACTERISTICS OF CHINESES-STYLE SAUSAGES MADE FROM DIFFERENT RAW MATERIALS AND STORED UNDER REFRIGERATION

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Key Words: Chinese-style sausage; hot-boned; chilled, frozen; quality

Introduction

Traditionally, in Taiwan it was common to use hot-boned meat that was sold in traditional markets to make sausages. However, due to sanitary concerns, possible lacking of good temperature-control and also increasing numbers of modern meat processing facilities, more and more chilled or frozen meat are used to make sausage products. Also the supply of frozen meat imported from overseas is increasing. Raw materials from various sources, such as hot-boned, chilled or frozen meat, might have different physic-histological characteristics, and influences the qualities of processed products. Freezing applied to preserve meat has many advantages, such as holding for a longer period of time between production and use, but unfortunately, also has some disadvantages. Chilled meat currently is commonly used in making many sausage products. Hot-boning of animal carcasses and processing of pre-rigor meat have been reported to have some advantages, such as higher emulsifying capacity, water-holding capacity, and etc. How to choose appropriate meat and thus make high quality products is always a major concern for the meat industry.

Objectives

The objective of this study was to evaluate the quality characteristics of Chinese-style sausages made from various raw materials, including hot-boned, chilled, and frozen pork, during refrigerated storage.

Methodology

Fresh pork was ground through a 9.5 mm plate. Hot-boned meat was made into sausages immediately, while the chilled group raw materials were first stored at 4°C for 3 days, then making into sausages; frozen group raw materials were stored at -18°C for 7 days, and then thawed at 4°C for 1 day before making into sausages. Raw materials including 80% pork ham and 20% pork back fat, were mixed with 8% sugar, 3% water, 1.3% salt, 1% MSG, 0.2% white pepper powder, 0.15% polyphosphate, 0.05% sodium erythorbate, 0.012% NaNO₂, 0.01% five-spice powder, and 0.01% cinnamon powder.

The meat mixture was stuffed. Raw sausages were linked and dried in a preheated oven at 50°C for 1 hr, 60°C for 2 hr. Following drying, sausages were cooled, vacuum packaged, and then stored at 4°C. At days of 0, 7, 14, 21, and 28, samples were analyzed for pH, L*, a*, and b*, TBA, VBN, and TPC. Three trials were conducted. Data were analyzed using SAS GLM with a 5% level of significance. Means were separated using Duncan's new multiple range test.

Results & Discussion

The data show that the pH values of the samples decreased from 6.45 to 6.30 during 21-days refrigerated storage (Table 1). This result agreed with other studies (Kuo and Chu, 2003; Wu, 2003). The decrease of pH values of the sausages was probably due to lactic acid producing bacteria fermenting sucrose added to the sausages. Less pH reduction in this study was probably because of less sugar (8%) added in this study compared to 10% sugar added in the previous studies. In this study, there was no significant difference of pH values of the sausages among the treatments. The results indicated that "L*" values of the sausage samples slightly increased with storage time (Table 2). No significant difference of "L*" values of the sausages among the treatments was observed during refrigerated storage. In this study, the "a*" values of the samples increased along with storage time for each treatment, and the "a*" and "b*" values of the sausages made from either chilled or frozen meat were significantly higher than the ones made from hot-boned meat. This result agreed with the study done by Lin and Chao (2001).

The TBA values of all sausage samples increased with storage time (Table 3). In this study, at days 0 and 7, the frozen-meat sausage samples had significantly higher TBA values than the samples prepared with other meats. This result was probably because ice crystals formed during frozen storage, partially disrupted muscle cell structure, thus increased the possibilities of oxygen entering the tissue and which would increase lipid oxidation, and result in higher TBA values of the frozen samples. After day 7, all the three groups of samples in vacuum packages had similar TBA values throughout refrigerated storage at 4°C. Table 3 illustrates VBN values of the three sausage samples significantly increased when storage time increased. In this study, at day 0, frozen-meat sausage samples had significantly higher VBN values than the other two groups, while no significant difference was observed after 7-days storage. Also, all the samples for each group in this study did not exceed 20 mg/100 g, which is the value that is often described as the level necessary to detect meat spoilage.

No significant difference of TPC of the samples among the treatments was observed during refrigerated storage, even though the sausages prepared from hot-boned meat had higher TPC than the other two groups (Table 4). In this study, total plate counts of all refrigerated samples met an even more restrict standard which is $\leq 3 \times 10^6$ CFU/g for frozen non-fully cooked meat products that was established as the Chinese Agricultural Standard.

Conclusions

In conclusion, Chinese-style sausages made from meat, hot-bone, chilled, or frozen pork, were acceptable as evaluated by microbiological and physicochemical characteristics analyzed in this study. Manufacturers should select appropriate raw materials to make sausages according to feasibility, economic concerns, and other factors. However, the importance of high quality raw materials and good sanitary processing conditions should not be overemphasized when making products.

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Tables and Figures

Table 1. The pH values of the Chinese-style sausage made from various raw materials and stored at 4°C

Meat type	Storage time (day)				
	0	7	14	21	28
Hot-boned meat ¹	6.45±0.16	6.47±0.13	6.48±0.18	6.46±0.16	6.30±0.23
Chilled meat ²	6.45±0.13 ^{ab}	6.47±0.12 ^a	6.41±0.17 ^{ab}	6.48±0.17 ^a	6.25±0.33 ^b
Frozen meat ³	6.47±0.14	6.46±0.16	6.48±0.09	6.34±0.16	6.30±0.33

¹Hot-boned meat: Meat from hot carcasses from a local meat market was made into sausages immediately.

²Chilled meat: Meat was storage at 2°C for 3 days, and then made into sausages.

³Frozen meat: Meat was storage at -18°C for 7 days, thawed at 4°C for 1 day, and then made into sausages.

^{ab}Means within a row have different superscripts are significantly different (p<0.05).

Table 2. Color evaluation of the Chinese-style sausage made from various raw materials and stored at 4°C

Meat type	Storage time (day)				
	0	7	14	21	28
	L values				
Hot-boned meat	37.73±1.24	37.25±1.00	38.59±2.48	37.34±2.00 ^y	39.05±3.47
Chilled meat	36.95±1.39	36.60±2.22	38.07±1.88	37.16±1.98 ^y	38.89±3.43
Frozen meat	37.90±1.71	37.65±2.83	39.67±2.32	39.50±1.92 ^x	38.85±2.55
	a values				
Hot-boned meat	9.46±0.84 ^{by}	11.03±1.15 ^a	10.24±1.17 ^{aby}	11.33±0.61 ^{axy}	11.32±1.41 ^a
Chilled meat	10.73±1.35 ^x	11.14±1.39	11.36±1.33 ^x	11.98±0.86 ^x	11.73±1.13
Frozen meat	10.78±0.91 ^{bx}	11.82±0.96 ^a	11.13±0.49 ^{abxy}	11.16±0.62 ^{aby}	11.37±1.29 ^{ab}
	b values				
Hot-boned meat	8.22±0.65 ^{ab}	7.86±0.52 ^{by}	8.86±0.75 ^{ay}	8.80±0.84 ^{axy}	8.88±1.33 ^{ay}
Chilled meat	8.62±0.77 ^b	8.11±0.94 ^{by}	8.86±0.91 ^{by}	8.52±1.04 ^{by}	10.15±0.83 ^{ax}
Frozen meat	8.16±0.85 ^c	9.74±0.63 ^{abx}	10.25±1.03 ^{ax}	9.41±0.62 ^{bx}	10.07±0.60 ^{abx}

^{xy}Means within a column for the same test have different superscripts are significantly different (p<0.05).

^{abc}Means within a row have different superscripts are significantly different (p<0.05).

EFFECT OF TRANSGLUTAMINASE AND PHOSPHATES ON THE QUALITY OF LOW-SALT CHICKEN MEAT HAM

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Key Words: transglutaminase, phosphates, chicken meat, low-salt ham

Introduction

A microbial transglutaminase (E.C. 2.3.2.13) has the ability to polymerize and cross-link proteins by forming covalent bonds between glutamine and lysine residues and thereby to improve physical properties of meat and poultry products (Nielsen 1995, Kuraishi et al. 1998). Sodium chloride and phosphates are the components of curing brines, which are most frequently used in meat processing. These substances have great influence on texture, flavour and sensory characteristics of meat products (Rutkowski & Gwiazda 1993, Scharner & Proehl 1996, Ruusunen & Poulanne 2004). Thanks to the action of transglutaminase on proteins, deterioration of texture of low-salt products could be avoided and substitution of phosphates is possible (Kuraishi et al. 1997, 1998).

Objectives

The purpose of the study was to determine the effect of transglutaminase (“Activa WM”, 0.0, 0.2 or 0.3% per product weight) and phosphates (0.0 or 0.15% as P₂O₅ per product weight) on the quality of low-salt chicken meat ham.

Methodology

The experimental material used in this study was chicken tight meat chilled 48 h post mortem. Six variants of ham were produced. Levels of components tested are shown in Table 1.

Chicken meat was ground in a laboratory grinder through a kidney plate and mixed with curing brine containing NaCl, 0.015% NaNO₂ and 0.05% sodium ascorbate (% per product weight) dissolved in one third of the total amount of added water. After curing (4-6 °C for 24 h) it was mixed with the rest of added water and other brine components (% per product weight): 0.3% sodium glutamate, 1.5% soy isolate Supro 545, 0.1% saccharose, 0.4% seasonings, phosphates (Tari P31, BK Giulini) and transglutaminase (Activa WM, Ajinomoto). The total brine addition was 30% in relation to the weight of meat. For better brine absorbing meat chunks were tumbled in a laboratory tumbler (1.5 h, speed: 9 r.p.min). After stuffing into termoshrinkable polyamide casings (63 mm in diameter, 20 cm length) and clipping all hams were hung for 1 h at room temperature. Hams were subjected to heat treatment in hot water (70-72 °C), temperature reached in

the core was 68 °C. Product yield after heat treatment was 100%. Hams were chilled in ice water and then in chilling room at 4-6 °C for 24 h. The analyses performed on the final product comprised the determination of basic chemical composition (total protein, water and fat content), nitrite residue and nitrosyl heme pigments (i.g. pigments of cooked cured meat) to total heme pigments ratio. Objective colour values (CIE L* a* b*) of ham slices were determined using a Minolta CR-200 colorimeter. Objective textural characteristics of hams (hardness, chewiness, springiness, cohesiveness, maximal penetration force) were evaluated using a Zwicki 1120 universal testing machine. Sensory evaluation of colour, aroma, taste and texture of hams was carried out using a 5-point scale by six panelists (Baryłko-Pikielna 1975). The experiment was performed in four replications. The statistical evaluation of the results was conducted using the Statgraphics for Windows program ver. 4.1 Plus.

Results & Discussion

Basic chemical composition of low-salt chicken meat hams was not affected by addition of transglutaminase preparation and / or phosphates (Table 2). Nitrite residue was below 0.0100% (threshold stipulated by Polish Standard) in all hams. Nitrosyl heme pigments to total heme pigments ratio is useful for evaluation of the result of curing process of meat. It ranged from 56.6 to 58.4% and did not depend on transglutaminase and / or phosphates addition (Table 2). Values above 50% are commonly accepted in such products. Both transglutaminase and phosphates did not affect colour values (L*, a*, b*) of chicken meat hams (Table 2). Reduction of sodium chloride content from 2.0 to 1.5% did not cause deterioration of colour of the final product. As in our study an addition of pig plasma transglutaminase in the 0-1% range did not produce significant differences in colour of low-salt chicken meat balls (Tseng et al. 2000). Nielsen et al. (1995) reported that raw meat with transglutaminase addition had significantly lower a* values than the controls (P<0.05). Hammer (1998) indicated decrease in yellowness of finely comminuted Bruehwurst sausages with increasing microbial transglutaminase concentration (0-0.2%). Addition of transglutaminase and / or phosphates favourably affected the textural characteristics of low-salt chicken meat hams (Table 2, Fig. 1). Hams processed with addition of transglutaminase and phosphates exhibited significantly (P<0.05) higher hardness, chewiness, cohesiveness and maximal penetration force (formulas 5 and 6) in relation to ones manufactured without the enzyme. Transglutaminase did not exhibited an influence on springiness of examined hams. Effects of transglutaminase on the texture are well documented for raw, restructured meat (Kuraishi et al. 1997, 1998, Nielsen et al. 1995). Hammer (1998) also reported that finely comminuted sausages containing 0.2% transglutaminase were harder and firmer in comparison to sausages produced without the enzyme addition. The studies of Tseng et al. (2000) showed, that the gel strength of low-salt chicken meat balls increased with increasing transglutaminase levels from 0 to 1%, with levels above 0.2% being significantly higher than the controls. The results of the study of Pietrasik and LiChan (2002) indicated that salt was critical in ensuring good binding and textural characteristics of cooked pork batter gels. Samples containing 0.5% transglutaminase exhibited greater hardness, cohesiveness, springiness, fracturability and chewiness than those produced without enzyme addition. However, application of transglutaminase (0.0-

0.6) was not able to improve textural parameters in low-salt products (<2%) to the same levels as the high-salt products. Mueller (2003) found, that to manufacture a good quality pork ham from PSE meat, combined addition of 0,05% transglutaminase and 0,05% phosphates should be recommended. Thanks to the positive influence of those additives on proteins desired muscle binding was obtained and deterioration of textural characteristics was avoided. Sensory evaluation of low-salt chicken meat hams showed that colour, aroma and taste were not significantly influenced ($P<0.05$) by transglutaminase and / or phosphate addition (Table 2). All hams were scored very high for mentioned sensory characteristics (average scores not less than 4.6). There was, though, a significant effect ($P<0.05$) of transglutaminase on sensory evaluated texture of low-salt hams (formulas 4-6). In opinion of panelists, transglutaminase addition caused deterioration of juiciness. Although the scores for texture were very high, ranging from 4.5 to 4.9, it was observed that transglutaminase induced gel strengthening was not fully accepted by panelists. In other studies Tseng et al. (2000) found that low-salt chicken meat balls manufactured with 1,0% transglutaminase addition were scored significantly ($P<0.05$) higher for texture, juiciness and overall acceptability than controls (without enzyme).

Conclusions

There is possibility to manufacture high quality chicken meat ham with reduced salt content (from 2.0 to 1.5% per product), but combined addition of transglutaminase “Activa WM” (0.2%) and phosphates (0.15% as P_2O_5 per product weight) is recommended.

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Tables and Figures

Table 1. Composition of low-salt chicken meat ham formulas

Formula	Chicken leg meat (%)	Water* (%)	NaCl** (%)	Transglutaminase** (%)	Phosphates** (%)
1	100.0	30.0	2.0	--	--
2	100.0	30.0	1.5	--	--
3	100.0	30.0	1.5	--	0.15
4	100.0	30.0	1.5	0.2	--
5	100.0	30.0	1.5	0.2	0.15
6	100.0	30.0	1.5	0.3	0.15

* per meat weight

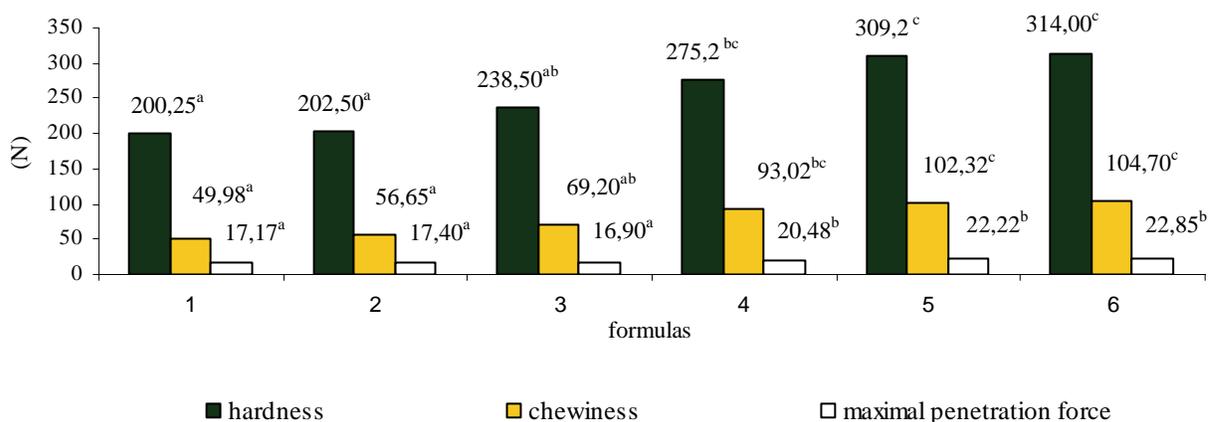
** per final product weight

Table 2. Effect of transglutaminase and phosphates on selected quality characteristics of low-salt chicken meat hams

	Formulas					
	1	2	3	4	5	6
Basic chemical composition						
Protein (%)	18.4 ^a ± 1.08	18.1 ^a ± 1.18	18.1 ^a ± 0.90	18.2 ^a ± 0.62	18.1 ^a ± 0.60	18.7 ^a ± 0.82
Fat (%)	8.1 ^a ± 0.85	8.3 ^a ± 0.52	8.5 ^a ± 0.68	8.0 ^a ± 0.41	8.3 ^a ± 0.26	8.1 ^a ± 0.17
Water (%)	70.5 ^a ± 1.56	70.9 ^a ± 2.02	70.8 ^a ± 1.77	70.1 ^a ± 1.55	70.6 ^a ± 1.60	70.4 ^a ± 1.59
Flow of curing process						
NHP/THP (%)	58.4 ^a ± 2.98	56.8 ^a ± 5.33	56.6 ^a ± 6.96	58.3 ^a ± 5.57	57.8 ^a ± 2.60	57.0 ^a ± 4.30
Colour values						
L* value	65.60 ^a ± 1.71	67.12 ^a ± 0.36	64.98 ^a ± 2.32	66.62 ^a ± 0.78	65.20 ^a ± 1.06	65.42 ^a ± 2.86
a* value	10.02 ^a ± 1.50	9.42 ^a ± 2.19	6.68 ^a ± 2.13	10.08 ^a ± 1.45	9.92 ^a ± 1.23	8.80 ^a ± 1.76
b* value	8.58 ^a ± 0.94	8.72 ^a ± 1.78	8.28 ^a ± 0.83	8.78 ^a ± 1.03	8.88 ^a ± 0.84	8.98 ^a ± 1.49
Textural characteristics						
Springiness	0.80 ^a ± 0.00	0.72 ^a ± 0.05	0.75 ^a ± 0.06	0.78 ^a ± 0.05	0.78 ^a ± 0.05	0.80 ^a ± 0.00
Cohesiveness	0.30 ^a ± 0.00	0.38 ^{ab} ± 0.05	0.38 ^{ab} ± 0.05	0.45 ^c ± 0.06	0.42 ^{bc} ± 0.05	0.40 ^{bc} ± 0.00
Sensory characteristics						
Colour	4.8 ^a ± 0.17	4.9 ^a ± 0.12	4.8 ^a ± 0.15	4.8 ^a ± 0.13	4.8 ^a ± 0.17	4.7 ^a ± 0.21
Aroma	4.7 ^a ± 0.21	4.8 ^a ± 0.17	4.7 ^a ± 0.24	4.7 ^a ± 0.06	4.8 ^a ± 0.25	4.67 ^a ± 0.33
Taste	4.7 ^a ± 0.09	4.8 ^a ± 0.13	4.7 ^a ± 0.29	4.6 ^a ± 0.19	4.8 ^a ± 0.15	4.7 ^a ± 0.29
Texture	4.9 ^a ± 0.06	4.8 ^{ab} ± 0.06	4.9 ^a ± 0.10	4.5 ^b ± 0.10	4.8 ^{ab} ± 0.18	4.7 ^b ± 0.10

NHP/THP – nitrosyl heme pigments to total heme pigments ratio

^{a, b} – means in rows with different superscripts are significantly different at P<0.05



^b – means with different superscripts are significantly different at P<0.05

Fig. 1. Effect of transglutaminase and phosphate on hardness, chewiness and maximal penetration force of low-salt chicken meat hams

Table 3. TBA and VBN values of the Chinese-style sausage made from various raw materials and stored at 4°C

Meat type	Storage time (day)				
	0	7	14	21	28
	TBA (mg malonaldehyde/kg)				
Hot-boned meat	2.09±0.15 ^{bxy}	2.11±0.10 ^{by}	2.19±0.12 ^a	2.13±0.11 ^{ab}	2.14±0.14 ^{ab}
Chilled meat	2.02±0.10 ^{by}	2.07±0.05 ^{by}	2.19±0.21 ^a	2.22±0.22 ^a	2.19±0.15 ^a
Frozen meat	2.11±0.12 ^{bx}	2.27±0.24 ^{ax}	2.18±0.24 ^{ab}	2.17±0.11 ^{ab}	2.18±0.10 ^{ab}
	VBN values (mg/100g)				
Hot-boned meat	8.78±1.06 ^{ax}	11.09±1.58 ^{bx}	12.94±1.90 ^{cx}	14.55±1.28 ^{dx}	15.17±1.11 ^{dx}
Chilled meat	8.55±1.28 ^{ax}	10.63±1.06 ^{bx}	12.86±1.24 ^{cx}	13.71±1.25 ^{dx}	15.25±1.34 ^{ex}
Frozen meat	9.78±0.75 ^{ay}	11.01±0.89 ^{bx}	13.01±1.18 ^{cx}	14.02±1.15 ^{dx}	15.32±1.38 ^{ex}

^{xy}Means within a column have different superscript are significantly different (p<0.05).

^{ab}Means within a row have different superscript are significantly different (p<0.05).

Table 4. The TPC of the Chinese-style sausage made from various raw materials and stored at 4°C

Meat type	Storage time (day)				
	0	7	14	21	28
Hot-boned meat	5.00±0.15 ^x	5.00±0.57	4.53±0.41	5.03±0.59	5.07±0.63
Chilled meat	4.93±0.56 ^x	4.65±0.10	4.61±0.35	4.70±0.46	4.91±0.29
Frozen meat	4.46±0.35 ^y	4.84±0.31	4.90±0.66	4.85±0.57	4.92±0.23

^{xyz}Means within a column for the same test have different superscripts are significantly different (p<0.05).

^{abc}Means within a row for the same test have different superscripts are significantly different (p<0.05).

EFFECT OF STORAGE ON THE TEXTURE OF LEVERPATÉ AND EMULSION SAUSAGE

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Key Words: Texture, storage, fat, rheology, meat product

Introduction

The BSE crisis in Europe in the last decade has resulted in numerous changes in the procedures used during the slaughter process, meat processing and in the composition of feed. Animal fat is no longer used as an ingredient in animal feed and has been replaced with lipids originating from vegetables. Vegetable lipids differ from animal lipids in the fatty acid composition. The former normally has a higher content of unsaturated fatty acids, which have a significant influence on the physical characteristics of the fat, e.g. melting point and hardness. The physical characteristics of fat often influence the texture of the final product. More unsaturated fat, the softer the resulting product will be. The fatty acid composition of feed is known to be reflected in the adipose tissue of monogastric animals like pigs. Thus, the fatty acid composition of feed might have a major impact on the texture of products containing large amounts of pork fat.

Besides the fatty acid composition the physical characteristics of fat are determined by the crystallization process. Normally triglycerides crystallize into three different forms: α , β' , β , with increasing order of packing of the crystal lattice, resulting in increasing stability and melting points. In a former study we found that fast cooling of lard resulted in a higher content of α -crystals compared to slow cooling (Svenstrup et al., 2004, 2005). The unstable crystal forms rearrange through an irreversible phase transition into more stable crystal forms with higher melting points. The timeframe of this rearrangement in meat products is not known. However, if the rearrangement of unstable crystals is a slow process running for days or weeks, the melting point of fat incorporated in meat products increases during storage which thereby changes the texture of the final meat product

Objectives

The objective of the study is primarily to investigate the effect of storage on the texture of liver pâté and emulsion sausage. Secondly to investigate the effect of two different fat sources derived from animals fed various fat types and the effect of cooling rate on the texture of an emulsion sausage during storage. The results presented are obtained from two independent, ongoing experiments.

Methodology

Experiment 1: Twelve freshly produced liver pâtés from the same batch were obtained from a commercial producer (Table 1, Fransk Postej, Stryhns A/S). The pâtés were stored at 5°C. At day 0, 1, 3, 5, 8, 10, 15, 18 and 25 after production, a randomly selected pâté was selected for determination of the complex modulus (G^*) and the phase angle using small amplitude oscillatory shear. For the determinations a Bohlin C-VOR rheometer (Malvern Instruments, Malvern, UK) equipped with serrated parallel plates (diameter 25 mm) was used. A sample of the pâté was mounted between the plates and allowed to relax for 15 minutes before the measurement was started. During measurement, the rheological properties of the pâté were measured at a strain of 0.002 and a frequency of 1 Hz at temperatures from 5 to 40 °C. The temperature of the sample during measurement was accurately controlled and increased with an Asphalt Peltier element (Malvern Instruments, Malvern, UK) designed for the rheometer. Every time the temperature was increased, the sample was let to equilibrate for 2 minutes before the rheological properties were measured.

Experiment 2: Forty pigs, half females and half castrated males, were at an age of 35 days allotted to one of two treatments consisting of standard feed that contained either 3 % palm oil or 3 % rapeseed oil. Pigs were slaughtered at 100 kg live weight. After three days post-mortem, 500 gram of back fat were individually vacuum sealed and stored frozen at -20°C for up till 3 months prior to processing. The fat samples were pooled into two groups either fed palm oil or rapeseed oil.

Emulsion sausages were prepared using a standard formulation (**Table 1**). Lean pork was ground through a 4,5 mm orifice plate, presalted with NaCl/nitrite and kept at 4°C for 18 h. The batter was prepared using a bowl chopper. Presalted meat was chopped while adding phosphate and ¼ of ice-water at low chopping speed. Dry milk and ice water were added. Fat, wheat flour and ice water were added at high speed gradually adding the remaining ice-water and spices. Chopping time was controlled so that the temperature of the batter did not exceed 11°C. The total chopping time was 13 min. The batter (190 g) was stuffed into aluminum cans using a manual filling machine. The filled cans were kept for 2 hours at 5°C to allow the curing reaction to complete. The sausages were cooked in a convection oven at 78°C to an internal temperature of 72°C.

After cooking the sausages were cooled using two cooling rates by immersing the cans into a water bath. The samples cooled rapidly were placed in ice-water (0°C) for 3 hours. Slow cooling was obtained using stepwise cooling. The cans were placed in a water bath at 40°C for 1 h, 20°C for 1 hour and finally in ice-water for 1 hour. After cooling the cans were stored at 4°C until analysis.

Four cans from each treatment (fat, cooling rate, storage time) were tested for Young's modulus and penetration force, each sample being punched 5 times. A cylindrical stainless-steel plunger (diameter 9,8 mm) attached to a 100-N cell connected to the crosshead of the Instron machine, crosshead speed 50 mm/min was used.

Statistical analysis was carried out with the Statistical Analysis System version 8.02 (SAS Institute, Cary, NC, USA). The mixed procedure was applied when calculating least squares means (LSM) and standard errors (SE), and significant differences between LSM were evaluated using the option Pdiff. Degrees of freedom were estimated with the Satterthwaite method. The models for Young's modulus included fixed effects of fat type, storage time and cooling rate and random effect of can nested within fat type,

storage time and cooling rate. Two-way interactions were included in the models if significant.

Results & Discussion

Experiment 1. The complex modulus (G^*) can be interpreted as a measure for the total stiffness of the samples (**Figure 1**). Freshly produced liver pâté had a G^* of 25 kPa measured at 5°C, which increased to 43 kPa after 10 days storage. Thus, the total stiffness of the samples can be interpreted to increase approximately 70%, during the first 10 d of storage when G^* is measured at 5°C. No further changes in stiffness occurred in the remaining storage period. When G^* was determined at higher temperatures two effects were observed: the value at day 0 decreased with increasing measuring temperature and the effect of storage decreased. No differences in G^* were detected during storage at a measuring temperature of 35°C.

Liver pâté contains a large proportion of lard/fat (Table 1). In a former study we showed that the rate of fat melting in liver pâté increases when the temperature is increased to 32°C. At this temperature the rate of fat melting was at a maximum and at 48°C all fat was melted (Svenstrup et al., 2005). Melting of fat can therefore explain the effect of the measuring temperature on G^* presented in figure 1. At 5°C a major part of the fat is in a solid state and this part decreased with increasing temperature making the pâté less stiff.

The effect of storage seemed to fade when G^* was measured at higher temperature. Since the major part of lard melts within the temperature range of the measurements (Svenstrup et al., 2005), the increase in stiffness during storage might be related to the fat fraction of the product. A plausible explanation is, that during cooling of the freshly produced liver pâtés a major part of the fat crystallize into unstable crystal forms. During storage these are rearranged into more stable crystal forms. The more stable crystal forms of the fat probably create a more ridged network in the microstructure of the liver pâté. This gives the product a stiffer appearance in the rheological measurements.

Experiment 2. Young's modulus (E_u) can also be interpreted as a measure of total stiffness of the samples (**Figure 2**). There was an effect of feed ($P < 0.001$), cooling rate ($P < 0.0001$) and storage time ($P < 0.0001$). At day 1, sausages produced from pigs fed palm oil and cooled fast were approximately 15% stiffer than sausages cooled slowly (**Figure 2A**). At day 27, there was no difference in stiffness between the samples. Sausages produced from pigs fed rapeseed oil and cooled fast were approximately 8% stiffer than sausages cooled slowly (**Figure 2B**).

The effect of storage is similar to the results obtained in experiment 1, i.e. the sausages become stiffer during storage. A rearrangement of unstable fat crystals into more stable crystals might also explain the results obtained in experiment 2. However, the effect of cooling rate disagrees with this explanation. The order of packing of single tri-glycerides into the crystal lattice is time dependent. Thus, the faster the cooling rate, the less stable the crystals will be. The fast cooled sausages were expected, initially, to have a higher fraction of unstable fat crystals compared to the slowly cooled sausages. Therefore, it was expected that the fast cooled sausages would be less stiff and that the stiffness would increase more during storage compared to the slowly cooled sausages. However, the opposite effect was observed (**Figure 2**).

At this stage, we do not have an explanation for this contradiction. It might be related to the protein gel-network, starch gelation and/or the emulsion of fat. For more than 2 hours, the temperature of the slowly cooled sausages was above 20°C during the cooling procedure. Since a part of the fat is in liquid form above 20°C, it might influence the emulsion stability. This could lead to coalescence of fat which will influence the texture.

Conclusions

The stiffness of both liver pâté and emulsion sausage increases during storage. For liver pâté changes in the fat fraction was clearly responsible for the increased stiffness of the product. Sausages produced from pigs fed palm oil are stiffer than sausages produced from pigs fed rapeseed oil. The cooling rate has an effect on the stiffness. Fast cooling resulted in stiffer sausages. The effect of storage might be related the crystallization of fat.

Acknowledgements

Stryhns A/S are acknowledged for supplying material used in experiment 1 and Danish Meat Research Institute for supplying the fat used in experiment 2.

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Tables and Figures

Table 1. Composition of liver pâté and emulsion sausage.

Liver pâté		Emulsion sausage	
Liver	33 %	Lean pork	52,7 %
Back fat	30 %	Back fat	19,7 %
Skimmed milk	25 %	Dry milk	2,4 %
Wheat flour	4 %	Wheat flour	1,98 %
Margarine	3 %	Dry onion	0,22 %
Onion	2 %	Phosphate mix	0,34 %
NaCl	1,5 %	Ice/water	21,9 %
Spices	1 %	NaCl	0,38 %
Sugar	0.5 %	White pepper	0,16 %

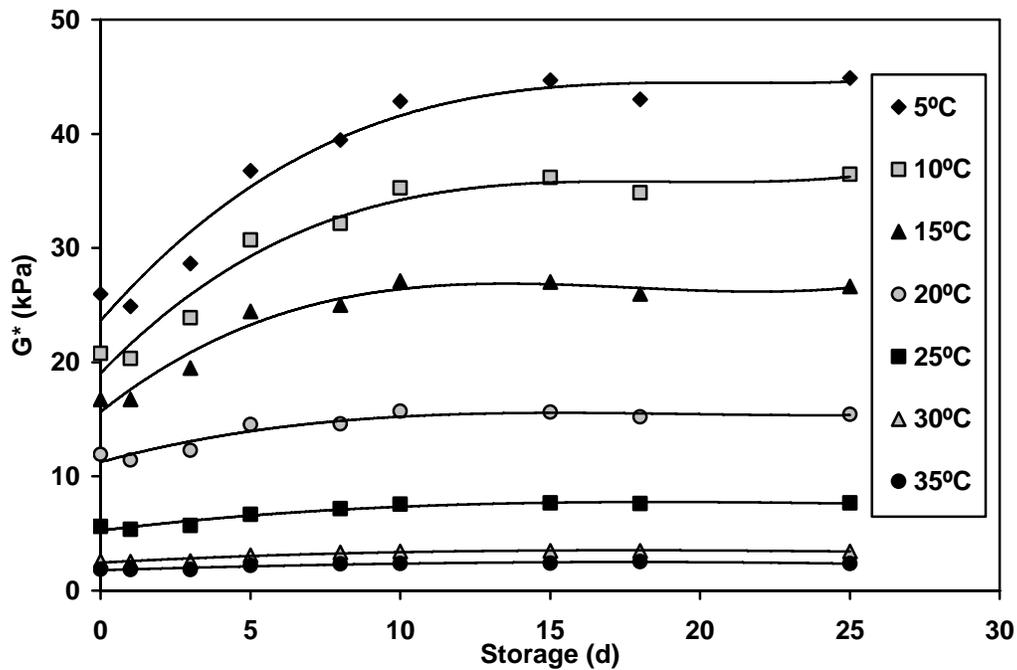


Figure 1. Complex modulus (G^*) of liver pâté during storage at 5°C for up to 25 days. G^* was determined at seven different temperatures. Lines are guidance to the eye.

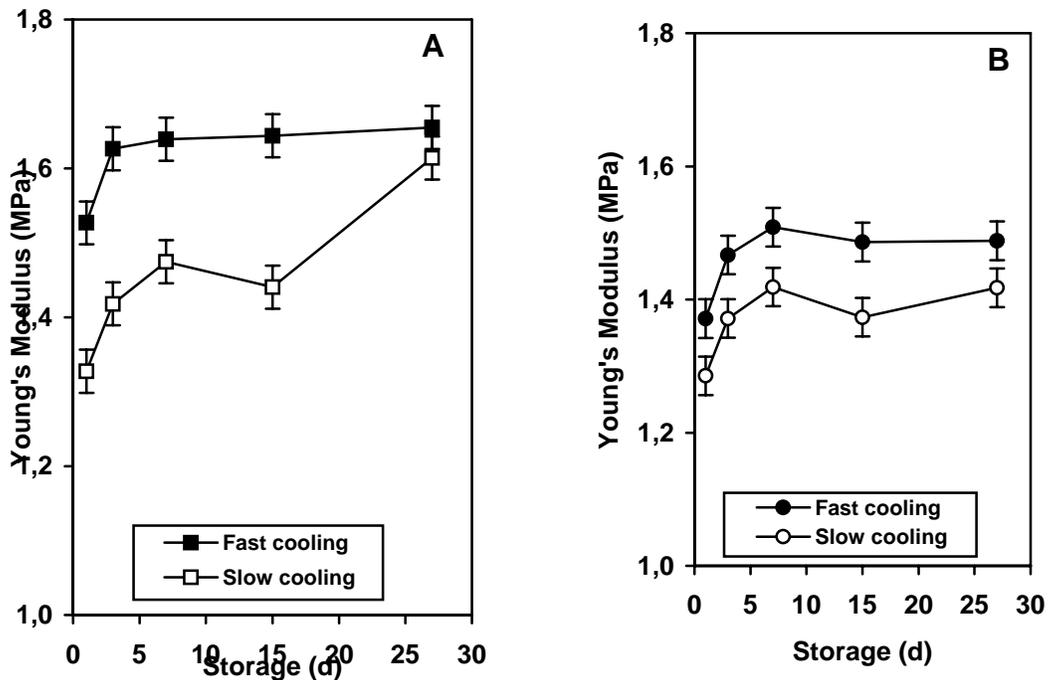


Figure 2. Young's Modulus (E_u) of emulsion sausages during storage for 27 days at 4°C. Fat is obtained from pigs fed either 3% palm oil (A) or 3% rapeseed oil (B). Sausages have been cooled either slowly ($\sim 0.5^\circ\text{C}/\text{min}$) or fast ($\sim 1^\circ\text{C}/\text{min}$).

EFFECT OF PARTIAL REPLACEMENT OF BEEF FAT WITH PRE-EMULSIFIED OLIVE OIL ON SOME QUALITY CHARACTERISTICS OF TURKISH FERMENTED SAUSAGE (SUCUK)

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Key Words: Fermented meat, sausage, sucuk, olive oil, fat, quality

Introduction

Turkish fermented sausage, which is called sucuk, is one of the most popular traditional dried meat product in Turkey. It is produced mainly from beef meat and/or mutton. Beef or lamb fat are also used for sucuk production and are rich in saturated fatty acids and cholesterol (Kayaardı and Gök, 2003). The relationship between dietary fat and the development of cardiovascular disease and hypertension (NCEP, 1988) has prompted consumers to be more aware of and concerned about the amount and characteristics of fat in their diet (O'Neil, 1993). In dry fermented sausages, reduction of fat has been shown to be difficult because it is often cause of technological and sensory problems (Wirth, 1988).

The reduction of fat led to an increase in the weight loss, hardness and firmness of the product and cause colour defects (Muguerza et al., 2002). Modification of the lipid fraction is one of the main strategies applied has been the reduction of the fat content. Thus the incorporation of olive oil in meat products may have a positive effect on consumer health (Bloukas et al., 1997). The substitution of animal fat by olive oil has been researched in different kind of fermented sausages (Bloukas et al., 1997; Muguerza et al., 2001; Muguerza et al., 2002; Kayaardı and Gök, 2003).

Objectives

The objective of this study was to investigate the effect of replacing beef fat with pre-emulsified olive oil on the chemical and sensory quality characteristics of sucuk.

Methodology

Four batches of sucuk formulations about 6 kg each were prepared. The control batch (C) was prepared with beef fat. Olive oil pre-emulsified with simplese (1 part simplese, 2 part olive oil) was added to next three batches by replacing 15% (O15), 30% (O30) and 50% (O50) of beef fat respectively. Each treatment was formulated to contain 80% beef and 20% total fat. The following ingredients were added per kg of meat mixture. 27.5 g spice mix, 28 g curing mix, 10 g garlic and 0.5 g starter culture mix. The prepared sucuk batters were stuffed into collagen casings, than fermented at 20-21°C and relative humidity (RH) 88% until the pH decreased to pH 5.4, after this step, sucuk samples were

left for six hours at 19-20°C, 80% RH. Drying was carried out at 23-24°C with a relative humidity 80% until the pH decreased to 5.0, and at 14-15 °C and 70% RH for the rest of drying period.

Moisture (AOAC, 1990), fat (Flynn and Bramblet, 1975) protein (Anonymous, 1979), pH (Landvogt, 1991) and TBA (Shahidi, 1985), colour (L^*, a^*, b^*, C, H) measurements were carried out on sucuk samples before and after ripening period. pH and moisture changes were measured on the 0, 3, 6, 9, and 12 th day of ripening period. The firmness of sucuk samples was measured with penetrometer. Sensory analyses of sucuk samples were carried out by using 5 point scoring scale. Data analysis was carried out with one-way ANOVA and Student-Newman-Keuls multiple comparisons methods. Software used was SPSS version 11.0.

Results & Discussion

No differences ($p > 0.05$) were found in moisture, fat, protein contents. TBA values of sucuk batters were almost the same. No significant differences were observed in L^* and b^* values of sucuk batters but substituting %50 beef fat with olive oil resulted higher b^* values ($P < 0.05$).

Figure 2-3 show the changes in moisture and pH during the ripening period. Fermentation and ripening period had a significant effect on moisture content of sucuk samples ($P < 0.05$). During the initial stages of ripening period no differences were observed in moisture content of sucuk samples. At the 9th day of ripening period control samples had the lowest moisture content. On day 12 C samples had 37.2% moisture content, O50 samples had 44.1% moisture content. It is possible that pre-emulsified olive oil could have covered the meat particles and prevented the release of moisture (Bloukas et al., 1997). Also the effect of simplese to binding water could be the reason of the high moisture content of samples containing high amounts of olive oil. All treatment groups showed decrement in pH during ripening ($P < 0.05$), but no differences were observed among the treatment groups ($P > 0.05$). pH values of samples significantly affected by processing time. Incorporation of olive oil into the sucuk formulation had no significant effect on colour parameters (L^*, a^*, b^*, C, H) of the end products ($P > 0.05$). As a result of moisture loss, the fat content increased with processing time of sucuk samples. The final fat contents ranged from 29.8% to 31.5%. No differences were found in the fat and protein content of end product ($P > 0.05$). Replacement of beef fat with olive oil resulted differences in product firmness. O50 and O30 samples were softer than control samples (higher penetration values). No significant differences were observed in the TBA values among the four types of treatments. No difference was found in weight losses of treatment groups between the beginning and at the end of the processing time ($P > 0.05$).

Usage of olive oil in sucuk formulation had a significant ($P < 0.05$) effect on external appearance, slice appearance, slice colour and overall acceptability but had no effect on external colour, flavour and texture ($P > 0.05$). O50 samples had lowest external appearance, slice appearance and slice colour scores ($P < 0.05$). According to sensory evaluation results O15 sucuk samples had similar sensory characteristics with control samples.

Conclusions

This study suggested that pre-emulsified olive oil can be successfully used in Turkish fermented sausage (sucuk)

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Tables and Figures

Table 1. Chemical composition and TBA values of sucuk batters

Treatment	Fat (%)	Protein (%)	Moisture (%)	TBA (mg Ma/kg)
O15	21.64	18.36	58.34	0.41
O30	23.60	17.92	55.41	0.41
O50	21.53	17.81	57.90	0.39
C	18.11	17.09	61.63	0.31

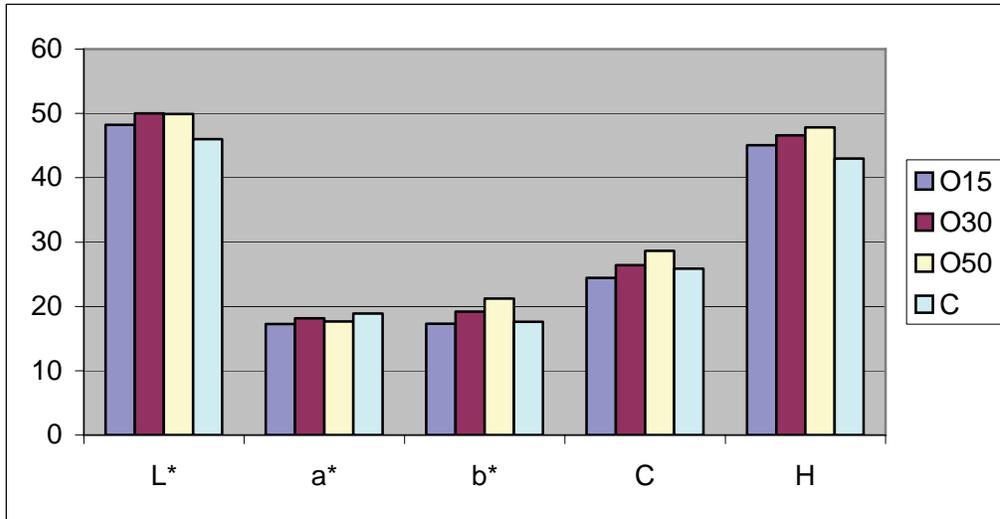


Figure 1. Colour parameters of sucuk batters

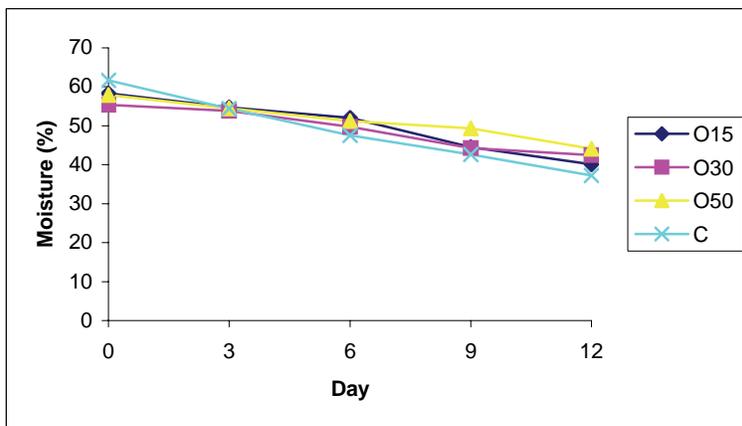


Figure 2. Moisture (%) changes during ripening period.

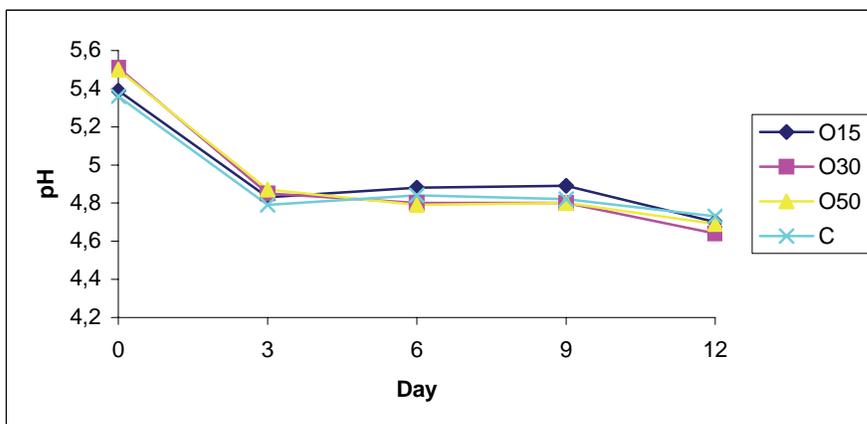


Figure 3. Changes in pH values during ripening period

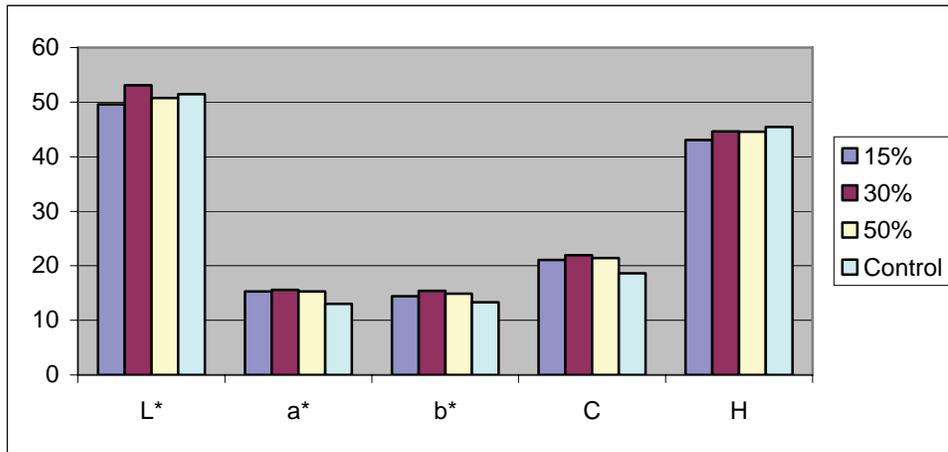


Figure 4. Effects of Replacing Beef Fat with Olive Oil on Colour Parameters of sausages

Table 2. Chemical composition, TBA, penetrometer values and weight loss of sucuk samples.

Sample	Fat (%)	Protein (%)	Moisture (%)	Penetrometer Value (mm)	Weight Loss (%)	TBA (mg Ma/kg)
O15	30.9	26.1	40.1ab	4.7ab	26.0	0.53
O30	31.5	23.7	42.5b	5.8b	23.57	0.56
O50	30.8	25.7	44.1b	6.6b	19.2	0.54
C	29.8	30.2	37.2a	3.3a	29.9	0.54

Table 3. Sensory properties of sucuk samples

Sample	Outside Surface Appearance	Slice Appearance	Outside Surface Colour	Slice Colour	Flavour	Texture	General Acceptability
O15	4.7b	4.5c	4.8	5.0a	4.5	4.3	5.0c
O30	4.2b	3.9b	4.0	4.5b	3.8	4.0	3.8b
O50	3.1a	3.2a	3.7	3.8a	3.3	2.8	3.2a
Control	4.5b	4.7c	4.8	4.8b	4.5	4.3	4.8c

Different letters in the same column indicate significant differences ($P < 0.05$).

CHARACTERIZING QUALITY OF RENDERED DUCK FAT

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Keywords: duck fat; tocopherol; fatty acid composition; oxidative stability; PV; AV.

Abstract

Characteristics of rendered duck fat (RDF) were compared to other commercial sources of fats and oils (e.g. butter, tallow, lard, soybean oil, and olive oil). Tocopherol content was determined by HPLC. Fatty acid composition and conjugated linoleic acid (CLA) content was determined using GC. The oxidative stability was determined by measuring peroxide values (PV) and p-anisidine values (AV) during storage at 50 °C in the dark. Under these conditions, the lengths of time prior to severe oxidation were as follows: RDF (8 days), lard (40 days), tallow (32 days), butter (36 days), soybean oil (10 days), and olive oil (38 days). The range of total tocopherol concentrations in the fats and oils varied from 0.02 to 27.8 mmol α -tocopherol/kg lipid. The relative polyunsaturation index varied from 6.0 to 119.4. No CLA was found in RDF.

Introduction

Rendered duck fat, a by-product of duck meat production, has potential for increased use in various food applications. However, little is known about its composition and oxidative stability. Conjugated linoleic acid (CLA) content is of interest because of potential impacts on human health. CLA has been reported to decrease the risk of cancer (Ip, C., 1997 and Banni, S., 1999). There are anecdotal claims that rendered duck fat (RDF) is resistant to oxidation at room temperature, but supporting evidence is lacking. Proper marketing claims are needed to promote RDF. Thus the objective of this work was to determine the oxidative stability of RDF compared to competing sources of cooking oils and fats. Basic research into the quality characteristics of duck fat will benefit the duck industry by establishing the unique properties associated with the duck compared to other sources of fats and oils. A better understanding of factors affecting lipid oxidation of duck fat could also provide the basis for improving product quality and shelf life.

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Objectives

1. Determine oxidative stability of fresh raw duck fat and rendered duck fat compared to other fat sources.
2. Determine fatty acid composition and conjugated linoleic acid (CLA) content in the various fats.
3. Examine tocopherol contents in duck fat and other fat sources.

Materials and Methods

Chemicals and materials: Isooctane, glacial acetic acid, chloroform, methanol, ammonium thiocyanate, iron(II) sulfate, barium chloride, p-anisidine from Sigma Chemical Co.(St. Louis, MO). Methanolic-HCl-3N (Supelco); Methyl alcohol anhydrous 99.8% (Acros); GC reference standard FAME mixture #463, and CLA isomers #UC58M from Nu-Chek-Prep Inc. (Elysian, MN). Rendered duck fat and raw duck fat were supplied by Maple Leaf Farms TM. Tallow, lard, butter, soybean oil, and olive oil were bought from local store (tallow was rendered after purchase). Fats and oils were stored at -20 before experiments.

Methods

Peroxide value (PV)

To prepare the iron(II) chloride solution, 0.4g barium chloride dihydrate was dissolved in 50 mL water. This solution was added slowly and with constant stirring to an iron (II) sulfate solution (0.5g FeSO₄·7H₂O dissolved in 50mL water). Two milliliters of 10N hydrochloric acid was added to the resulting solution. The barium sulfate precipitate was filtered off to give a clear iron(II) solution, which was stored in a brown bottle and kept in the dark. (Shantha N. C. and Decker E.A. 1994).

To prepare the ammonium thiocyanate solution, 30g ammonium thiocyanate was dissolved in water, and the volume was up to 100 mL.

To determine the peroxide value, the sample (0.05-0.3g, depending on the extent of peroxidation) was mixed in a disposable glass tube with 9.8mL chloroform-methanol (7:3, v/v) on a vortex mixer for 2-4 s. 100µL Ammonium thiocyanate solution was added, and the sample was mixed on a vortex mixer for 2-4 s. Then, 100µL iron (II) solution was added, and the sample was mixed on a vortex mixer for 2-4 s. After 5 min incubation at room temperature, the absorbance of the sample was determined at 500nm against a blank that contained all the reagents except the sample by using a spectrophotometer (UV-2401 PC, Shimadzu Co.). The entire procedure was conducted in subdued light and completed within 10 min.

The peroxide value, expressed as milliequivalents of peroxide per kilogram of sample, was calculated by using the following formula:

$$\text{Peroxide value} = (A_s - A_b) \cdot m / (55.84 \cdot m_0 \cdot 2)$$

A_s -- absorbance of the sample; A_b -- absorbance of the blank; m —slope, obtained from the calibration curve= 41.52; m_0 – mass in grams of the sample; 55.84—atomic weight of iron.

Anisidine Value (AV)

Oil sample (0.5-1 g) was weighed into a 25 mL volumetric flask and made up to the mark with isooctane. The absorbance (A_1) was measured at 350nm against a pure isooctane blank using spectrophotometer. 5mL aliquots of the oil solution or 5mL isooctane (blank) were then transferred to each of three 10mL test tubes and 1mL para-anisidine solution (0.25 w/v solution in glacial acetic acid) was added to each tube. The test tubes were stoppered, shaken, and allowed to stand for 10 minutes. The absorbance (A_2) was measured at 350 nm against the isooctane blank containing para-anisidine (Rossell, J. B. 1986). The AV was calculated by using the following equation:

$$\text{Anisidine value(AV)} = 25 \cdot (1.2A_2 - A_1) / \text{sample weight}$$

Fatty Acid Analysis

Acid-catalyzed methylation procedure: Weigh 5-30 mg of test portion into a screw-capped vial. Dissolve test portion in 1 mL toluene, add 2 mL of anhydrous 4% HCl/methanol, and heat for 20 min at 60 . Add H₂O to make a 95:5 mixture of methanol/H₂O that expels hexane. Add 2-3 mL hexane and mix well, allow layers to separate, and remove hexane layer containing the FAME. Dry hexane layer over Na₂SO₄ and use directly for GC analysis.

Gas chromatograph system equipped with FID, split/splitless injection ports, autosampler (Hewlett-Packard, model 7673), and a Hewlett-Packard ChemStation software data system was used. CP Sil 88 flexible fused column (100 m×0.25mm i.d. ×0.2um film thickness) was employed. 1- 3 L volumes sample were injected (Kramer J. K.G. et al 2002). Percentage of each fatty acid was calculated by normalization of total fatty acid methyl esters (Livisay, S. A. et al. 2000 and Emken, E. A. et al. 2002).

Tocopherol determination by HPLC

Tocopherol contents in the oils were separated and quantified by a high-pressure liquid chromatography (HPLC) system, Agilent 1100 series HPLC, incorporating Alltech Alltima silica 5u (4.6*250 mm) column, fluorescence detector, and DAD detector. 1% 2-propanol in hexane was used as mobile phase at flow rate 1ml/min. The amount of injection of sample was 20uL. The effluent was monitored with the fluorescence spectrophotometer set at an excitation wavelength (λ_{ex}) 295nm and emission wavelength (λ_{em}) 325nm and DAD detector set at wavelength 295nm (Carpenter, A.P. 1979, Petillo, D. et al 1998 , Nesaretnam K. et al, 2004).

Results and Discussion

Oxidative stability of the various fats and oils

In order to investigate oxidative stability, the different fats and oils were subjected to an accelerated storage condition (50 °C, dark) which is representative of the autoxidation process during normal shelf life (Hrncirik, K. 2005). The PV and AV of oils were determined once every two days. The results are shown in Table 1 and 2 and expressed as mean ± SD (n=3). Lard and butter were much more resistant to PV and AV formation compared to RDF. Soybean oil was slightly more resistant to PV and AV formation compared to RDF. Tallow was more resistant to PV and AV formation compared to rendered duck fat. Olive oil was more resistant to PV and AV formation than both rendered duck fat and tallow during storage. However, oxidation values in olive oil samples were somewhat elevated between day 0 and 6 compared to rendered duck fat and tallow.

Table 1. PV (meq./kg) of oils and fats during accelerated storage (50 °C, dark)

Oil & fats/days	0	6	8	10	12	14	16	20	32	36	38	40	44	50	60	80
RDF	2.08	11.4	41.3	85.3	145	216										
Lard	6.06	6.9	7.48	5.49	8.86	8.43	10.1	13.2				34.2	87.7	164		
Butter	0.24	0.74	1.01	1.15	1.48	1.6	2.3	3.03				56	195			
Soybean oil	0.84	2.04	8.26	29.5	58.5	60.3	175	204								
Olive oil	4.12	10.3	11.7	12.9	14.1	16.3	17.8	20.6				36.9	39.4	50.3	55.9	67.7
Tallow	1.28	1.41	1.65	1.83	2.08	2.31	2.59	3.45	36.9	151						

Table 2. AV of oils and fats during accelerated storage (50 °C, dark)

Oil & fats/days	0	6	8	10	12	14	16	18	20	24	40	44	50	58	80
RDF	2.2	3.88	10.1	20.6	33.9	53									
Lard	0.52	1.36	1.33	1.1	1.6	1.36	1.6		1.91	5.10	9.42	80.9			
Butter	3.60	0.20	0.73	0.86	1.24	0.89	1.09	1.24	1.36	44.1	47.6				
Soybean oil	2.30	1.57	2.56	3.39	5.65	7.67	10.8	14.7	18.4	42.0					
Olive oil	1.90	5.84	5.73	5.90	5.71	6.28	5.98		6.16	5.67	5.43	5.64	6.87	7.00	
Tallow	0.55	0.77	0.89	0.92	0.72	0.95	1.02		1.26	1.55	35.5	39.4			

According to the peroxide value of the oils during incubation at 50 °C, we selected the time (day) when the PV of oil increased suddenly (exceeded 30-35 meq./kg) as an index of the oxidative stability of the oil (Fig. 1).

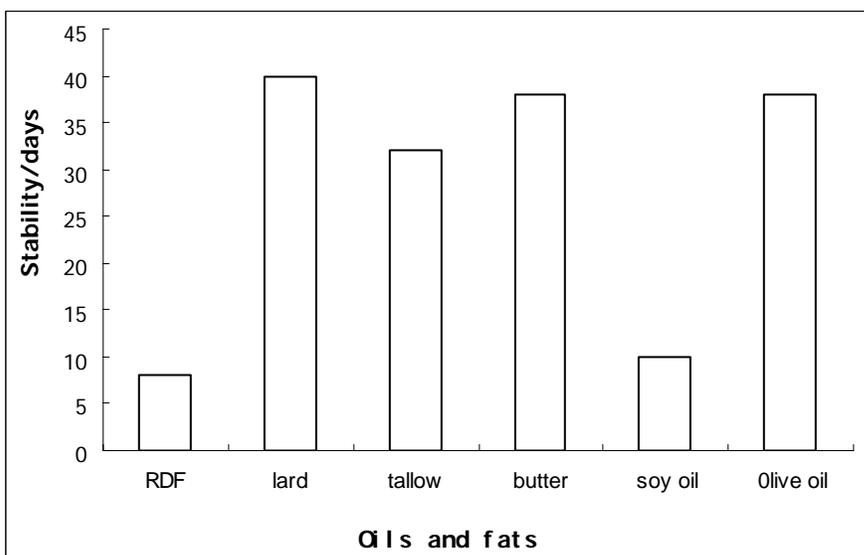


Fig. 1. Oxidative stability of various fats and oils based on time of elevation in peroxide value beyond 30 meq/kg.

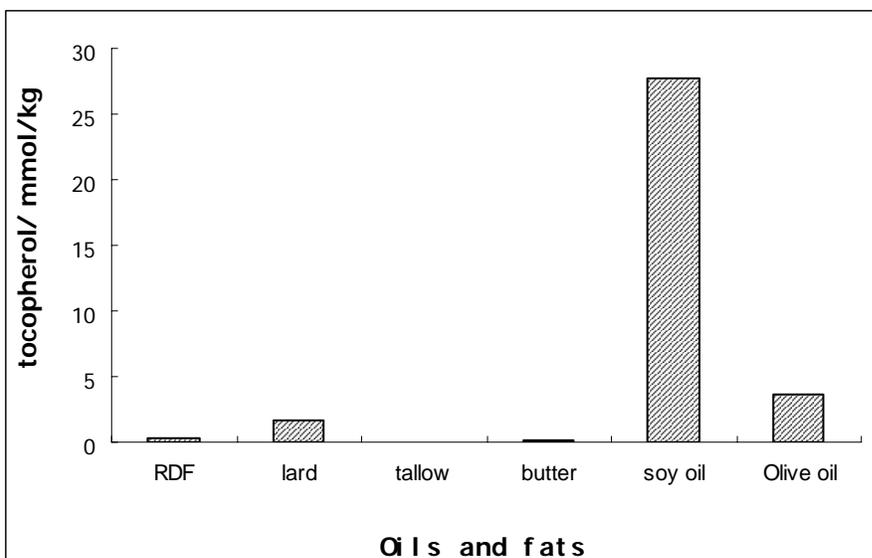


Fig. 2. Concentration of V_E (as α -tocopherol) in oils and fats

Tocopherol content and fatty acid composition

In order to help explain the varying oxidative stabilities in the different fats and oils, we determined the tocopherol content (Fig. 2) and fatty acid composition (Table 3). Soybean oil had a very high tocopherol content (27mmol/kg) followed by olive oil (3.7mmol/kg) while duck fat, lard, tallow, and butter had lower tocopherol contents.

Fatty acid composition datum is shown in Table 3. It indicated that there were higher unsaturated fatty acid contents, especially polyunsaturated fatty acids such as linoleic

acid (18:2), in duck fat (20.49%) and soybean oil (49.35%). Fatty acid composition of duck fat was similar to vegetable oil: high unsaturated fatty acids: 16:1(3.67%), 18:1(41.61%), high polyunsaturated fatty acids: 18:2 (20.49%), 18:3 (1.05%), and low saturated fatty acids: 18:0 (6.27%). Additionally, total 18:3 fatty acids in duck fat (1.05%) are higher than other oils except soybean oil.

Table 3. Fatty Acid Composition of fats and oils.

Oil/FA	duck fat	lard	tallow	butter	olive oil	soybean oil
5:0			1.72			
8:0			1.09			
10:0			2.42			
12:0			2.78			
14:0	0.76	1.27	3.25	9.64		
14:1	0.67	0.37	1.24			
16:0	22.83	19.25	25.90	28.79	14.45	11.99
16:1	3.67	1.98	2.03	1.56	0.92	
17:0	0.20	0.54	1.12	0.70		0.12
18:0	6.27	18.96	30.57	14.25	4.08	5.70
18:1	41.61	39.45	29.90	26.09	65.4	23.52
18:2	20.49	12.92	2.66	2.12	10.74	49.35
CLA		0.14	0.34	0.71		
18:3	1.05	0.62		0.57	0.66	6.90
20:0		0.32	0.23	0.24	0.58	0.48
20:1	0.52			0.16	0.30	0.24
20:2	0.17	0.57				
20:3	0.15	0.20		0.10		
22:0				0.20	0.63	
22:1	0.39	0.21		0.14		
22:4	0.19	0.11				
24:0					0.16	
Other	1.79	2.70	3.23	4.58	2.67	0.91

From the results of fatty acids composition in Table 3, we calculated total polyenes by the following equation: Total polyenes = Σ percentage of polyunsaturated fatty acids \times number of double bond.

The total polyenes results are shown in Table 4. Soybean oil had the highest polyene value (119.4) while rendered duck fat had a polyene value of 45.68. Polyene value was lowest in tallow (6.0). CLA contents of lard, tallow, and butter were 0.14%, 0.34%, and 0.71% respectively. No CLA was detected in rendered duck fat, olive oil and soybean oil.

Table 4. Total Polyene, and CLA content in the various fats and oils

Oil	duck fat	lard	tallow	butter	olive oil	soybean oil
Σ Polyene	45.68	30.16	6.0	6.25	24.06	119.4
CLA (%)	ND	0.14	0.34	0.71	ND	ND

ND- Not detected

Duck fat was easily oxidized (Fig. 1) because of its high percentage of polyunsaturated fatty acids (Table 4) and low content of tocopherol (Fig. 2) relative to the other fats and oils examined. Soybean oil had similar oxidative stability compared to duck fat (Fig. 1). The high content of tocopherol probably increased the oxidative stability of soybean oil in spite of its high content of linoleic acid. The relatively low percentage of 18:2 fatty acid and elevated tocopherol content in olive oil produced high oxidative stability.

Conclusion

The oxidative stability of rendered duck fat (RDF), lard, tallow, butter, olive oil, and soybean oil were 8, 40, 32, 36, 38, and 10 days, respectively. Our test results indicated that duck fat was not resistant to oxidation. Both tocopherol content and fatty acids composition impact oxidative stability of oils. Tocopherol appeared to inhibit oil oxidation. High percentage of 18:2 fatty acids in RDF and soybean oil appeared to accelerate oxidation. Only olive oil contained more 18:1 than RDF. Saturated fatty acid content was low in RDF. Only soybean oil contained more 18:3 than RDF. These attributes provide a fatty acid profile in RDF that should be perceived as healthy. No CLA was found in duck fat. More research works need to be done to extend shelf life of duck fat products when processing in industry.

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ON MAKING IT FEASIBLE TO ANALYZE AND RELATE THE ACTIVITIES OF LYSOSOMAL PROTEASES NON-DESTRUCTIVELY TO THE FINAL QUALITY OF DRY CURED HAMS

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Key Words: dry cured ham, enzyme analysis, fluorescence, light scatter, myoglobin

Introduction

Cutting of the ham before salting is critical as it determines important characteristics like direction and time needed for salt and water diffusion. Trimming of raw hams before processing is therefore a standardized procedure. The trimming generally makes it possible to collect small pieces of muscles, some from highly relevant ham muscles, for future characterization of the raw material.

In an ongoing project (Thauland et al., 2003), where the method of X-ray computed tomography is developed for interval monitoring of salt content and distribution in ham, the small pieces from *Gluteus medius* (GM) and *Semimembranosus* (SM) were collected as possible future indicators of the final quality of dry cured hams. As the enzyme activity is salt dependent, relevant assay should be carried out once the correct salt concentration is known for a specific dry cured ham. The suitability of the trimmings for lysosomal enzyme activity determination was evaluated here as the activities of such enzymes have been indicated as important determinants of end quality of dry cured hams produced over longer (9-12 months) time periods (see for example Garcia-Garrido et al., 2000).

At the same time some aspects related to using a fluorescence assay for enzyme analysis in a quantitative manner on widely different (age and breed) animals were studied.

The aspects studied were: the effect of myoglobin absorbance and the light scattering from the fat present in the collected muscle pieces on the emitted fluorescence intensity.

Objectives

The objectives were to examine the suitability of the small muscle pieces (GM and SM) trimmed from widely different green hams, for lysosomal enzyme assays.

Methodology

Materials: 55 large hams (11-14 kg) were bought from a slaughterhouse as regular hams with shank, and trimmed for dry cured ham production by expert trimmers at The Norwegian Meat Research Center. Pieces of SM and the lighter part of GM were collected and weighted.

In addition, pieces from 16 widely different hams, removed as above, were used for determination of enzyme activity in a screening experiment. These samples were selected from different breeds (Norwegian Landrace/Yorkshire; Noroc (=Norwegian Landrace/Yorkshire/Duroc) and Duroc) and ages (5-36 months). Obvious fat tissue was removed from meat samples. 5 g meat samples, frozen in liquid nitrogen, stored at -80°C were analyzed during a 6 week period.

Methods: Enzyme assay: The enzyme (cathepsin B and B+L) activities were analysed according to the principles of Etherington et al. (1987) and Parreño et al. (1994). Extraction buffer (0.05 M sodium citrate buffer with 1 mM Na₂-EDTA and 0.2% w/v Triton X-100, pH 6.0) to meat was 10:1. Homogenisation: 3 x 30 sec using polytron. Standard centrifugation lasted 15 min at 4°C at 21 000 g_{max}. Thereafter filtration was performed using Whatman filter 4. This extract was used for scatter determination; for several measurements of total protein content, and for some myoglobin and cathepsin determinations. Extended centrifugation lasted 1 hr at 21 000 g_{max}, and then only the extract below the upper lipid layer was used. Essentially this extended centrifugation method removed most of the lipid material from the extract. Cathepsin B and B+L were assayed with substrates N-CBZ-L-arginyl-L-arginine-7-amido-4-methylcoumarin (Z-Arg-Arg-Nmec, Sigma Aldrich C5429)) and N-CBZ-L-phenylalanyl-arginine-7-amido-4-methylcoumarin (Z-Phe-Arg-Nmec, Sigma Aldrich C3282)), respectively. NMec was used as standard (Sigma Aldrich, A9891). Substrates and standards were used at 5 µM; the assay was at 37°C for 30 min (cathepsin B) or 25 min (cathepsin B+L), respectively. *Total protein:* Protein was determined by the biuret method (<http://www.hamline.edu/depts/biology/courses/biocon2/biuret.html>) using bovine serum albumin (96 % pure) as standard. Total protein concentrations were used to calculate specific enzyme activities.

Myoglobin concentration: Myoglobin concentration was estimated using the absorbance of the extracts at 525 nm after extended centrifugation. Commercial myoglobin (Sigma Aldrich, MO630-16) was used as standard. *Light scattering:* The absorbance (525 nm) before and after extensive centrifugation, was taken as scattered light. *Instrumentation:* Perkin Elmer LS 50 was used; excitation was at 342 nm, bandwidth 2.5 nm; scanning rate 250 nm/min. Large cuvettes were used. Spectral measurement was at 21.5 °C. Spectra from 360-600 nm were acquired.

Statistics: The computer program from MINITAB Statistical Software (version 14.12; Minitab Inc., State College, USA) was used to perform one-way analysis of variance, one-way multiple comparisons of mean, paired tests and regressions

Results & Discussion

Figure 1 shows a picture of a ham trimmed for production of dry cured ham. The muscles SM, BF (*Biceps femoris*) and the lighter part of GM are indicated in Figure 1. **Table 1** gives the weights of the three different muscle pieces. It is apparent that the

amount of SM removed can be very small. The variation in size was, however, large. Priority was given to comparing the enzyme activities of GM and SM as SM is the larger muscle inside the dry cured ham, while GM was easily assessable in large quantities. In addition, the activities of BF and SM have been compared earlier (Parreño et al., 1994).

Table 2 gives scattered light, myoglobin content and enzyme activities for the 16 extracts analyzed. Light scattering seemed due to lipid-emulsification during work up of the extract and could be difficult to remove. Light scattering was typically most problematic with the Noroc/Duroc breeds; breeds known for their higher levels of intramuscular fat. Myoglobin level was significantly related to animal age ($p < 0.05$). Total protein concentrations of the extracts differed; SM having the higher mean of total protein (SM: 7.7 mg/ml; GM: 7.1 mg/ml; $p < 0.01$).

Two SM muscles were selected for setting up the enzymatic assay (**Figure 2**). These muscles were chosen because they gave the higher amount of myoglobin in the extract (animal 1; Table 2) and the higher amount of light scattering during standard work-up procedure (animal 2; Table 2). These muscles were characterized by being 1) from an older animal and 2) of the breed Duroc with a high intramuscular fat content, respectively. Myoglobin absorbance was severe only for wavelengths below 460 nm when the meat extraction fraction (meat extract: total assay solution) was $\leq 16.2\%$ (Figure 3). As an example, the reduction in emission intensity at 460 nm using a myoglobin concentration of 0.026 mg/ml was 6%. Scattering (Figure 2, animal 2), if present, seemed prominent at all wavelengths. It could be surmised that the actual position from where the SM sample was obtained, would make it difficult to remove all fat (see Figure 1).

The presence of absorbance and scattering will also reduce the possibility of obtaining a high correlation between the disappearance (at 360 nm) of emission from the substrate and the appearance (at 460 nm) of emission from the product. In our case the correlation was $r = 0.82$ and $r = 0.69$ for GM and SM, respectively, when meat fractions up to 16.2% were used. Lack of a better relationship is suggested to be due to analytical errors accompanying the assay when working at low muscle extract fractions, and bias due to scattering and absorbance. It should be memorized that the impact of myoglobin absorbance cannot be removed from the assay, unless myoglobin, or any other interfering pigment, is removed from the extract for example by chromatography.

The fate of the exciting light in terms of scattering/absorbance/emission will of course depend on the ratio between fluorochrome and muscle extract. However, this ratio can only be controlled at the beginning of the assay.

The enzyme analysis (Table 2) were therefore carried out with meat extract fractions $\leq 4.2\%$. This low fraction will secure that the effect of myoglobin absorbance should not affect the emission spectrum too much at 460 nm; a wavelength typically used for measuring enzyme activity.

No significant difference in the mean enzyme activities of cathepsin B and B+L for the 16 samples of the two muscles was found. The mean activities being $2.5 \mu\text{M} \times \text{min}^{-1} \times \text{g}^{-1}$ and $3.9 \mu\text{M} \times \text{min}^{-1} \times \text{g}^{-1}$ for cathepsin B and cathepsin B+L, respectively (mean specific activities were 0.37 and $0.54 \mu\text{M} \times \text{mg}^{-1}$ protein, respectively).

In contrast, significant differences between animals were detected (Table 2). Two Noroc breeds had significantly higher cathepsin B in GM than the other animals had; no significant differences were found for SM from the different animals. For cathepsin B+L

it also seemed more difficult to obtain a significant differentiation between animals using SM compared to GM. However, significant differences between animals were found for both muscles (Table 2).

A larger experiment involving X-ray computed tomography analysis throughout long term processing of hams is now underway. The experiment that will be supported by the biochemical methods described above.

Conclusions

The *mean* cathepsin B and B+L activities determined on meat pieces removed from *Semimembranosus* and *Gluteus medius* of 16 different animals were not differentiated.

The small pieces of *Semimembranosus* removed from Noroc/Duroc breeds were difficult to measure due to light scattering, and it was also more difficult to differentiate between animals.

The highest cathepsin B activity was measured in *Gluteus medius* from a Noroc breed.

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Tables and Figures

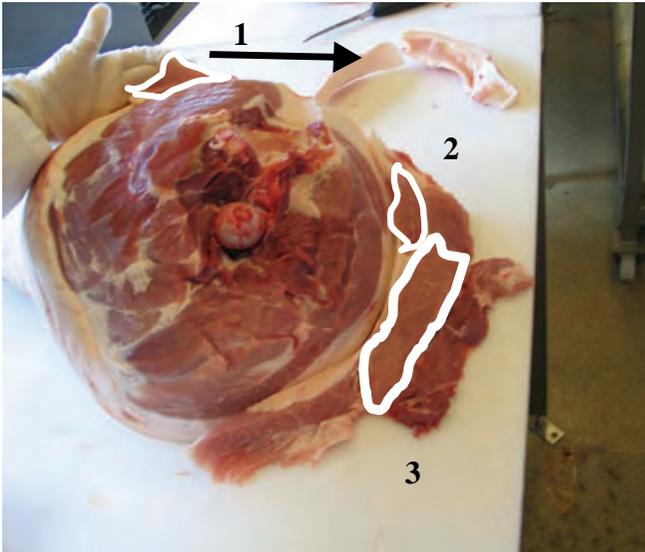


Figure 1. The figure shows a ham trimmed for dry cured ham production. Smaller parts of three muscles were collected. These muscles were 1) Semimembranosus 2) Biceps femoris and 3) Gluteus medius (lighter part). The arrow points at the origin of Semimembranosus, i.e. just beneath the surface of the subcutaneous fat.

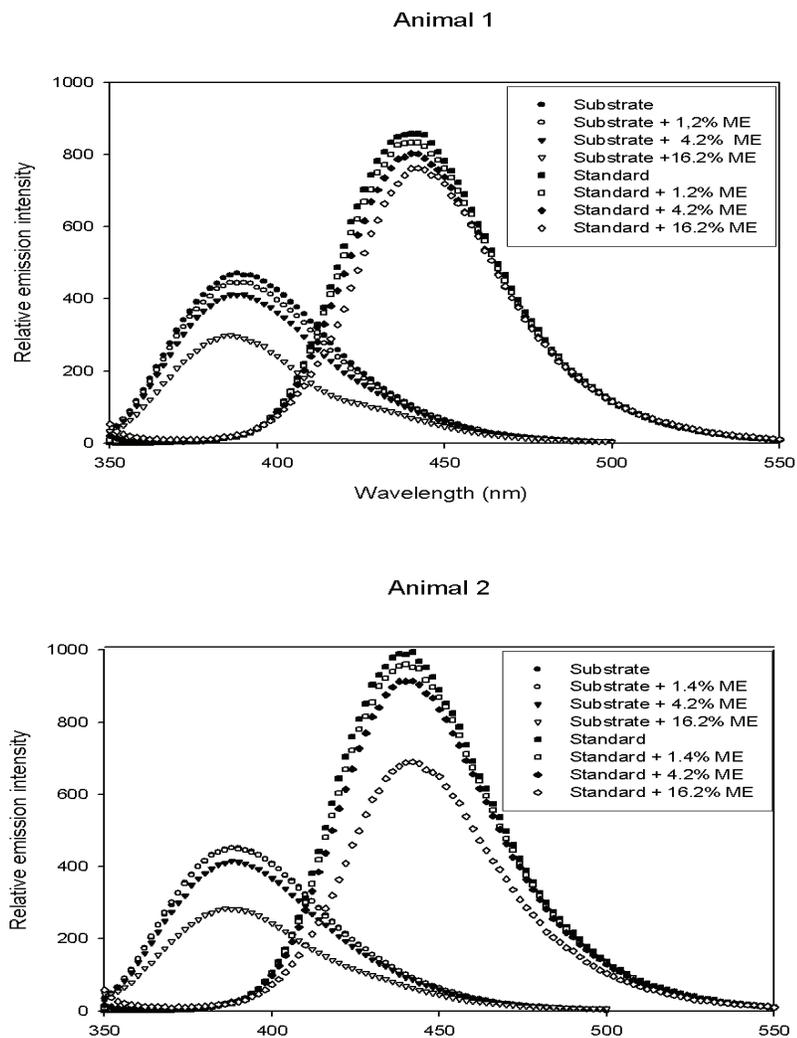


Figure 2. Emission spectra of substrate (to the left) and standard (to the right) obtained for two (1, upper panel; 2, lower panel) different animals as a function of muscle extract (ME) fraction. The enzyme activity was stopped by the presence of 0.72 % mono chloroacetate.

Table 1. Weight of pieces of muscle removed upon trimming 55 hams for dry cured ham production.

Muscle	Mean (g)	Standard deviation (g)	Minimum (g)
<i>Gluteus medius</i>	208	97	107
<i>Biceps femoris</i>	75	32	21
<i>Semimembranosus</i>	39	20	7

Table 3. The characteristics of the 16 different meat pieces from *Semimembranosus* (SM) and *Gluteus medius* (GM) that were screened for lysosomal enzyme activity (*expressed as activity in $\mu\text{M} \times \text{min}^{-1} \times \text{g}^{-1}$ meat sample).

The same letters, by row, indicate no significant difference ($p > 0.05$).

Breed	Landrace/ Yorkshire	Duroc	Landrace/ Yorkshire																						
Age(yrs)	3	0.42	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Animal number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16									
Scattered light-SM	0.008 ^a	0.469 ^c	0.077 ^a	0.001 ^a	0.062 ^a	0.02 ^a	0.003 ^a	0.03 ^a	0.391 ^c	0.089 ^b	0.308 ^c	0.271 ^c	0.029 ^b	0.043 ^b	0.038 ^a	0.067 ^a									
Scattered light-GM	0.019 ^a	0.21 ^c	0.066 ^{ab}	0.009 ^a	0.068 ^{ab}	0.019 ^a	0.011 ^a	0.095 ^{bc}	0.243 ^c	0.099 ^{ab}	0.136 ^{bc}	0.251 ^c	0.052 ^a	0.043 ^a	0.046 ^a	0.024 ^a									
Myoglobin(mg/ml)-SM	0.45 ^{cd}	0.29 ^b	0.41 ^{cd}	0.29 ^b	0.43 ^{cd}	0.37 ^{bc}	0.29 ^b	0.48 ^d	0.36 ^{bc}	0.32 ^b	0.35 ^{bc}	0.26 ^b	0.35 ^{bc}	0.29 ^b	0.29 ^b	0.37 ^{cd}									
Myoglobin(mg/ml)-GM	0.37 ^{bc}	0.26 ^a	0.39 ^{cd}	0.40 ^{cd}	0.40 ^{cd}	0.35 ^{bc}	0.34 ^c	0.37 ^{bc}	0.32 ^b	0.32 ^b	0.33 ^b	0.34 ^b	0.37 ^{bc}	0.26 ^a	0.28 ^a	0.39 ^{cd}									
Cathepsin B activity-SM*	1.43 ^a	2.30 ^a	1.99 ^a	1.38 ^a	0.87 ^a	1.51 ^a	1.67 ^a	2.19 ^a	3.09 ^a	3.45 ^a	2.02 ^a	2.92 ^a	1.45 ^a	4.47 ^a	4.58 ^a	1.57 ^a									
Cathepsin B activity-GM*	1.20 ^a	2.18 ^{ab}	1.51 ^{ab}	1.70 ^{ab}	1.36 ^a	1.29 ^a	1.65 ^{ab}	1.71 ^{ab}	2.30 ^{ab}	9.07 ^b	4.58 ^b	3.56 ^{ab}	2.66 ^{ab}	1.43 ^a	2.38 ^{ab}	2.12 ^{ab}									
Cathepsin B+L activity-SM*	2.39 ^a	4.49 ^{ab}	3.32 ^{ab}	2.05 ^a	1.91 ^a	3.73 ^{ab}	3.34 ^{ab}	3.91 ^{ab}	3.83 ^{ab}	7.83 ^b	5.03 ^{ab}	5.56 ^{ab}	5.75 ^{ab}	4.17 ^{ab}	4.15 ^{ab}	2.72 ^{ab}									
Cathepsin B+L activity-GM*	2.21 ^a	4.45 ^a	3.76 ^a	4.98 ^a	2.00 ^a	1.78 ^a	3.32 ^{ab}	3.81 ^{ab}	3.91 ^{ab}	2.93 ^{ab}	4.64 ^{ab}	5.25 ^{ab}	6.83 ^b	2.86 ^{ab}	6.86 ^b	4.75 ^{ab}									

Meat Processing and Packaging

**COLOR STABILITY OF CHILLED BEEF PACKAGED IN AN ATMOSPHERE
CONTAINING LOW LEVEL OF CARBON MONOXIDE**

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Key words: Chilled beef, myoglobin, modified atmosphere packaging (MAP)

Introduction

The main purpose of MAP of chilled meat is to extend the microbiological shelf life and the color stability of the packaged meat. The gas composition normally used in MAP is 70% O₂/30% CO₂ or 60% O₂/30% CO₂/10% N₂. The 70% O₂/30% CO₂ gives the product an extended shelf life compared to air Gill,1996 .Although the elevated concentration of oxygen prolong color stability of the meat, it is also expected to increase the rate of lipid oxidation (zhao, et al 1994) which causes a rancid off-flavor in meat. The 60% O₂/30% CO₂/10% N₂ further extend the shelf life of packaged meat, but because of the non-oxygen atmosphere and elevated CO₂ concentration, the meat become dark pink or pale brown after packaging. CO is a colorless, odorless and tasteless gas, which can binds strongly to the meat pigment myoglobin to form stable ,cherry red carboxymyoglobin. The technological, hygienic and toxicological aspects of using CO in MAP for meat have recently reviewed with the conclusion that CO used in concentrations up to 1% does not present a toxic hazard to the consumer (Sorheim,Aune and Nesbakken,1997). To obtain a stable red color for the meat while prevent the meat lipid from oxidizing, low concentrations of CO can be introduced into the package which contain no oxygen.

Objectives

The objective of present study was to compare different MAP (with and/or without CO) and vacuum packaging on the shelf life and the color stability of chilled beef, and eventually to obtain 21 days shelf life and red color stability for beef packaged in low level carbon monoxide without the application of oxygen.

Methodology

Gas composition

The original gas mixtures were designed as 0.5%CO/60%CO₂/39.5%N₂ 70%O₂/30%CO₂ and 60%CO₂/40%N₂. The actual gas mixtures used in the study were supplied by Beijing Haipu Gas company and consisted of 0.5%CO/60.4%CO₂/39.1%N₂ (referred to as CO group), 69.9%O₂/30.1%CO₂ (referred to as HO group) and 62.8%CO₂/37.2%N₂ (referred to as NO group). And the vacuum packaged group referred to as VA group.

Samples preparation

Beef *Longissimus dorsi* muscle which had aged at 1±1 for 48 hours were used. Before packaging, the muscles were trimmed of external fat and cut into 1.5 cm thick steaks of about 100g and randomly grouped into 4 groups, each has 18 samples. To limited the discoloration caused by bacteria, all the hands, instruments and knives were sterilized using 75% ethanol before the cutting.

Packaging parameters and storage conditions

One group of sample steaks were vacuum packaged in polyethylene bags using Multivac machine (Wolferschwenden,Germany) , and the other three groups of sample steaks were placed on polystyrene tray of size 18×24×3cm with the top film made of polyethylene and polyamide laminated(water vapor permeability 8g/m²·24h at 23 ,oxygen permeability 40-50ml/m²·24h at 23).Then the trays were flushed with selected gas mixtures(HO, CO, NO) using MAP machine(Ross Industry Inc), respectively. Immediately after packaging, the samples were kept at 1±1 until sampled.

Sampling

Three samples were collected from each group at an interval of 7 days for measurements of redness a* value, MMb% ,TBA values and sensory panel evaluations.

MMb%

MMb% of the samples were measured by HITACHI 557 Reflectance Spectrophotometry according to Judd (1975).

TBA

Lipid oxidation was measured using the 4 mm top layer of each sample, homogenized with a kitchen blender. The lipid oxidation was estimated as TBARS by the extraction method of Witte(1970)

Color

Color was measured on the meat surface immediately after opening the package using a TC-P G-Colormeter (Beijing optical instrument Co.) and expressed as CIE a* value (redness).

Meat samples were evaluated by a seven-member expert panel. The attributes studied were: Red color, Discoloration and Fresh meat odor. All three attributes were scored using a 5-point scale. For “Red color”, 5 denoted extremely high and 1 denoted extremely low. Scores for “Discoloration” referred to percentage of discolored surface, according to

Luno(2000):1=none, 2=0-10%, 3=11-20%,4=21-60%,and 5=61-100%. Scores for “Fresh meat odor” were:1=excellent,, not different from fresh meat; 2=good, but slightly poorer than fresh meat; 3=acceptable, but obviously poorer than fresh meat; 4=hardly acceptable as fresh meat; and 5=non acceptable. Samples were scored by each panel member, and mean values were agreed there of after discussion by all seven panelists.

Statistical analysis

Analysis of variance by Tukey’s multiple comparisons test was performed using SPSS software (Version8.0).Significance was defined at $p<0.05$.

Results & Discussion

The color changes during storage

As showing in Fig.1, during the first week of storage, the color of samples in the HO group were bright red, but these samples began to discolor during the second week and at the 14th day of storage, MMb% exceed 50% and at the 35th day of storage, the sample were totally discolored. The result indicated: the high level of oxygen(69.9%O₂) in the HO group can cause lipid oxidation during storage, the free radicals produced from lipid oxidation accelerated the formation of metmyoglobin, which give the meat brown color. The color of samples in VA group were remain dark red during the entire 4-week study. The color of samples in the NO group became pale immediately after packaging, the redness a* value consistently lower than that of the VA group $p<0.05$; The color of samples in the CO group kept bright red and the redness a* value maintained very high through the entire 4-week study.

In the vacuum package where the oxygen was totally depleted, the meat pigment maintain in the reduced form, thus give the meat dark red color during the entire 4-week study. In the NO group, because of the high concentration of CO₂, which can dissolve in the meat and lower the pH value of the meat surface, give meat a pale color, and the redness a* value of NO group lower than the VA group through the entire 4-week study $p<0.05$.The introduce of low level of carbon monoxide to the package made the CO group maintained bright red through the entire 4-week study.

The changes of MMb% in the surface of the chilled beef presented in fig.2.At the end of the study, the difference of the redness a* value of the four different groups were significant($p<0.01$),the order of the redness a* value from high to low were CO VA NO HO. Because there is a high corelationship between redness a* value and MMb%, so the higher the redness a* value, the lower the MMb%.

The lipid oxidation during storage

As showing in the Fig.3, during the 35 days of storage, the TBA value of samples in the CO, NO and VA group maintain very low, and at the 35th day of storage, all the three groups had TBA values lower than 0.5mg/kg. The TBA value of HO group increased slowly in the first 2 weeks, from 14th days of storage, it increased quickly and at 21st days, the rate of the increase was slow down. During the 4-week study, the TBA value of

HO group increased from 0.37 to 4.93, the variation is significant compared with the other three group $p < 0.01$.

The change of the TBA value indicated that high concentration of oxygen can give the chilled beef bright red color, but the color stability time was limited, only 4 to 6 days. At the same time, the high concentration of the oxygen could increase the lipid oxidation, the radicals produced from the oxidation can increase the discoloration. So HO package only suitable for short shelf-life chilled beef products. For those whose shelf life is expected to be 1 week or more, it was not recommended to use the HO MAP. The result also suggested that the atmosphere of NO, CO and VA group can inhibit lipid oxidation. Besser and Kramer (1972) reported that CO is an enzyme inhibitor and can inhibit lipid oxidation, which can delay the Mb and Hb oxidation. But in our study, because of the low CO concentration, we did not find any significant difference in lipid oxidation in these three groups.

The TVB-N changes during storage

As showing in Fig.4, The TVB-N value of HO group increased very fast after packaging, and at the 14th day of storage, the value has exceeded 15mg/kg, which indicate the end of its shelf life. Several studies indicated that most of the micro flora which cause the putrefaction of the meat are aerobic bacteria, in the presence of O₂, the bacteria grow very quickly and decomposed protein and other nutrients into peptides, amino acid and other small N-containing organic compounds, that why the TVB-N value of the HO group increased very quickly during the study. The TVB-N value of other three groups increased very slowly at the first three weeks, and then kept relatively stable. There are may be two reasons for this: first, there were no O₂ in the gas mixtures of VA, NO and CO groups, so the main bacteria survived was LAB, who can produce lactic acid during growth which can lower the pH of the beef. Second, there were high concentration of CO₂ in these three groups, and the solubility of CO₂ in water and in lipid are very high (Gill, C.O. 1988), which can form carbonic acid on the surface of the beef, so the TVB-N value was not increased after 21 days of storage.

The panel scores during storage

The panel scores, including redness, discoloration, fresh flavor were collected during the 4-week study and presented in Table 1.. The samples of HO group maintained bright red for one week, and then discolored completely; the color of the CO group maintained bright red throughout the entire 4-week study; The NO group discolored very quickly in the first week; the VA group maintained dark red during the whole storage period, but the drip losses of the VA group can be as high as 6%, which make the meat less acceptable to consumers.

For the fresh meat flavor, at the 14 day, the sample in the HO group have rancid flavor after cooking, while the samples in the CO, NO, VA group have no off-flavor at this time of storage. At the 21st day storage, the flavor of CO and NO groups began to deteriorate and at 28th days, the flavor deteriorating of VA group is one week later than the CO and NO groups, this indicated that the low concentration of CO has no effect on the flavor change of the packaged beef.

El-Badawi (1966) reported that CO can bond to Mb to form CO-Mb, who has similar light reflectance with MbO₂. Clark(1976), Luno(1998) reported that 1%CO+25%N₂+24%O₂+50%CO₂ can keep the bright red color of the beef. Kropf(1980),Sorheim(1997)reported that 0.5-2%CO can keep chilled beef bright red. In our studies, we only applied 0.5%CO+/60.4%CO₂+39.1%N₂ in the gas mixtures but the color preserve effect is significant. Danity and Mackey (1992) reported that 10%CO can keep beef color, at the same time can inhibited the microbial growth. But in our studies we did not found any bacteria static effect of the CO due to the low concentration of CO in the gas mixture.

Conclusions

Beef stored in 0.5%CO/60.4%CO₂/39.1%N₂ kept bright red and had TVB-N value lower than 20 mg/100g at 28th days of storage. Beef stored in 70%O₂/30%CO₂ could keep bright red for one week and then the lipid would be oxidized and strong off-odor was produced. Beef packaged in 60%CO₂/40%N₂ or vacuum could keep TVB-N value lower than 20mg/100g at 28th days of storage, but the color would be brown or dark pink and would not be accepted. The studies suggested it was feasible to extend the shelf life and color stability of the chilled beef to 21 days or more by modified atmosphere packaging with gas mixtures of 0.5%CO/60%CO₂/39.5%N₂.

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Tables and Figures

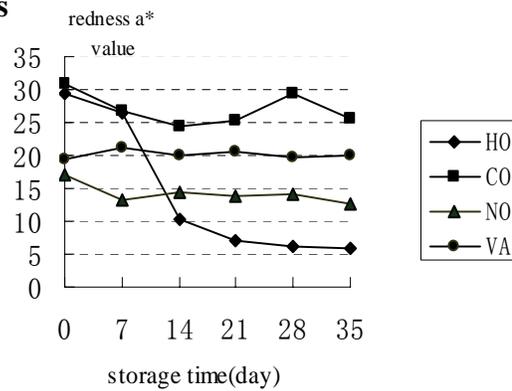


Fig. 1 The redness a* value of chilled beef packaged in different atmosphere during storage at 1±1

Fig2 The MMb% of chilled beef packaged in different atmosphere during storage at 1±1

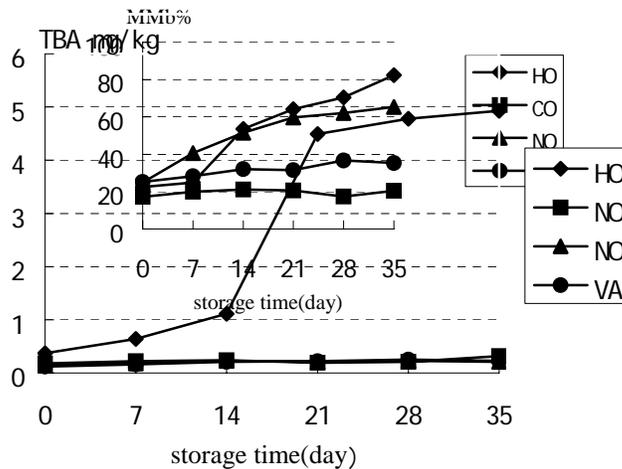


Fig.3 TBA value of chilled beef packaged in different atmosphere during storage at 1±1

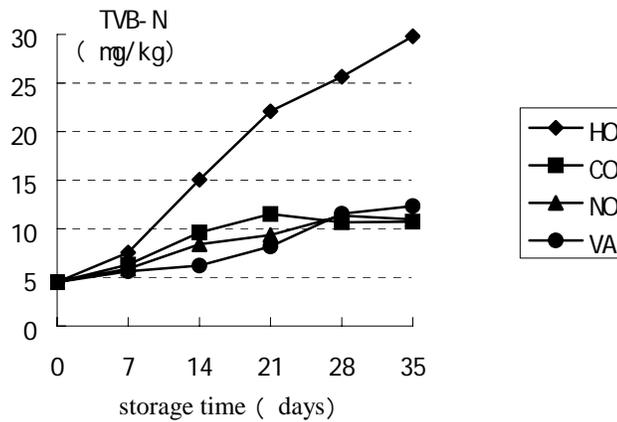


Fig4 The TVB-N value of chilled beef packaged in different atmosphere during storage at 1±1

Table 1 Mean sensory value of chilled beef steak

value	Group	Storage time(day)					
		0	7	14	21	28	35
redness	HO	5	4	2	1		
	CO	5	5	5	5	5	5
	NO	5	3	2	2	1	
	VA	5	4.2	4	4	3.5	3.5
discoloration	HO	1	3	5			
	CO	1	1	1	1	1	2
	NO	1	3	4	4	5	
	VA	1	1	1	1.5	2	2
flavor	HO	1	2	4	5		
	CO	1	1	1	2	3	3
	NO	1	1	1	2	3	3
	VA	1	1	1	1	2	3

“Red color”, 5 denoted extremely high and 1 denoted extremely low.

“Discoloration” 1=none, 2=0-10%, 3=11-20%,4=21-60%,and 5=61-100%.

“Fresh meat odor”:1=excellent,, not different from fresh meat; 2=good, but slightly poorer than fresh meat; 3=acceptable, but obviously poorer than fresh meat; 4=hardly acceptable as fresh meat; and 5=non acceptable.

CARBON MONOXIDE AS A COLORANT IN DRY FERMENTED SAUSAGES

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Key Words: Carbon monoxide, nitrite, color, spectra, beef, dry fermented sausage

Introduction

Finding alternative colorants in dry cured meat products for nitrite (NaNO₂) and nitrate (NaNO₃), a precursor to nitrite, has been a long time challenge. Carbon monoxide (CO) is successfully used for improving the color of packaged fresh meat and heme-containing fish (Sørheim, 2005). A recent experiment showed that CO could substitute for nitrite in cooked beef and pork sausages, utilizing the high heat denaturation temperature of carboxymyoglobin (COMB) (Sørheim et al., 2004). The initial color of CO-treated cooked sausages was bright red, but the color stability was insufficient compared to nitrite sausages. CO also has the possibility of replacing nitrite in dry cured sausages, depending on the stability of COMB at the low pH in fermented products.

Objectives

To study the effect of substitution of nitrite with CO on the color of dry fermented beef sausages.

Methodology

The main experiment of dry fermented beef sausages consisted of 4 treatments: Raw materials pre-treated with 1 % CO/ 99 % N₂ (CO-R), direct flushing of the batters with 1 % CO/ 99 % N₂ (CO-D), addition of 120 ppm NaNO₂ to the batters (N), and control with no CO or NaNO₂ (C). All treatments, except the control, were produced with and without addition of 500 ppm ascorbic acid. The experiment was repeated, giving a total of 14 batches.

The basic recipe consisted of 91.5 % beef semimembranosus muscles, 4.5 % NaCl, 0.5 % glucose, and starter culture (Biocarna™ Ferment CXX, Danisco, Copenhagen, Denmark) with 0.3 g per batch dissolved in 3.5 % water. Each batch weighed 1.5 kg.

Fresh meat was ground twice through a 4 mm plate. Meat for the CO-R treatment was placed in polyamide pouches, compressed to < 0.5 mm, packaged in 1 % CO / 99 % N₂ with < 0.2 % residual O₂, and stored at 3 °C for 5 days. Batters were prepared in a small Stephan UM5 chopper (A. Stephan u. Söhne, Hameln, Germany) with a lid and double bladed knives. The total chopping time was 2 min. For CO-R, pretreated meat with CO was used without additional supply of CO in the chopper. Batters for CO-D were flushed with 1 % CO/ 99 % N₂ at 2 bars during the chopping. All batters were filled in fibrous casings of 43 mm x 50 cm (Viskase, Willowbrook, IL, USA). The fermentation started at 22°C and 95 % RH, were reduced to 18 °C and 90 % RH at 3 days, and was held at 15°C and 85 % RH for the remaining 9 days. The sausages were not smoked. pH and weight loss were recorded during the production.

CIE L*a*b* values (lightness, redness, yellowness) of the sausages were measured with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) with 8 mm viewing port, 2° viewer angle and illuminant D65. Visual color evaluation was performed by three trained assessors using a 5 point scale of 1 = very red to 5 = extremely gray/brown. Instrumental and visual color was recorded 0, 15 and 60 min. after slicing of the sausages. Significant differences in color were evaluated by a multivariate method described by Langsrud (2002). Denaturation of myoglobin in CO-R and N sausages was analysed by the method of Krzywicki (1979). pH of batters and sausages was measured with an InLab 427 gel electrode (Mettler-Toledo, Urdorf, Switzerland).

Spectra of COMB and nitrosomyoglobin (NOMB) were obtained by using 0.2 % equine myoglobin (Sigma Chem. Co., St. Louis, MO, USA) dissolved in 0.5 M phosphate buffers at pH 5.6 and 4.7. The solutions were first converted to reduced deoxymyoglobin with solid sodium dithionite (Na₂S₂O₄). CO (100 %) was lightly flushed through the solutions for 30 sec., and NaNO₂ (100 ppm) was added. The solutions were scanned in the range 450 to 650 nm with 1 nm intervals using an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Waldbronn, Germany). At pH 5.6, the solutions were scanned after 30 min., and at pH 4.7 after 30 min. and 20 hrs. Due to partial denaturation, the NOMB pH 4.7 solution at 30 min. and both pH 4.7 solutions at 20 hrs were filtered before scanning.

Results & Discussion

The color scores of the sausages immediately, 15 min. and 60 min. after slicing are shown in Fig. 1. Sausages of CO-R were more red than N initially, but the red color of CO-R sausages faded at 15 and 60 min. of air exposure. The CO-R batters were relatively firm and dense, minimizing CO loss via exchange with oxygen during chopping. CO-D was less efficient in preserving color of these sausages than CO-R. The finished CO-treated sausages were discolored at the rim, probably due to O₂ uptake through the casings. Fig. 2 with a* redness values confirms the visual color scores. In addition, data of Fig. 2 reveals a beneficial color effect of the antioxidant ascorbic acid for the CO-R and N treatments (p<0.05). For all 4 treatments, L* lightness values decreased with display time, but b* yellowness values were not much affected (results not shown).

Initial pH of the batters was 5.6, and final pH of the fermented sausages was 4.7. Weight losses of the sausages ranged from 32 to 35 %. Denaturation of myoglobin was measured to be 61 – 65 % for CO-R and 84 – 90 % for N sausages. The high degree of

denaturation of the N sausages shows that nitrosoheme is a colorful hydrophobic pigment.

Fig. 3 demonstrates two characteristic and similar absorbance maxima at pH 5.6 for COMB (541 and 577 nm) and NOMB (547 and 578), in agreement with Nam & Ahn (2002). Low pH had no effect on the intensity of the COMB spectra, as they were virtually identical at pH 5.6 and 4.7. However, at pH 4.7 the NOMB spectra had lower absorbance at all wavelengths, compared to pH 5.6. The lower NOMB concentration at pH 4.7 was probably due to a more rapid conversion of nitrite ions and nitrous acid to nitric oxide (NO), which may have escaped as a gas before it could react with myoglobin. To minimize NO losses, Rust (1975) recommends nitrite and ascorbate curing brines to be held at alkaline or only slightly acidic conditions. Slinde (1987) found that during ripening of salami sausages the amount of extractable hydrophilic pigments decreased to zero. In our model experiment, the absorbance of COMB at low pH was only slightly lower at 20 hours, indicating that CO stabilizes myoglobin towards denaturation. It is likely that only the 6th position is occupied by CO and that the 5th position of the iron is still bound to the globin chain. The heme group as such may add one or two ligands of CO or NO. To what extent one or two molecules of CO and NO are bound to the denatured form needs to be studied further.

Conclusions

Dry fermented beef sausages with pH 4.7 were initially red after treatment of raw materials with a gas mixture with 1 % CO, and more red than sausages with 120 ppm nitrite added. Direct flushing of the batters with 1 % CO was less efficient for color formation than gas pretreatment. In accordance with previous findings of CO-treated cooked sausages, the color stability of both CO-R and CO-D samples upon air display was insufficient compared to nitrite sausages, and needs to be studied in more detail. Solutions of COMB and NOMB had absorbance maxima at similar positions at pH 5.6. COMB was more stable than NOMB at pH 4.7, indicating loss of NO at acidic conditions.

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Figures

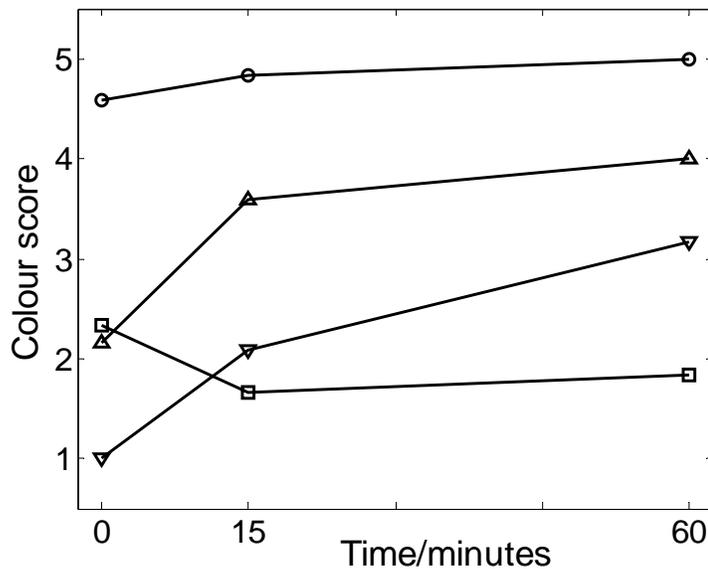


Fig. 1. Visual color score of sliced dry fermented sausages exposed to air for 60 minutes. Symbols: ∇ = CO pretreatment of raw materials (CO-R), Δ = CO flushing of batters (CO-D), □ = nitrite (N), ○ = control with no CO/nitrite (C). Color scale: 1 = very red, 2 = some red, 3 = slightly red, 4 = some gray/brown, 5 = extremely gray/brown.

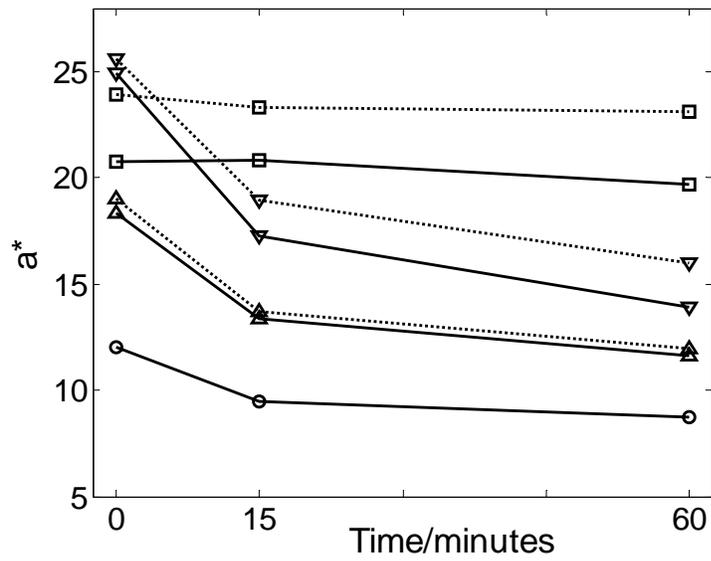


Fig. 2. CIE a^* redness values of sliced dry fermented sausages exposed to air for 60 min. For symbols, see Fig. 1. No ascorbic acid = solid lines. With ascorbic acid = dotted lines.

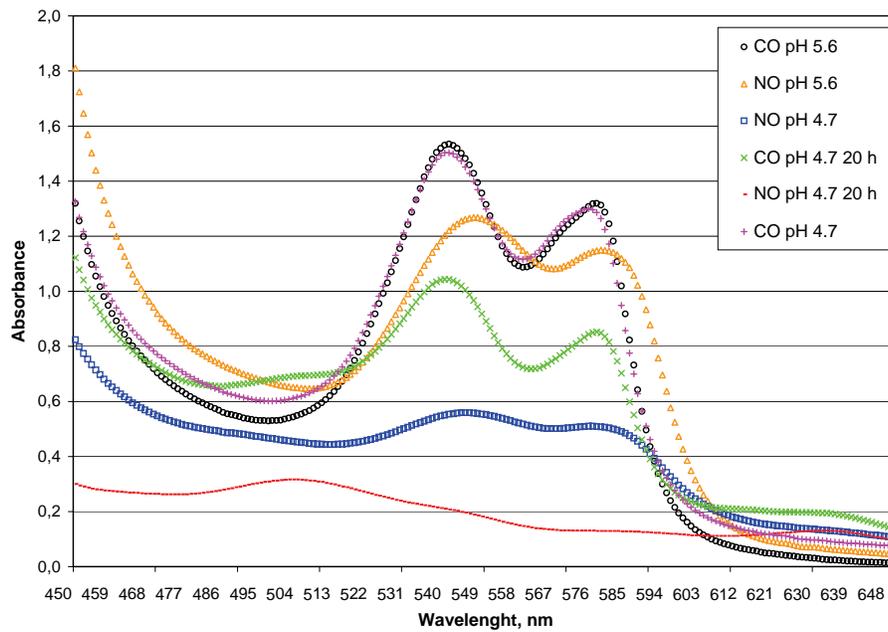


Fig. 3. Absorption spectra of carboxymyoglobin (CO) and nitrosomyoglobin (NO) in solutions with pH 5.6 and 4.7 at 30 minutes and 20 hours (20h).

**THE UTILIZATION OF MODIFIED ATMOSPHERIC-PACKAGING (MAP)
ASSOCIATED WITH OXYGEN SCAVENGERS TO PREVENT THE
TRANSIENT DISCOLORATION OF RUMP AND STRIPLOIN STEAKS**

Venturini, A.C.; Contreras, C.J.C.*; Sarantópoulos, C.I.G.L.

Retail slices of rump (*Gluteus medius*, GM) and striploins (*Longissimus dorsi*, LD) steaks were master packaged under CO₂ and stored at 1±1°C for 14, 28, 35, and 42 days. A commercial oxygen scavenger (ATCO HV1000[®]) was also tested to achieve an oxygen-free atmosphere. Controls without O₂ scavengers were prepared in the same manner. At each storage time, two master-packs with and two without the oxygen scavenger were opened, and the trays were placed in a retail-display case at 4±2°C for 1, 24, and 48h. The low growth rate of aerobic psychrophilic flora on the beefsteaks clearly demonstrated the bacteriostatic effect of CO₂ during storage. The maximum level of bacterial growth reached during retail display was approximately 10⁶ UFC/g after 42 days of storage in MAP. The steaks stored in master-packs with the oxygen scavenger bloomed to the desired red color associated with freshly cut meat in the display case for the entire storage period, except in the case of GM steaks that showed a cycle of transient discolorations. The rump and striploins steaks stored without oxygen scavenger failed to bloom. After 42 days of storage, the acceptability of the rump and striploins steaks stored in oxygen-free atmosphere was 49% and 77%, respectively.

Key Words: modified atmosphere, CO₂, transient discoloration, rump and striploins steaks

Introduction

The color of fresh meat is the most important quality parameter to attract the consumer at the time of purchase. A bright-red color is desired for beef offered for retail sale. The coloration of fresh beef depends on the relative amounts of three forms of myoglobin: reduced myoglobin (ruby), oxymyoglobin (red), and metmyoglobin (brown). The atmosphere of the packaged meat can affect the color. A transient discoloration can occur during the initial storage period in packaging with a modified atmosphere that has a low concentration of oxygen. If the amount of residual oxygen is not excessive (<0.06%), the transient discoloration of beef striploins will be resolved in two days (Gill & McGinnis, 1995). However, slices of tenderloins, which have a lower stability to color oxidation, undergo two cycles of transient discoloration when stored in a CO₂ atmosphere for 4 and 7 days due to the presence of residual oxygen (Tewari, Jayas, Jeremiah et al., 2001). This difference in color stability within different meat cuts is the result of metmyoglobin-reducing activity (MRA) or other reducing factors (O'Keeffe & Hood, 1981a,b).

Objectives

The objective of the present study was to assess, under pilot plant conditions, the efficacy of a commercial self-activating oxygen scavenger to prevent transient discoloration of fresh beef with different oxidative color stability, such as rump and striploins beefsteaks stored under pure CO₂ in master-packs at 1±1°C.

Methodology

Vacuum-packaged eye of rump and striploins were cut into approximately 10-15-mm-thick slices and were placed in an expanded polystyrene tray on a liquid soak pad. Each tray was over-wrapped with clear PVC thermosealed film (11µm, Goodyear®) presenting a high O₂ transmission rate of about 12,232 cm³ (STP)/m²/day at 25°C, 75% R.H. and 1 atm. After sealing, four trays with rump and four with striploins were placed in an alternated manner inside of a master-pack (434 x 582 mm) with an O₂ transmission rate of 19 mL (STP)/m²/day at 25°C, 75% R.H. and 1 atm. The master-packs filled with 3.5 L of pure CO₂/kg of meat were evacuated using a 'double vacuum-flush' cycle, and sealed using a gas flushing system (A300, CVP Systems Ltd., USA). Immediately before sealing, a commercial oxygen scavenger formulated to reduce 1000cm³ of O₂ (ATCO® HV1000, Standa Industrie, France) was placed into each master-pack. Moreover, master-packs without O₂ scavengers were prepared in the same way to represent the residual oxygen effects on the meat color. All packs were stored in a cold chamber in the dark for up to 42 days at 1±1°C. After 14, 28, 35, and 42 days of storage, the master-packs were removed from the chamber. The O₂ concentration (%) was analyzed immediately before opening the master-packs using an O₂ gas-analyzer (PBI Dansensor). After the gas analysis, the rump and striploin trays were taken from the master-packs and randomly placed in an illuminated (fluorescent light) retail-display case in ambient air at 5±1°C for 48h for subjective and objective color evaluations. The data was obtained through the transparent shrink film on each day of retail display. Color instrumental measurement was carried out with a portable spectrophotometer (model CM 508d, Minolta Co. Inc., New Jersey, USA). The average of eight readings was recorded for each steak of tray. The reflectance port size was 25mm. The illuminant was D₆₅, the observer angle was 10° and the specular component was excluded.

The red visual color of steaks was evaluation on a non structured scale of 9 cm, anchored with colored chips from Munsell's Atlas which followed the color changes occurring in fresh meat, where 0 cm = none red color; 5.5 red; 7 = brownish red and 9 cm = reddish brown color. Lean discoloration was scored on a six-point scale where 1 = no discoloration, 2 = 5% discoloration, 3 = 5 - 15% discoloration, 4 = 15-25% discoloration, 5 = 25-35% discoloration, 6 = 35-100% discoloration and global appearance was anchored on seven point scale where 1 = extremely unacceptable, 2 = moderately unacceptable, 3 = slightly unacceptable, 4 = neither acceptable nor unacceptable, 5 = slightly acceptable, 6 = moderately unacceptable and 7 = extremely unacceptable.

The acceptance of the meat color by consumers was evaluated 42 days after storage under MAP through the application of a Test of Central Localization.

The effects of packaging system (with and without scavenger system), storage time (14, 28, 35 e 42 days) and display time (1 and 48h) and that of their interaction effects were evaluated by analysis of variance – ANOVA (SAS Institute Incorporated, Cary, North Carolina, USA, 1988). When $p < 0.05$, significant effects were observed. The experiment was replicated 8 times.

Results & Discussion

On the processing day, the meat showed a normal pH (5.5 ± 0.12). However, during the storage under MAP (0, 14, 28, 35, and 42 days), the rump and striploin steaks packed with and without oxygen scavengers showed low pH variations (< 0.03). A non-significant quantity of air was kept inside of a few master-packs after the thermosealing. The residual concentration of oxygen in the master-packs with oxygen scavengers was lower than 0.1% after 14 days post-storage. The Table 1 summarises the results of the variance analysis (ANOVA) for color parameters of steaks stored into different packaging systems (with or without scavengers). The saturation index (C^*), which is an indicator of color intensity, clearly showed the need for oxygen scavengers in the master-pack for the occurrence of the re-blooming of the color. During the MAP storage all steaks stored with oxygen scavengers had highest C^* , visual red and appearance (< 0.000) when compared to steaks stored without scavengers, where the oxidation of the red color on the beef surface was followed by a significant reduction of these parameters. Within the CIELAB system, the re-blooming with time for rump steaks is seen clearly in a large increase in C^* and R_{630} - R_{580} with decrease in L^* , h^* values. Rump steaks stored under MAP in the presence of oxygen scavengers had a discoloration percentage significantly inferior to the steaks stored without scavengers (< 0.000) (Table 1), although up to the 14th day under MAP, these steaks had a similar discoloration percentage when compared those products stored without oxygen scavengers that had the visual color and the overall appearance seriously impaired. This discoloration was resolved in the subsequent storage periods when the concentration of oxygen was $< 0.1\%$.

Beef striploins stored under MAP with oxygen scavengers bloomed to the desired red color associated with freshly cut meat 1h after displaying in air and had almost no discoloration despite of the measurable oxygen residual, whereas steaks without oxygen scavengers showed a superior discoloration ($p > 0.05$) and failed to bloom for all storage times. The irreversible discoloration observed in these steaks stored without oxygen scavenger was probably the result of excessive residual O_2 in these master-packs during the storage period under MAP (0.7-3.2%).

The color parameters did not differ significantly during the aerobic exposure (48 h) after each storage period under MAP.

After 42 days of storage under MAP, the trays with rump with oxygen scavengers were considered acceptable by 49% and striploins by 77% of the 80 consumers that responded 'probably would buy' or 'certainly would buy' these products. The fat of the steaks stored under MAP with oxygen scavengers was infiltrated with the meat pigment, and it conferred a pinkish color to the tissue exposed to air, thus influencing positively the acceptance of the products.

The perception of a greater volume of exudates, by certain consumers, in the trays with rump steaks probably had a significant impact at the purchase time. A greater

volume of liquid in trays can be prevented with a pad that has a better absorption capacity.

Conclusions

Self-activating oxygen scavenger in association with MAP under CO₂ was more successful for LD than GM steaks in preventing transient discoloration and extending the shelf life of fresh beef. Effective removal of air is critical for extending the storage life of retail-ready fresh beef products in master-packaged under a CO₂ atmosphere. Better results could be obtained by evacuating the master-packs within a machine with vacuum chamber, which is designed to optimize the removal of air from the master-packs at the time of sealing.

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Tables and Figures

Table 1. Results of the variance analysis on color characteristics.

Effect		L*	C*	h*	C	visual red ^b	Discolor (%) ^d	Global appearance
Packaging		**	***	*	***	***	***	***
Storage		***	***	***	***	NS	NS	***
Display		NS	NS	NS	*	***	***	***
Packaging *Storage	GM	NS	***	NS	***	NS	NS	**
Packaging * Display		NS	*	NS	NS	***	***	***
Storage*Display		NS	NS	NS	**	NS	NS	**
Packaging *Storage* Display		NS	NS	NS	NS	NS	NS	***
Packaging		*	***	NS	NS	***	***	***
Storage		NS	NS	NS	NS	***	*	***
Display		NS	NS	NS	NS	NS	*	***
Packaging *Storage	LD	NS	*	NS	NS	***	***	***
Packaging * Display		NS	NS	NS	NS	NS	NS	NS
Storage*Display		NS	NS	NS	NS	***	*	*
Packaging *Storage* Display		NS	NS	NS	NS	NS	NS	*

Packaging system: Masterpack system with or without oxygen scavengers.

L*=lightness; $C^* = (a^{*2} + b^{*2})^{1/2}$; h*: $\arctan(b^*/a^*)$

Visual red (lean): 0 cm = none; red color = 5.5; brownish red = 7 and 9 cm = reddish brown

Discoloration %: 1 = no discoloration, 2 = 5% discoloration, 3 = 5-15% discoloration, 4 = 15-25% discoloration, 5 = 25-35% discoloration, 6 = 35-100% discoloration.

*** p<0.001

** p<0.01

* p<0.05

NS: not significant (p>0.05)

USE OF HIGH PRESSURE FOR APPLICATION OF CARBON MONOXIDE IN CASE-READY PACKAGING SYSTEMS FOR FRESH BEEF

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Key Words: Packaging, Carbon Monoxide, Pressure

Introduction

The color of fresh beef is a primary determinant of quality to the retail consumer. Kropf (1980) suggested that the single greatest factor determining the purchase of meat at retail was probably muscle color.

Several researchers have shown that the addition of carbon monoxide (CO) to fresh beef can improve and stabilize the bright red color associated with high quality fresh beef (Hunt et al., 2004; Jayasingh et al., 2001; Luno et al., 2000). These researchers have tested the use of CO at low concentrations (0.1 to 5.0%) in a modified atmosphere package (MAP) to stabilize beef color, delay metmyoglobin formation, and extend retail case life. Jayasingh et al. (2001) found that beef needed 24 h in a 5% CO atmosphere in order to stabilize the bright red color when repackaged in a vacuum package. Reducing the CO application time is necessary for high speed case-ready packaging systems because a CO application time of 24 h is not feasible in a high volume situation. Reducing the CO application time to seconds rather than hours would allow for case-ready packages with meat-to-film contact, such as vacuum packaging, which may be more appealing to consumers than MAP packages with 'head space' between the meat surface and film. It may be that 100% CO applied under high pressure could accomplish beef bright red color fixation in relatively short application times.

Objectives

Hypothesis: High pressure can be used for the application of carbon monoxide (CO) to fresh beef to minimize the time required for color fixation in a high speed case-ready packaging system.

Objective: Determine if high pressure could be used for the application of carbon monoxide (CO) to fresh beef in order to minimize the time required for color fixation in a high speed case-ready packaging system.

Methodology

Beef steaks were cut from USDA Select subprimals, and 100% CO gas was applied to each steak using high pressure chambers. Initial CO penetration was measured by

cutting a cross section of each steak immediately following CO application. Steaks were then immediately packaged in anaerobic MAP packages with a mixture of nitrogen and carbon dioxide. The MAP packages utilized shallow trays which resulted in meat-to-film contact (no head space in packages). On d1 following CO application and packaging, a^* was measured on the surface of each steak using a Minolta colorimeter Model CR-310 and a D65 illuminant. Our preliminary research showed that d1 a^* was highly correlated with subsequent a^* readings (d2 to d20) during retail display for CO-treated steaks packaged in anaerobic MAP with meat-to-film contact (Figure 1). Therefore, we report d-1 a^* in this paper because d-1 a^* is an indicator of beef color across all days of retail display in CO-treated fresh beef in anaerobic packages.

Phase 1 – Effect of pressure and duration.

In Phase 1, 32 beef steaks were cut from longissimus muscle (LM) and used in a 2 x 3 factorial treatment design with two pressures (1724 and 3447 kPa) and three durations (30, 60, and 90 sec).

Phase 2 – Effect of duration and muscle using enhanced steaks.

In Phase 2, 109 beef steaks, enhanced to 110% of green weight with a solution of water, phosphate and salt, were used and CO was applied at 3447 kPa using a 3 x 3 factorial treatment design with three muscles (LM, psoas major = PM, semimembranosus = SM) and three durations (15, 22.5 and 30 sec).

Results & Discussion

Phase 1 – Effect of pressure and duration.

Steaks treated at 3447 kPa had greater initial CO penetration than steaks treated at 1724 kPa (Table 1). Steaks treated for 60 or 90 sec had greater initial CO penetration than steaks treated for 30 sec. Therefore, CO penetration was a function of both pressure and duration of CO application. Steaks treated at 3447 kPa had higher d-1 a^* than steaks treated at 1724 kPa (Table 2). Duration had no effect on d-1 a^* ($P > 0.05$). Through case life monitoring in preliminary studies, we had determined that a minimum of approximately 1.00 mm of initial CO penetration and a minimum d-1 a^* of 25 to 27 were required for color stability in an anaerobic package. Given these minimums, it appeared from Phase 1 that color stability could be accomplished with either a 60-sec duration at 1724 kPa or a 30-sec duration at 3447 kPa (Table 1 and 2). We wondered if color stability could be accomplished at 3447 kPa in less than 30 sec; hence, shorter durations were tested in Phase 2.

Phase 2 – Effect of duration and muscle using enhanced steaks.

As duration of CO application increased from 15 sec to 22.5 sec to 30 sec, initial CO penetration increased (Table 3). Steaks from SM had a greater initial CO penetration than steaks from PM which had a greater initial CO penetration than LM. As duration of

CO application increased from 15 sec to 22.5 sec to 30 sec, d-1 a* increased (Table 4). Day-1 a* was highest for PM steaks, intermediate for LM steaks and the deep side of SM steaks, and lowest for the superficial side of SM steaks. Carbon monoxide penetration was deepest for SM steaks, but SM steaks were less red than the other muscles. Steaks from SM may require a deeper CO penetration than other muscles. Assuming a minimum d-1 a* of 25 to 27, CO application at 3447 kPa would require a minimum application duration of 30 sec for LM and SM steaks and a minimum application duration of 15 to 22.5 sec for PM steaks. These durations required for color fixation are considerably less than those required when CO is used in low concentrations in MAP packaging (Jayasingh et al., 2001).

Conclusions

High pressure (3447 kPa) application of CO was effective at fixing fresh beef color in 30 sec or less for application in anaerobic MAP systems. This short duration required for color fixation when using high pressure CO application may make it feasible to use in a high speed, high volume case-ready operation. Stabilizing color with CO would allow for retail beef to be sold with bright red color while using vacuum packaging or anaerobic MAP with meat-to-film contact.

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Tables and Figures

Figure 1. Effect of d-1 a* readings of CO-treated beef longissimus steaks packaged in anaerobic MAP with meat-to-film contact on subsequent a* readings during d 2 to 20 of retail display.

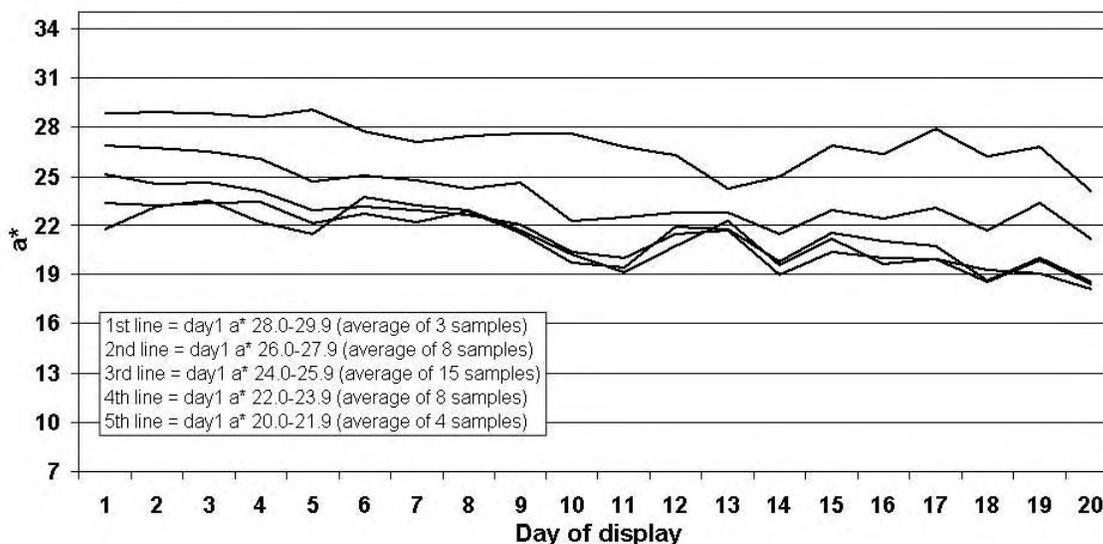


Table 1. Effects of pressure and duration of CO on initial color penetration (mm) in non-enhanced beef longissimus steaks (pressure P = 0.0476, duration P = 0.0001, pressure x duration P = 0.3796; means lacking a common letter differ, P < 0.05).

Application duration	Pressure		Average
	1724 kPa	3447 kPa	
30 sec	0.76	0.95	0.86a
60 sec	1.07	1.27	1.17b
90 sec	1.27	1.27	1.27b
Average	1.03a	1.16b	

Table 2. Effects of pressure and duration of CO on d-1 a* in non-enhanced beef longissimus steaks (pressure P = 0.0477, duration P = 0.1448, pressure x duration P = 0.4848; means lacking a common letter differ, P < 0.05).

Application duration	Pressure		Average
	1724 kPa	3447 kPa	
30 sec	23.2	27.7	25.4
60 sec	27.3	28.5	27.9
90 sec	27.2	29.0	28.0
Average	25.9a	28.4b	

Table 3. Effects of muscle and duration of CO application at 3447 kPa on initial color penetration (mm) in enhanced beef steaks (muscle P = 0.0001, duration P = 0.0172, muscle x duration P = .8480; means lacking a common letter differ, P < 0.05).

Application duration	Pressure			Average
	Longissimus	Psoas major	Semimembranosus	
15.0 sec	0.84	0.95	1.34	0.88a
22.5 sec	0.78	1.12	1.52	1.10b
30.0 sec	1.02	1.24	1.78	1.39c
Average	1.05a	1.14b	1.52c	

Table 4. Effects of muscle and duration of CO application at 3447 kPa on d-1 a* in enhanced beef steaks (muscle P = 0.0001, duration P = 0.0001, muscle x duration P = 0.0733; means lacking a common letter differ, P < 0.05).

Application duration	Pressure				Average
	Longissimus	Psoas major	Semimembranosus (superficial)	Semimembranosus (deep)	
15.0 sec	23.9	25.3	20.9	22.3	23.1a
22.5 sec	24.8	28.7	21.1	24.5	24.8b
30.0 sec	25.5	29.1	25.4	26.6	26.6c
Average	24.8b	27.7c	22.5a	24.5b	

EFFECT OF DIETARY SUPPLEMENTATION OF VITAMIN E ON CHARACTERISTICS OF LAMB MEAT OF AIR AND MODIFIED ATMOSPHERE PACKAGED

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Key Words: Vitamin E, Air packaged, Modified atmosphere packaged, Color stability, Lamb meat

Introduction

Meat color is one of the most important qualities that customers use to purchase meat. Meat color affects the perception of freshness and determines the retail shelf life of meat. Extending this period should improve retail salability. The color of meat depends of many factors such as concentration of haeminic pigments, principally of myoglobin, and the chemical state of these pigments. The color of meat is due to a balance between oxymyoglobin, which is thought to indicate freshness and considered attractive to the consumer, and metmyoglobin, which is brown and unattractive (Renner, 1990).

Change in meat color is closely associated with lipid and pigment oxidation (Buckley et al., 1995), as well as with bacterial load. Nevertheless, for meat packed under aerobic conditions lipid oxidation is not a limitation for storage because it occurs at a slower rate than discoloration and microbial growth (Jakobsen et al., 2000). Meat packed under high-oxygen modified atmosphere in refrigeration retards significantly the undesirable formation of metmyoglobin and the surface oxymyoglobin layer is thicker and the meat appears redder (Taylor et al., 1990) and prevents microbial growth of anaerobic pathogens (Ogrydziak et al., 1982). A disadvantage is that lipid oxidation increases and is one of the primary causes of quality loss in meat during such storage (Renner et al., 1993).

Dietary supplementation with antioxidants produces an increase in lipid and pigment stability. Vitamin E is the primary lipid-soluble antioxidant in biological systems, since it breaks the chain of oxidative processes. Dietary supplementation with vitamin E increases the amount of α -tocopherol deposited in muscle and fat tissue (Jensen et al., 1998). The deposition of α -tocopherol in cell membranes allows it to act directly and effectively in control of lipid oxidation and, indirectly, in color deterioration in many species (Faustman et al., 2000).

Objectives

The aim of this study was to analyze whether the supplementation with vitamin E in the diet of lamb during the whole or in the last two weeks of the fattening period had an

effect on meat color, pigments content and lipid oxidation under air and modified atmosphere packaged.

Methodology

Twenty-two weaned male Manchego breed lambs were randomly assigned to two experimental groups of 11 lambs each. The dietary regimes of the lambs were: 1) concentrate diet supplemented with 270 mg of vitamin E/kg feed during the whole fattening period (32.2 ± 0.7 days), from an initial live weight of 14.5 ± 0.2 to a slaughter weight of 26.1 ± 0.3 kg; 2) concentrate diet containing 20 mg of vitamin E / kg feed since 14.7 ± 0.2 kg until lambs weighed 20.7 ± 0.1 kg, 18.7 ± 1 days, after that lambs received the supplemented diet with 270 mg of vitamin E/kg feed until animals reached the slaughter weight of 26.6 ± 0.3 kg, 14.3 ± 0.8 days. The lambs were housed in individual pens (1 m²). Feed, water and barley straw were offered *ad libitum*. Twice per week, feed intake and live weight were recorded. When animals reached the fixed slaughter weight between 26 and 27 kg, lambs were slaughtered in a commercial abattoir. After 24 h postslaughter, m. *longissimus dorsi* from the left-half carcass was dissected and cut in 7 slices which were randomly assigned to each types of packaged, air and modified atmosphere and each storage times, 2, 6 and 12 days. The seventh slice was vacuum packed and frozen at -20°C for subsequent α -tocopherol analysis. Muscles slices in air packaged were placed on fiberboard trays and overwrapped with oxygen permeable (10,000 cm³ O₂/m²/24 h) polyvinyl chloride (PVC) film. Muscle slices in modified atmosphere packaged were packed using BB41 Pouches (150 μ m, polyamide/polyethylene, 50/100, Cryovac) with low gas permeability (7 cc/m²/24 h O₂, 150 cc/m²/24 h CO₂, water vapor transmission rate was 1.5 g /m²/24 h) and flushed with 70 % O₂ and 30 % CO₂ with a 2:1 gas volume to meat ratio in each pack, using a packing machine type EV-15-1-CD-SC (Tecnotrip, S. A. Barcelona Spain). Meat samples were stored in darkness at $2 \pm 1^\circ\text{C}$.

The concentration of α -tocopherol in muscle was determined using the procedure of Cayuela et al. (2003). The results were expressed in mg α -tocopherol / kg muscle. Color measurements were done by reflectance using CM-2600d spectrophotometer (Minolta). The results were expressed as lightness (L*), redness (a*) and yellowness (b*). The relative content of myoglobin, oxymyoglobin and metmyoglobin was calculated according to Krzywicki (1979). Lipid oxidation in meat samples was assessed by the 2-thiobarbituric acid method of Maraschiello et al. (1999), evaluating thiobarbituric reactive substances (TBARS). The results are expressed in mg of malonaldehyde (MDA) / kg muscle.

Data were analyzed statistically using PROC MIXED of SAS version 8.2. (SAS Inst. Inc, Cary, NC). A factorial design was used in which dietary regimen was examined as main factor and type of packaged and storage time, a repeated measurement. The lowest Bayesian Information Criterion (BIC) was used to choose the matrix of the error structure. The matrix of error used was compound symmetry and unstructured, since they displayed the lowest BIC.

Results & Discussion

Tissue accumulation of α -tocopherol in vitamin E-supplemented animals appears to occur in a dose- and duration-dependent manner (Gatellier et al., 2001). Lambs fed 270 mg of vitamin E /kg of feed during the entire fattening period had a concentration of α -tocopherol in muscle of 2.59 ± 0.16 mg / kg muscle, which was higher ($P < 0.001$) than lambs fed 270 mg / kg during last two weeks of fattening period, that had a concentration of α -tocopherol of 1.87 ± 0.17 mg / kg. Turner et al. (2002) reported that longissimus α -tocopherol concentration from lambs fed 300 IU of vitamin E /kg of DM for 21 days was higher than that from lambs fed 300 IU/kg for 7 days. Their longissimus α -tocopherol concentrations (2.89 and 1.91 mg / kg muscle for 21 and 7 days, respectively) were similar to our results, though the time of supplementation was lower the weight of the lambs was twice the our experiment (47 kg).

The meat color parameters, pigments content and TBARS for the dietary regimes and types of packaged during storage time are presented in figure 1. The significance of the main effects and interactions in the full model are presented in table 1. The L* color (a higher number indicates more white than black) was affected by dietary regimen ($P < 0.05$). Lambs supplemented during whole fattening had higher value than the other group. Besides, a* (indicating an increase in green color relative to red) and b* (indicating an increase in blue color relative to yellow) were also higher for lambs supplemented during whole fattening. This results showed that meat from lambs supplemented during whole fattening period was clearer and redder than those supplemented in the last half of the fattening, it could be the higher concentration of α -tocopherol in meat of lambs supplemented the whole fattening, increases color stability and maintains more suitable color of meat (Turner et al., 2002). There was a significant interaction for a* ($P < 0.05$) and b* ($P < 0.001$) between type of packaging and storage time, MAP reduced lightly a* and b* parameters during storage, whereas air packaged meat increased these values during storage.

In relation to pigments content, metmyoglobin was not affected by dietary regimen and showed a significant interaction ($P < 0.01$) between type of packaging and storage time, increasing more rapidly in air-packed meat than in MAP, independently of dietary regimen. Oxymyoglobin content also had a significant interaction ($P < 0.05$) between type of packaging and storage time. Its proportion was higher in MAP than in air packaged. MAP maintained the proportion of oxymyoglobin during storage whereas air packaged increased its proportion during storage. In this sense, Kerry et al. (2000) reported that MAP with high oxygen proportion improved color of meat, reducing metmyoglobin formation and maintaining the desirable bright-red color of meat due to oxymyoglobin. In air-packaged, the oxygen penetration inside meat is lower, because the oxygen pressure is lower than MAP, and the accumulation of metmyoglobin is enhanced (Ledward, 1970).

Although TBARS showed a significant effect of dietary regimen and storage time, it also showed a significant interaction ($P < 0.001$) between both effects. The lipid oxidation was only lower after 12 days of storage in meat from lambs supplemented during the whole fattening period than in the other dietary regimen (figure 1). These results agree with Formanek et al., (1998) who reported that TBARS values in α -tocopherol acetate-supplemented minced beef were reduced in aerobic and MAP packs compared to not supplemented beef following refrigerated storage for 10 days. Gatellier et al., (2001) observed lower TBARS at the end of storage time such as air-packaged as MAP in beef

supplemented with 1000 mg α -tocopheryl acetate/animal/day for 111 days before slaughter respect to not supplemented beef.

Conclusions

Increase of tissue accumulation of α -tocopherol in vitamin E supplemented lambs appears to occur in a duration-dependent manner. The dietary regimen of vitamin E supplementation did not affect the general characteristics of meat, though meat from lambs supplemented during the whole fattening period had a light color and reduced the lipid oxidation at the end of storage. MAP prolonged a desirable color of meat during storage whereas air packaged induced lower color stability in meat during storage.

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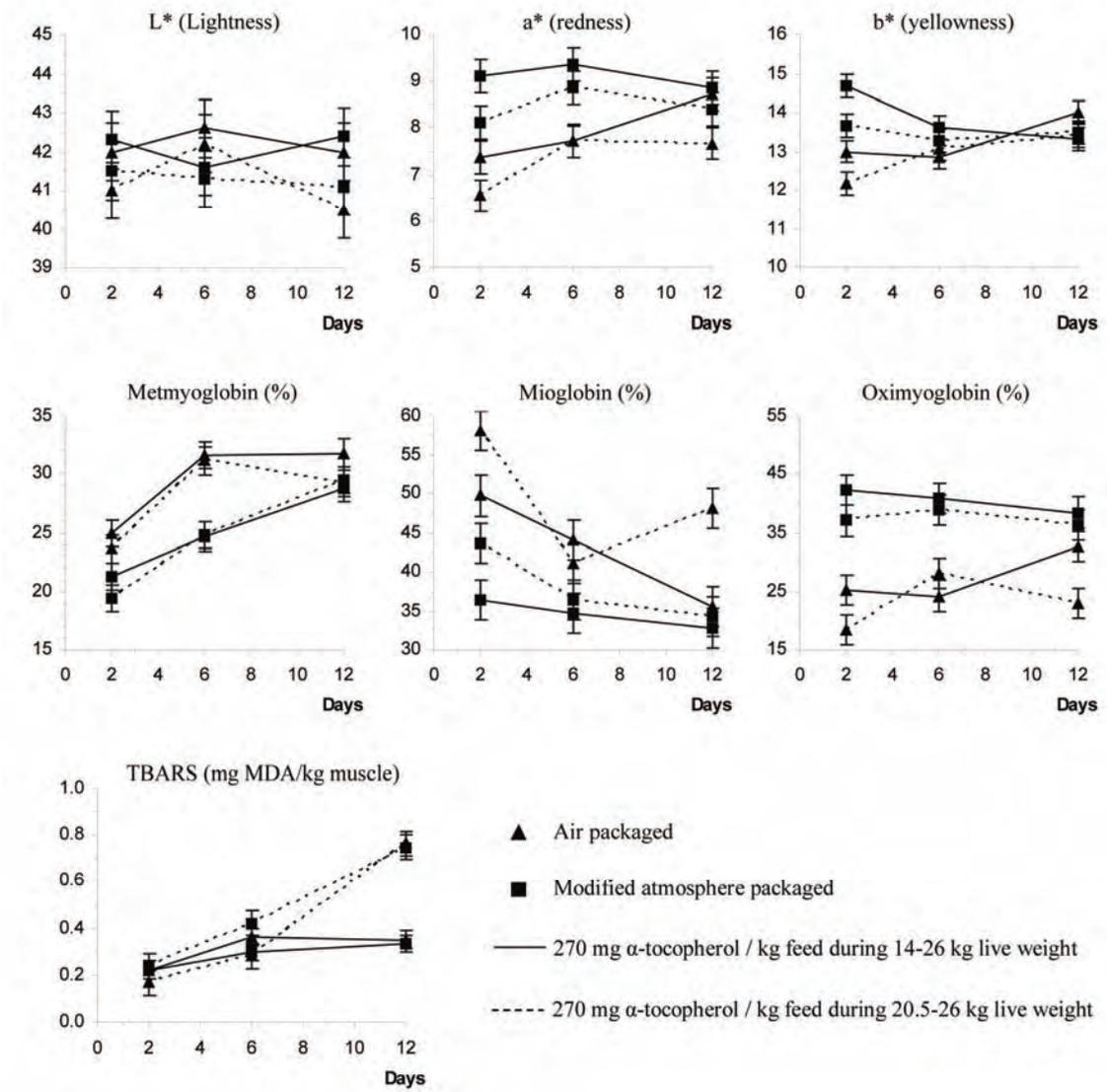
Tables and Figures

Table 1. Summary table of the significance of the main effects and their interactions for meat color parameters, pigment content and TBARS.

Variables	Main effects in the full Model						
	Dietary regimen (DR)	Type of packaging (TP)	Storage time (ST)	DRxTP	DRxST	TPxST	DRxTPxST
<i>Color</i>							
L*	*	NS	NS	NS	NS	NS	NS
a*	**	***	**	NS	NS	*	NS
b*	*	***	NS	NS	NS	***	NS
<i>Pigments (%)</i>							
Metmyoglobin	NS	***	***	NS	NS	**	NS
Myoglobin	**	***	***	NS	*	NS	NS
Oximyoglobin	**	***	NS	NS	NS	*	NS
TBARS							
(mg MDA/ kg muscle) ***	NS	***	NS	***	NS	NS	

NS: No significant; * P<0.05; ** P<0.01; *** P<0.001

Figure 1. Evolution of meat color parameters, pigment content, TBARS and meat discoloration for the dietary regimes and types of packaged during storage time (mean \pm S.E.)



SHELF LIFE OF DIFFERENT COLOUR BREAST TURKEY MEAT UNDER MODIFIED ATMOSPHERE PACKAGE

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Key Words: Poultry, MAP, Shelf life, colour, turkey

Introduction

The use of CO₂ enriched atmospheres extends the shelf life of raw poultry by inhibiting the psychrotrophic gram-negative bacteria and *Pseudomonas spp.* (Blakistone, 1999). The success of MAP technology application on meat depends mainly of its initial microbiological contamination (influenced by slaughtering and deboning hygienic conditions) and of temperature control during storage and distribution, however the growth of microorganisms is also influenced by intrinsic meat factors (Lucke *et al.*, 1995, Borch *et al.*, 1996, Dainty *et al.*, 1996).

An important part of turkey breast meat presents different characteristics of colour, pH and water holding capacity being classified as PSE-like meat (Barbut, 1996, Barbut, 1997, Santé *et al.*, 1998, Owens *et al.* 2000). Boulianne *et al.* (1998) also observed darker poultry meat with a lower **L** and a higher pH than normal meat. Fletcher *et al.* (2000) observed different colour characteristics on broiler breast meat, establishing three colour groups with different values of **L**, **a** and pH. Fraqueza *et al.* (2001) stated that 23% of the turkey breast muscles measured were evaluated as being too dark or too light. These colour categories of meat with different characteristics could be sliced and packaged under modified atmosphere with gas mixtures usually composed by CO₂ and N₂ being the products shelf life influenced by intrinsic characteristics associated with colour as the pH.

Objectives

The aim of this study was to determinate the evolution of spoilage flora in sliced turkey meat under modified atmosphere packaging 50%CO₂/50%N₂ along a storage period time at 0°C clarifying the relation between meat colour and respective shelf life.

Methodology

The sampling of breast muscles with different colour was performed on different days from turkey carcasses (BUT 9 and BIG 6) slaughtered and deboned under plant conditions. Colour (Minolta Colorimeter CR-300, Minolta, Osaka, Japan; using the L, a, b, co-ordinates, CIELab colour system), and pH 24h *post-mortem* (pH_{24h}, Sentix Sp, WTW, A991409014) were measured on the *Pectoralis major* muscles after carcasses

deboning. The breast muscles were selected according to Lightness (L) value and pH: $L \geq 51$ and $pH < 5.8$ for Lighter colour, $43 < L < 51$ for Intermediate colour, $L \leq 43$ and $pH > 5.8$ for Darker colour. Breast muscles of different colour categories were sliced. Less than one hour after transportation under refrigeration to the laboratory the sliced meat were individually packaged in an aerobic atmosphere (polystyrene trays wrapped in an oxygen permeable polyvinyl film) and on modified atmosphere (MAP) containing 50% N_2 and 50% CO_2 in "HBX-070" bags (a multilayer film EVOH-based) sealed with a packaging machine (EVT-7-CD, Tecnoprip, Barcelona). The aerobic atmosphere and MA packaged meat have been immediately stored ($0 \pm 1^\circ C$ in the dark) for 12 and 25 days respectively. At least five replicates were performed for each study condition.

On days 0, 5, 12, 19 and 25 the following Microbiological analyses were carried out: Total mesophilic aerobic counts (Plate Count Agar, Sharlau, Spain) at $30^\circ C$ for 2 days, total psychrotrophic aerobic counts (Plate Count Agar, Sharlau, Spain) at $7^\circ C$ for 10 days, anaerobic count at $7^\circ C$ for 10 days (Brewer Anaerobic Agar, Merck, Germany), *Enterobacteriaceae* counts in Violet Red Bile agar (VRB agar, Merck, Germany) at $37^\circ C$ for 2 days, *Pseudomonas spp.* counts (CFC agar base, Oxoid, UK) after incubation at $30^\circ C$ for 2 days, lactic acid bacteria (LAB) counts on Man Rogosa Sharpe Agar (Oxoid, UK) incubated at $30^\circ C$ for 3 days and *Brochothrix thermosphacta* count in streptomycin, actidione, thallos acetate agar (STAA, Oxoid, UK) incubated for 2 days at $30^\circ C$. Counts were expressed as log cfu/g.

Statistical analysis: Data was analysed using SPSS 11.5 for Windows. The comparison between different package condition and different colour quality meat samples, for microbial parameters, was performed by model adjustment of a one-way ANOVA for each day. If *F* test from ANOVA was significant, a LSD test post hoc multiple comparisons for observed Means has been performed. The comparison between days, considering each package and colour meat condition, was made by t-test for dependent samples.

Results & Discussion

The characteristics of pH and L from breast turkey samples selected and categorized as Lighter, Intermediate and Darker colour are presented on Table 1. Darker turkey meat is characterized by significantly ($p < 0.001$) lower lightness (L) values and higher pH values than Intermediate and Lighter colour meat.

The microbial flora evolution during storage of sliced turkey meat under MAP (50% CO_2 and 50% N_2) and aerobic package is represented on Figures 1 to 4.

The total mesophilic and psychrotrophic aerobic counts on turkey meat under MAP after five days of storage are not significantly different from those observed initially, the gas mixture have an inhibitory effect on microbial flora which present a longer lag phase related to the presence of CO_2 (Dixon *et al.*, 1989, Blakistone, 1999). The inhibitory effect of CO_2 is evident when we observe the significant reduction ($p < 0.001$) obtained on microbial counts of aerobic gram-negative flora in meat under MAP conditions (Figure 1 and 2) compared to those obtained in meat under aerobic package.

After the initial storage time at $0^\circ C$ was observed on darker meat colour under MAP a significant increase ($p < 0.05$) of total mesophilic counts (from 4.88 to 5.95 log cfu.g⁻¹) and total psychrotrophic aerobic counts (from 4.97 to 5.56 log cfu.g⁻¹) despite the

anaerobic conditions created and the inhibitory effect of CO₂. Between the different periods of analysis (12th, 19th and 25th days) the growth difference of mesophylic and psychrotrophic aerobic flora (Figure 1) was of 2 log cfu.g⁻¹ in Darker turkey meat and of 1 log for Lighter and Intermediate. After 25th days of storage a higher total mesophylic and psychrotrophic aerobic counts (p<0.05) of approximately 2 log ufc.g⁻¹ was registered in the darker meat compared the other colour categories of meat. After this storage time the difference of microbial population between meat colour categories could be not relevant because all are over the limits of hygienic acceptability (Figure 1) but the darker meat will have off-odors and slime sooner than the others meat colour categories.

Pseudomonas spp. and *Enterobacteriaceae* growth was inhibited during meat storage time under MAP 50% CO₂ and 50% N₂ (Figure 2).

After 12 days of storage the anaerobic psychrotrophic counts increased significantly (p<0.05) on sliced meat under MAP 50% CO₂ and 50% N₂ (Figure 3). Their growth is more effective in dark colour meat than on the others colour meat categories however this difference was not significant between colour meat categories. There is anaerobic facultative flora that seems to be not inhibited by CO₂ (Figure 3). The lactic acid bacteria counts was higher on Intermediate and Darker colour meat than on Lighter, respectively with 4.14, 4.03 and 3.06 log ufc.g⁻¹ after twelve days of storage under MAP (Figure 4). After this period of storage the LAB counts difference of 1 log disappears; the increase of counts related to storage time was not significantly different between colour meat categories (≈5 log cfu.g⁻¹).

Brochotrix thermosphacta counts was similar on different colour turkey meat categories under aerobic and MAP 50% CO₂ and 50% N₂ conditions (Figure 4) and increased significantly (p<0.05) during storage time. There is an inhibitory effect of the anaerobic conditions of this gas mixture on the growth of *Brochotrix thermosphacta* however the sensitivity of these microaerophylic bacteria to the absence of O₂ was more pronounced on lighter and intermediate meat colour.

The Darker turkey meat presented higher counts of psychrotrophic aerobic flora than the Lighter samples (p<0.05) after 12th day of storage in aerobic conditions at 0°C. This tendency was observed on the *Pseudomonas spp.* counts. The increased development of the flora on Dark poultry meat compared with that observed in Lighter colour samples is related to differences on pH and temperature which influence the development of aerobic psychrotrophic counts particularly *Enterobacteriaceae* and *Pseudomonas spp.* (Allen *et al.*, 1997 and 1998, Fraqueza *et al.*, 2002). When other hurdles are created as in MAP these previous referred bacterial groups are inhibited. The inhibitory effect of CO₂ is related with gram-negative bacteria particularly *Pseudomonas spp.* (Blakistone, 1999). The dissolution of CO₂ in meat water phase with carbonic acid formation change the internal pH of bacterial cells disturbing the biological systems equilibrium and inducing cell inactivation without interruption of their walls (Erkmen, 2000) also the aerobic flora without the presence of O₂ will be inhibited. Lactic acid bacteria are responsible by spoilage of turkey meat under MAP since this slower growing group is not inhibited by the anoxic conditions (Santé *et al.*, 1994). The pH associated with meat colour has no effect on their development. However other aerobic facultative psychrotrophic bacterias have grown on darker meat under MAP with 50% CO₂ and 50% N₂ when *Pseudomonas spp.* and *Enterobacteriaceae* were inhibited, being responsible by meat spoilage. Their development is promoted by intrinsic conditions related to darker meat with a pH value

equal or higher than 6 and its nutrients content. According to Boulianne *et al.* (1998) the darker meat is richer in iron. This nutrient is very important for non-siderophores species and rapidly utilized without energetic loss by siderophores species (Champomier *et al.* 1996, Gram *et al.*, 2002) promoting their growth.

The shelf life of sliced dark turkey meat under MAP with 50% CO₂ and 50% N₂ is shorter than the Lighter and Intermediate turkey meat. According to hygienic microbial standards (Unnamed, 1998), sliced turkey dark meat under MAP (50% CO₂ and 50% N₂) after 12 days of storage at 0°C, will be over the limit of quality hygienic standards and not recommended to be consumed. The microbial shelf life period of MAP sliced turkey meat is increased of one more week to Intermediate and Lighter colour turkey meat under this study conditions.

Conclusions

The shelf life of sliced Darker turkey meat under MAP (50% CO₂ and 50% N₂) at 0°C is one week shorter than the Lighter and Intermediate colour meat. This difference is attributed to intrinsic characteristics of Darker meat promoting the development of spoilage aerobic facultative psychrotrophic flora.

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Tables and Figures

Table 1: Characteristics of breast turkey samples (*Pectoralis major*) selected.

Breast samples	Lighter n=6	Intermediate n=6	Darker n=5	Sig.
pH	5.69±0.07 ^a	5.85±0.03 ^b	6.05±0.13 ^c	***
L	51.73±0.60 ^c	46.78±1.47 ^b	41.33±1.31 ^a	***

^{abc}Means within a row with different superscript letters are significantly different;
*** p<0,001

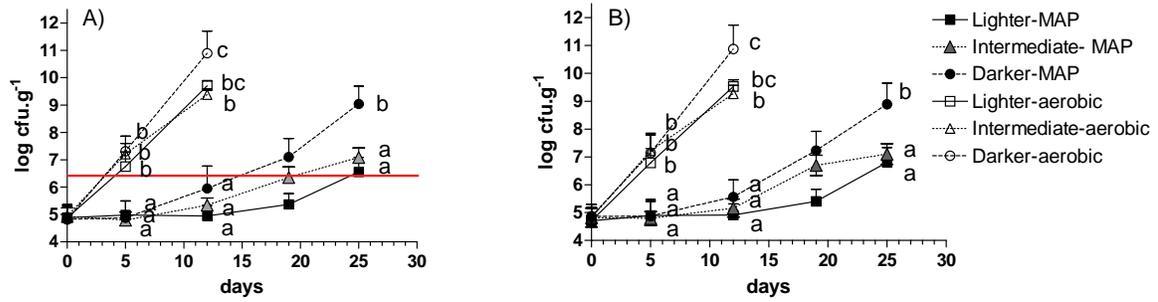


Figure 1: Total mesophylic (A) and psychrotrophic aerobic (B) counts evolution on Lighter, Intermediate and Darker colour sliced turkey meat during storage time (^{abc} different letter for the same day are significantly different).

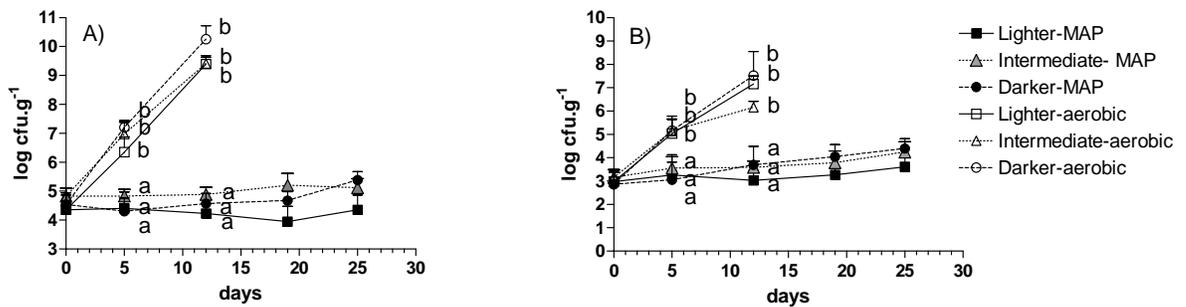


Figure 2: *Pseudomonas* spp. (A) and *Enterobacteriaceae* (B) evolution on Lighter, Intermediate and Darker colour sliced turkey meat during storage time (^{ab} different letter for the same day are significantly different).

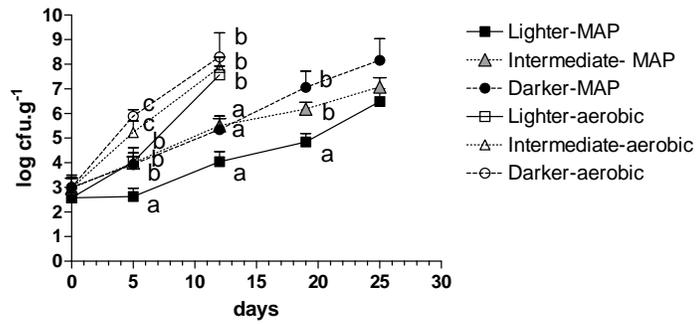


Figure 3: Total anaerobic flora evolution on Lighter, Intermediate and Darker colour sliced turkey meat during storage time (^{abc} different letter for the same day are significantly different).

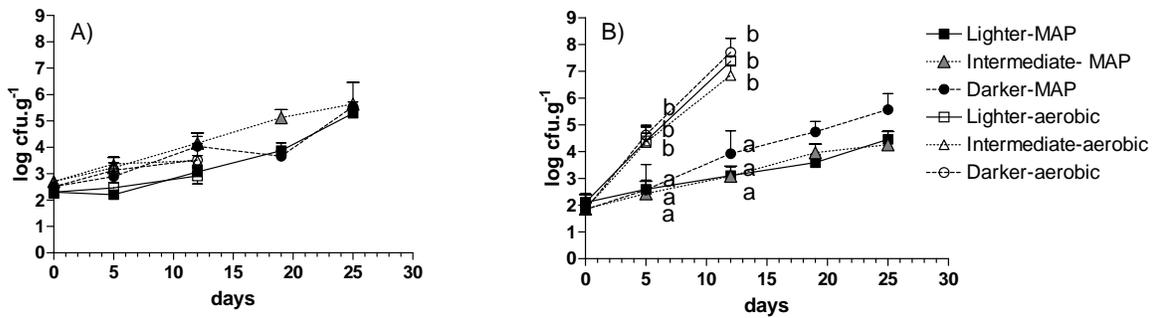


Figure 4: Lactic acid bacteria (A) and *Brochotrix thermosphacta* (B) evolution on Lighter, Intermediate and Darker colour sliced turkey meat during storage time (^{ab} different letter for the same day are significantly different).

EFFECT OF ULTIMATE PH AND PACKAGING ON MICROFLORA OF BEEF OF MARONESA BREED STORED AT 4°C

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Key Words: beef, packaging, spoilage microflora, ultimate pH

Introduction

Meat is a good support for bacterial growth because of its composition: 75% water and many different metabolites such as amino-acids, peptides, nucleotides and sugars (Labadie, 1999). Spoilage of meat is the result of the microbial activity of several microorganisms whose survival and growth is dependent on the characteristics of the product and the way it is processed and stored, affecting the qualitative and quantitative composition of the spoilage microflora (Huis in't Veld, 1996). The predominant bacteria associated with spoilage of refrigerated beef are *Brochothrix thermosphacta*, *Carnobacterium* spp., *Enterobacteriaceae*, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens* (Borch et al., 1996).

Among the parameters that affect the growth of microorganisms in meat, it is assumed that the ultimate pH and the gaseous composition of the packaging have an important role (Newton and Gill, 1978; Walker and Betts, 2000). The preservation of foods in modified atmosphere packaging (MAP) generally consists in the enclosure of the food product in gas-barrier materials, in which the gaseous environment as been changed (Gould, 1996) This procedure inhibits spoilage agents minimizing the loss of products and maintaining a higher quality in perishable food during its normal shelf-life or extending it. Nevertheless, MAP should be associated with strict temperature control to achieve maximum microbial inhibition (Tewari et al. 1999).

MAP generally uses three different gases: oxygen, carbon dioxide and nitrogen each with a specific function. Packages containing up to 80% oxygen and 20% carbon dioxide (CO₂) (high oxygen MA) will preserve fresh meat colour and prevent anaerobic spoilage but will have only a slightly increase in the shelf-life (Borch et al., 1996). Atmospheres with high concentrations of CO₂ are used to benefit from the bacteriostatic effect of this gas; nevertheless it is usually associated with rapid discoloration of meat surface causing sensorial rejection. Vacuum packaging prevents the growth of a few groups of spoilage microorganisms, due mainly to the low availability of oxygen (Church and Parsons, 1995).

Objectives

The aim of the present work was to determine the influence of different meat packaging: (air, vaccum and modified atmospheres with different gaseous compositions)

on spoilage microflora usually associated with spoilage of refrigerated beef (Total viable counts, Lactic Acid Bacteria, Enterobacteriaceae, Pseudomonas spp. and Brochothrix thermosphacta) and the influence of ultimate pH (Normal and High) of beef of maronesa breed on same microflora.

Methodology

M. longissimus thoracis et lumborum of bovine maronesa breed with high ultimate pH values (≥ 6.2 , $n=6$) and with normal pH values (< 5.8 , $n=6$) were cut from carcasses 24h post mortem and kept in cold storage. Muscles were cut into pieces weighing approximately 70g and packed in 5 different types of packaging namely: air (A); vacuum packaging (V); 70%O₂ + 20%CO₂ + N₂ (MAP70/20); 50%O₂ + 40%CO₂ + 10%N₂ (MAP50/40) and 30%O₂ + 60%CO₂ + 10%N₂ (MAP30/60).

Air: Meat cuts were tray-packaged in air overwrapped with polyethylen film.

Vacuum: Beef cuts were individually vacuum packaged in COMBITHERM bags (WIPAK Walsrode, HAFRI) which have an oxygen transmission rate of $63\text{cm}^3\text{ m}^{-2}\text{ d}^{-1}\text{ atm}^{-1}$ at 23°C, 0% r.h. and water vapor transmission (WVT) of $1\text{g m}^{-2}\text{ d}^{-1}$ at 23°C, 85% r.h, using a SAMMIC V-420 SGA.

Modified atmosphere packaging: Meat cuts were individually placed in COMBITHERM XX bags (WIPAK Walsrode, HAFRI) 0,115 mm thick and OTR of $1\text{cm}^3\text{ m}^{-2}\text{ d}^{-1}\text{ atm}^{-1}$ at 23°C, 0% r.h. and WVT of $1\text{g m}^{-2}\text{ d}^{-1}$ at 23°C, 85% r.h.. The atmosphere in the MA packages was first removed and then flushed with the appropriate gas mixture (Praxair, Portugal) using a SAMMIC V-420 SGA. The final gas to meat ratio was approximately 3:1.

Following packaging, meat samples were stored at $4\pm 1^\circ\text{C}$ and examined at intervals of 3, 7, 10, 14 and 21 days post mortem (the last not made for packaging A) for microbiological analysis. One extra sampling period (28 days) were made for vacuum and MAP30/60.

Microbiological analysis: Meat cuts were sampled aseptically at each interval. Samples were homogenized with tryptone salt (tryptone 0.3% and NaCl 0.85%) in a Stomacher for 90s. Serial decimal dilutions were prepared in the same solution for microbiological determinations.

Total aerobic counts were determined on Plate Count Agar (OXOID CM325) (30°C 3 days), Lactic acid bacteria (LAB) on double layer on MRS agar (OXOID CM361) (30°C 3 days).

Enterobacteriaceae, Brochothrix thermosphacta and Pseudomonas were determined basically according to ISO 5552(1997), ISO 13722 (1996) and NF V 04-504 (1998) on double layer VRBG (OXOID CM485) (37°C 24h), Brochothrix thermosphacta on STAA agar (CM881, SR151; OXOID) (25°C 2 days) and Pseudomonads counts on CFC agar (CM559, SR103; OXOID) (25°C 2 days), respectively.

Statistical analysis

The data were subjected to analysis of variance with GLM procedure in order to test the effect of pH, packaging and interactions for each day of storage using the Systat programme 10.2 (Systat Software Inc., 2002). Tukey test was used to locate differences between means at 5% level of probability.

Results & Discussion

Results of microbial analysis are presented in tables 1 and 2, according the packaging regime and the pH group respectively.

According to results presented in table 1, it is possible to observe that LAB are generally unaffected by the packaging, as indicated by the similar ($P \geq 0.05$) counting along all the period studied.

For the TVC, *B. thermosphacta*, Enterobacteriaceae and Pseudomonas, the same pattern was generally observed. Thus, the air packaging presents always higher counts than any other packages. The effect of the composition of MAP observed was not as intense as it was expected, namely regarding the similar counts observed in high CO₂ packaging for different spoilage groups. The type of packaging has influenced the growth of BT, with significant differences ($P < 0.001$) in day 7 and day 14, and significant differences ($P < 0.05$) in 10 day post mortem. The higher counts values were obtained in air (6.60 log ufc cm⁻²) at day 14 post mortem and in MAP70/20 (6.27 log ufc cm⁻²) and in MAP50/40 (6.07 log ufc cm⁻²) both at day 21 post mortem. These results are in accordance with the resistance of this bacterium to high concentrations of CO₂ (Newton and Rigg, 1979). The effect of higher CO₂ concentration was noted also in the counting of Pseudomonas at 7, 10 and 21, days, resulting in lower counting. Considering the aerobic character of Pseudomonas it was expected a stronger effect of packaging.

Among all microorganisms, Enterobacteriaceae presented the lowest level of growth in all storage times and type of packaging, being the higher value 3.81 log cfu cm⁻² in vacuum at day 28.

Generally, it was observed higher counts – significantly different - of all the groups of microorganisms screened in meat presenting a higher ultimate pH (table 2), except for LAB. That difference is usually pointed by several authors based on the shorter lag phase of spoilage bacteria in those meats. Additionally, the implications that overgrowth have in the sensorial spoilage perception is due also to the smallest amount of available glucose that the meat with high pH presents, inducing bacteria to a rapid shift in metabolism to the use of amino acids. The exception observed in LAB is related to its known tolerance to low pH (Gill, 1983; Walker and Betts, 2000). As observed for the effect of packaging, it was also in the group of BT and Pseudomonas that this effect was more evident, particularly after 7 days pm.

Conclusions

According to the results of the present work it is possible to conclude that the packaging strategy used have implications on the spoilage microflora. The main differences were observed between air, vacuum and the other MAPs. Differences between MAPs were found for the counts of Pseudomonas and Brochothrix thermosphacta. The effect of increasing carbon dioxide concentration on the packaging was not evident on the growth of LAB, Enterobacteriaceae and TVC.

The effect of the ultimate pH of the meat was evident for almost all spoilage groups studied, except for LAB. Higher pH was associated to higher counts.

The packaging more suitable for each type of meat, considering the ultimate pH, should be established, from the microbiological data, as those obtained with this work, but also from the evaluation of the sensory consequences of its growth.

Acknowledgment

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Tables and Figures

Table 1. Microflora (log cfu/g, means (standard deviation) isolated from beef of different type of packaging.

Microflora	Packaging	Storage time (days)						
		1	3	7	10	14	21	28
Lactic Acid Bacteria	Air	1.05 (1.16)	0.95 (0.90)	2.71 (1.86)	4.05 (2.35)	4.75 (1.64)	nd ²	nd
	Vacuum	«	0.79 (1.06)	1.58 (1.83)	2.98 (1.71)	4.16 (2.05)	4.77 (2.04)	5.37 (2.30)
	MAP _{70/20}	«	1.11 (1.10)	1.62 (1.41)	2.95 (2.10)	4.37 (1.89)	4.87 (1.98)	nd
	MAP _{50/40}	«	1.14 (1.21)	1.58 (1.18)	2.64 (2.05)	3.42 (2.15)	5.07 (1.27)	nd
	MAP _{30/60}	«	1.29 (0.98)	1.59 (1.14)	2.99 (1.68)	3.62 (2.18)	5.21 (1.72)	5.33 (2.24)
	sig ¹			ns	ns	ns	ns	ns
<i>Brochothrix thermosphacta</i>	Air	0.35 (1.21)	1.13 (1.69)	4.29 a ³ (2.12)	5.31 a (2.56)	6.60 a (1.14)	nd	nd
	Vacuum	«	0.19 (0.66)	2.18 b (1.94)	3.40 ab (2.30)	5.52 ab (1.22)	5.99 81.43)	5.78 (1.79)
	MAP _{70/20}	«	0.55 (1.00)	2.07 b (1.97)	3.57 ab (2.10)	5.07 ab (1.97)	6.27 (2.49)	nd
	MAP _{50/40}	«	0.47 (1.12)	1.18 b (1.82)	2.69 b (2.31)	4.25 bc (2.03)	6.07 (1.62)	nd
	MAP _{30/60}	«	0.22 (0.78)	0.69 b (1.38)	2.08 b (2.41)	2.97 c (2.27)	5.31 (2.42)	5.29 (2.26)
	sig			ns	***	*	***	ns
Enterobacteriaceae	Air	0.12 (0.43)	0.48 (0.91)	1.50 a (1.47)	2.52 a (2.03)	3.78 a (2.17)	nd	nd
	Vacuum	«	0.46 (0.94)	0.45 b (1.04)	1.28 ab (1.99)	2.99 ab (2.39)	3.45 a (2.67)	3.81 a (2.95)
	MAP _{70/20}	«	0.00 (0.00)	0.08 b (0.29)	0.67 b (1.00)	1.09 bc (1.77)	2.07 ab (2.09)	nd
	MAP _{50/40}	«	0.16 (0.55)	0.11 b(0.38)	0.15 b (0.51)	0.56 b (1.34)	1.33 b (1.62)	nd
	MAP _{30/60}	«	0.45 (0.68)	0.55 ab(0.74)	0.62 b (1.68)	1.03 bc.(1.31)	1.31 b (1.28)	0.75 b (1.37)
	sig			ns	**	**	***	*
Total viable counts	Air	2.52 (0.60)	2.95 (0.80)	6.54 a (1.09)	8.21 a (0.99)	8.79 a (0.63)	nd	nd
	Vacuum	«	2.48 (0.70)	3.41 b (1.29)	4.64 b (1.25)	6.14 b (1.23)	6.69 (1.33)	7.22 (1.11)
	MAP _{70/20}	«	2.50 (0.61)	3.21 b (0.96)	4.25 b (1.39)	5.81 bc (1.47)	7.38 (1.24)	nd
	MAP _{50/40}	«	2.77 (0.48)	3.11 b (1.10)	3.91 b (1.45)	4.88 bc (1.48)	6.28 (1.72)	nd
	MAP _{30/60}	«	2.56 (0.49)	2.86 b (0.83)	3.67 b (1.43)	4.76 c (1.75)	6.32 (2.01)	6.21 (1.62)
	sig			ns	***	***	***	ns
<i>Pseudomonas</i> spp.	Air	1.33 (1.48)	2.28 a (1.56)	6.85 a (1.01)	7.93 a (0.96)	8.70 a (0.75)	nd	nd
	Vacuum	«	1.08 ab (1.24)	2.99 b (1.64)	4.40 b (1.26)	5.08 b (1.11)	5.45 ab (0.83)	5.69 a (0.88)
	MAP _{70/20}	«	1.09 ab (1.04)	2.29 b (1.14)	3.22 bc(1.37)	4.41 b (1.63)	6.41 a (1.33)	nd
	MAP _{50/40}	«	0.92 b (1.13)	2.04 b (1.42)	2.30 c (1.40)	2.73 c (1.56)	4.26 bc (1.64)	nd
	MAP _{30/60}	«	1.10 ab (1.18)	1.57 b (1.14)	1.79 c (1.53)	3.09 c (1.60)	3.09 c (1.60)	3.17 b (1.64)
	sig			*	***	***	***	***

¹Significance: ns - not significantly different ($P \geq 0.05$); * - significantly different ($P < 0.05$); ** - very significantly different ($P < 0.01$); *** - high significantly different ($P < 0.001$).

²nd – not determined.

³In the same row means without common letters are significantly different ($P < 0.05$).

Table 2. Microflora (log cfu/g, means (standard deviation) isolated from beef of the two pH groups (Normal pH<5.8 and High ultimate pH ≥6,2)

Microflora	pH group	Storage time (days)						
		1	3	7	10	14	21	28
Lactic Acid Bacteria	Normal	0.94 (0.99)	1.03 (0.99)	1.53 (1.37)	2.60 (1.83)	4.36 (1.08)	4.70 (0.96)	5.79 (1.00)
	High sig ¹	1.15 (1.24)	1.08 (10.9)	2.10 (1.65)	3.64 (2.03)	3.77 (2.58)	5.26 (2.44)	4.92 (2.98)
<i>Brochothrix thermosphacta</i>	Normal	0.00 (0.00)	0.26 (0.79)	1.31 (1.97)	2.68 (2.52)	4.08 a (2.20)	5.08 (2.18)	4.96 (2.00)
	High sig	0.70 (1.59)	0.77 (1.34)	2.86 (2.14)	4.14 (2.32)	5.68 b (1.71)	6.74 (1.45)	6.11 (1.92)
Enterobacteriaceae	Normal	0.00 (0.00)	0.19 (0.51)	0.34 (0.70)	0.74 (1.26)	1.13 (1.67)	1.33 (1.73)	1.54 (2.21)
	High sig	0.25 (0.56)	0.43 (0.86)	0.73 (1.23)	1.36 (1.83)	2.65 (2.41)	2.75 (2.45)	3.01 (3.10)
<i>Pseudomonas</i> spp.	Normal	0.48 (0.67)	0.67 (0.98)	2.80 (2.11)	3.50 (2.56)	3.93 (2.75)	4.38 (1.68)	4.14 (1.77)
	High sig	1.91 (1.66)	1.91 (1.30)	3.50 (2.44)	4.36 (2.51)	5.32 (2.44)	5.23 (1.94)	4.72 (1.90)
Total viable counts	Normal	2.44 (0.38)	2.57 (0.51)	3.38 (1.49)	4.29 (2.11)	5.37 a (1.94)	6.04 (1.48)	6.48 (1.27)
	High sig	2.60 (0.72)	2.74 (0.74)	4.27 (1.85)	5.58 (1.93)	6.79 b (1.77)	7.29 (1.53)	6.95 (1.64)

¹Significance: ns - not significantly different ($P \geq 0.05$); * - significantly different ($P < 0.05$); ** - very significantly different ($P < 0.01$); *** - high significantly different ($P < 0.001$).

EFFECT OF RETAIL-PACKAGING METHODS ON PREMATURE BROWNING OF COOKED BEEF PATTIES

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Key Words: Ground beef, Premature browning, Retail packaging, Color, Meat juice.

Introduction

Consumers are advised to use the color of internal meat or of meat juice to determine when cooked beef patties are well done. However, several experiments have shown that meat packed in a high concentration of oxygen develops premature browning (PMB), which means that the meat appears well done at lower temperatures than expected. The proportion of ground beef being packed centrally in modified atmosphere with a high concentration of oxygen is increasing, which causes a risk of PMB. This implies that the color of the cooked meat is no longer a reliable indicator for when the meat is well done and when the pathogens, if any, are killed. Furthermore, some Danish consumers have a preference for 1.5-2.5 cm thick patties with a pink internal color where the meat juiciness is high. Persuing only to achieve a pink cooked color irrespective of the achieved centre temperature can mislead the consumers.

Objective

To examine the effect of the oxygen level in retail packaging systems of ground beef on the development of premature browning of meat and meat juice during cooking of beef patties.

Methodology

Loosely ground beef (8-10% fat) was portioned into 250 g retail packages and packed with three different headspace oxygen concentrations (0%, 20% and 80%). The meat was modified atmosphere (MA) packed (tray-sealed) in high oxygen packaging (80% O₂/20% CO₂) at a Danish commercial meat packaging plant. Furthermore, trays were packed in oxygen permeable wrap film (20% O₂/80% N₂) and anaerobic Mapackaging using low oxygen permeable bags (OTR:40-45cm²/m²/24h/23°C/85% RH,300x400PA/PE20/70, Walten-hofen, Germany) filled with 100% nitrogen (N₂) and sealed using a Multivac A300/16 packing machine (100 mbar vacuum, 750 mbar filling).

The meat packages were subsequently stored at 2°C for 24 hours (20% O₂), 2 days (0% O₂) and 3 days (80% O₂). Beef patties weighing 125g were formed in a template (diameter = 9.7 cm, height = 1.5-1.7 cm) and equilibrated after storage at room temperature to approx. 15°C. Four patties were cooked in a pre-heated frying pan (180°C) and were turned every 3 minutes until internal temperatures of 55°C, 65°C, 71°C

and 75°C, respectively, was reached. Time and temperature data were registered (logging every 15 sec.) during and after heating using a Grant-1205 time and temperature logger.

Two color charts were developed from standardized pictures of the internal color of beef patties and meat juice. The charts represent the variation within different packaging methods and the centre temperature. On the basis of the color charts, two 5-point color gamuts were designed: one for meat juice color (figure 1) and one for meat color (figure 2). They represent the development in color from raw meat to cooked meat, which is well done. After cooking the internal color of one cross-sectioned patty were evaluated by a trained expert using the gamut. Meat juice was obtained from three patties by a pipette after a conic section in the centre of the patty. The juice was then centrifuged at 1300 rpm for 2 min. and chilled in a water bath. Five drops on a white filter paper were used for immediate evaluation of meat juice color by the expert using the gamut in figure 2.

Results & Discussion

When the temperature increases, the color of the meat juice changes from dark red, through to pink ending up in a yellow/clear color (figure 3), irrespective of the oxygen concentration in the packages. The difference between the packages is the temperature at which the color change (from reddish shades to a clear shade) occurs. The temperature-point is very much dependent on the headspace oxygen concentration in the packaged. For packages with 0% oxygen (N₂), the meat juice remained faint pink all the way to 75°C and never cleared. When the meat was packed in atmospheric air (wrap - 20% oxygen), the meat juice was still red at 65°C and the denaturation of the pigment including clearing of the meat juice took place at around 71°C. In headspace containing even higher oxygen concentrations (80% oxygen) the meat juice cleared already between 55-65°C.

For meat color, the same tendency is seen in the development of well-done appearance. Increasing centre temperature scores are related to well-done appearance. When packing in 0% oxygen (N₂), the color remained faint rose-pink all the way to 75°C. Whereas packing in wrap resulted in a faint rose-pink at 65°C and had a well-done appearance at 71°C. If the meat was packed in 80% oxygen, a well-done appearance was obtained already at 55°C, whereas the meat juice cleared at 65°C. Overall, the higher the oxygen concentration in the headspace of the pack, the greater the tendency for the ground meat to develop a well-done appearance at lower temperatures. The early color changes can be explained by the phenomenon of PMB. PMB is defined as meat having a well-done appearance at lower temperatures than expected (Hauge et al., 1994).

Premature browning occurs as the pigment heat-denaturates, which is related to the oxidative state of the pigment (Warren et al., 1996). In anaerobic environments the pigment (myoglobin) is present as deoxymyoglobin and in oxygen containing environments as oxymyoglobin, the oxidized form metmyoglobin is present at very low oxygen concentrations at 0,5-1% (Ledward, 1970). Deoxymyoglobin is the most heat stable form of the pigment, and blooms when heated to approx. 68-69°C. Both oxymyoglobin and metmyoglobin are relatively more heat labile and obtain a brown and well-done appearance at a centre temperature of around 55°C, and even more distinct at around 65°C (Hunt et al., 1999).

If ground beef is cooked on a grill, in a frying pan or in an oven, calculations by Jacobsen (2004) shows that a heat treatment to reach 65°C in the centre is sufficient to obtain microbiologically safe cooked ground beefs. These calculations are based on time/temperature studies, and z-values for *Salmonella*, *L. monocytogenes* and *E. coli*.

Conclusions

Because of premature browning, ground beef packed in a high oxygen concentration obtains a well-done appearance already at 55°C. Beef patties produced from traditional wrap-packed ground beef (20% oxygen) obtains the same well-done appearance around 71°C.

Hence, from a safety point of view present recommendations - to cook ground beef until the meat juice clears, or the meat is no longer pink - are insufficient if microbiological food safety is to be considered. However, from an eating quality point of view cooking to 75°C (Danish recommendations) or 71.1°C (US recommendations) seems unnecessary since cooking to 65°C centre temperature ensures a sufficient killing effect to eliminate pathogenic bacteria. At the same time cooking to 65°C will result in a more juicy beef patty.

Perspective

New public recommendations must be applied for safe cooking of ground beef with emphasis on cooking time and/or centre temperature. The thickness of the patty and the temperature of the frying pan should also be considered.

To ensure that a beef patty is both safe and tasty, the recommendation could be as follows: Ground beef should be cooked to a centre temperature of 65°C at a high temperature (180°C-200°C). For normal sized patties (125g/1.5-1.7 cm thick) the estimated cooking time is 13-15 minutes and for thick patties (175g/2.3-2.5 cm) the estimated cooking time is 17-20 minutes.

It may be possible to develop a mathematical model for calculating the cooking time dependent on specific conditions like beef thickness, meat temperature and temperature of frying pans, fats, and the required final centre temperature. The developed model should be customized for consumer use and available on the Internet for easy consumer access.

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Tables and Figures

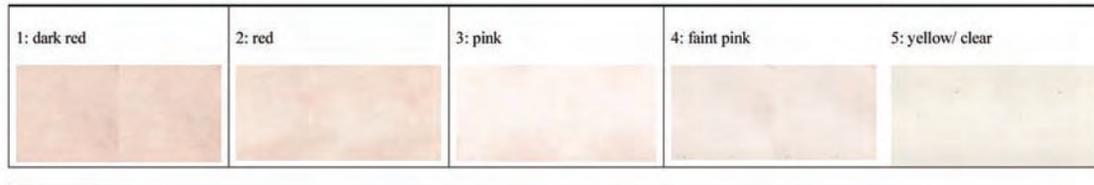


Figure 1. Five-point gamut for evaluation of meat juice color

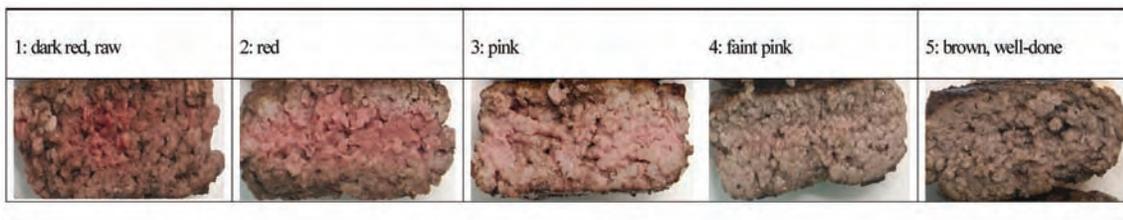


Figure 2. Five-point gamut for evaluation of internal meat color

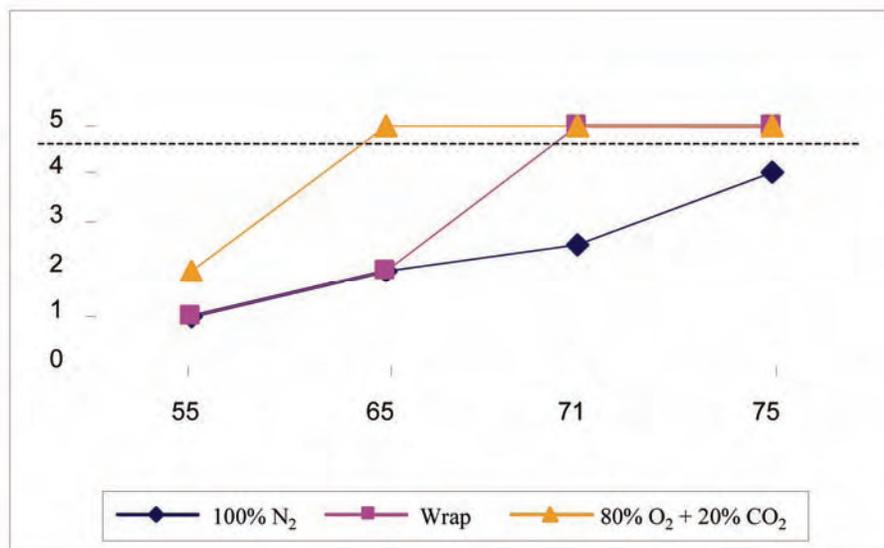


Figure 3. Meat juice color of beef patties for different packaging methods and end point cooking temperatures evaluated using a 5-point color gamut; 1= dark red, 2= red, 3= pink, 4 = faint pink, 5 = yellow/clear. The broken line indicates the point of change when the meat juice clears (n=4).

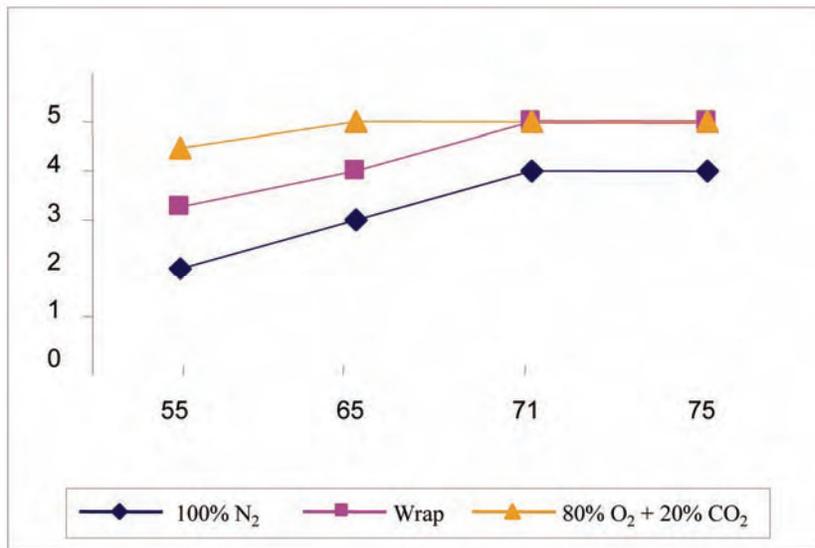


Figure 4. Meat color of beef patties for different packaging methods and end point cooking temperatures evaluated using a 5-point color gamut; 1= dark red, 2= red, 3= pink, 4 = faint pink, 5 = brown. The broken line indicates the point of change when the meat reaches a well-done appearance (n=4).

SENSORY EVALUATION OF GROUND BEEF STORED IN DIFFERENT ATMOSPHERES

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Introduction

It is well established that sensory properties of beef loin steaks such as tenderness and juiciness decrease during storage in a modified atmosphere pack (MAP) containing high oxygen and that a rancid/oxidized/warmed over flavour (WOF) may develop (Seideman et al., 1979; Tørngren, 2003; Clausen, 2004; Sørheim et al., 2004). In contrast, the effect of MA packing on the sensory properties of ground beef has not been documented to the same extent (Jayasingh et al., 2002). Case ready meat packaging is a fast growing segment (Zilbermann, 2003), and when choosing a packing technology it is essential to ensure that it will maintain the eating quality of the meat during storage. MAP with high O₂ also increases the amount of oxymyoglobin, which gives rise to a well-done appearance at temperatures much lower than expected (Hunt et al., 1999). This might lead to an increase in food born illness if people use colour of meat as indicator of meat temperature, as pointed out by Tørngren & Madsen (2005).

An alternative to high O₂ MAP is CO₂, N₂ and CO alone or in combination. CO₂ is added to the gas mixture because of its antimicrobial properties (Jakobsen & Bertelsen, 2002) and thereby increases the shelf life. But CO₂ has also shown adverse effect on beefsteak quality. Increasing the CO₂ level to more than 20% will increase the development of pores and fissures after cooking caused by rapid release of CO₂ from the meat (Bruce et al., 1996; Penny, 1999; Kerry & Ledward, 2002; Sørheim et al. 2004). Increasing the amount of CO₂ may also lead to snug down (low pressure) because the CO₂ is absorbed by the meat. N₂ is an inert gas, but beef will appear purple (deoxymyoglobin (DMb)), if O₂ is excluded from the gas mixture, or brown (metmyoglobin (Mb)), if a low concentration of O₂ (½-1%) is left in the gas mixture. Low concentrations of CO (0.4%) will give the meat the desirable shiny red colour (oxymyoglobin (OMb)) corresponding to meat exposed to O₂. However, it is not allowed to use CO in the EU at the moment, but since it has been allowed in the USA, perhaps it may be an option in the future. 0.3-0.5% CO in a gas mixture is estimated not to constitute any health risk (European Commission, 2001). However, little is known about the sensory quality of meat stored in MAP containing CO.

Objectives

The purpose of the present study was to examine sensory quality of ground beef stored in five different atmospheres and cooked as patties.

Methodology

Source of meat: 30 x 500 g ground beef packs (ground twice through 6.0 and 2.5 mm holes, 10% fat), was sampled in a random standardized manner from a commercial packing plant. Six packs (replicates) were assigned to each of the following MA combinations at a commercial plant.

MA-combination	Abbreviation
25% CO ₂ / 75% O ₂	High O ₂
60% CO ₂ / 40% N ₂	High CO ₂
60% CO ₂ / 39,6% N ₂ / 0,4% CO	High CO ₂ + CO
30% CO ₂ / 70% N ₂	Low CO ₂
100% N ₂	Nitrogen

Packaging: Tray (13 x 18 x 6 cm), (O₂ permeability: 15 cm³/m²/d, bar) covered with transparent film, (O₂ permeability: 0.5 cm³/m²/d, bar), Cryovac. Sealed Air Corporation, top sealed (Mondini). The storage temperature was 2°C. All packages were kept in light surroundings (800-900 lux) for 2 days prior to the analysis.

Atmosphere content was measured at the time of packing in extra pack and prior to sensory analysis of all packs (Check Mate 9900, BPI Dansensor). Batches 1, 2 and 3 were stored for 7 days and batches 4, 5 and 6 for 8 days before sensory analysis.

Cooking and sensory evaluation: After storage the meat was equalized at room temperature (approx. 20°C) to an internal temperature of max. 15°C. 500 g minced meat from each pack were shaped into 5 patties using a template (thickness: 1.5 cm: diameter: 9.5 cm). The patties were fried on a preheated frying pan (155°C), turned every 2 minutes until an internal temperature of 76 ± 1°C was reached. The patties were halved and evaluated by 8 trained assessors using a 15-point non-structured line (where 0 = slight and 15 = intense). The attributes assessed were WOF, meat flavour, sour flavour, juiciness, spongy texture, firmness, gumminess, crumbling and doneness (internal colour). Cooking loss (%) was recorded by weighing 5 patties before and after cooking.

Statistics: Data were used for an analysis of variance model (mixed procedure, SAS version 8.2). The fixed effect in the model was the main effect.

Sensory data: $Y_{ijk} = \mu + \text{packing gas } i \text{ (fixed)} + \text{batch } j \text{ (random)} + \text{assessors } k \text{ (random)} + \text{packing gas} * \text{assessors } ik \text{ (random)} + \text{packing gas} * \text{batch } ij \text{ (random)} + x \text{ batch} * \text{assessors } jk \text{ (random)} + e_{ijk}$

Cooking loss: $Y_{ijk} = \mu + \text{packing gas } i \text{ (fixed)} + \text{batch } j \text{ (random)} + \text{packing gas} * \text{batch } ij \text{ (random)} + e_{ij}$

Results & Discussion

Results of the sensory evaluations are shown in Table 1.

Flavour. Patties prepared from meat stored in high O₂ for 7 to 8 days scored high for WOF (7.4 points) whereas meat stored without O₂ scored between 2.6-3.5 points (P<0.01). WOF is normally related to cooked, stored and reheated meat and is mainly caused by oxidation of fatty acid (Konopka and Grosch, 1991). Other investigations have also shown an increase in WOF or in TBARS during storage of raw meat in high O₂ (Jacobsen & Bertelsen, 2000; Tørngren, 2003; Clausen, 2004; John et al., 2004; Sørheim

et al., 2004; Seyfert et al., 2005). In this study only three of the trained assessors could identify a substantial difference in WOF in meat stored with or without O₂ (a difference larger than 6 points). Two assessors identified a minor difference (approx. 1 point) and three assessors found no difference. We therefore assume that not all people are sensitive to WOF.

Meat stored in 100% N₂ had the lowest score for intensity of meat flavour (4.9 points) and meat stored with CO₂ had the highest score (6.1-6.5 points)(P<0.05). In between lies meat stored in high O₂ with a score of 5.7 points. This is surprising since other investigations have shown that beefsteaks stored without O₂ scored higher in meat flavour than meat stored in high O₂ (Tørngren, 2003; Clausen, 2004). The explanation is probably a pronounced microbiological growth in packs with 100% N₂. This assumption is supported by a high score for sour/acidic flavour in meat packed in 100% N₂.

Juiciness: The meat stored in high O₂ or high CO₂ had the lowest score in juiciness (6.1–6.4 points) and meat stored in 100% N₂ or 30% CO₂/70% N₂ scored highest (7.0-7.2 points)(P<0.05). Clausen (2004), Sørheim (2005) and Jayasingh et al. (2002) showed that meat stored in 80% O₂/20% CO₂ had a reduced juiciness compared to meat stored without high O₂ and CO₂. Tørngren (2003) found that storage 16 days storage in 50% CO₂/50% N₂ did not result in a reduced juiciness compared to storage in a vacuum packaging. More research is required to elucidate whether it is O₂ only that is the cause of the decreasing juiciness or whether it is a combination of both O₂ and CO₂.

Texture: No significant differences were found in the attributes spongy texture, firmness, gumminess and crumbling. Using a statistic design with all effects fixed, the spongy texture was significantly higher in meat stored in 100% N₂. Jayasingh et al. (2002) found minced meat stored in 80% O₂/20% CO₂ to be less tender than fresh minced meat. Other studies have shown that beefsteaks become less tender during storage in high O₂ MAP (Tørngren, 2003; Clausen, 2004; Sørheim, 2004).

Internal colour: The assessors found significant differences in internal colour of the cooked meat (P<0.001). Meat that had been stored in high O₂ appeared most well done (11.8 points) followed by meat stored without O₂ and CO (10.9-11.3 points) and least well done was meat stored in 0.4% CO (7.3 points). Hunt et al. (1999) have demonstrated that OMB looks well done at 55°C and DMb looks well done at 75°C. Anyway, at 75°C patties with DMb had a significantly lower visual score (less brown) than the patties with OMB. John et al. (2004) showed that patties stored in 0.4% CO and cooked to 79°C remained somewhat red even at the internal temperature of 79°C. Packaging ground beef in MA containing CO could lead the consumer to cook the patties to a higher internal temperature than usual to obtain a well done colour. This will lead to higher cooking loss and less juicy meat (Martens et al., 1982). Similarly Tørngren & Madsen (2005) have demonstrated that different packaging methods with more or less O₂ largely influence the cooked appearance of ground beef and that neither internal colour nor colour of meat juice can be used as indicators for safe cooking. Thus measurement of the core temperature of ground beef patties is necessary to obtain a safe cooking procedure, which at the same time ensures the optimal eating quality.

Cooking loss: The mean cooking loss varied from 28.7-29.7% between the different storage atmospheres, and there were no significant differences between the different packaging methods. Sørheim et al. (2004) found that minced beef that had been stored in 50-80% CO₂/20-50% N₂ lost more juice during cooking than meat stored in 100% N₂ or

vacuum packed. However, Bentley et al. (1989) did not find any difference in cooking loss when stored in 100% CO₂ or 100% N₂.

Gas composition after storing: The gas composition in the packages was measured after 7 to 8 days storage (Table 2). At the time of packing approx. ½% O₂ was left in the package, but after 7 to 8 days of storage the amount of O₂ was reduced to zero probably due to microbiological growth. CO₂ had decreased during storage due to absorption in the meat.

Conclusions

Patties of ground beef stored for 7 to 8 days in MA containing high O₂ (80% O₂/20% CO₂) scored higher points for WOF, were less juicy and looked more well done compared to meat stored in 100% N₂. Patties of ground beef stored in high CO₂ were less juicy than meat stored in low CO₂. Patties of ground beef stored in CO looked less well done compared to meat stored in MA with or without O₂. Otherwise meat from the CO packs did not differ significantly with respect to sensory attributes from packs without CO. Patties of ground beef stored in 100% N₂ scored higher points for sour flavour and less for meat flavour compared to meat stored in other types of MA packaging. The explanation is probably pronounced microbiological growth. In conclusion, this study showed that packaging with high concentrations of O₂ also has a negative impact on the eating quality of cooked ground beef.

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Tables and Figures

Table 1. Mean (incl. min. and max.) sensory score (8 trained assessors using a non-structured line scale, anchored to the extremes; 0= slight, 15=intense) of beef patties prepared from ground beef meat stored in different MAP (n=6).

Modified Atmosphere	Warmed over flavour	Meat Flavour	Sour/acid Flavour	Juiciness	Internal Colour	Doneness	Gumminess
25%CO ₂ /75%O ₂	7,4 _a 6,8-8,0	5,7 _{ab} 4,9-6,5	0,6 _c 0-0,9	6,1 _c 4,2-5,3	11,8 _a 11,3-12,2		3,1 1,7-4,4
60%CO ₂ /40%N ₂	2,6 _b 1,2-3,3	6,5 _a 5,4-7,7	1,8 _{bc} 0,5-3,2	6,4 _{bc} 5,4-7,1	11,3 _b 10,7-11,9		3,7 1,9-5,8
60%CO ₂ /40%N ₂ /0,4% CO	2,6 _b 1,2-4,5	6,2 _a 5,3-7,4	2,7 _b 1,4-4,1	6,4 _{bc} 6,0-7,0	7,3 _c 6,9-7,7		3,1 2,0-5,6
30%CO ₂ /70%N ₂	2,9 _b 2,1-4,2	6,1 _a 4,8-7,7	3,7 _b 1,5-6,2	7,2 _a 6,8-7,7	11,1 _b 10,8-11,3		3,1 2,4-4,2
100% N ₂	3,5 _b 2,8-4,0	4,9 _b 3,8-5,9	5,8 _a 3,9-9,5	7,0 _{ab} 6,6-7,3	10,9 _b 10,4-11,4		4,2 3,4-4,7
Significance	**	**	***	*	***		ns

*: P<0.05 **: P<0,01 ***: P<0,001 ns: not significant Within columns, means with different letters differ (P<0.05)

Table 2. Mean (incl. min. and max.) O₂ and CO₂ content after 7 to 8 days MAP

Modified Atmosphere	O ₂ %	CO ₂
25% CO ₂ / 75%O ₂	67,7 62,9-72,4	23,5 21,5-26,6
60% CO ₂ / 40% N ₂	0,0 0,0-0,4	39,9 34,8-43,0
60% CO ₂ / 40% N ₂ / 0,4% CO	0,0 0,0-0,0	40,3 34,4-43,2
30% CO ₂ / 70% N ₂	0,0 0,0-0,0	22,8 21,5-23,9
100% N ₂	0,0 0,0-0,0	11,5 10,2-14,0

EFFECT OF DISPLAY CONDITIONS AND ASCORBIC ACID ON COLOR, LIPID OXIDATION AND SENSORY PROPERTIES OF FRESH PORK LOIN CHOP

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Key Words: pork loin chop, fresh meat, color, lipid oxidation, lighting, display

Introduction

The key to retail meat display is to present pork products in an attractive and saleable format. Effective sales depend on fresh appearance, acceptable product quality with an absence of abnormal traits including an unattractive color, excess drip in the package or dehydration. The role of lighting is to show the true quality of the pork product, without detracting from appearance or deceiving the customer about product quality. Lighting can speed up product discoloration but is essential for marketing and presentation of pork, both for traditional and for case-ready sales. The essential philosophy is the satisfied customers are repeat and loyal costumers. Display is defined as the offering of product under lighting in the retail case, usually under refrigeration. Display is not the same as storage, which implies keeping the product in the dark and usually not for sale. Some use the term storage when they really mean display.

Display lighting effects appearance or rate of discoloration of meat, which could result from temperature elevation at the meat surface, photochemical effects and/or differences in color fading because of different spectral energy distribution (Kropf, 1998). Several studies have examined the effect of light on discoloration of fresh meat, although conflicting results have been reported. Djenane et al. (2001) found that the use of a lamp emitting UVA radiation (360 nm) was severely detrimental to the retail life of meat, while the absence of UV radiation, either by lighting with a UV-free lamp or by using a polycarbonate filter, significantly extended beef meat retail life.

The color of meat is a very important quality attribute, and is the primary attribute of fresh meat that affects consumer purchase decision. Consumers expect a uniform appearance within a group of similar pork products and relate any color differences within similar products lower product quality (Faustman and Cassens, 1990; Hood, 1980). Thus, any process that negatively affects the color of fresh meat can lead to lower consumer appeal and marketability.

Lipids are present in muscles as structural components of the muscle membranes, as storage droplets of triacylglycerol between muscle fibers and as adipose tissue (marbling fat). These lipids, or more precisely their fatty acids, contribute to a wide range of quality attributes. For fresh meat these are color stability, drip loss and the development of oxidative rancidity.

With increased consumption of prepackaged raw meat, control of oxidation has become increasingly important. A great variety of substances and conditions may be considered as exerting antioxidant activities (Ladikos and Lougovois, 1990).

In the last 15-20 years, particular attention has been paid to the use of natural antioxidants, because the use of synthetic antioxidants has become less acceptable (Mielche and Bertelsen, 1994). Regarding vitamin C, Wheeler et al. (1996) indicated that it can be very effective for stabilizing color when added to steak surfaces; vitamin C was capable of extending display life even at the highest (9°C) temperature used. Vitamin C has been used as a steak surface treatment either alone or in combination with other antioxidant (Mitsumoto et al., 1991a; Mitsumoto et al., 1991b) to stabilize meat color during display. Depending on conditions, ascorbic acid can act as a prooxidant or an antioxidant (Elliot, 1999).

Objectives

The purpose of this study was to determine the effects of display conditions and addition of ascorbic acid on color fading, lipid oxidation and sensory properties of fresh pork loin chop over-wrapped.

Methodology

Meat

Pork loin chops from carcasses were obtained from the abattoir 48 h post slaughter. Chops were cut at 1.5 cm thick, and exposed to air for 1 h at 2°C to allow blooming; this operation was done aseptically. After blooming, samples were divided into two groups. Each chop in two groups was sprayed with L-ascorbic acid (500 ppm) solution. The other two groups were not sprayed, and named as controls.

Packaging and display conditions

Chops with ascorbic acid and control, were placed on polystyrene trays, before to be over-wrapped. Trays with samples (treated with ascorbic acid and untreated) were displayed at 1°C in a cabinet divided into two sections. One section was illuminated by a standard supermarket fluorescent lamp (F32T8-TL83, Philips Universal Hi Vision, USA). The second section was illuminated by a low-UV, color balanced lamp (Promolux® Platinum L36w, Market Group Ventures Inc., Canada). Packs were displayed up to 17 days. Samples were removed from the cabinet at day 3, 6, 10, 13 and 17 for analysis. All samples were exposed to lighting continuously at 1000 lux at the surface. Light intensity was measured using a Sper Cientific light meter (model 840020, Sper Cientific, Taiwan).

Purge loss

Pork loin chops were weighed at beginning and end of storage. Purge loss was calculated as follows:

$$\% \text{ Purge loss} = \frac{\text{Initial weight} - \text{Post storage weight}}{\text{Initial weight}} \times 100$$

pH

The meat pH was measured using a pH meter (Corning Ion Analyzer 255, Corning Science Products, Corning, N.Y., USA) with an General Purpose Combination electrode (Corning Science Products, Corning, N.Y., USA). Three grams of meat was mixed with 27 ml of water and homogenized before measurements were taken. Three readings were obtained from each pork chop portion.

Water holding capacity

Water holding capacity (WHC) was determined using the method described by Honikel and Hamm (1994).

Color measurement

Color changes on the surface of fresh meat samples each 3 or 4 days during display were monitored. CIE L*, a* and b* values were measured 30 min after package opening using a Minolta Spectrophotometer (CM2600d model).

Lipid oxidation

Lipid oxidation in displayed meat samples was measured as thiobarbituric acid-reactive substances (TBARS) as described by Pfalzgraf *et al.* (1995). TBARS values were calculated from standard curve and expressed as mg malondialdehyde kg⁻¹ meat.

Sensory analysis

A random color, off-odor and appearance test was conducted to evaluate whether panelists could distinguish between treatments. Semi-trained panelists were recruited to carry out the evaluation. Prior to each session meat color and appearance pictures were given to each panelist so they could designed a score. Sensory panelists scored chops for color on a 6-point scale (1= soft and 6= dark red), for off-odor on a 6-point scale (1= very soft and 6= very intense), for appearance on a 7-point scale (1=very unpleasant and 7= pleasant) (Forrest, 2002).

Statistical analysis

The significance of differences among samples at each day of storage was determined by analysis of variance (ANOVA) using the Least Square Difference method of the General Linear Model procedure of SPSS (SPSS 1995). Differences were considered significant at the p<0.05 level.

Results & Discussion

Purge loss (%) was significantly increased ($p < 0.05$) in all treatments during display time (results not shown). Purge losses were below 4% at end of storage time. Treatments with more purge loss were those displayed under fluorescent light, with 3.67 and 3.79%, with and without antioxidant respectively.

In the present experiment no difference was found in the pH of the samples ($p > 0.05$).

The WHC values for all samples were decreased as the display time increased ($p < 0.05$) (results not shown). Type of lighting had no effect ($p > 0.05$) on WHC values.

Respect to color, before packaging, the pork chops were darker and less red. In general, as the storage time increases the meat tends to have a paler appearance, L^* values are increased. This trend is generally and clearly displayed in the results where, at packaging time (day 0), L^* values for samples over-wrapped show higher values ($p < 0.05$), except for end of storage time, when all samples were lower (results not shown). For a^* values (Figure 1), following a considerable initial increase, generally decreased ($p < 0.05$) after exposing the meat to the lighting. Between samples exposed to both lamp types, there were differences along displaying time ($p < 0.05$). Pork chops exposed to Promolux® had higher a^* values. Bertelsen and Skibsted (1987) reported on the comparative effects of certain wavelengths upon photo-oxidation of solutions of oxymyoglobin. Their work provided convincing evidence that ultraviolet wavelengths strongly encourage discoloration. Therefore, reducing the amount of ultraviolet wavelengths that come in contact with the surface meat can result in improved display life. Addition of ascorbic acid like antioxidant was not showed effect ($p > 0.05$).

Values of TBARS are shown in Figure 2. TBARS formation was most intense in pork chops displayed under fluorescent light, which showed increasing significant differences ($p < 0.05$) with respect to other display condition from the 13th day of display until the end of the display time. Illumination with Promolux® resulted in a significant ($p < 0.05$) inhibition of lipid oxidation; however, this effect was evident only after 13 days of display. TBARS formation was not clearly inhibited by treatment with ascorbic.

Results of sensory analysis of pork chops, including evaluation of color, off-odor and appearance, are summarized in Figure 3. Concerning color (Fig. 3a), sensory scores showed that samples subject to Promolux® light and with antioxidant added preserved a good color until day 13 of storage. These results are in agreement with instrumental color measurements. Fresh meat odor (Figure 3b) was preserved for until 10 days under Promolux® light and with addition of ascorbic acid. At day 13 of display only samples with antioxidant preserved fresh meat odor and at end of display time, samples displayed under Promolux® showed best odor. For appearance (Figure 3c), results showed that decreased with similar pattern of evolution to those of odor and color.

Conclusions

According to the results discussed above, it may be concluded that the use of lamp with absence of UV radiation, like Promolux®, together with treatment with an antioxidant significantly could extend meat display life.

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Tables and Figures

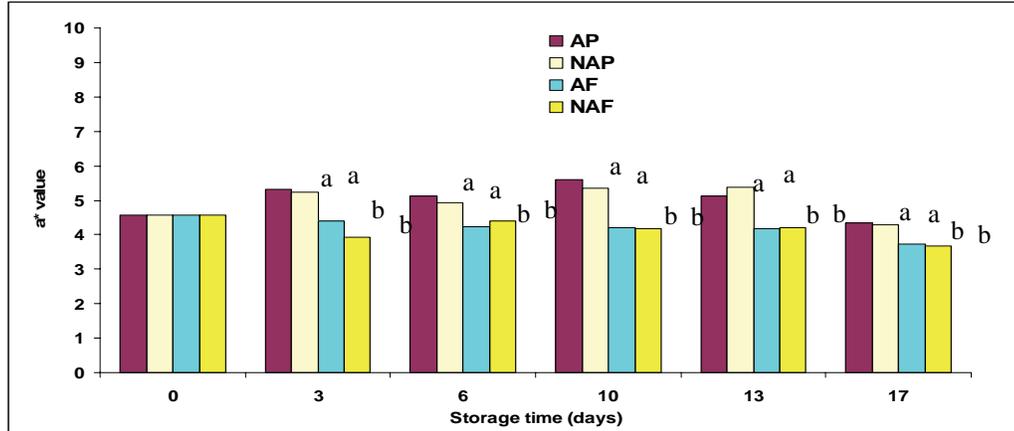


Figure 1: Effect of display conditions and storage time on a* values (redness) of over-wrapped fresh pork loin chops. AP: antioxidant + Promolux® lamp; NAP: no-antioxidant + Promolux® lamp; AF: antioxidant + fluorescent lamp; NAF: no antioxidant + fluorescent lamp. (Columns in the same day of display with different letters differ significantly (P<0.05)).

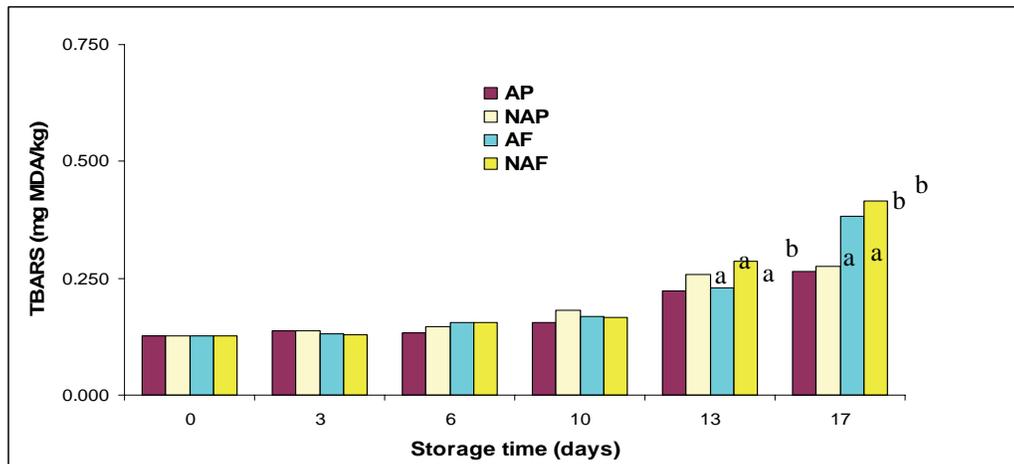


Figure 2: Effect of display conditions and storage time on TBA formation of over-wrapped fresh pork loin chops. AP: antioxidant + Promolux® lamp; NAP: no-antioxidant + Promolux® lamp; AF: antioxidant + fluorescent lamp; NAF: no antioxidant + fluorescent lamp. (Columns in the same day of display with different letters differ significantly (P<0.05)).

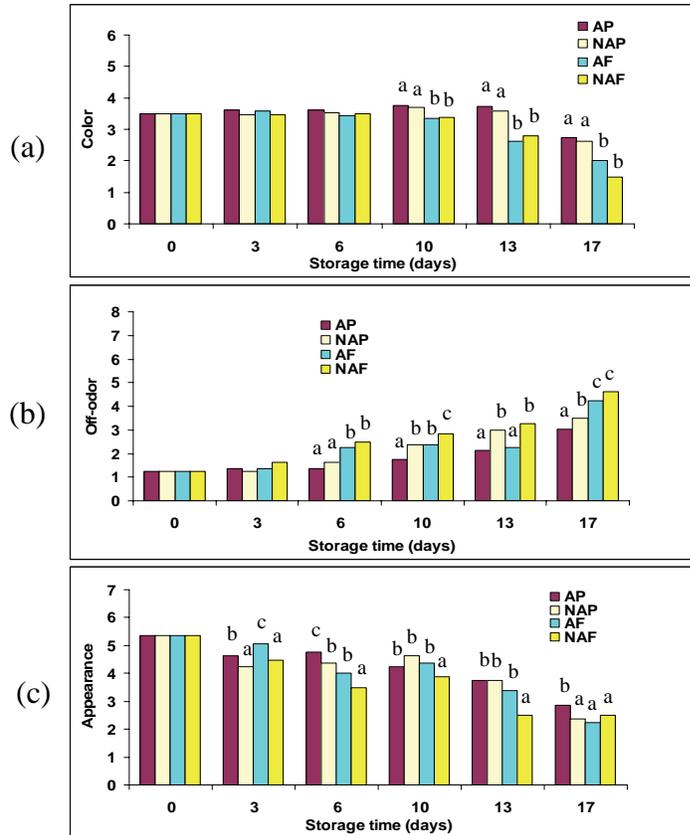


Figure 3: Effect of display conditions and storage time on sensory attributes: a) color, b) off-odor and c) appearance, of over-wrapped fresh pork loin chops. AP: antioxidant + Promolux® lamp; NAP: no-antioxidant + Promolux® lamp; AF: antioxidant + fluorescent lamp; NAF: no antioxidant + fluorescent lamp. (Columns in the same day of display with different letters differ significantly (P<0.05)).

ASCORBIC ACID AND ORIGANOX™ IN DIFFERENT PACKAGING SYSTEMS TO PREVENT THE INCIDENCE OF PORK LUMBAR VERTEBRAE DISCOLORATION

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Key Words: Pork bone, Discoloration, Antioxidant

Introduction

Meat color is widely recognized to be one of the most influencing characteristics for fresh meat purchasing decisions. Understandably, bone color of bone-in fresh meats can serve in a similar role as an indicator of wholesomeness to consumers (Gill, 1996). Innovations such as modified atmosphere packaging (MAP), and standard packaging such as polyvinyl chloride (PVC) film overwrap, are ways to maintain desirable red color in case ready meats; however, bone discoloration is problematic.

Recent research has addressed beef bone blackening by evaluating various interventions in preventing this problem. Mancini et al. (2004) found the application of 2.5% ascorbic acid (AA) minimized beef lumbar vertebrae discoloration. Similarly, Grobbel et al., (2005) indicated that 2.5% AA or ultra-low oxygen (ULOx) MAP effectively reduced beef bone discoloration.

Gill (1996) suggested that bone darkening occurs when hemoglobin is disrupted and accumulates at the cut bone surface where oxyhemoglobin darkens to methemoglobin. It should be considered that oxidation-reduction potential is higher for bone marrow than for muscle because bone marrow contains more pro-oxidative iron (Calhoun et al., 1998). Accordingly, Grobbel et al. (2005) showed that beef bone marrow containing more total iron and hemoglobin is more prone to discoloration.

Objectives

Investigations exploring interventions to the ‘black-bone’ problem have primarily focused on beef bones. This condition can be a problem in bone-in fresh pork, and our objective was to investigate means to prevent it. Topical antioxidants including ascorbic acid (AA) and Origanox™ (OG), a natural antioxidant, were used as possible interventions, along with high-oxygen (HiOx) MAP, ULOx MAP, and PVC packaging systems.

Methodology

Forty-eight pork backbones were obtained from a commercial abattoir, from which six 2.54 cm thick cross-sections of lumbar vertebra were cut at 6 d postmortem. Cut

sections from each backbone were treated with a 0.5 ml aliquot of 1 of 5 antioxidant treatments: 1.25%, 1.875%, and 2.5% AA; combination treatments of 0.15% OG + 0.30% AA and 0.225% OG + 0.45% AA; and control with no treatment applied. Both Grobbel et al. (2004) and Mancini et al. (2004) identified that 2.5% AA prevented darkening of beef bones. We used lower AA concentrations because pork marrow has lower oxidation-reduction potential than beef (Calhoun et al., 1998).

Vertebrae were packaged such that 6 vertebral sections in 1 package came from 1 backbone and represented all 6 treatments. Three packaging systems were used: 1) HiOx MAP (80% O₂, 20% CO₂); 2) ULOx MAP (70% N₂, 30% CO₂) with an activated oxygen scavenger; and 3) PVC overwrap film on foam trays.

Packages were displayed under continuous fluorescent lighting (2153 lux, 3000K, CRI 85) for 8 d at 2°C in an open retail display case and rotated twice daily to maintain random placement.

Six trained panelists scored porous marrow of the cut surface once daily on 6 d: from d 0 to d 5 and once on d 8 of display. A seven-point scale was used for HiOx MAP and PVC packages: 1) bright reddish-pink to red, 2) dull reddish-pink, 3) slightly grayish-pink or –red, 4) grayish-pink or –red, 5) moderately gray, 6) all gray or grayish-black, and 7) black discoloration. ULOx MAP samples were evaluated using a different seven-point scale: 1) bright purplish-red or –pink, 2) dull purplish-red or –pink, 3) slightly grayish-purple or –pink, 4) grayish-purple or –red, 5) moderately gray, 6) all gray or grayish-black, and 7) black discoloration.

Duplicate instrumental CIE L*a*b* measurements were taken on each cut vertebral section using a 0.64 cm aperture (Illuminant A) and averaged. These instrumental measurements were taken from all samples on day 0, from 24 opened packages on day 2, and from 24 opened packages on day 8. L* corresponds with lightness, a* with redness, and b* with yellowness. The a*/b* ratio is an indicator of discoloration, with lower ratios indicating more discoloration.

Data were analyzed with SAS PROC MIXED (SAS Institute, Inc., Cary, NC). Pairwise comparisons of least squares means were used to determine significant differences ($P < 0.05$).

Results & Discussion

Because the same visual scale was used, PVC and HiOx MAP packaged bones can be compared (Table 1). Control lumbar vertebra packaged in PVC and HiOx MAP did not exhibit graying ($P < 0.05$) until d 3 and d 4 of display, respectively. From d 2 to d 8, antioxidant treated bones in HiOx MAP had better ($P < 0.05$) visual scores than those packaged in PVC or the HiOx MAP control vertebrae. By d 5 of display, bones packaged in PVC and treated with both OG + AA treatments, or with 1.25% AA exhibited graying ($P < 0.05$). On d 5, the least desirable ($P < 0.05$) lumbar vertebrae were the PVC packaged controls, whereas HiOx MAP antioxidant-treated bones had the most desirable ($P < 0.05$) visual color. On d 8 of display, bones in HiOx MAP treated with 1.875% or 2.5% AA had superior ($P < 0.05$) visual scores compared to both OG + AA treatments, 1.25% AA treated bones were intermediate. All antioxidant treated bones in HiOx MAP had superior visual scores on d 8 compared to all bones in PVC and the HiOx control

bones. Among PVC packaged bones, 1.875% and 2.5% AA resulted in more desirable d 8 visual scores.

An antioxidant effect was not observed ($P > 0.05$) for bones in ULOx MAP; however, a day effect was observed ($P < 0.05$; Table 2). Visual color scores declined ($P < 0.05$) from d 0 to d 3, maintained ($P > 0.05$) from d 3 to d 5, and declined again from d 5 to d 8 ($P < 0.05$).

The HiOx MAP bones increased in L^* from d 0 to d 2, and decreased from day 2 to d 8 ($P < 0.05$; Table 3). The PVC bones underwent a similar decline. The L^* values declined for antioxidant treated bones in ULOx MAP from d 2 to d 8 ($P < 0.05$). By d 8, control HiOx MAP bones had the lowest L^* values, and PVC bones treated with either 1.875% or 2.5% AA had the highest ($P < 0.05$) L^* values. Among antioxidant treated bones in HiOx MAP, those treated with 1.875% AA had the highest ($P < 0.05$) L^* values. Nominal differences in d 8 L^* value were observed among ULOx bones.

We observed a marked increase ($P < 0.05$) in a^* values (more red) for HiOx MAP antioxidant-treated bones from d 0 to d 8 (Table 4). For HiOx control bones, however, a^* decreased ($P < 0.05$) from d 2 to d 8, and also for bones packaged in PVC and treated with 0.15% OG + 0.30% AA, 1.25% AA, and the control. Other antioxidant treated bones in PVC underwent an increase ($P < 0.05$) in a^* from d 0 to d 2, but did not change ($P > 0.05$) from d 2 to d 8. All bones packaged in ULOx MAP increased ($P < 0.05$) in a^* from d 2 to d 8, although the increase was not as great as that observed for HiOx MAP antioxidant treated bones. The HiOx MAP bones treated with 2.5% AA had the highest ($P < 0.05$) d 8 a^* values.

Antioxidant treated bones in HiOx MAP had higher ($P < 0.05$) a^*/b^* ratios than all PVC packaged bones (Table 5). Among antioxidants used in HiOx MAP, the highest a^*/b^* ratio was observed for 0.225% OG + 0.40% AA; ratios of those treated with 0.15% OG + 0.30% AA, 1.25% and 2.5% AA were the lowest; and 1.875% AA was intermediate. All PVC packaged bones and HiOx MAP control bones had lower ratios. The lowest ratio ($P < 0.05$) for PVC bones was observed for those treated with 1.875% AA. The ULOx MAP bones treated with either 1.875% or 2.5% AA had the least a^*/b^* discoloration.

Conclusions

Our experiment utilized antioxidants at varying levels coupled with different packaging systems to evaluate their effectiveness at preventing pork bone discoloration. Visual and instrumental values indicated that bones packaged in HiOx MAP benefit from the application of an antioxidant, with higher concentrations of ascorbic acid (1.875% and 2.5%) generally yielding more desirable results. The ULOx MAP appeared to be an effective means to prevent bone discoloration and, according to instrumental measures, also benefits from the application from a higher concentration of ascorbic acid. Origanox™ combined with ascorbic acid was not as effective as the ascorbic acid-only treatments. The application of a topical antioxidant appears to reduce bone discoloration and may be of value for use in bone-in fresh pork cuts.

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Tables and Figures

Table 1. Visual color scores^a of pork lumbar vertebrae packaged in high-oxygen MAP and PVC overwrap treated with antioxidants and displayed 8 d

Packaging	Antioxidant	Day						
		0	1	2	3	4	5	8
HiOx	0.15% OG+0.30% AA	1.36 ^{b,u}	1.54 ^{c,uv}	1.75 ^{de,vw}	1.94 ^{d,wx}	2.13 ^{e,xy}	2.25 ^{f,y}	2.76 ^{f,z}
HiOx	0.225% OG+0.45% AA	1.37 ^{b,v}	1.53 ^{c,vw}	1.72 ^{e,w}	1.98 ^{d,x}	2.14 ^{e,xy}	2.29 ^{f,y}	2.72 ^{f,z}
HiOx	1.25% AA	1.35 ^{b,v}	1.51 ^{c,vw}	1.70 ^{e,wx}	1.95 ^{d,xy}	2.07 ^{e,y}	2.18 ^{f,y}	2.59 ^{f,g,z}
HiOx	1.875% AA	1.34 ^{b,v}	1.48 ^{c,ww}	1.74 ^{de,w}	2.01 ^{d,x}	2.07 ^{e,xy}	2.19 ^{f,y}	2.39 ^{g,z}
HiOx	2.5% AA	1.37 ^{b,v}	1.50 ^{c,vw}	1.68 ^{e,wx}	1.94 ^{d,xy}	2.03 ^{e,yz}	2.07 ^{f,yz}	2.24 ^{g,z}
HiOx	Control	1.23 ^{b,t}	1.65 ^{bc,u}	2.05 ^{cd,v}	2.60 ^{c,w}	3.17 ^{c,x}	3.76 ^{c,y}	4.79 ^{b,z}
PVC	0.15% OG+0.30% AA	1.45 ^{b,t}	1.80 ^{bc,u}	2.29 ^{bc,v}	2.59 ^{c,w}	3.08 ^{c,x}	3.43 ^{cd,y}	3.77 ^{d,z}
PVC	0.225% OG+0.45% AA	1.38 ^{b,t}	1.83 ^{bc,u}	2.33 ^{bc,v}	2.68 ^{c,w}	3.13 ^{c,x}	3.42 ^{cd,y}	3.90 ^{d,z}
PVC	1.25% AA	1.32 ^{b,t}	1.67 ^{bc,u}	2.18 ^{bc,v}	2.45 ^{c,w}	2.86 ^{cd,x}	3.26 ^{d,y}	3.75 ^{d,z}
PVC	1.875% AA	1.30 ^{b,v}	1.67 ^{bc,w}	2.20 ^{bc,x}	2.44 ^{c,x}	2.75 ^{d,y}	2.81 ^{e,y}	3.24 ^{e,z}
PVC	2.5% AA	1.42 ^{b,v}	1.84 ^{bc,w}	2.29 ^{bc,x}	2.48 ^{c,x}	2.77 ^{d,y}	2.90 ^{e,y}	3.39 ^{e,z}
PVC	Control	1.44 ^{b,u}	1.92 ^{b,v}	2.48 ^{c,w}	3.24 ^{b,x}	3.81 ^{b,y}	4.30 ^{b,z}	4.31 ^{c,z}

^a 1=bright reddish-pink to red, 2=dull reddish-pink, 3=slightly grayish-pink or -red, 4=grayish-pink or -red, 5=moderately gray, 6=all gray or grayish-black, and 7=black discoloration

^{b,c,d,e,f,g} Means with different superscript letters within columns differ ($P < 0.05$)

^{t,u,v,w,x,y,z} Means with different superscript letters within rows differ ($P < 0.05$)

Table 2. Visual color scores^a of pork lumbar vertebrae packaged in ultra-low-oxygen MAP displayed 8 d pooled across treatments

Score	Day						
	0	1	2	3	4	5	8
	1.95 ^b	2.32 ^c	2.80 ^d	3.13 ^e	3.41 ^e	3.51 ^e	3.74 ^f

^a 1=bright purplish-red -pink, 2=dull purplish or -pink, 3=slightly grayish-purple or -pink, 4=grayish -purple or -red, 5=moderately gray, 6=all gray or grayish-black, and 7=black discoloration

^{b,c,d,e,f} Means with different superscript letters differ ($P < 0.05$)

Table 3. L* values of pork lumbar vertebrae treated with different antioxidants and displayed 8 d

Packaging	Antioxidant	Day		
		0	2	8
HiOx	0.15% OG+0.30% AA	48.87 ^{cd,y}	52.71 ^{abc,z}	44.22 ^{ghi,x}
HiOx	0.225% OG+0.45% AA	49.39 ^{bcd,y}	51.30 ^{abcd,z}	42.36 ^{hi,x}
HiOx	1.25% AA	49.42 ^{bcd,y}	53.33 ^{ab,z}	45.30 ^{efg,x}
HiOx	1.875% AA	48.79 ^{cd,y}	52.69 ^{abc,z}	48.62 ^{bc,y}
HiOx	2.5% AA	48.69 ^{d,y}	53.81 ^{a,z}	45.77 ^{efg,x}
HiOx	Control	48.60 ^{d,y}	51.49 ^{abcd,z}	41.09 ^{i,x}
PVC	0.15% OG+0.30% AA	49.53 ^{abcd,y}	51.31 ^{abcd,z}	47.15 ^{cde,x}
PVC	0.225% OG+0.45% AA	48.61 ^{d,z}	49.50 ^{defg,z}	46.85 ^{def,y}
PVC	1.25% AA	48.54 ^{d,y}	50.71 ^{cde,z}	49.42 ^{ab,yz}
PVC	1.875% AA	49.04 ^{cd,y}	50.67 ^{cdef,z}	50.73 ^{a,z}
PVC	2.5% AA	49.45 ^{abcd,z}	51.07 ^{bcde,z}	50.02 ^{ab,z}
PVC	Control	48.73 ^{cd,y}	50.56 ^{cdef,z}	48.83 ^{bc,y}
ULOx	0.15% OG+0.30% AA	50.10 ^{abc,y}	48.03 ^{g,y}	44.17 ^{ghi,z}
ULOx	0.225% OG+0.45% AA	51.34 ^{a,y}	48.30 ^{fg,y}	43.89 ^{ghi,z}
ULOx	1.25% AA	50.59 ^{abc,y}	49.85 ^{defg,y}	44.20 ^{ghi,z}
ULOx	1.875% AA	51.06 ^{ab,y}	50.71 ^{cde,y}	45.67 ^{efg,z}
ULOx	2.5% AA	50.67 ^{ab,y}	49.51 ^{defg,y}	44.28 ^{fg,z}
ULOx	Control	51.20 ^{ab,y}	49.15 ^{efg,y}	45.12 ^{efg,y}

^{a,b,c,d,e,f} Means with different letters within a column differ ($P < 0.05$)
^{x,y,z} Means with different letters within a row differ ($P < 0.05$)

Table 4. a* values of pork lumbar vertebrae treated with different antioxidants and displayed 8 d

Packaging	Antioxidant	Day		
		0	2	8
HiOx	0.15% OG+0.30% AA	23.09 ^{abc,x}	28.91 ^{a,y}	36.19 ^{b,z}
HiOx	0.225% OG+0.45% AA	22.70 ^{abc,x}	29.42 ^{a,y}	37.34 ^{b,z}
HiOx	1.25% AA	23.40 ^{ab,x}	29.89 ^{a,y}	37.21 ^{b,z}
HiOx	1.875% AA	23.61 ^{ab,x}	29.86 ^{a,y}	36.07 ^{b,z}
HiOx	2.5% AA	23.14 ^{abc,x}	29.03 ^{a,y}	39.66 ^{a,z}
HiOx	Control	22.64 ^{abc,y}	30.84 ^{a,y}	26.97 ^{de,z}
PVC	0.15% OG+0.30% AA	22.27 ^{bc,x}	29.01 ^{a,z}	26.72 ^{ef,y}
PVC	0.225% OG+0.45% AA	22.90 ^{abc,y}	28.76 ^{a,z}	27.10 ^{de,z}
PVC	1.25% AA	23.47 ^{ab,x}	29.47 ^{a,z}	25.73 ^{ef,y}
PVC	1.875% AA	22.50 ^{abc,y}	29.00 ^{a,z}	29.09 ^{cd,z}
PVC	2.5% AA	22.37 ^{abc,y}	29.01 ^{a,z}	28.29 ^{cde,z}
PVC	Control	23.82 ^{a,x}	29.37 ^{a,z}	25.91 ^{ef,y}
ULOx	0.15% OG+0.30% AA	22.68 ^{abc,y}	19.79 ^{cd,x}	27.49 ^{de,z}
ULOx	0.225% OG+0.45% AA	21.98 ^{abc,y}	20.66 ^{bc,y}	28.38 ^{cde,z}
ULOx	1.25% AA	22.76 ^{abc,y}	20.34 ^{c,x}	29.43 ^{c,z}
ULOx	1.875% AA	22.22 ^{bc,y}	17.91 ^{d,x}	24.29 ^{f,z}
ULOx	2.5% AA	21.52 ^{c,y}	18.74 ^{cd,x}	28.14 ^{cde,z}
ULOx	Control	22.25 ^{bc,y}	21.27 ^{b,y}	28.91 ^{cde,z}

^{a,b,c,d,e,f} Means with different letters within a column differ ($P < 0.05$)
^{x,y,z} Means with different letters within a row differ ($P < 0.05$)

Table 5. a*/b* values of pork lumbar vertebrae treated with different antioxidants and displayed 8 d

Packaging	Antioxidant	Day		
		0	2	8
HiOx	0.15% OG+0.30% AA	1.26 ^{a,z}	1.25 ^{ab,z}	1.14 ^{d,y}
HiOx	0.225% OG+0.45% AA	1.25 ^{ab,z}	1.24 ^{abc,z}	1.24 ^{abc,z}
HiOx	1.25% AA	1.25 ^{ab,z}	1.25 ^{ab,z}	1.16 ^{d,y}
HiOx	1.875% AA	1.26 ^{a,z}	1.25 ^{ab,z}	1.20 ^{cd,y}
HiOx	2.5% AA	1.26 ^{a,z}	1.23 ^{abc,z}	1.15 ^{d,y}
HiOx	Control	1.27 ^{a,z}	1.28 ^{a,z}	1.02 ^{ef,y}
PVC	0.15% OG+0.30% AA	1.24 ^{ab,z}	1.21 ^{bcd,z}	1.03 ^{ef,y}
PVC	0.225% OG+0.45% AA	1.26 ^{a,z}	1.21 ^{bcd,z}	1.02 ^{ef,y}
PVC	1.25% AA	1.20 ^{bc,z}	1.23 ^{abc,z}	0.99 ^{f,y}
PVC	1.875% AA	1.25 ^{ab,z}	1.23 ^{abc,z}	1.07 ^{e,y}
PVC	2.5% AA	1.24 ^{a,z}	1.21 ^{bcd,z}	1.04 ^{ef,y}
PVC	Control	1.26 ^{a,z}	1.26 ^{a,z}	1.02 ^{ef,z}
ULOx	0.15% OG+0.30% AA	1.22 ^{ab,y}	1.14 ^{de,x}	1.27 ^{ab,z}
ULOx	0.225% OG+0.45% AA	1.19 ^{c,z}	1.13 ^{e,y}	1.20 ^{cd,z}
ULOx	1.25% AA	1.22 ^{ab,z}	1.14 ^{de,y}	1.23 ^{bc,z}
ULOx	1.875% AA	1.21 ^{bc,y}	1.12 ^{e,x}	1.31 ^{a,z}
ULOx	2.5% AA	1.21 ^{bc,y}	1.14 ^{de,x}	1.30 ^{a,z}
ULOx	Control	1.19 ^{c,z}	1.18 ^{cde,z}	1.23 ^{bc,z}

^{a,b,c,d,e,f} Means with different superscript letters in column differ ($P < 0.05$)
^{x,y,z} Means with different superscript letters in row differ ($P < 0.05$)

CHARACTERIZATION, EFFECTS OF PACKAGING, AND ANTIOXIDANT PREVENTION OF BEEF BONE MARROW DISCOLORATION

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Key Words: Bone marrow, Discoloration, Packaging, Antioxidant

Introduction

Bone marrow discoloration to a gray-black color and its occurrence in modified-atmosphere packaged (MAP), bone-in, beef retail cuts has been observed by meat retailers. Consumers could perceive bone discoloration as unwholesome, and it might affect their acceptance of a fresh meat product (Gill, 1996). Bone marrow discoloration has been reported in high-oxygen MAP beef and pork, and also in cuts packaged in polyvinyl chloride film (PVC).

Gill (1996) suggested that bone blackening occurs when bone is cut and hemoglobin is released to the surface, where it will accumulate when the red blood cells are disrupted. Over time and through exposure to air, hemoglobin on the surface of the bone turns from red to brown to black (Gill, 1996).

Mancini et al. (2004) found that treating beef lumbar vertebrae with 1.5 or 2.5% ascorbic acid was effective in minimizing marrow discoloration, with the 2.5% ascorbic acid treatment being the most effective through a 5-d display.

Objectives

The objectives of Experiment 1 were to determine the prevalence of marrow discoloration in beef humeri, ribs, thoracic vertebrae, and scapulas in different packaging systems and to determine factors that may cause marrow discoloration. Experiment 2 was designed to evaluate the effects of applying different antioxidant treatments in preventing beef lumbar vertebrae marrow discoloration from occurring in different packaging systems.

Methodology

Experiment 1: Thirty-six beef humeri, ribs, scapulas, and thoracic vertebrae from two replications of 18 different USDA Select and Choice carcasses were obtained from a commercial abattoir, and cut into 2.54-cm-thick sections at 4 d postmortem. Cross-sections of the humeri (shaft), ribs, and thoracic vertebrae (medial to lateral through the main body of the vertebrae), and cross-sections perpendicular to the spinous process of scapulas were cut. Bones were packaged into one of three package types: 1) PVC overwrap; 2) high-oxygen (80% O₂, 20% CO₂) MAP; and 3) ultra-low-oxygen (70% N₂,

30% CO₂) MAP. One each of a humerus, scapula, and thoracic vertebra, and two rib bone sections were placed in each package.

Experiment 2: Seventy-two beef backbones containing lumbar vertebrae from USDA Select and Choice carcasses were obtained from a commercial abattoir and held at 2°C in a cardboard box for either 6 or 14 d postmortem. Vertebrae were then cut similar to Experiment 1. Before packaging, bone sections were treated with one of the following antioxidant treatments: control with no treatment application; 1.25% or 2.5% ascorbic acid; 0.1% or 0.2% rosemary; or a combination treatment of 0.15% Origanox™ and 0.3% ascorbic acid. Origanox™ is a natural antioxidant extracted from the edible-herb family *Labiatae*. A 1-ml aliquot of the given antioxidant solution, dissolved in distilled-deionized water, was pipetted onto the marrow cut surface of individual bones.

Packages were displayed under continuous fluorescent lighting (Experiment 1: 1614 lux, 3000K; Experiment 2: 2153 lux, 3000K) for 5 d at 2°C.

Ten trained visual panelists scored bone-marrow color once daily for 5 d, beginning on d 0 about 1 h after packaging for both experiments. Mancini et al. (2004) developed the 7-point color scale used to score bones in high-oxygen MAP and PVC. Bone sections in high-oxygen MAP and PVC packages were scored according to the seven-point scale: 1) bright reddish-pink to red, 2) dull pinkish-red, 3) slightly grayish-pink or grayish-red, 4) grayish-pink or grayish-red, 5) moderately gray, 6) all gray or grayish-black, and 7) black discoloration. We developed the 7-point scale for ultra-low-oxygen MAP bones: 1) bright purplish-red or purplish-pink, 2) dull purplish-pink or purplish-red, 3) slightly grayish purple or pink, 4) grayish-purple or grayish-red, 5) moderately gray, 6) all gray or grayish-black, and 7) black discoloration. Panelists were instructed to score the porous portion of the bone marrow in half-point increments.

Instrumental CIE L*a*b* measurements were taken by using a 0.64-cm aperture (Illuminant A) on a Hunter labscan 2 in both experiments. Instrumental color measurements were taken on d 0, 2, and 4 of display.

Upon completion of display, bones were cleaned of all meat and connective tissue, vacuum packaged, and stored at -80°C until further analysis. As determined by previous research in our laboratory, scapulas did not have enough bone marrow to extract for chemical analysis. In Experiment 1, humeri, ribs, and thoracic-vertebrae marrow samples were pooled (three humeri, six ribs, or six thoracic vertebrae marrow samples) within bone and packaging type to obtain enough marrow to conduct the laboratory analyses. Marrow was not pooled in Experiment 2.

Bone marrow was extracted by using a modified procedure of Calhoun et al. (1998). Humeri, ribs, and vertebrae marrow (10-g, 1-g, and 1-g samples, respectively) were used to determine TBARS content on d 0 and 4 of display by using a slightly modified method described by Witte et al. (1970). Hydrophobic interaction HPLC was used to determine myoglobin and hemoglobin (Hb) pigment concentrations. Total iron (AOAC method 968.08) was measured with an atomic absorption spectrophotometer at 248.3 nm. Phosphorus content was measured at 700 nm. In Experiment 2, marrow was extracted and TBARS were measured on d 0, 2, and 4 of display.

Statistical analysis was completed by using the PROC MIXED procedure of SAS®. Means were separated by using Fisher's Protected LSD with Prasad-Rao-Jeske-Kackar-Harville standard errors and the Kenward-Roger degrees of freedom. Highest-order

interactions were reported; main effects were reported when no interactions were significant. Significance was determined at probability values of $P < 0.05$.

Results & Discussion

Ribs, scapulas, and thoracic vertebrae marrow discolored in PVC and high-oxygen MAP packaging according to visual color scores (Table 1). Oxidation was considerably less for humeri marrow than for ribs and thoracic vertebrae marrow and did not change over display time (Table 2). Marrow from humeri had much less ($P < 0.05$) total iron and Hb than marrow from ribs and thoracic vertebrae. Myoglobin was greater ($P < 0.05$) in marrow from ribs than in thoracic vertebrae and was undetected in humeri (Table 3). Lumbar vertebrae treated with 0.1 and 0.2% rosemary, and control treatments discolored ($P < 0.05$) in PVC and high-oxygen MAP. The combination of 0.15% Origanox™ + 0.3% ascorbic acid maintained desirable color through d 2 of display in PVC and high-oxygen MAP. Ascorbic acid treatments of 1.25 and 2.5% were equally effective ($P > 0.05$) in preventing bone discoloration in lumbar vertebrae packaged in high-oxygen MAP (Tables 4, 5, and 6). In all three packaging systems, bones held 14 d postmortem discolored more and had larger TBARS values than did those held 6 d. Instrumental color results, especially a^* and chroma, supported visual color scores for both experiments (data not shown). Overall, ascorbic acid treatments were most effective in minimizing TBARS changes throughout display (data not shown).

Conclusions

Distinct bone marrow discoloration occurred in ribs, scapulas, and thoracic vertebrae packaged in PVC or high-oxygen MAP but was minimal in ultra-low oxygen MAP. Discoloration was not an issue in humeri. Bone marrow discoloration is likely caused primarily by oxidation of Hb but also by heme-catalyzed lipid oxidation. Untreated lumbar vertebrae discolored to relatively dark gray when packaged in PVC and high-oxygen MAP, but not when packaged in ultra-low-oxygen MAP. Ascorbic acid treatments, particularly the 2.5% application, were very effective in preventing bone-marrow discoloration, and were superior to other treatments.

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Tables and Figures

Table 1. Means^a of visual color scores^b for bone marrow from different bones in different package types from d 0 to 4 of display

Bone	Package ^c	Day				
		0	1	2	3	4
Humeri	PVC	1.5 ^v	2.0 ^w	2.2 ^x	2.3 ^y	2.6 ^z
Humeri	High	1.4 ^w	1.8 ^x	2.1 ^y	2.2 ^y	2.5 ^z
Humeri	Ultra-low	1.8 ^w	2.5 ^x	2.9 ^y	2.8 ^y	3.0 ^z
Ribs	PVC	1.7 ^{ev}	4.6 ^{dw}	5.0 ^{dx}	5.2 ^{dy}	5.3 ^{dz}
Ribs	High	1.4 ^{dw}	4.6 ^{dx}	5.1 ^{dy}	5.2 ^{dy}	5.3 ^{dz}
Ribs	Ultra-low	2.1 ^w	2.5 ^x	2.8 ^y	3.0 ^z	3.1 ^z
Scapulas	PVC	2.1 ^{ev}	4.2 ^{dw}	4.9 ^{dx}	5.1 ^{dy}	5.5 ^{dz}
Scapulas	High	1.8 ^{dv}	4.6 ^{ew}	5.2 ^{ex}	5.4 ^{ey}	5.6 ^{dz}
Scapulas	Ultra-low	2.3 ^v	3.1 ^w	3.4 ^x	3.6 ^y	3.7 ^z
Thoracic vertebrae	PVC	2.2 ^{ew}	5.3 ^{dx}	5.8 ^{dy}	5.8 ^{dy}	6.1 ^{dz}
Thoracic vertebrae	High	1.6 ^{dw}	5.2 ^{dx}	5.6 ^{dy}	5.8 ^{dyz}	5.9 ^{dz}
Thoracic vertebrae	Ultra-low	2.7 ^w	3.1 ^x	3.3 ^y	3.4 ^{yz}	3.5 ^z

^aStandard error for all means = 0.14

^bHigh-oxygen and PVC color scale: 1=bright reddish-pink to red, 2=dull pinkish-red, 3=slightly grayish-pink or grayish red, 4=grayish-pink or grayish red, 5=moderately gray, 6=all gray or grayish-black, and 7=black discoloration; Ultra-low-oxygen color scale: 1=bright purplish-red or purplish-pink, 2=dull purplish-pink or purplish-red, 3=slightly grayish purple or pink, 4=grayish-purple or grayish-red, 5=moderately gray, 6=all gray or grayish-black, 7=black discoloration

^cPVC = polyvinyl chloride overwrap film; High = high-oxygen modified atmosphere packaging; and Ultra-low = ultra-low oxygen modified atmosphere packaging

^{d,e}Means with different superscript letters within bone type and within columns (PVC vs High) differ ($P < 0.05$)

^{v,w,x,y,z}Means with different superscript letters across rows (days) differ ($P < 0.05$)

Table 2. Means^a of 2-thiobarbituric reactive substances^b for bone marrow from different bones in different package types from d 0 and 4 of display

Bone	Package ^c	Day	
		0	4
Humeri	PVC	0.03 ^z	0.06 ^z
Humeri	High	0.03 ^z	0.06 ^z
Humeri	Ultra-low	0.03 ^z	0.04 ^z
Ribs	PVC	0.74 ^z	0.77 ^{ez}
Ribs	High	0.74 ^y	0.84 ^{ez}
Ribs	Ultra-low	0.74 ^y	0.65 ^{dz}
Thoracic vertebrae	PVC	0.67 ^y	1.04 ^{ez}
Thoracic vertebrae	High	0.67 ^y	1.01 ^{ez}
Thoracic vertebrae	Ultra-low	0.67 ^y	0.75 ^{dz}

^aStandard error for humeri on d 0 and d 4 = 0.02; for ribs on d 0 and d 4 = 0.03; and for thoracic vertebrae on d 0 and d 4 = 0.03

^bmg malonaldehyde/ 1000 g sample

^cPVC = polyvinyl chloride overwrap film; High = high-oxygen modified atmosphere packaging; and Ultra-low = ultra-low oxygen modified atmosphere packaging

^{d,e}Means with different superscript letters within bone types and within columns differ ($P < 0.05$)

^{y,z}Means with different superscript letters across rows (days) differ ($P < 0.05$)

Table 3. Main-effects means^a of total iron, phosphorus (P), hemoglobin (Hb), and myoglobin (Mb) for humeri, ribs, and thoracic vertebrae bone marrow

Bone	Total Iron			
	ppm	P ppm	Hb mg/g	Mb mg/g
Humeri	8.12 ^x	867.92 ^z	4.5 ^y	— ^b
Ribs	237.35 ^z	847.41 ^z	159.5 ^z	0.530 ^z
Thoracic Vertebrae	219.08 ^y	574.32 ^y	153.0 ^z	0.313 ^y

^aStandard error for humeri total iron = 0.54, P = 92.59, and Hb = 0.45; for ribs total iron = 10.8, P = 43.72, Hb = 13.2, and Mb = 0.05; for thoracic vertebrae total iron = 8.93, P = 42.10, Hb = 6.36, and Mb = 0.02

^bNot detected in humeri bone marrow

^{x,y,z}Means with different superscript letters within columns differ ($P < 0.05$)

Table 4. Means^a of visual-color scores^b for different antioxidant treatments of lumbar vertebrae packaged at 6 or 14 d postmortem in polyvinyl chloride overwrap and displayed from d 0 to 4

Antioxidant treatment	6 d Postmortem				
	Display day				
	0	1	2	3	4
Control	2.3 ^{ew}	5.1 ^{ewx}	5.3 ^{fx}	5.7 ^{fy}	5.9 ^{ez}
1.25% Ascorbic acid	1.5 ^{cdv}	2.0 ^{cdw}	2.5 ^{dx}	3.7 ^{dy}	4.6 ^{dz}
2.5% Ascorbic acid	1.5 ^{cdw}	1.7 ^{cwx}	2.0 ^{cx}	2.7 ^{cy}	3.1 ^{cz}
0.1% Rosemary	2.0 ^{ew}	4.9 ^{ex}	5.2 ^{fy}	5.4 ^{fyz}	5.8 ^{ez}
0.2% Rosemary	1.9 ^{dex}	5.0 ^{ey}	5.1 ^{fy}	5.5 ^{fz}	5.8 ^{ez}
0.15% Origanox TM +	1.3 ^{cv}	2.3 ^{dw}	3.8 ^{ex}	4.7 ^{ey}	5.1 ^{dz}
0.3% Ascorbic acid					
Antioxidant treatment	14 d Postmortem				
	Display day				
	0	1	2	3	4
Control	3.7 ^{ew}	5.1 ^{fx}	5.4 ^{gy}	5.7 ^{eyz}	5.9 ^{fz}
1.25% Ascorbic acid	1.7 ^{cdv}	2.2 ^{cw}	3.3 ^{dx}	4.2 ^{dy}	4.9 ^{dz}
2.5% Ascorbic acid	1.8 ^{cdw}	2.1 ^{dwx}	2.3 ^{cx}	3.1 ^{cy}	3.9 ^{cz}
0.1% Rosemary	3.2 ^{ew}	4.8 ^{efy}	5.3 ^{fgz}	5.4 ^{ez}	5.4 ^{efz}
0.2% Rosemary	3.0 ^{dew}	4.5 ^{ex}	4.9 ^{fy}	5.3 ^{ey}	5.7 ^{fz}
0.15% Origanox TM +	1.4 ^{cv}	2.8 ^{dw}	4.0 ^{ex}	4.6 ^{dy}	5.1 ^{dez}
0.3% Ascorbic acid					

^aStandard error for all means = 0.20

^bSee footnote ^b Table 1

^{c,d,e,f,g}Means with different superscript letters within columns within postmortem age differ ($P < 0.05$)

^{v,w,x,y,z}Means with different superscript letters across rows within postmortem age differ ($P < 0.05$)

Table 5. Means^a of visual-color scores^b for different antioxidant treatments of lumbar vertebrae packaged at 6 or 14 days postmortem in high-oxygen modified-atmosphere packaging and displayed from d 0 to 4

Antioxidant treatment	6 d Postmortem				
	Display day				
	0	1	2	3	4
Control	1.4 ^{cx}	5.1 ^{ey}	5.5 ^{fz}	5.6 ^{ez}	5.8 ^{ez}
1.25% Ascorbic acid	1.3 ^{cw}	1.5 ^{cwx}	1.8 ^{cxxy}	1.8 ^{cyz}	2.2 ^{cz}
2.5% Ascorbic acid	1.4 ^{cx}	1.5 ^{cxxy}	1.8 ^{cxxyz}	1.8 ^{cyz}	2.0 ^{cz}
0.1% Rosemary	1.4 ^{cx}	4.7 ^{dey}	5.2 ^{efz}	5.2 ^{ez}	5.5 ^{ez}
0.2% Rosemary	1.4 ^{cw}	4.5 ^{dx}	5.0 ^{ey}	5.3 ^{eyz}	5.5 ^{ez}
0.15% Origanox™ +	1.4 ^{cv}	1.9 ^{cw}	3.1 ^{dx}	3.6 ^{dy}	4.1 ^{dz}
0.3% Ascorbic acid					
Antioxidant treatment	14 d Postmortem				
	Display day				
	0	1	2	3	4
Control	2.4 ^{ew}	4.4 ^{dx}	5.1 ^{ey}	5.5 ^{ez}	5.8 ^{ez}
1.25% Ascorbic acid	1.9 ^{cdx}	1.9 ^{cx}	2.0 ^{cxxy}	2.4 ^{cyz}	2.6 ^{cz}
2.5% Ascorbic acid	1.7 ^{cx}	1.8 ^{cxxy}	2.0 ^{cxxy}	2.1 ^{cyz}	2.4 ^{cz}
0.1% Rosemary	2.4 ^{dew}	4.6 ^{dx}	5.1 ^{ey}	5.4 ^{eyz}	5.6 ^{ez}
0.2% Rosemary	2.3 ^{dew}	4.8 ^{dx}	5.4 ^{ey}	5.6 ^{eyz}	5.8 ^{ez}
0.15% Origanox™ +	1.8 ^{cw}	1.9 ^{cw}	2.6 ^{dx}	3.2 ^{dy}	3.7 ^{dz}
0.3% Ascorbic acid					

^aStandard error for all means = 0.20

^bSee footnote ^b Table 1

^{c,d,e,f}Means with different superscript letters within columns within postmortem age differ ($P < 0.05$)

^{v,w,x,y,z}Means with different superscript letters across rows within postmortem age differ ($P < 0.05$)

Table 6. Means^a of visual-color scores^b for different antioxidant treatments of lumbar vertebrae packaged at 6 or 14 d postmortem in ultra-low oxygen modified-atmosphere packaging and displayed from d 0 to 4

Antioxidant treatment	6 d Postmortem				
	Display day				
	0	1	2	3	4
Control	2.2 ^{cw}	2.5 ^{cwx}	2.6 ^{cxy}	2.9 ^{cyz}	3.2 ^{cz}
1.25% Ascorbic acid	2.1 ^{cx}	2.4 ^{cxy}	2.5 ^{cyz}	2.7 ^{cyz}	2.9 ^{cz}
2.5% Ascorbic acid	2.1 ^{cx}	2.3 ^{cxy}	2.4 ^{cxy}	2.7 ^{cyz}	2.9 ^{cz}
0.1% Rosemary	2.1 ^{cx}	2.5 ^{cy}	2.6 ^{cyz}	2.7 ^{cyz}	2.9 ^{cz}
0.2% Rosemary	2.1 ^{cw}	2.4 ^{cwx}	2.5 ^{cxy}	2.8 ^{cyz}	3.0 ^{cz}
0.15% Origanox TM +	2.0 ^{cw}	2.2 ^{cwx}	2.5 ^{cxy}	2.7 ^{cy}	3.0 ^{cz}
0.3% Ascorbic acid					
Antioxidant treatment	14 d Postmortem				
	Display day				
	0	1	2	3	4
Control	2.7 ^{dy}	3.7 ^{dz}	3.7 ^{ez}	3.6 ^{ez}	3.6 ^{dz}
1.25% Ascorbic acid	2.1 ^{cx}	2.3 ^{cxy}	2.5 ^{cy}	3.0 ^{cdz}	2.9 ^{cz}
2.5% Ascorbic acid	2.1 ^{cx}	2.4 ^{cxy}	2.5 ^{cy}	2.7 ^{cyz}	2.9 ^{cz}
0.1% Rosemary	2.6 ^{dy}	3.5 ^{dz}	3.5 ^{dez}	3.5 ^{ez}	3.4 ^{dz}
0.2% Rosemary	2.8 ^{dy}	3.5 ^{dz}	3.5 ^{dez}	3.7 ^{ez}	3.5 ^{dz}
0.15% Origanox TM +	2.3 ^{cdx}	2.5 ^{cx}	3.1 ^{dy}	3.4 ^{dez}	3.5 ^{dz}
0.3% Ascorbic acid					

^aStandard error for all means = 0.20
^bSee footnote ^b Table 1; standard error for all means = 0.20
^{c,d,e}Means with different superscript letters within columns within postmortem age differ ($P < 0.05$)
^{w,x,y,z}Means with different superscript letters across rows within postmortem age differ ($P < 0.05$)

QUALITY OF CHUB-PACKAGED FRESH PORK SAUSAGE DURING REFRIGERATED DISPLAY

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Introduction

The United States Code of Federal Regulations describes fresh pork sausage as being prepared with fresh or frozen pork, seasonings and not more than 3% water or ice which may be added to facilitate chopping or mixing (CFR, 2003). The product is typically utilized as a breakfast item after pan or oven grilling. Three forms of stuffed fresh pork sausage predominate: chub-packaged, patties, or small diameter links. Marketing as tray packaged preformed patties and sausage links requires additional handling and display in non-barrier films which leads to shelf life expectations in the range of 14-21 days (Anon., 2004; Sebranek et al., 2004). Bulk packaged sausage in chub form of approximately 0.45 kg units are generally expected to have a longer display life due to less handling and use of casings with oxygen barrier properties.

Objectives

The purpose of this study was to evaluate oxidative, microbial, and sensory properties of chub-packaged fresh sausage quality during a 10-week display period at 4°C.

Methodology

Three production lots of fresh pork sausage stuffed in 6.25 cm diameter casings and linked into 0.45 kg chub-style packages were each manufactured in a different week at a regional processing plant operating under USDA inspection. The chubs were blast frozen (approximately -30°C), boxed, and shipped overnight to our laboratory where they were stored for 4 days at -20°C. On day 5 all chubs were thawed at 4°C for approximately 36 hr with initial sampling on day 6 (week 1). Chubs not used initially were held refrigerated at 4°C until used for weekly evaluations. Sausage mixes had been stuffed in 3 different casings made of co-extruded polyethylene/polyvinylidene chloride (PE/PVDC) with an oxygen transmission rate (OTR) of 11±3 cc/m²/24 hr, laminated PE/PE-PVDC with an OTR of 22±6 cc/m²/24 hr, or laminated PE/metallized (Al foil) PE/PVDC with an OTR of 4±2 cc/m²/24 hr. Two chubs of each lot-film combination were removed at weekly intervals and analyzed as stated below.

Oxidative stability of sausages was determined from 2-thiobarbituric acid (TBA) reactive substances (TBARS). Samples were analyzed in duplicate following the

distillation method of Tarladgis et al. (1960) modified by analyzing distillates from 10 g samples in 100 ml of the HCl-water solvent. TBARS values were expressed as mg malonaldehyde per kg sample.

For each chub, total aerobic bacteria counts were determined following APHA (1992) methods. An 11 g center cross-section sample was aseptically removed, homogenized in a stomacher for 2 min with 99 ml of 0.1% peptone, serial diluted and then duplicate pour plates were prepared for incubation. Plates were incubated at 35°C for 48 hr and the counts were expressed as log₁₀ colony forming units (CFU/ml) per g of sample.

After removing samples for TBA values and microbiological analysis, the remaining sausage of both chubs was sliced into patties and oven grilled using a slotted grill for fat drainage and served warm to each of 24 untrained panelists. A 9-point hedonic marked line scale was used to rate each sample's overall taste/ flavor with 9=very good, 7=good, 5=average, 3=bad and 1=very bad. Although no training was conducted, panelists were familiar with this type product. Product was evaluated only through 8 weeks of refrigerated display.

Main effects of film OTR, display time (week) and their interaction were used in a general linear model analysis of variance (SAS, 1996) with replication effect and remaining interaction used as the error term. Response means were separated utilizing the lsmeans command of SAS.

Results & Discussion

TBARS values did not differ ($P>0.05$) due to type of film or replication. The increases in TBARS values with weeks of display indicate very small yet statistically significant ($P\leq 0.05$) changes in the oxidative quality of the sausage after 4 weeks (Figure 1). Sensory rancidity is generally not expected unless the raw pork when cooked yields a TBA value above the range of 0.5-1.0 (Younathan and Watts, 1960; Gray and Pearson, 1987). Murphy et al. (2004) reported very low TBARS values (estimated at 0.5 – 1.5) for a European commercial full-fat (18.7%) fresh breakfast sausage during 12 days of lighted display. Their sausages were small diameter link style whereas the chub-packaged sausages in this study are considered “bulk” packaged. The chub-packaged sausages were approximately 31% fat and did not contain any antioxidant.

Initial microbial counts were 4.3 log₁₀ for mesophilic aerobic bacteria (Figure 2) and 3.4 log₁₀ for lactic acid bacteria (not shown). These are below the 10⁵ maximum cited in Roller et al. (2002) for total bacteria counts as a Good Manufacturing Practice recommendation by the Institute of Food Science and Technology (IFST, London). With good sanitation programs and proper handling, low microbial counts were usually found on examination of fresh pork sausage produced in USDA inspected meat plants (Surkiewicz *et al.*, 1972). Total aerobic bacteria counts were below 7 log₁₀ until 7 weeks of display. When chubs were examined at 7 and 8 weeks of display, the appearance and odor observed during forming of patties for cooking and sensory evaluation were normal with no slime development, no abnormal color and no off-odor being detected. Even though no defects associated with spoilage were noticed, after 8 weeks of display sensory panel evaluation was discontinued. Other researchers have stated that spoilage (off-odor, off-flavor, sliminess, color deterioration) is generally noticeable when bacterial counts attain levels of 10⁶ – 10⁷ (Jay, 1998).

There was no overall effect ($P>0.05$) on sensory ratings of taste/flavor over 8 weeks of display due to type of film used for chub manufacture. Panel rating means show that sausage packaged in the laminated films with OTRs of 4 and 22 cc/m²/24 hr rated, on average, approximately 6, between “average (5)” and “good (7)” taste/flavor during the 10 week period (Figure 3). Pork patties from sausage packaged in the co-extruded film with an OTR of 11 cc/m²/24 hr decreased from a panel rating of approximately 6 (“average” to “good”) after week 6 in display to a mean rating of 5 (“average”) by week 8. All of these films are considered oxygen barrier films. Panel evaluations were discontinued at 8 weeks of display due to microbial outgrowth exceeding 10⁷ – 10⁸ cfu/g. No panelist listed any oxidative or flavor defect in the comments section of their panel sheets. Murphy et al. (2004) reported no association between TBARS or microbial counts and sensory acceptability scores for low fat (18.7%) pork sausage in small casing form during 12 days of display. Similar results were reported for acceptability of small diameter sausages evaluated by Roller et al. (2002). The bulk encased sausage in this study maintained quality for a longer period (approximately 8 weeks) as determined by oxidative, microbial and sensory evaluation.

Conclusions

No differences in oxidative stability or microbiological outgrowth were found for chub-packaged fresh pork sausage in films varying in oxygen transmission rate from 4 to 22 cc/m²/24 hr during 10 weeks of display at 4°C. Panelists rated the taste/flavor of cooked patties up to 8 weeks of display as being between “average” and “good” (rating of 6 on a 9 point scale) except for sausage packaged in a co-extruded film with an OTR of 11 cc which declined to “average” after 6 weeks of display. Bulk style pork sausage appears to maintain quality characteristics longer than similar link or preformed pattie style products as based on prior literature reports.

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Tables and Figures

Figure 1 – Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde/kg product) of chub-packaged pork sausage in films differing in oxygen transmission rate (OTR) and displayed at 4°C for 10 weeks.

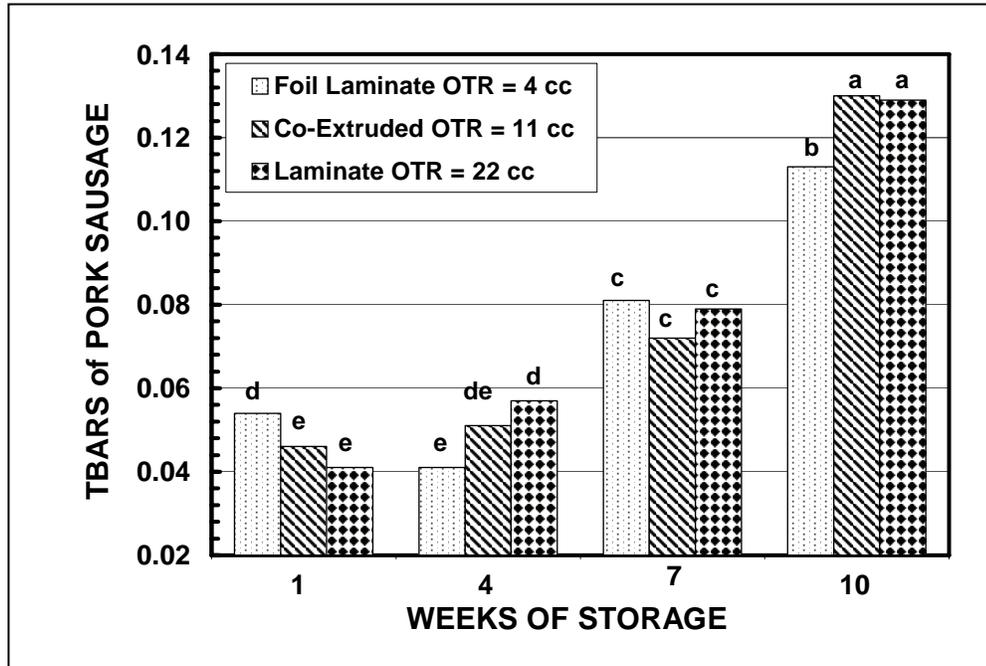


Figure 2 – Total aerobic plate counts of chub-packaged pork sausage in films differing in oxygen transmission rate (OTR) and displayed at 4°C for 10 weeks.

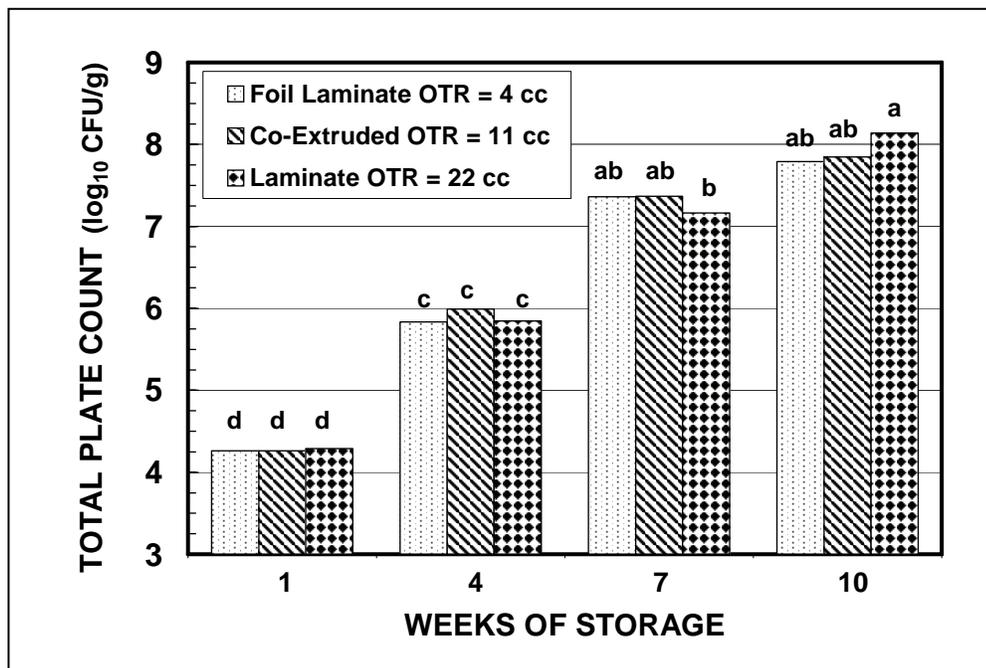
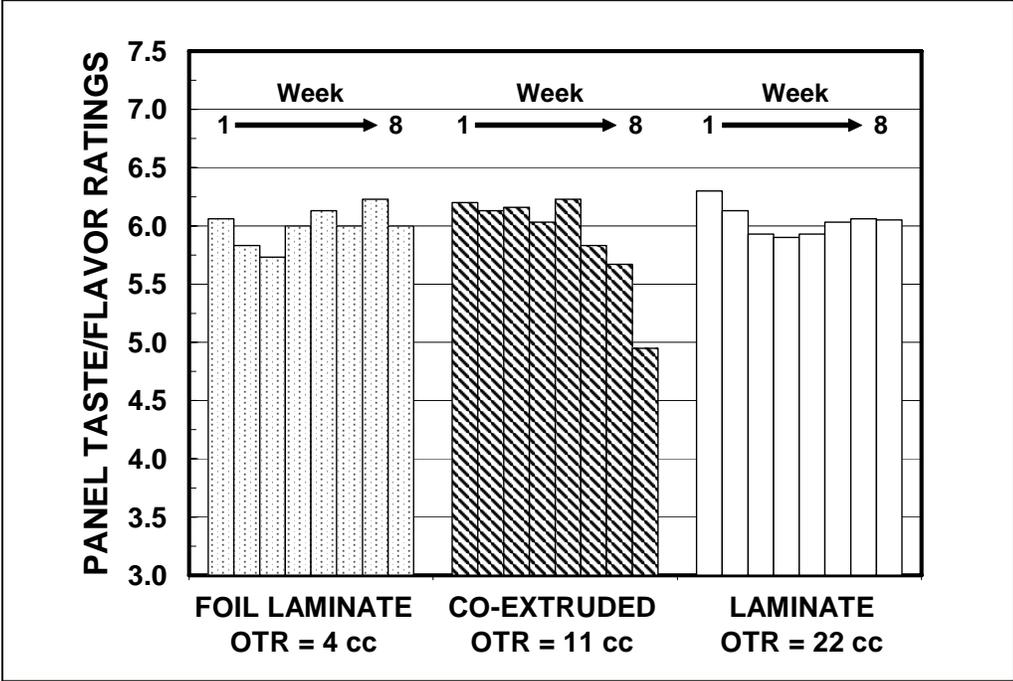


Figure 3 – Panel evaluations of taste/flavor of oven grilled patties from chub-packaged pork sausage in films differing in oxygen transmission rate (OTR) and displayed at 4°C for 8 weeks.



IMPROVEMENT OF SAFETY AND SHELF-LIFE OF THE FERMENTED SAUSAGE BY USING ELECTRON-BEAM IRRADIATION AND ANTIOXIDANTS

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Key Words: electron-beam irradiation, fermented sausage, packaging, antioxidants, lipid oxidation

Introduction

Recently, the interest in irradiation has been increased in meat industry because irradiation is one of the most effective technologies for inactivating food-borne pathogens and improving the microbial safety of meat (Gants, 1996). In addition, irradiation aimed at improving food safety also effectively reduces the number of spoilage organisms, resulting in an extension of the shelf life, particularly, of muscle foods (Min et al., 1997). The FDA approved irradiation for red meats and poultry to control foodborne pathogens and extend the product shelf-life (Gants, 1998). Fermented sausages can primarily be produced by lactic acid bacteria, which reduce the pH of the sausage within a few days. One of the major concerns in irradiating meat is its effect on the generation of off-odor and lipid oxidation, either of which can impact negatively on acceptance of such treated meat products in the marketplace (Ahn et al., 2000). Although irradiation has been studied for about 50 years and bactericidal effects have been demonstrated in fresh and cooked meat products, very few studies have been done on fermented sausages by using electron-beam irradiation, especially following the emergence of *E. coli* O157:H7 as a major pathogen of concern in these products (Sommers et al, 2004, Dickson & Maxcy, 1985). It has become clear that packaging is a critical factor that affects the quality of irradiated meat (Nam and Ahn, 2003). There have been many studies showing that the addition of antioxidants has been used to retard lipid oxidation in meat and meat products (Decker & Xu, 1998). However, there has been few attempt to combinate packaging and effect on the generation of off-odor in fermented sausages by using irradiation. In this respect, this research was conducted to examine the combined effect of electron-beam irradiation and antioxidants on the shelf -life of fermented sausages during cold storage

Objectives

The aim of this study was not only to improve of safety and shelf-life of an irradiated fermented sausage, but also to control the off-odor and lipid oxidation of the irradiated fermented sausage as related to packaging during cold storage

Methodology

Semi-dry type (Thuringer) fermented sausage was made which contained (wt%) the following: pork lean meat(74.73%), pork back fat(18.27%), starter culture(3.32%)(RosellacTM, Canada), sodium chloride(2.33%), NaNO₂(0.008%), ginger(0.06%), pepper(0.29%) and glucose(1%). The raw meat and ingredients were blended to form a batter. This mix is stuffed into cellulose casings (15 mm diameter). The samples were vacuum-packaged and then irradiated at 2, 4, 6 kGy using RF Accelerator (Korea Atomic Energy Research Institute, Daejeon, Korea). The energy and electric current were 2 MeV, 10 mA, respectively. Non-irradiated samples (i.e., control) were kept at cold temperature during the irradiation process. After irradiation, these samples were taken to lab and then fermented and aged in a chamber (Bastra, Germany) until aging(below pH 5.3). The following conditions of relative humidity (RH) and temperature were applied : day 0 until day 3, 80-94% RH and 25 °C. According to irradiation dose, these samples were used for microbiological analysis and determinations of pH. After the fermented sausage aged, samples were divided into aerobic and vacuum packaging and then these samples were stored at 4±1 °C for 2weeks. The irradiated samples were stored together with the non- irradiated samples (i.e., control). The microbial quality (total aerobic microbes, lactic acid bacteria, E-coli), pH, the color (Colorimeter, CR-210, Minolta, Japan), VBN, TBA values (Witte et al, 1970), sensory characteristics of the fermented sausage were measured after 0, 7 and 14 days of storage. To control off-odor and lipid oxidation in the fermented sausage by using irradiation, four antioxidants treatment were formulated as follows: (1) control; (2) Ascorbic acid at 1000 ppm; (3) dl- a-tocopheryl Acetate at 1000 ppm (4) Ascorbic acid + dl- α -tocopheryl Acetate at 1000 ppm (5) Rosemary powder at 1000 ppm. Also, TBA values(Witte et al, 1970), meat color, sensory characteristics(descriptive analysis with scaling) of the fermented sausage were measured after 0, 7 and 14 days storage

Results & Discussion

The pH value of fermented sausage was decreased (below pH 5.3) regardless of irradiation until aging (Table 1). American Meat Institute requires the final pH of semi-dry type sausage to fall to 5.3 within times determined by the fermentation temperature (e.g. 80 hrs at 24 °C). These criteria are presumed to prevent the growth of *S. aureus*. As shown in Table 1, the final pH value of irradiated and non-irradiated sausage corresponded to these criteria.

Table 2 and 3 show the effects of irradiation dose on microbial quality of sausages. Total aerobic counts and lactic acid bacteria count reflected the addition of the starter culture (Dickson and Maxcy 1985). As the fermentation progressed, total aerobic counts closely paralleled the lactic acid bacteria counts. The population of the total aerobic and lactic acid bacteria increased slowly regardless of irradiated and non-irradiated samples until aging. Dickson and Maxcy (1985) reported that the population of coliforms and Staphylococci could be reduced by irradiation but the growth of lactic acid bacteria increased slowly after irradiation. As shown in Table 2 and 3, irradiation with 2kGy was effective in the survival of lactic acid bacteria.

The results suggested that irradiation of 2kGy in producing fermented sausages was more effective in reducing the population of the total aerobic than non-irradiated sausage during cold storage (Table 4 and 5). The population of the total aerobic and lactic acid bacteria showed lower value with the vacuum packaging than these for aerobic packaging. Results indicated that irradiation coupled with vacuum packaging may enhance the microbial safety of fermented sausage during cold storage.

TBARS values of 2kGy-irradiated samples were significantly higher than those of non-irradiated samples during cold storage. And also, TBARS value of 2kGy-irradiated samples increased in aerobic packaging during storage, but those in vacuum packaging decreased a little (Table 6). Previous studies showed that irradiation increased lipid oxidation in aerobically packaged meat and developed off-flavors (Ahn, Nam, Du, & Jo, 2001; Patterson & Stevenson, 1995). Recently, Ahn et al. (2001) suggested that aerobic packaging would be more beneficial in reducing the characteristic irradiation off-odor during cold storage than vacuum packaging, unless lipid oxidation is a problem.

Redness (a value) of non-irradiated samples became higher than that of 2kGy-irradiated samples and also Redness (a value) of vacuum packaging samples became higher than that of aerobic packaging one as the storage period increased. Yellowness (b value) of non-irradiated samples became higher than that of 2kGy-irradiated samples and also yellowness (b value) of aerobic packaging samples became higher than that of vacuum packaging one as the storage period increased (Table 7 and 8).

Studies reporting irradiation effects on meat color have been inconsistent. An increase in redness of pork color due to irradiation has been reported (Grants and Patterson, 1991). Recently, Nam and Ahn (2002) suggested that aerobic packaging would be more desirable for the irradiated meat color than vacuum packaging, if lipid oxidation was not considered.

In the descriptive analysis, Aroma, off-flavor and color values were higher in irradiated samples than in non-irradiated samples, but overall acceptability values were lower in irradiated samples than in non-irradiated samples (Table 9)

The effects of antioxidants addition to retard lipid oxidation and off-odor in irradiated fermented sausage are shown in Table 10 and 11.

Jo and Ahn (2000) reported that the radiolytic degradation of amino acids, especially sulfur amino acids, was the main mechanism of off-odor production in irradiated meat.

In the descriptive analysis with scaling for fermented sausage (D-14), flavor, off-flavor and tenderness were better in rosemary treatment than others, and also, rosemary treatment gone highest values in the overall acceptance (Table 10). TBARS value of all treatments were lower than control treatment, especially, TBARS values of rosemary treatment samples was the showed lowest as the storage period increased (Table 11).

Conclusions

Combinations of irradiation of 2kGy and vacuum packaging in producing fermented sausage were more effective in surviving lactic acid bacteria during aging than other treatments. Also, The addition of rosemary powder could help to control off-odor and lipid oxidation of the fermented sausage during cold storage. From this result, it can be concluded that irradiation coupled with the addition of rosemary may enhance the safety and quality of the fermented sausage during cold storage

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Tables and Figures

Table 1. The pH change of the fermented sausage irradiated with electron beam

Irradiation dose (kGy)	Days			
	0 day	1 day	2 day	3 day
control	6.37±0.02 ^{ab}	6.22±0.13 ^b	6.43±0.08 ^a	5.24±0.07
2kGy	6.37±0.02 ^b	6.52±0.04 ^a	5.93±0.17 ^b	5.23±0.11
4kGy	6.42±0.05 ^a	6.52±0.05 ^a	5.76±0.07 ^b	5.23±0.07
6kGy	6.43±0.02 ^a	6.41±0.05 ^a	5.95±0.23 ^b	5.16±0.11

a~b Means ±S.D were significantly different in the same column(p<0.01)

Table 2. Changes of total plate counts(log CFU/g) of the fermented sausage irradiated with electron beam

Irradiation dose (kGy)	Days			
	0 day	1day	2 day	3 day
control	7.37±0.12 ^a	7.98±0.22 ^a	7.91±0.41	7.92±0.26 ^a
2kGy	6.24±0.27 ^b	6.83±0.31 ^c	7.99±0.27	7.97±0.10 ^a
4kGy	6.20±0.20 ^b	7.30±0.72 ^b	7.81±0.28	7.56±0.31 ^b
6kGy	6.12±0.12 ^b	5.77±0.15 ^d	7.62±0.15	7.83±0.14 ^a

a~d Means ±S.D were significantly different in the same column(p<0.01)

Table 3. Changes of lactic acid bacteria(log CFU/g) of the fermented sausage irradiated with electron beam

Irradiation dose (kGy)	Days			
	0 day	1 day	2 day	3 day
control	6.89±0.09 ^a	7.60±0.17 ^a	7.96±0.14 ^a	7.97±0.14 ^a
2kGy	6.02±0.09 ^b	6.41±0.35 ^b	7.73±0.23 ^b	7.94±0.30 ^a
4kGy	5.76±0.15 ^c	7.39±0.64 ^a	7.63±0.16 ^b	7.65±0.23 ^a
6kGy	5.51±0.09 ^d	5.66±0.60 ^b	7.63±0.15 ^b	7.68±0.21 ^b

a~d Means ±S.D were significantly different in the same column(p<0.01)

Table 4. Changes of total plate counts(log CFU/g) of differently packaged sausages irradiated during storage 4

Packaging	Irradiation dose	Storage(day)		
		0	7	14
Aerobic	0kGy	8.72±0.31 ^A	8.60±0.33 ^{Aa}	7.97±0.13 ^{Ba}
	2kGy	8.64±0.12 ^A	7.94±0.14 ^{Bb}	7.91±0.13 ^{Ba}
Vacuum	0kGy	8.70±0.21 ^A	7.88±0.15 ^{Bb}	7.90±0.35 ^{Ba}
	2kGy	8.58±0.23 ^A	7.83±0.43 ^{Bb}	7.49±0.24 ^{Ba}

a~b Means ±S.D were significantly different in the same column(p<0.01)

A-B Means ± S.D were significantly different in the same row (p<0.01)

Table 5. Changes of lactic acid bacteria(log CFU/g) of differently packaged sausages irradiated during storage 4

Packaging	Irradiation dose	Storage(day)		
		0	7	14
Aerobic	0kGy	8.53±0.23	8.50±0.14 ^a	7.97±0.23
	2kGy	8.57±0.12	8.10±0.14 ^b	7.94±0.13
Vacuum	0kGy	8.62±0.22 ^A	7.99±0.15 ^{Bb}	7.76±0.13 ^B
	2kGy	8.57±0.41 ^A	8.00±0.16 ^{Bb}	7.50±0.44 ^C

a~b Means ±S.D were significantly different in the same column(p<0.01)

A-C Means ± S.D were significantly different in the same row (p<0.01)

Table 6.TBARS values of differently packaged sausages irradiated during storage at 4

Packaging	Irradiation dose	Storage(day)		
		0	7	14
Aerobic	0kGy	0.44±0.04 ^b	0.42±0.03 ^c	0.47±0.04 ^c
	2kgy	0.80±0.04 ^a	1.79±0.05 ^a	1.43±0.03 ^b
Vacuum	0kGy	0.43±0.04 ^b	0.43±0.12 ^c	0.52±0.57 ^c
	2kgy	0.79±0.04 ^a	1.61±0.18 ^b	1.30±0.15 ^d

a~d Means ±S.D were significantly different in the same column(p<0.01)

Table 7. a-values of differently packaged sausages irradiated during storage at 4

Packaging	Irradiation dose	Storage(day)		
		0	7	14
Aerobic	0kGy	7.15±0.02 ^a	5.60±0.20 ^B	4.87±0.08 ^C
	2kgy	6.16±0.19 ^a	5.00±0.22 ^C	3.74±0.12 ^D
Vacuum	0kGy	7.15±0.02 ^b	6.87±0.16 ^A	5.89±0.18 ^A
	2kgy	6.16±0.19 ^b	5.46±0.09 ^C	5.44±0.11 ^B

a~b Means ±S.D were significantly different in the same column(p<0.01)

Table 8. b-values of differently packaged sausages irradiated during storage at 4

Packaging	Irradiation dose	Storage(day)		
		0	7	14
Aerobic	0kGy	4.79±0.13 ^a	3.94±0.06 ^A	3.66±0.06 ^a
	2kgy	4.46±0.16 ^b	3.73±0.17 ^a	3.33±0.32 ^{ab}
Vacuum	0kGy	4.79±0.13 ^a	3.79±0.13 ^a	3.04±0.33 ^b
	2kgy	4.46±0.16 ^b	3.39±0.09 ^b	3.03±0.17 ^B

a~b Means ±S.D were significantly different in the same column(p<0.01)

A~D Means ±S.D were significantly different in the same column(p<0.01)

Table 9. Descriptive analysis with scaling of vacuum packaged sausages irradiated during storage at 4

	Aroma	Off-flavor	Color	Acceptability
0 kGy	4.35±1.8 ^B	4.55±2.4	5.00±1.3	4.60±1.6
2 kgy	5.95±1.8 ^A	5.90±2.2	5.95±1.9	4.00±1.9

A~B Means ±S.D were significantly different in the same column(p<0.01)

Table 10. Descriptive analysis with scaling of vacuum packaged sausages irradiated by antioxidants (D-14)

	Aroma	flavor	Off-flavor	Color	juiciness	tenderness	Acceptability
control	6.9±1.55	7.4±1.19	5.0±2.33	6.1±1.13	5.8±1.83	5.8±1.39	4.1±2.03
vit.C	6.8±1.91 ^{AB}	6.6±1.19 ^A	5.5±2.14 ^{BC}	6.4±1.41 ^{AB}	5.8±2.55 ^{ABC}	6.3±1.98 ^{ABC}	4.5±2.45 ^C
vit.E	6.4±2.33	6.5±1.85	4.4 ±2.45	5.3±2.19	5.3±1.91	6.4±1.41	4.6±1.77
vit.C+vit.E	6.6±0.92	6.0±0.93	5.0 ±1.93	5.4±0.74	5.0 ±1.77	6.1±1.25	4.9±2.23
rosemary	6.4±1.19 ^A	5.8±1.49 ^{AB}	3.9 ±2.03 ^B	5.5±1.31 ^{AB}	4.1±1.55 ^B	5.6±2.00 ^{AB}	5.6±2.07 ^{AB}

A-B Means ± S.D were significantly different in the same row (p<0.01)

Table 11. TBARS values of vacuum packaged sausages irradiated by antioxidants

	D-0	D-7	D-14
control	1.61±0.50	1.93 ^a ±0.31	1.78±0.33
vit.C	1.56 ±0.32	1.44 ^b ±0.19	1.46±0.13
Vit. E	1.43 ±0.13	1.47 ^b ±0.18	1.41±0.22
vit c+vit.E	1.50 ±0.28	1.33 ^b ±0.13	1.34±0.12
Rosemary	1.55 ±0.33	1.49 ^b ±0.23	1.38±0.26

a~b Means ±S.D were significantly different in the same column(p<0.01)

NEURAL NETWORK BASED SAFE PROCESS TIME PREDICTOR FOR SINGLE SIDED PAN FRYING OF BEEF PATTIES

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Key Words: Meat processing; Cooking; Hamburger; Pan frying; Neural network; Modeling; Simulation

Introduction

Hamburger (meat patty) is one of the most popular foods in North America and elsewhere. Eating of undercooked ground beef may result in a type of food poisoning that is commonly called hamburger disease. Hamburger disease is caused by *E. coli* O157:H7, which lives in the intestines of cattles, and can be transferred to the outer surface of carcasses when cattles are slaughtered. The Food and Drug Administration and U.S. Department of Agriculture have recommended a minimum target cooking temperature of 68.3°C with 16 s holding time for foodservice operations to enhance food safety (USDA-FSIS 1993; FDA 1999). However, it is difficult to implement these standards in a restaurant or at home due to the complexities and difficulties in measuring the internal temperature, and the non-homogenous composition of the patties. Consequently, patties are usually overcooked resulting in poor textural quality. Therefore, to ensure reliable quality and safety of cooked patties, an accurate prediction of the temperature variations within the patties is necessary. Pan-frying, a very popular cooking method, imparts an aromatic, savoury flavor to the patties (Gisslen, 1999). Neural network (NN) is an information processing system, which learns from input/output data to determine the relationships between input/output data, and is used in pattern recognition, classification, etc. In recent years, NN has attracted researchers in many disciplines of science and engineering, since it is capable of correlating large and complex data sets.

Objectives

The objective of this research is to develop NN based prediction of safe cooking time for single-sided pan-frying of frozen and unfrozen beef patties.

Methodology

Heat and Mass (moisture and fat) Transfer Model Development

Moisture and fat transfers were assumed to be due to the capillary flow of water and fat due to shrinkage of the patty and pressure increase at increased temperature. The heat transfer was modeled using Fourier's laws, and energy balances. The models were solved with finite difference approach. Single-sided pan-frying involves patty's flipping time which can be odd and even. The equations of boundary conditions and each node are

essentially the same except that there is a reversal of nodes after flipping. The predicted temperature at each node in the patty was used to calculate the destruction rate of a microorganism. The process time was calculated for 12 log reductions of *E. coli* O157:H7 (Ou and Mittal 2005a,b).

Experimental Model Validations

Beef patties were from the same batch to ensure similar composition. Patties were pan-fried on a combo health grill. Additionally on/off proportional controller and solid state relay were used to control the frying temperature of 160°C. During the cooking, the patty was flipped 3 times. The flippings were at an interval of 120 s. Moisture and fat contents during cooking were determined at different cooking times. The temperature histories at the geometric centre, top and bottom layers of the selected patties were also measured using thermocouples with diameter of 0.25 mm, and recorded every 30 s.

The model input parameters include physical and thermal properties of hamburger patties, heating conditions, related coefficients for mass transfer, and thermal resistance of microorganisms. The simulation program was written in MATLAB language and executed on a PC computer. For unfrozen and frozen patties, the root mean squared errors (RMSE) were as: for average moisture and fat contents – 0.10 to 0.17 db and 0.064 to 0.078 db respectively; and central temperatures – 2.3 to 3.0°C. These models predicted various parameters satisfactorily.

Network Training and Testing

By taking pan temperature: 140°C, 160°C and 180°C, product thickness: 8.5, 9.5, 10.5, 11.5 and 12.5 mm, and flipping interval: 1, 1.5, 2, 2.5, and 3 min, 75 datasets were obtained using validated process models. By repeating for unfrozen and frozen patty conditions, total datasets obtained were 150. A NN with 4 input elements (pan temperature, product thickness, flipping interval and patty condition) and 1 output element was build for process time prediction (Fig. 1). Unfrozen patty condition as 0 and frozen condition as 1 were denoted. Taking 22 datasets for testing and 22 datasets for production, remaining 106 datasets were used to train the NN. After every data set training, NN weights were adjusted. Testing datasets were fed to test trained NN after training for 150 epochs. Testing errors were recorded. In the beginning of training, testing error decreased with training process. Training was continued until testing error did not decrease. If testing error was greater than the minimum testing error, then testing was continued until 200 000 epoch training was reached. After training, 22 production sets were used to verify NN performance.

Results & Discussion

First, different hidden layer arrangements and combinations of learning rate 0.5 and momentum 0.5 were chosen to examine the production results. In 22 production sets, percentage of prediction with relative error < 5% was 86.4% using 10 hidden elements NN. Then prediction errors for different learning rate and momentum values using 10 hidden elements were calculated. The maximum percentage of prediction with relative

error <5% was 90.9%, using learning rate of 0.7 and momentum of 0.7. Mean absolute error and mean related error were 17.2 s and 3.2% respectively.

Prediction by 3 layer NN could get appreciated accuracy, but sometimes 4 layer NN will get better result. Therefore, prediction results were obtained with different elements in hidden layers and with different leaning rate and momentum. The best result by 4-4 hidden layer NN with learning rate 0.9 and momentum 0.5 provided mean absolute error and mean related errors of 15.6 s and 2.7% respectively. Percentage of prediction with relative error <5% was 95.5%. Thus, prediction errors have not improved using 4 layer NN compared to 3 layer NN.

Examining all of input and output data, the output range was much bigger. It may affect the prediction accuracy. To reduce output ranges, values of process time were transformed using logarithmic function. Two NNs with $\ln(t)$ and $\log_{10} t$ as outputs were developed, respectively. After training NN, power functions (e^x or 10^x) were used to transfer predicted data to original process time, and absolute and related errors were calculated. The optimum results were given by \log_{10} function with 3 layer NN having 10 elements in hidden layer (Tables 1-2). Learning rate was 0.5 and momentum was 0.7. Mean absolute error and mean relative error were 12.70 and 2.27% respectively. Percentage of prediction with relative error <5% was 95.4%, Fig. 2 shows the relationship between original process time and predicted time using trained NN.

Conclusions

A simple 3 layer architecture of a neural network was able to predict the safe cooking time for single sided pan frying of beef patties with reasonable accuracy. A neural network is simple to use as compared to simulation process models. The NN with 4 input elements (pan temperature (140 to 180°C), patty thickness (8.5 to 12.5 mm), flipping interval (1 to 3 min) and patty condition (0 for frozen and 1 for unfrozen)) and 1 output element was built for safe process time predictions. The optimum results were obtained when output was transformed by \log_{10} function with 3 layer NN and 10 elements in the hidden layer. Learning rate was 0.5 and momentum was 0.7. The mean absolute error and mean relative error were 12.7 s and 2.3% respectively. The percentage of prediction with relative error less than 5% was 95.4%.

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Tables and Figures

Table 1. Effect of transferred process time data by \log_{10} function on prediction errors for process time using 3 – layer NN (learning rate 0.5 and momentum 0.5)

Elements in hidden layer→	5	7	10	12	14
Mean absolute error, s	18.9	17.6	17.1	14.8	17.7
s.d., s	13.3	12.6	11.6	10.9	12.6
Mean related error, %	3.5	3.2	3.2	2.9	3.4
s.d., %	1.6	1.2	1.8	1.9	2.2
% datasets within 5% relative error	81.8	77.3	86.4	90.9	72.7

Table 2. Effect of learning rate and momentum using \log_{10} function on prediction errors of process time using 3 – layer NN (10 elements in hidden layer)

Learning rate and momentum→	0.5, 0.5	0.5, 0.7	0.5, 0.9	0.7, 0.5	0.7, 0.7	0.7, 0.9	0.9, 0.5	0.9, 0.7	0.9, 0.9
Mean absolute error, s	14.8	12.7	12.9	18.3	13.7	12.7	12.8	13.6	17.4
s.d., s	10.9	12.5	13.7	13.0	11.4	13.9	10.9	11.1	14.3
Mean related error, %	2.9	2.3	2.3	3.3	2.5	2.3	2.4	2.5	3.1
s.d., %	1.9	1.9	2.0	1.9	1.9	2.1	2.0	1.9	2.0
% datasets within 5% relative error	90.9	95.4	90.9	81.8	95.4	90.9	90.9	86.4	77.3

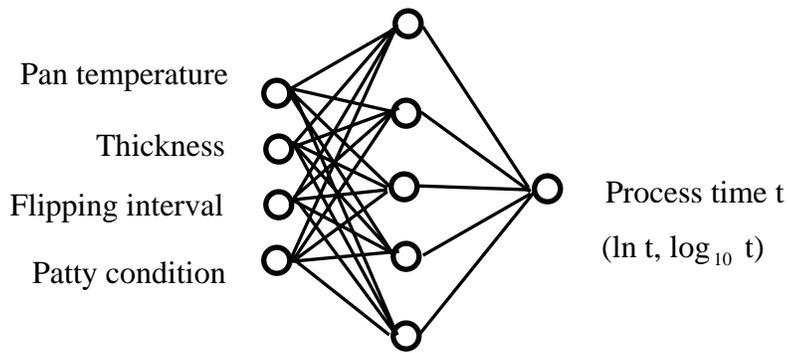


Fig 1. Neural network architecture for predicting safe process time for single sided pan frying of beef patties

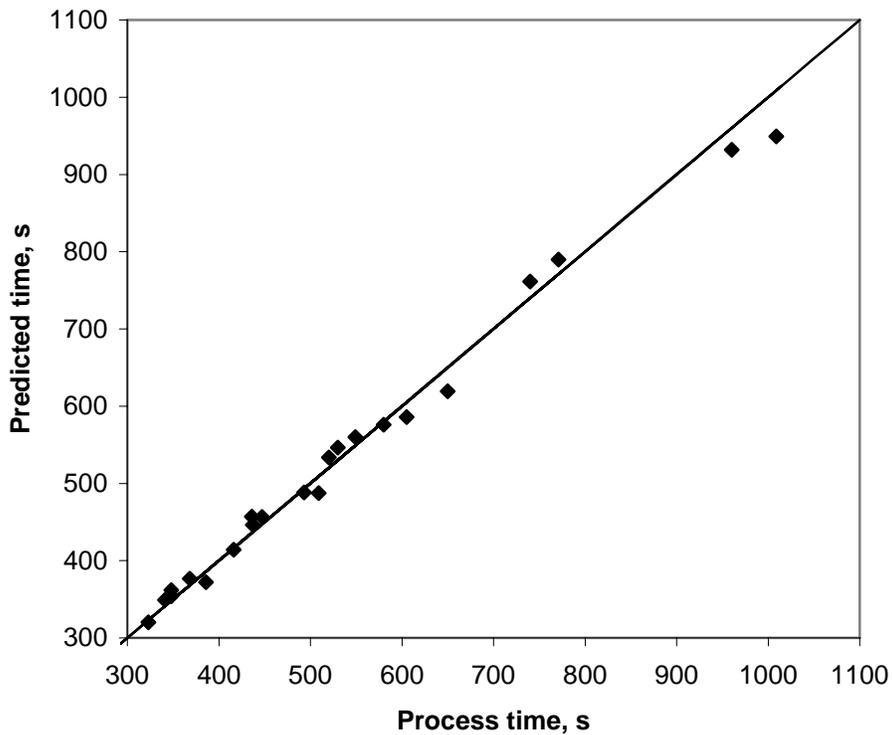


Fig. 2. Process time prediction with 3 layer NN using \log_{10} function transferred process time data (10 elements in hidden layer with learning rate 0.5 and momentum 0.7)

STUDY ON THE OPTIMIZATION OF HYDROLYSIS CONDITIONS FOR POULTRY BONE

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Introduction

China leads the world for the production of poultry and livestock. Although the resource of bones is abundant, the utilization of this high quality and nutritional substance is less emphasized. Every year, tons of bones from livestock and poultry are treated as industry materials and animal food with low value. The protein and other nutritional elements of the bones are not be fully utilized. Enzymatic hydrolysis is an effective way to utilize the bones properly with a procession that will change collagen protein of bones into peptide and L-amino acid in normal temperature and a short time. Enzymatic hydrolysis will improves the nutritional value and functional characteristic of bones, which facilitates the effective utilization of bones rather than treating as wastes. Better and more complete utilization of bones from these animals can perhaps reduce the economic loss of enterprise and bring more dollars back to the producers. Up to now, various bones had been hydrolyzed with enzymes. Surowka and Fik (1994) optimized the conditions of nitrogen recovery from chicken heads using pepsin and analyzed the amino acid composition of protein hydrolysis. Linder et al (1995) used enzymatic hydrolysis to recover protein from veal bones. Soottawat Benjakul (1997) used enzyme to hydrolyze the protein from *Pacific Whiting* solid wastes. Wang et al (2001) reported the hydrolysis of fresh swine bones with trypsin to study the optimal conditions.

The objective of the present study was to optimized the hydrolysis conditions for protein of poultry bones with enzyme Alcalase using a quadratic rotation perpendicular regressive design with three factors and five levels.

Materials And Methods

Poultry bones were ground, dried and defatted before being used.

The enzyme that was used was Alcalase 2.4 L, a food grade preparation of Novozymes, Beijing, China.

All chemicals / reagents used in this work were food-grade of reagent or reagent-grade.

Enzymatic hydrolysis

The defatted bone sample was mixed with water that made the substrate concentration was 8%. The hydrolysis was performed under different conditions with respect to temperature, pH and E/S. The pH of the mixture was kept constant by continuous addition of a 6M NaOH solution to the reaction mixture. The reaction was stopped by lowering the pH to 3-4 and temperature was 90 °C, waiting 10 min in order to deactivate the enzyme.

Nitrogen recovery (NR)

The nitrogen recovery (NR) was calculated according to the method of Linder (1995):

$$\text{NR}\% = \frac{\text{total nitrogen in supernatant (mg)}}{\text{total nitrogen in substrate (mg)}} \times 100\%$$

Degree of hydrolysis (DH)

The degree of hydrolysis (DH) was calculated according to the method of Adler-Nissen (1986):

$$DH = \frac{h}{h_{\text{tot}}} \times 100 = \frac{B \times N_b \times 100}{\alpha \times M_P \times h_{\text{tot}}}$$

Optimized Designing

The data were analyzed according to the model of quadratic rotation perpendicular regressive design that was described by Xu (1988)

Results And Discussions

Modeling

Total of twenty-three experiments were carried out with three times according to the three factors and five levels quadratic rotation perpendicular regressive design, in order to estimate residual variance for the condition of hydrolysis duration. Table 1 described that the optimal time for hydrolysis was 60 min.

The rotation design was used with three independent variables (E/S, pH, temperature), The explanatory model equation for NR% was as follows:

$$y = 71.1718 + 2.8639x_1 + 1.7778x_2 + 2.3704x_3 + 0.4962x_1x_2 + 0.0512x_1x_3 - 1.6938x_2x_3 - 1.1869x_1^2 - 1.7172x_2^2 - 2.267x_3^2 \quad (\text{a})$$

Table 1 Responses of Dependent Variables to the Hydrolysis Conditions with Alcalase

Test number	X ₁	X ₂	X ₃	E / S %	pH	T	Y	NR%
1	1	1	1	3	9	65	71.78	
2	1	1	-1	3	9	45	70.06	
3	1	-1	1	3	7	65	71.39	
4	1	-1	-1	3	7	45	62.74	
5	-1	1	1	1	9	65	66.32	
6	-1	1	-1	1	9	45	64.65	
7	-1	-1	1	1	7	65	67.76	
8	-1	-1	-1	1	7	45	59.47	
9	1.682	0	0	3.682	8	55	73.07	
10	-1.682	0	0	0.318	8	55	60.38	
11	0	1.682	0	2	9.68	55	69.04	
12	0	-1.682	0	2	6.32	55	61.41	
13	0	0	1.682	2	8	72	67.25	
14	0	0	-1.682	2	8	38	60.09	
15	0	0	0	2	8	55	72.83	
16	0	0	0	2	8	55	71.09	
17	0	0	0	2	8	55	72.45	
18	0	0	0	2	8	55	70.30	
19	0	0	0	2	8	55	71.56	
20	0	0	0	2	8	55	71.90	
21	0	0	0	2	8	55	70.72	
22	0	0	0	2	8	55	69.24	
23	0	0	0	2	8	55	70.83	

F-test

The model adequacy for the equation was tested by F-test. The result was showed in Table 2.

Table 2 Result of F-test of Nitrogen Recovery

Source	square sum	free degree	F-ratio	critical value
Regress	405.892	9	F ₂ =17.9765	F _{0.01} (9,13)=4.19
Residual	32.614	13		
Imitate	22.743	5	F ₁ =2.6866	F _{0.05} (5,8)=3.69
Error	9.871	8		
Summation	438.506	22		

F₁-test verified that three factors namely E/S, pH-value and temperature were the main factors that affected the result of Alcalase for hydrolyzing proteins of bones. F₂-test indicated that the model was adequate.

T-test

T-test was referred by the regression coefficients of the model. The result was as follows:

$$t_0=134.8905, t_1=6.6819, t_2=4.1484, t_3=5.5304, t_{11}=2.9870, t_{12}=0.8862, t_{13}=0.0915, \\ t_{22}=4.3216, t_{23}=3.0246, t_{33}=5.7052$$

Coefficients except of $t_{12}=0.8862$ and $t_{13}=0.0915$ were bigger than $t_{0.05}(13)=2.160$. This result indicated that b_{12} and b_{13} were not prominence. The best explanatory model equation for NR% was:

$$y=71.1718+2.8639x_1+1.7778x_2+2.3704x_3-1.6938x_2x_3-1.1869x_1^2-1.7172x_2^2-2.267x_3^2$$

b

The model was verified to reflect theoretically intrinsic rule on protein hydrolysis of bone according to the F-test and T-test results.

The evaluation of the effects of contribution ratio of x_1 , x_2 and x_3 was 1.8655 2.3337 and 2.3819 respectively. This indicated that temperature x_3 was the most important variable from the three codes, which affected NR and had the highest regression coefficient, followed by pH x_2 and E/S x_1 .

Interaction effects on Nitrogen Recovery(y) were observed between E/S x_1 and pH x_2 , E/S x_1 and T x_3 and pH x_2 and T x_3 .

Effect of technologic conditions on NR(y)

Effects of E/S x_1 and pH x_2 interaction on NR y

When the temperature (x_3)of model b was fixed at 0 coded level, we could get the model of E/S x_1 and pH x_2 on NR y.

$$y=71.1718+2.8639x_1+1.7778x_2-1.1869x_1^2-1.7172x_2^2$$

A three-dimensional response surfaces and contour plots were protracted based on this model (Fig.1).

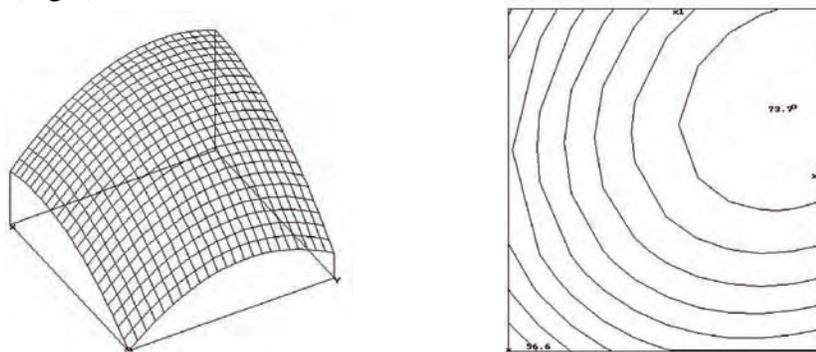


Figure 1 Effects of E/S and pH Interaction on the Amount of NR

In Figure 1 when E/S x_1 and pH x_2 were moderate, NR got maximum. The contour plot indicated that interaction effects of x_1 and x_2 were mutual. The NR was same with high E/S and low pH-value or low E/S and high pH-value. It was useful for optimizing the resultant in practice.

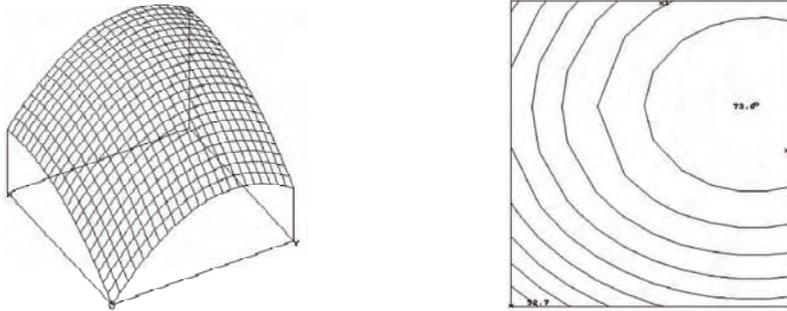


Figure 2 Effects of E/S and T Interaction on the Amount of NR

Effects of E/S x_1 and T x_3 interaction on NR y

When pH x_2 of model b was fixed at 0 coded level, we got the model of E/S x_1 and T x_3 on NR y .

$$y=71.1718+2.8639x_1+2.3704x_3-1.1869x_1^2-2.267x_3^2$$

Three-dimensional response surfaces and contour plots were protracted based on this model Fig.2 .

In Figure 2 when E/S x_1 and T x_3 were moderate, NR got maximum. The contour plot indicated that interaction effect of x_1 and x_3 were mutual.

Effects of pH x_2 and T x_3 interaction on NR y

When E/S x_1 of model b was fixed at 0 coded level, we could get the model of pH x_2 and T x_3 on NR y .

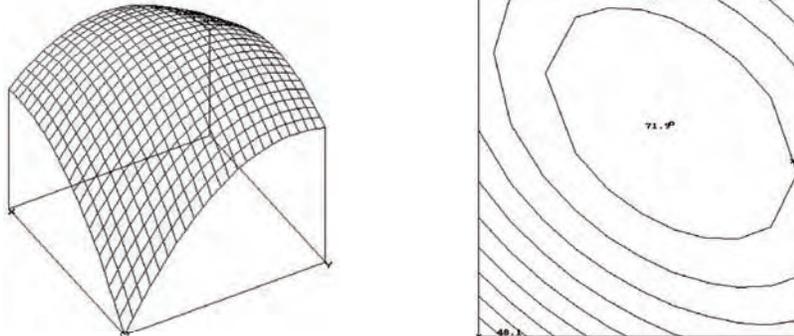


Figure 3 Effects of pH and T Interaction on the Amount of NR

Three-dimensional response surfaces and contour plots were protracted based on this model Fig.3 .

In Figure 3 when E/S x_1 was fixed at 0 coded level, the three-dimensional response surfaces of x_2 and x_3 interaction effects were presented as a parabola. At optimum pH x_2 and T x_3 , the amount of NR increased from contour plot: NR reached the maximum when pH x_2 and T x_3 were moderate. We could get a definite NR with low x_2 and high x_3 or high x_2 and low x_3 .

Optimization of hydrolysis time

According to the optimized hydrolysis condition, when the condition was E/S=3.33%, pH=8.54, temperature=58 °C, nitrogen recoveries of hydrolyzed protein in different periods of hydrolysis were showed in Figure 4.

From the curve, during the optimum reaction time (0-60 min), the amount of nitrogen recovery increased as reaction time increased, and remained constant after 90 min.

Therefore, the optimal hydrolysis time was 90 min.

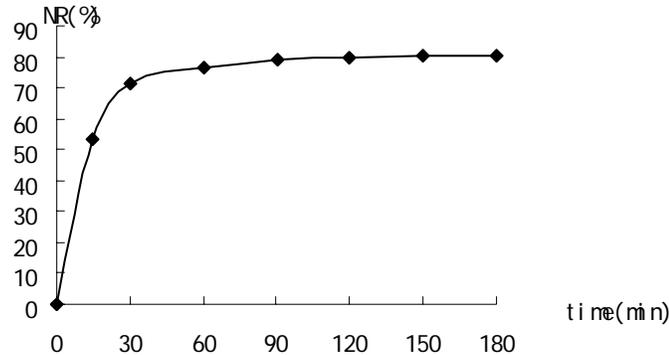


Figure 4 Dependence of Nitrogen Recovery on the Length of Hydrolysis Period

Conclusions

The regression model of quadratic rotation perpendicular regressive design with three factors and five levels was used in this study, when concentration of substrate was 8%. According to the F-test and T-test, the model reflected theoretically intrinsic law on protein hydrolysis of poultry bone. The results obtained from this pattern coincided in the actual experiment results and reflected the intrinsic relationship of hydrolysis process. With the design of rotation regression, three factors, namely E/S, pH-value and temperature were confirmed to be the main factors that affected the hydrolysis of the protein of poultry bone with Alcalase. The contributions of the three factors to nitrogen recovery ratio were: temperature > pH-value > E/S. The optimal hydrolysis conditions of hydrolyzing the protein of poultry bone with Alcalase were: concentration of enzyme (E/S)=3.33%, pH=8.54, hydrolyzing temperature=58°C, hydrolysis time=90 min and NR>75%.

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Abstract

STUDY ON THE OPTIMIZATION OF HYDROLYSIS CONDITIONS FOR POULTRY BONE

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China leads the world for the production of poultry and livestock. Although the resource of bones is abundant, the utilization of this high quality and nutritional substance is less emphasized. Every year, tons of bones from livestock and poultry are treated as industry materials and animal food with low value. The protein and other nutritional elements of the bones are not be fully utilized. Enzymatic hydrolysis is an effective way to utilize the bones properly with a procession that will change collagen protein of bones into peptide and L-amino acid in normal temperature and a short time. Enzymatic hydrolysis will improves the nutritional value and functional characteristic of bones, which facilitates the effective utilization of bones rather than treating as wastes. Better and more complete utilization of bones from these animals can perhaps reduce the economic loss of enterprise and bring more dollars back to the producers. The objective of the present study was to optimized the hydrolysis conditions for protein of poultry bones with enzyme Alcalase using a quadratic rotation perpendicular regressive design with three factors and five levels. According to the F-test and T-test, the model reflected theoretically intrinsic law on protein hydrolysis of poultry bone. The results obtained from this pattern coincided in the actual experiment results and reflected the intrinsic relationship of hydrolysis process. With the design of rotation regression, three factors, namely E/S, pH-value and temperature were confirmed to be the main factors that affected the hydrolysis of the protein of poultry bone with Alcalase. The contributions of the three factors to nitrogen recovery ratio were: temperature > pH-value > E/S. The optimal hydrolysis conditions of hydrolyzing the protein of poultry bone with Alcalase were: concentration of enzyme (E/S)=3.33%, pH=8.54, hydrolyzing temperature=58°C, hydrolysis time=90 min and NR>75%.

DEVELOPMENT AND CONSUMER ACCEPTANCE OF PRE-COOKED LAMB LEG ROASTS

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Key Words: Lamb, Pre-cooked roasts, Warmed-over flavor, Reheating, Phosphates

Introduction

With the ever-increasing trend of both females and males working outside of the home, a demand for convenience and a fast, palatable meal has been on the rise (Salvage, 1999). The red meat industry, especially the beef and pork industries, has taken steps to develop products that are not only low in fat, but also quick, easy, and convenient to prepare to accommodate the changing lifestyles of consumers (Nayga, 1993). Between 1970 and 1989 red meat consumption in the United States fell, especially lamb, decreasing by nearly 50% (Nayga, 1993). It is obvious that the lamb industry needs to regain market share and needs to find a way to rebuild consumer confidence in lamb products.

The biggest change in retail markets between 1988 and 1998 is toward convenience items (Nunes, 1998). Unfortunately, little effort has been focused on pre-cooking lamb. With most supermarkets now having a variety of ready-to-eat foods and frozen prepared foods ready for heating (Nayga, 1993), a pre-cooked lamb product seems to be economically marketable and sustainable. The field of ready-to-eat products has been expanded greatly over the past few years in order to meet the convenience demands of consumers. Therefore, the lamb industry needs a product that can achieve market share in home meal replacement to increase the consumption of lamb and reverse the negative opinion that some consumers have about the overall eating satisfaction of lamb.

Objectives

The objective of this study was to determine the most acceptable reheating method and the spices and flavorings most acceptable with lamb to develop a palatable, convenient, pre-cooked lamb leg roast.

Methodology

Preparing the Roasts

Lamb legs (n = 60) were purchased from Pak Marketing in San Angelo, Texas. First, the patella was removed from the legs and any excess subcutaneous fat was trimmed. The legs were cut into four 3.81 cm roasts with a bandsaw for a total of 240 uniform roasts.

All roasts were then trimmed free of any external fat and the seam fat containing the popliteal lymph node was removed. All roasts were injected using a Gunther Pickler Injector (model P1632, Koch Supplies, Inc., Kansas City, MO) with a 15% injection of a brine mixture of water, phosphate, and salt. The control roasts were injected in order to allocate consistency between all roasts upon reheating. After the roasts were injected, they were allowed to drain. Three spice blends were formulated (60 roasts/spice blend), and 60 roasts were used as a control group. The roasts were assigned to one of four treatments: control (CON), Italian, Mexican, and prime rib. Roasts within each leg were randomly assigned to one of four treatment groups to achieve an equal number of roasts from each leg location within each treatment. The roasts were cooked and smoked in a smokehouse (model 1000, Alkar Corporation, Lodi, WI) to an internal temperature of approximately 63°C to achieve a medium-rare degree of doneness (AMSA, 1995). After cooking, the roasts were chilled to 2°C, vacuum packed, and frozen at -10°C.

Trained Sensory Panel

Trained sensory panel analysis was conducted on 120 roasts (30 roasts/treatment) to determine the ideal reheating method and to detect differences between spices for differing palatability characteristics. The roasts were thawed and reheated one of three ways (10 roasts/treatment/reheat method); conventional oven, microwave, and boiling to an internal temperature of approximately 63°C. A conventional oven was preheated to 163°C, four roasts were placed in an aluminum pan, and 250 mL of distilled water was placed in the bottom of the pan. Two roasts were placed on paper plates, covered with wax paper, and reheated in a microwave (model JES1036PWH, General Electric, Louisville, KY). Boiling involved placing each individual roast into unsealed cryovac bags and placing them into pots containing two liters of distilled water. Roasts were cut into 1 cm × 1 cm × 3.81 cm pieces and placed into serving pans to keep them warm. Samples were served warm to a seven-member panel trained according to Cross et al. (1978). Panelist evaluated the samples based on an 8-point hedonic scale involving initial and sustained juiciness, initial and sustained tenderness, flavor intensity, characteristic lamb flavor, and overall acceptability (8 = extremely juicy, tender, intense, characteristic lamb flavor, and like extremely; 1 = extremely dry, tough, bland, uncharacteristic lamb flavor, and dislike extremely). Panelist also evaluated the samples for warmed over flavor based on a 5-point hedonic scale (1 = no WOF; 5 = extreme WOF). Samples were served under red lights to mask color differences and panelists were given apple juice and water to cleanse their palates between samples. Results from the trained panel were used to determine the most appropriate reheating method for the consumer panel.

Consumer Panel

The remaining 30 roasts per treatment were used for consumer panels. Consumer panels were conducted to determine which spice blend was preferred. Each consumer (n = 199) tasted samples from each treatment. Roasts were thawed and then reheated for 3.5 min using a microwave (model JES1036PWH, General Electric, Louisville, KY). Roasts were cut into 1.5 cm × 1.5 cm × 3.81 cm pieces and placed into serving pans to keep them warm. Panelist tasted each sample to determine juiciness, tenderness, flavor, and overall liking (6-point scale from “like extremely” to “dislike extremely”). The last

attribute of the samples panelist were asked to evaluate was the likelihood to buy the roast (5-point scale from “definitely would buy” to “definitely would not buy”) if it was available in a grocery store. After tasting all four samples, consumers were asked which sample was preferred the least and the most. In addition, consumers were asked to answer demographic questions including: marital status, gender, ethnicity, age, household income level, and how many times they have consumed lamb in the last month.

Statistical Analysis

Data from the trained sensory panel were analyzed using the GLM procedure of SAS, as a 3 x 4 factorial design (3 cooking methods and 4 spice blends) with individual roast as the experimental unit.

Data from the consumer panel were analyzed using the GLM procedure of SAS as a completely randomized design with spice blend as the treatment and individual roast sample as the experimental unit. Comparisons of frequencies from consumer panelists' responses were tested for significance ($\alpha \leq 0.05$) using Chi-Square tests.

Results & Discussion

Trained Sensory Panel

No differences ($P > 0.05$) were found between reheating methods for initial and sustained juiciness and tenderness, flavor intensity, characteristic lamb flavor, overall acceptability, and WOF. This result is similar to Boles and Parrish (1990), who found microwave reheated pre-cooked pork roasts to be palatable. In contrast with the results of the current study, Lyon and Ang (1990) found that pre-cooked chicken patties varied in their off-flavor development when heated in either a microwave or a convection oven. This could be because lamb contains fewer polyunsaturated fatty acids and the chicken patties were refrigerated and the roasts for this study were vacuum packaged and frozen. No differences ($P > 0.05$) were found for initial and sustained juiciness and tenderness, flavor intensity, characteristic lamb flavor, overall acceptability, and WOF with a spice \times reheating method interaction. A significant difference existed between spices for initial and sustained juiciness and tenderness, flavor intensity, characteristic lamb flavor, overall acceptability, and WOF. For both initial and sustained juiciness, prime rib was the juiciest ($P < 0.05$), followed by Italian, Mexican, and the CON. According to Romans et al. (2001), the addition of phosphates helps to maintain a juicy product. Therefore, no differences should have been detected between spices for juiciness since all roasts were injected with the same brine percentage. Prime rib was more tender ($P < 0.05$) compared to the Mexican and the CON; and the CON was the toughest ($P < 0.05$) when compared to other treatments for both initial and sustained tenderness. The most intense flavor, characteristic lamb flavor, and WOF were associated with the CON group when compared to other treatments ($P < 0.05$). Smith et al. (1984) reported adding phosphates to pre-cooked roasts decreases the occurrence of an off-flavor development, and Boles and Parrish (1990) discovered when phosphates were added to roasts, they were more palatable. This indicates that the spices and seasonings used were able to mask lamb

flavor and helped to prevent WOF. Prime rib was rated the most acceptable overall, followed by Italian, Mexican, and the CON group ($P < 0.05$).

Consumer Panel

The 199 consumers who participated in the study showed a wide range of demographic characteristics. The percentages and numbers are based on all data provided; however, not all of the participants provided complete demographics. Sixty-seven percent of those surveyed were male while 33% were female. Sixty-eight percent of the consumers were married, and 32% were single. The most common ethnic groups represented were Caucasian and Hispanic totaling 98%, with Caucasian totaling 91% of the total consumers surveyed. American-Indian and other ethnic groups comprised the other 2%. Because of the overwhelming percentage of Caucasians in the study and the lack of ethnic diversity, the effect of ethnicity on consumer ratings was omitted. Seventy-two percent of consumers surveyed had consumed lamb zero times in the previous month followed by 15% and 7% consuming lamb once and twice respectively in the previous month.

Results from the consumer panel are similar to the results from the trained sensory panel for tenderness, juiciness, flavor, and overall liking of the spice blend treatments. Consumers rated prime rib the most tender, juiciest, most flavorful, and the highest for overall liking ($P < 0.05$) compared to all other treatment groups. The CON was lower ($P < 0.05$) for tenderness, juiciness, flavor, and overall liking compared with other treatments.

No differences ($P > 0.05$) in tenderness, juiciness, flavor, overall liking, and likelihood to buy between the treatments were found based on differences in demographic data (not shown in tabular form). Differences did exist, however, between treatments for all palatability attributes and percentages for each category on the hedonic scale. Prime rib was rated “like extremely” a greater percentage of the time ($P < 0.05$) compared to other treatments for tenderness. Consumers chose “like extremely” a higher ($P < 0.05$) percentage of the time and “like slightly” and “dislike slightly” a lower ($P < 0.05$) percentage of the time for prime rib compared to the other three treatments for juiciness. A higher percentage ($P < 0.05$) existed for the “dislike very much” category for the CON compared to other treatments. The top four categories comprised 88.9%, 94%, 92.5%, and 96% of the CON, Italian, Mexican, and prime rib responses, respectively. Prime rib received a higher ($P < 0.05$) percentage of responses for the “like extremely” category compared to other treatments for flavor. Prime rib was chosen significantly fewer times for “like slightly” compared to other treatments and a lower ($P < 0.05$) percentage of the time for “dislike slightly” and “dislike very much” compared to the CON. Prime rib was rated higher ($P < 0.05$) for “like extremely” compared to other treatments for overall liking. These results reinforce Cassard et al. (1965) who noted tenderness and flavor were the two most important factors in determining overall lamb satisfaction

Conclusions

The results of this study revealed roasts from lamb legs can be processed and retailed as a pre-cooked product to increase the value of these primal cuts. The addition of

phosphates to a brine injection can possibly help to reduce an off-flavor development during the reheating process. Certain spices have the ability to mask lamb flavor and improve palatability characteristics held in high regard to consumers.

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DETERMINATION OF BASELINE PRODUCT CHARACTERISTICS AND PRODUCTION METHODS IN SMALL AND VERY SMALL JERKY PLANTS

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Key Words: Jerky, water activity, salt concentration, jerky production

Introduction

Jerky products have long been a popular meat based food in the United States. For much of its history, these products have enjoyed production with minimal supervision or intervention by regulatory entities. However, in the light of a jerky related Salmonellosis outbreak during October 2003, new scrutiny has been turned to jerky production nationwide. The USDA FSIS (2004) response to this outbreak was the issuance of the “Compliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants,” which provide thermal processing guidelines, humidity requirements and end product recommendations that may be applied to any jerky production scenario. The USDA did not stop with simply issuing these new guidelines. In Spring 2004, USDA solicited proposals to conduct research focused on providing small and very small manufacturing plants a means for validating their HACCP plans, specifically by achieving a 6.5 log reduction in pathogenic microorganisms. In order to develop validation studies, there was a need to determine the current state of the jerky industry in these facilities.

Objectives

The objective was to develop a survey tool and collect information relative to manufacturing equipment, processing techniques, ingredients and end item characteristics typical of jerky products. The targeted segment of the jerky industry were small and very small manufacturers primarily in Kansas, Nebraska, Missouri, and Wisconsin. This information will be used as a baseline for the design of lethality challenge studies in whole muscle and ground and formed jerky.

Methodology

Development of a four page survey tool was a coordinated effort between Kansas State University and the University of Wisconsin, with input from the University of

Nebraska. It contained questions directed at detailing equipment, ingredients and processing techniques used in jerky manufacture to gain very specific information providing up to 168 data points. Surveys were mailed to 78 small and very small manufacturers of jerky that were identified through state meat processing associations, state and federally inspected plant lists and internet resources such as state or county health department listings. The packet mailed to processors included a cover letter, the four page survey, and a request to provide four samples of each jerky product they manufactured for complimentary NaCl, pH and water activity (a_w) analysis. Approximately one month after surveys were mailed, a follow-up telephone call was made to processors who had not yet returned the completed survey. In some cases, another copy of the survey was sent to processors if the first copy had been misplaced.

Survey data were compiled and means generated for quantitative data. Qualitative data were compared to determine if consensus existed among industry practices. Jerky samples that were returned with surveys were stored in original packaging at ambient temperature for up to two weeks before analysis. All lab analyses were done by KSU or UW, with one exception. NaCl analyses for jerky samples received by UW were done at KSU or a commercial lab. All samples were pulverized in a blender (Waring Blender 700 Model 33BL79, Waring Products Division, New Hartford, CT) with a 10 cm blending cup by two 30 sec bursts, pausing for 120 sec between bursts to help minimize heat buildup. Water activity was measured at ambient temperature using an AquaLab Series 3 TE (Decagon Devices, Pullman, WA), using standard seven ml disposable sample cups. Measurement of pH was based on a 10 g sample of pulverized sample material, blended with 110 ml distilled, deionized water for two min in a stomacher (Seward Stomacher 400 laboratory blender, Seward Medical, London, UK) and tested immediately with a pH meter (Accumet Portable model AP61 Fisher Scientific, Fair Lawn, NJ). NaCl content was determined using Quantab® titrators for chloride (0.05-1.0% NaCl, Hach Company, Loveland, CO), using five g pulverized sample material combined with 45 ml distilled, deionized water according to manufacturers instructions. Resulting measurements were plotted against one another and mean and standard deviation determined for each of the three parameters, by species and as a full lot. Results from whole muscle versus formed products were not separated. Upon completion of all analyses, each plant received their individual results plotted against results for all plants. Anonymity of plant results was maintained on all but their own sample data.

Results & Discussion

Of the 78 plants contacted, 37 contributed surveys for a 47% response rate. Of these, 33 plants contributed 61 product samples. Analytical results of product samples revealed a mean a_w of 0.74 (SD 0.10); mean NaCl of 6.85% (SD 2.5%); and mean pH of 5.85 (SD 0.33). Specific results broken down by species are shown in Table 1. Some key results garnered from the survey include thermal processing extremes and nearly a complete lack of relative humidity instrumentation. These ranged from processing jerky at 51.6C for 45 min followed by 3C increases every 45 minutes to reach an oven temperature of 62.8C maximum, to processing jerky at 93.3C for 6-7 h. Perhaps the single most important finding is the depth of variability within this industry. The survey tool provided the

means to quantify some of the extremes in this variability and in that way will serve as a pivotal source of information for developing challenge studies.

The survey instrument revealed that approximately 56% of all product produced is the whole muscle type and 44% is ground or chunked and formed. Total plant output was not requested nor reported. NaCl concentration in mixes ranged from 1% to more than 85%, and pickup of wet marinade ranged from 3% or less to 100%.

Survey results further revealed that 34 of 37 manufacturers (92%) used only a smokehouse for thermal processing, 3 of 37 (8%) used a commercial oven and one manufacturer (3%) used both a smokehouse and an oven for thermal processing. Within the thermal environment, nine manufacturers determined the wet bulb temperature to yield relative humidity and only one employed a relative humidity instrument. However, 35 of 37 manufacturers claimed they are able to control humidity. Controls employed are closing the dampers (35%), steam injection (8%), direct addition of water (8%), placing a pan of water in the house (3%) or a combination of these methods (43%). All respondents who claimed a combination of these methods used steam as one component, indicating that 51% of these manufacturers are using steam injection to increase their humidity. Manufacturers reported an average of 44% cooked yield.

Ingredients used in the production of jerky may be grouped into two broad categories, those with a functional purpose besides flavor and those added solely for flavor. Functional ingredients found included sodium nitrite, sodium erythorbate, lactic acid, and potassium sorbate. Thirty-two of 37 respondents (86%) indicated the use of sodium nitrite, however only 15 (41%) also indicated the use of sodium erythorbate as a cure accelerator. Those manufacturers not using a cure accelerator reported time spans ranging from 45 min (wet marinade) up to 7 d (dry cure) between the time seasonings are added until the drying process is begun. Only five (14%) manufacturers reported the use of potassium sorbate for mold inhibition and one (3%) reported using lactic acid to acidify the product. Ingredients used for their seasoning properties included soy sauce (62%), vinegar (8%), worcestershire sauce (16%) and tabasco sauce (5%). Lesser used ingredients included pineapple juice, teriyaki sauce, apple cider, garlic salt, and char oil (3% each).

Respondents indicated a variety of packaging methods were employed including vacuum (78%), no vacuum (32%), and gas flush (14%). Storage, distribution, and marketing were variable as well, with 32% reporting refrigerated storage, 3% frozen and 5 of 13 (38%) reported shelf-stable storage and distribution. However, examination of package markings upon receipt of samples indicated that 12 of 13 (92%) of sample packages lack any precautionary handling markings, and may be interpreted as shelf-stable.

Conclusions

In the Midwest, slightly more than half of the jerky produced by small and very small facilities that responded to the survey was whole muscle, while slightly less than half was ground and formed or chunked and formed. Jerky a_w and pH among different species was similar. NaCl levels among species may be similar, however an insufficient number of turkey samples were tested to conclude this similarity. This data provides sufficient correlation to proceed with lethality studies around a_w 0.74, NaCl 6.85% and pH 5.85,

with additional study around upper and lower limits of each parameter in combination to determine any synergistic affect between parameters.

Survey results confirmed the hypothesis that there is widespread variability in thermal processing and most other production methods from one manufacturer to another. Variables included processing time and temperature, processing environment relative humidity and method employed to control humidity. Extremes in thermal processing provide useful baselines under which to proceed with lethality studies. Challenge studies at thermal extremes, in the presence of mean product characteristics, can provide inactivation or protective effects for targeted microorganisms.

The survey revealed that, while a significant proportion of the industry has instituted the use of sodium nitrite into their product, many manufacturers may not be realizing any functional properties of this ingredient. Fifty-three percent of those using nitrite are not using a cure accelerator to speed the curing process. Among this group of products, product is allowed a lower limit of 45 minutes for the curing reaction to occur. With 86% of the industry using cure, challenge studies should be conducted with cure for most iterations, and without cure in a comparative study at the mean level of product and thermal processing conditions.

Slightly less widespread use of acidic ingredients are used in jerky production. Ingredients such as vinegar (pH 2.41), worcestershire sauce (pH 3.50), soy sauce (pH 4.83) and tabasco sauce (pH 3.20) are used by some manufacturers as flavor additives, primarily as a constituent of a wet marinade process. The pH of a marinade mixture and length of time held in marinade prior to thermal processing should be considered in a challenge study.

Packaging methods employed throughout the industry varied slightly with most manufacturers using vacuum packs or modified atmosphere packaging to extend shelf-life by preventing oxidation or mold growth. The use of potassium sorbate as a mold inhibitor was limited. One consideration in a challenge study would be the impact of packaging and subsequent shelf life on pathogen lethality during storage.

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Tables and Figures

Table 1. Mean NaCl content, a_w and pH of whole muscle, ground and formed, and chunked and formed jerky by species from 33 primarily Midwestern companies.

Species	% NaCl	SD¹	A_w	SD	pH	SD
Beef	6.90% (n=28)	2.54%	0.74 (n=49)	0.10	5.86 (n=49)	0.32
Pork	7.58% (n=5)	2.42%	0.71 (n=6)	0.09	5.87 (n=6)	0.35
Turkey	3.73% (n=1)	-	0.77 (n=4)	0.09	5.82 (n=4)	0.46
Buffalo	4.95% (n=1)	-	0.79 (n=2)	0.08	5.52 (n=2)	0.16
Maximum for all Products	11.54%		0.90			6.60
Minimum for all Products	2.10%		0.51			5.00

¹SD=Standard deviation.

EVALUATION OF THE FUNCTIONAL PROPERTIES OF NON-MEAT INGREDIENTS IN COOKED CHICKEN FILLETS

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Key Words: chicken, phosphate, non-meat ingredients.

Introduction

Poultry consumption has increased at a faster rate than other meat species due largely to its low fat content, health promoting image (Muguruma *et al.*, 2003) as well as convenience and ease of preparation (Anderson and Shugan, 1991). Salt and phosphate addition during marination of chicken has been shown to increase water holding capacity (Barbut 2002) and generates a more tender and juicy product with enhanced yields (Shahidi & Synowiecki 1997). However the presence of excessive amounts of phosphate can lead to organoleptic defects (texture and flavour) and negatively influence calcium, iron and magnesium balances in the body which can increase the risks of bone disease (Shahidi & Synowiecki 1997). Addition of alternative ingredients such as proteins and polysaccharides may also enhance meat cook yields and water holding capacity of poultry products (McKee and Alvarado, 2004; Swenson and Katen). Moreover, increasing EU consumer demands for meat products possessing a clean ingredient listing are leading to a shift in the use of artificial and chemically modified ingredients to more natural adjuncts. A study by Kerry (1996) showed that selected physically modified proteins and starches increase the water holding capacity of cooked cured ham products similar to phosphates and thus forms the basis for this research.

Objectives

To assess a range of test brines containing added proteins or starches in fresh chicken fillets and to compare final cooked yields of these test samples against controls with and without added tripolyphosphate.

Methodology

10 Chicken fillets (mean = 100g) were injected (15%) with either a control brine (A) consisting of water and sodium chloride (1.0% residual in the meat) or a control (B) containing water, NaCl and tripolyphosphate (TPP) with the latter two ingredients being incorporated in final meat products at residual levels of 1.0% and 0.3%, respectively. The % ingredients in the brine were calculated as follows: $[(100 + IR) * \% RIL] / IR$. (where IR = injection rate and RIL = residual ingredient level in the injected meat). A range of test proteins including Na caseinate, Ca caseinate, physically modified high gelling whey

protein concentrates –WPCs- 35% WPC A and 35% WPC B (Dairygold Co Operative Society Ltd. Mitchelstown, Co. Cork, Ireland.), 90% Soya isolate 548 (Protein International, St Louis, MO, USA.), 75% WPC (Denmark Proteins AS. Nr Vium, DK-6920, Videbaek, Denmark.) and 76% egg albumin (Lactosan (UK) Ltd, 5 Swingbourne Drive, Springwood Industrial Estate, Braintree, UK) were added to test brines as a replacement for TPP and compared against controls (A and B). Breast fillets were injected (Inject Star, model: BI-18) to a target of 15% with test ingredients at a residual level of 1.0%. with the exception of 35% WPCs (A and B) which were added at residual levels of 1, 2 and 3%. Similarly, a range of native test starches including: Pea A -inner fibre-, Pea B -outer fibre- (Cosucra SA., Rue de la Sucrierie 1, B-7740 Warcoing, Belgium), Potato A -physically modified- and Potato B -physically modified- (National Starch and Chemical Company, Prestbury Court, Greenscourt Business Park, 333 Styal Road, Manchester, M22 5LW, UK.) were assessed in test fillets at residual levels of 0.5% and 1.0% and compared against controls. After injection, chicken breasts were massaged for 10 min at low speed using a paddle mixer (Kenwood, model: Chef KM 300). Treatments were cooked at 110°C for 15 min to a minimum core temperature of 74°C in a steam cabinet (Zanussi, model: IOGN1/1) and subsequently cooled at 2°C x 16h prior to weighing. All treatments were carried out in triplicate. Yield and delta yield values were calculated according the following formulae:

$$\% \text{ Green Yield} = (\text{Cooked Weight} - \text{Original Weight}) \times 100$$

$$\text{Delta Yield (for each batch)} = \text{Test Ingredient Yield} - \text{Control Yield}$$

SPSS statistical computer software package (SPSS version 8, Ireland, 79 Old Kilmainham Road, Dublin 8, Ireland.) was employed in this study. This was a repeated Measures design with one “between-subjects” factor. The effects of a particular food/ingredient was investigated using a relevant SPSS output, which included tests for “within-subjects” effects (i.e. effect of cooking), tests for “between-subjects” effects and multiple comparisons within each day. Tukey’s test was used to adjust for multiple comparisons.

Results & Discussion

Cook yield values and delta values (test – control) are presented for test protein ingredients (Table 1) and polysaccharides (Table 2). Delta values were calculated in order to minimise the effect of cooking variability. Results showed that the addition of 0.3% residual TPP (Control B) increased cook yields on average by 10% over control A. With the exception of Na caseinate, all non meat proteins increased cook yields over control A (Table 1). However, only the physically modified high gelling 35% WPCs (A and B) at a 3% residual level and 75% WPC at a 2% residual level increased cook yields over control B. 75% Egg albumen at a 1% residual level also increased cook yield over controls (A and B) however it was quite difficult to hydrate and had a tendency to foam. In a number of instances test proteins were too difficult to solubilise or too viscous to inject/pump through the injector and were rejected (N/A). Na caseinate produced the most negative delta yield of all ingredients assessed and is in agreement with Kerry (1996). All test polysaccharides, increased cook yield versus control A, with pea starch A and potato starch A giving a greater yield than control B containing TPP (Table 2).

Conclusions

The addition of 0.3% residual TPP increase cook yields on average by 10%. Non meat proteins 35% protein WPCs (A and B) at a 3% level, 75% WPC at a 2% level gave yields equal to control B. Na caseinate gave the lowest cook yields of all test ingredients assessed. Polysaccharides also increased the yield of chicken fillets versus control A with pea starch A and potato starch A giving improved yields over control B. Results show that there is a potential to utilise physically modified proteins and polysaccharides as TPP replacers in cooked poultry processing.

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Tables and Figures

Table 1

Mean (n = 30) (i) % yield and (ii) delta (test – control) values of test chicken fillets containing added non meat proteins and controls containing 0 or 0.25% residual TPP (controls A and B, respectively).

% added	Sodium caseinate	Calcium caseinate	WPC 35 A	WPC 35 B	Soya	WPC 75	Pea	Egg Albumin
(i)								
Con A	77.1±2.1 ^a	80.2±3.1 ^a	78.4±2.6 ^a	78.6±1.5 ^a	79.1±2.5 ^a	77.0±3.0 ^a	77.2±3.8 ^a	77.1±3.1 ^a
Con B	86.9±3.3 ^b	91.1±4.2 ^b	89.1±2.4 ^b	89.1±2.0 ^b	90.3±2.0 ^b	86.0±2.8 ^b	86.4±3.4 ^b	86.4±2.1 ^b
1.0	68.8±3.3 ^c	81.6±2.7 ^a	82.3±2.6 ^b	84.2±3.0 ^b	86.2±1.6 ^b	84.6±2.4 ^b	84.9±2.1 ^b	88.1±2.4 ^c
2.0	N/A	N/A	85.1±3.3 ^b	88.6±2.8 ^b	N/A	86.4±1.9 ^c	N/A	N/A
3.0	N/A	N/A	89.5±3.5 ^b	89.8±2.1 ^c	N/A	N/A	N/A	N/A
(ii)								
Con B	9.9	10.9	10.7	10.5	11.2	9.0	9.2	9.2
1.0	- 8.3	1.4	3.9	5.6	7.1	7.6	7.7	11.0
2.0	N/A	N/A	6.7	10.0	N/A	9.4	N/A	N/A
3.0	N/A	N/A	11.1	11.2	N/A	N/A	N/A	N/A

Control A (Con A) = 1% residual NaCL, Control B (Con B) = 1% NaCL and 0.3% 0.3% residual phosphate

Different letters (a-c) within the same column indicate significant (p<0.05) differences

N/A = test ingredients insoluble or unacceptable for injection.

Table 2

Mean (n = 30) (i) % yield and (ii) delta (test - control) of test chicken fillets containing added polysaccharides and controls containing 0 or 0.25% residual TPP (a and b, respectively).

% added	Pea A (inner fibre)	Pea B (outer fibre)	Potato A	Potato B
(i)				
Con A	77.1±2.3 ^a	77.8±3.1 ^a	77.3±2.6 ^a	78.0±2.0 ^a
Con B	87.0 ±2.6 ^b	87.3±2.2 ^b	88.3±3.0 ^b	88.4±1.9 ^b
0.5	84.4±2.4 ^b	81.8±2.2 ^b	83.5±3.3 ^b	88.1±2.7 ^b
1.0	87.4±1.9 ^c	82.8±2.5 ^b	89.0±3.1 ^c	87.0±1.6 ^b
(ii)				
Con B	9.8	9.5	11.0	10.7
0.5	7.3	4.0	6.1	10.0
1.0	10.1	5.0	10.7	9.0

Control A (Con A) = 1% residual NaCL, Control B (Con B) = 1% NaCL and 0.3% residual phosphate

Different letters (a-c) within the same column indicate significant ($p < 0.05$) differences

N/A = test ingredients insoluble or unacceptable for injection.

Table 3

Mean (n = 30) (i) % yield and (ii) delta (test - control) of test chicken fillets containing added polysaccharides and controls containing 0 or 0.25% residual TPP (a and b, respectively).

% added	Pea A (inner fibre)	Pea B (outer fibre)	Potato A	Potato B
(i)				
Con A	77.1±2.3 ^a	77.8±3.1 ^a	77.3±2.6 ^a	78.0±2.0 ^a
Con B	87.0 ±2.6 ^b	87.3±2.2 ^b	88.3±3.0 ^b	88.4±1.9 ^b
0.5	84.4±2.4 ^b	81.8±2.2 ^b	83.5±3.3 ^b	88.1±2.7 ^b
1.0	87.4±1.9 ^c	82.8±2.5 ^b	89.0±3.1 ^c	87.0±1.6 ^b
(ii)				
Con B	9.8	9.5	11.0	10.7
0.5	7.3	4.0	6.1	10.0
1.0	10.1	5.0	10.7	9.0

Control A (Con A) = 1% residual NaCL, Control B (Con B) = 1% NaCL and 0.3% residual phosphate

Different letters (a-c) within the same column indicate significant ($p < 0.05$) differences

N/A = test ingredients insoluble or unacceptable for injection.

**EVALUATION OF PHYSICO-CHEMICAL AND TEXTURAL PROPERTIES,
AND SENSORY EVALUATION OF LOW-FAT/SALT RESTRUCTURED HAMS
MANUFACTURED WITH MILK PROTEINS**

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Key Words: sensory evaluation, low-fat/salt restructured hams, milk proteins

Introduction

Salt functions as improving water holding capacity, fat binding, attributing the desirable texture and sensory properties and increasing cooking yield. The combination of salt and phosphate decreased cooking loss of meats and meat products, and similar firmness were observed with lower sodium content when phosphate was used (Ruusunen et al., 2005). However, excessive sodium intake may be potential possibility to induce hypertension (Cortlandt, 2004). Thus, reducing sodium content in diet is highly recommended. But, salt reduction in the manufacture of meat products may have problems related to flavor, texture and sensory characteristics. Instead of completely removing the salt content, minimum level of salt may be required to produce meat products to keep cooking loss (Shults and Wierbicki, 1973) and protein solubility (Gordon and Barbut, 1992). Microbial transglutaminase (MTGase) has been successfully used to manufacture of low-fat, low-salt functional meat products for the improvement of textural characteristics and binding capacity (Chin and Chung, 2003). Improved enzymatic activities of MTGase were observed when the sodium chloride was added to the water. Replacing the salt with MTGase and other functional ingredients might be alternatives to reduce the salt or phosphate level in meat products (Muguruma et al., 2003; Serrano et al., 2004). However, no information is available how many level of salt could be reduced with the combination of MTGase and various milk proteins in the manufacture of restructured ham without quality defects.

Objectives

To complement the quality defects of restructured hams(Rh) due to salt reduction, the combination of 0.3% microbial transglutaminase (MTGase, Ajinomoto Activa-TG B) and 1% milk proteins (Sodium Caseinate and Whey protein concentrate) with reduced salt levels from 1.5 to 0.5% were added to the manufacture of Rh. Thus, the objectives of this study were to compare the low-fat/salt restructured hams with MTGase and milk proteins with the control with 1.5% salt and finally, select the most similar characteristics to those with control.

Methodology

The low-salt/fat restructured hams, which contained MTGase and milk proteins (sodium caseinate and whey protein concentrate), were manufactured followed by Lee and Chin (2004). Proximate analyses were measured the contents of moisture, fat and protein according to AOAC (1995) procedure. Color measurements were performed using a color meter and expressed by L, a and b values. Water holding capacity (WHC, %) and cookin loss (CL, %) were measured to detemine the functional proeprties. WHC was measured according to the modified method of Jauregui et al (1981) and CL was evaluated by a weigh difference of cooking before and after. Texture analyses were measrued by Instron Universal Testing Machine and expressed by hardness, springiness, gumminess, chewiness and cohesiveness (Bourne, 1978). Warner-Bratzler shear values were also measured. Sensory evaluation was performed by 7 sensory panels and evaluated the color, taste, texture and overall acceptability rating as #1 being a most like, and # 8 least like with 8-points hedonic scale. Statistical analyses were performed by one-way analysis of variance (ANOVA) using the SPSS 12.0 (2003) program with three replicates, and then the significant differences among tratments were evaluated by Student-Newman-Keuls multiple range test ($p < 0.05$).

Results & Discussion

pH and proximate composition of the low-salt/fat restructured hams(Rhs) manufactured with MTGase and milk proteins are shown in Table 1. pH values, moisture, fat and protein contents of Rhs were 6.07-6.22, 68-72%, 2-5%, 18-22%, respectively. Addition of MTGase and milk proteins with various salt levels did not affect ($p > 0.05$) pH values and proximate composition (Table 1). Hunter L, a, b values of Rhs were not affected by addition of MTGase and milk proteins ($p > 0.05$) either. Among treatments tested, expressible moisture (EM, %) were not different from each other ($p > 0.05$), whereas cooking loss (CL, %) were significantly affected ($P < 0.05$) by salt level and milk proteins ($p < 0.05$). As salt level increased, CL decreased and Rhs containing sodium caeinate (SC) had lower CL than those with whey protein concentrate(WPC) due to higher protein contents of SC rather than WPC (Table 2, $p < 0.05$). These results are confirmed with the previous report by Marsh (1983) who reported that myofibrillar proteins extracted by salt lead to enhance the protein solubility and water holding capacity. Especially, Rhs containing 1.0% WPC and 0.5% or 1% SC with either 0.5 and 1% salt had lower CL, whereas Rhs containing 1.0% salt and 1.0% SC were not different from control in CL. These results indicated that the addition of 0.3% MTGase with 1% SC had similar functionality to those with control (Table 2, $p > 0.05$). In texture profile analysis, no differences in hardness were observed among treatments ($p > 0.05$), whereas Rhs with 0.3% MTGase and milk proteins had significantly higher than the control in other textural characteristics ($p < 0.05$, Table 3). These results showed that addition of 0.3% MTGase and 1% milk proteins may contribute to enhance the binding capacity (Motoki, 1998). On the other hand, no differences in shear values were observed among treatments including the control ($p > 0.05$, Table 3). In sensory evaluation, Rhs containing 1.0% salt with 0.3% MTGase and milk proteins (SC, WPC) were similar to the control. Furthermore, Rhs containing 1% salt with 0.3% MTGase and 1.0% SC were similar to

those containing 1.5% salt with TGase and 1% SC(T3), and more acceptable than control(1.5% salt alone). Even, Rhs containing 0.5% salt in combined with 0.3% MTGase and 1% SC had similar sensory palatability to the control.

Conclusions

The minimum level of salt to manufacture of low-fat/salt Rhs was at least 1.0%. Rhs containing 1% salt with the combination of 0.3% MTGase and 1% SC had similar CL (%) to the control. The addition of 0.3% MTGase and 1% milk proteins increased most texture profile analysis values except hardness, resulting in improved textural characteristics as compared to the control. Sensory evaluation data showed that the combinations of 0.5% or 1% salt with 0.3% MTGase and 1% SC or 1% WPC were similar sensory characteristics to the control. In conclusion, the combination of 0.3% MTGase and 1% milk proteins compensated for the defects of Rhs with reducing salt levels, and SC was more efficient for the substrate of MTGase than WPC.

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Tables and Figures

Table 1. pH and proximate composition of restructured meat products as affected by the addition of TGase and milk proteins

Treatments* (Trt)	pH	Moist Fat Protein		
		------(%)-----		
Control	6.10±0.06	71.3±1.89	3.11±1.34	18.3±2.44
Trt 1	6.07±0.05	69.7±2.87	4.39±2.69	21.3±2.73
Trt 2	6.11±0.12	70.3±0.29	3.56±0.28	19.6±3.36
Trt 3	6.22±0.14	71.0±0.82	2.96±0.88	19.2±2.27
Trt 4	6.13±0.05	68.0±1.95	4.60±1.52	21.0±0.47
Trt 5	6.14±0.04	69.9±1.36	3.10±1.10	18.9±1.81

* Treatments (Trt): Control, Salt 1.5%; Treatment (Trt) 1, Salt 0.5%, Sodium Caseinate (SC) 1.0% + Transglutaminase (TGase) 0.3%; Trt 2, Salt 1.0%, SC 1.0% + TGase 0.3%; Trt 3, Salt 1.5%, SC 1.0% + TGase 0.3%; Trt 4, Salt 0.5%, Whey Protein Concentrate (WPC) 1.0% + TGase 0.3%; Trt 5, Salt 1.0%, WPC 1.0% + TGase 0.3%

Table 2. Hunter color and functional properties of restructured meat products as affected by the addition of TGase and milk proteins

Treatments* (Trt)	Hunter Color Values			Functional Properties	
	L	a	b	EM	CL
Control	66.6±1.40	13.2±1.31	4.19±0.25	22.8±2.03	7.82±1.06 ^d
Trt 1	65.8±1.15	14.4±0.63	5.35±1.11	20.4±4.54	14.1±2.88 ^{b*}
Trt 2	66.8±0.85	12.8±0.46	4.36±0.18	22.9±4.97	10.7±1.55 ^c
Trt 3	63.2±2.17	13.8±0.55	4.37±0.71	20.7±3.09	7.21±0.82 ^d
Trt 4	66.2±1.40	13.8±0.30	5.12±0.07	21.3±1.56	19.2±1.04 ^{a*}
Trt 5	65.5±3.79	13.0±0.85	4.54±0.08	19.4±3.60	14.2±1.10 ^{b*}

EM= expressible moisture (%); CL=cooking loss (%), * Treatments (Trts): See in Table 1

^{a-d} Means having same superscript within same column are not different (P<0.05)

*: Significant (P<0.05) are expressed by the asterisk (Dunnett's T-test)

Table 4. Textural properties of restructured meat products as affected by the addition of TGase and milk proteins

Treatments* (Trt)	Hardness (g)	Springiness (cm)	Cohesiveness	Gumminess	Chewiness	Shear value
Control	5377±212	0.28±0.02 ^b	0.24±0.02 ^b	1251±75 ^b	340±49 ^b	4.28±1.65
Trt 1	7367±1161	0.30±0.01 ^b	0.28±0.01 ^a	2057±262 ^a	594±56 ^a	2.38±0.54
Trt 2	7104±1066	0.35±0.03 ^{ab}	0.28±0.01 ^a	2000±278 ^a	694±47 ^a	2.94±0.57
Trt 3	7150±2193	0.37±0.05 ^a	0.28±0.03 ^a	1913±395 ^a	691±62 ^a	3.41±0.87
Trt 4	6715±1291	0.28±0.01 ^b	0.28±0.01 ^a	1905±351 ^a	538±118 ^a	3.87±1.49
Trt 5	6515±342	0.31±0.04 ^{ab}	0.28±0.01 ^a	1808±78 ^a	539±58 ^a	4.01±1.71

Treatments(Trts): See in Table 1, ^{a-b}: See in Table 2

A COMPARISON OF WILTSHIRE CURED PRE-RIGOR AND CONVENTIONALLY CHILLED PORK CUTS WITH ADDITIONAL CURING AGENTS

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Key Words: Wiltshire cure, hot-boned, cold-boned, rosemary, liquid smoke

Introduction

Pre-rigor or hot boning (HB) of meat has clear economic advantages over conventional or cold boning (CB). Losses during chilling, cost of refrigeration, labour and transportation can be considerably reduced (Pisula and Tysbury, 1996).

Wiltshire curing is a tank curing process developed in the United Kingdom over 300 years ago. Traditionally, Wiltshire curing is a lengthy process as it involves an injection, immersion and maturation stage before cooking, but results in quality products with a unique flavour.

The main functions of smoke on meat are to develop aroma, flavour and colour and also to preserve and protect from oxidation (Schwanke et al., 1996).

The use of smoke flavouring preparations offers some advantages over the traditional wood smoke. They are more economical and ecologically acceptable, they are easily applied to products and the concentration can be strictly controlled.

Rosemary contains a number of antioxidant compounds which have been shown to reduce lipid oxidation in meat (Fernandez-Lopez et al., 2005; McCarthy et al., 2001). This antioxidant activity in combination with a desirable and distinctive flavour has made rosemary the herb of choice for this study.

The present study was designed to take all of the above factors into account to produce a high quality cooked ham and cured loin with an acceptable shelf life.

Objectives

This task involved comparative analysis of Wiltshire cured pre-rigor and conventionally chilled pork primal cuts in terms of quality measurements as a function of time. Additional curing agents, rosemary oil and liquid smoke were incorporated into the hot and cold boning processes and their effects on product quality was assessed.

Methodology

Large White x Landrace pigs (n=24) were conventionally slaughtered. The leg and loin were removed from the right hand side of the carcass immediately after slaughter and

deboned. The left hand side entered the chill room (1°C) for 24 hours, after which time, the leg and loin were also removed and the bones removed. The hot-boned and cold-boned legs and loins were Wiltshire cured. This involved brine (18% NaCl, 0.10% NaNO₃, 0.07% NaNO₂) injection (w/v 10%) followed by immersion in a cover brine (24% NaCl, 0.30% NaNO₃, 0.10% NaNO₂, at 4°C) for 3 days and finally a maturation period where the meat was stacked and stored at 4°C for 4 days. Liquid smoke, which was a combination of an aqueous solution of food grade carbohydrates and natural wood smoke flavours (80:20 ratio of smok-EZ MB 12: P-50) and rosemary extract in sunflower oil (Guinness Chemicals Ireland Ltd., Portlaoise, Ireland) were then applied to specified treatment groups. The meat was dipped in liquid smoke and then held to remove excess. The rosemary extract was rubbed on by hand; making sure that full coverage of the meat was achieved. The legs were placed in shrink bags (Cryovac, BB4L), vacuum packed and cooked (dry heat, internal temp. 80°C for 8-9 hours). The loins were chilled to 0°C (to allow ease of slicing) and then sliced. Samples of cooked hams and cured loins were stored under MAP (70% N₂, 30% CO₂) in visual display units (4°C, 616 lux) for up to 28 days.

Colour measurements were made using a Cr-300 Chromameter (Minolta Co. Ltd, Japan) set on the Hunter colour scale and reported as the 'L' lightness, 'a' redness and 'b' yellowness values. Warner Bratzler shear force (tenderness) values were measured on a texture analyzer (Stable Micro Systems, UK) and results expressed in kg. Lipid oxidation was measured by the distillation method of Tarladgis *et al.* (1960) as modified by Ke *et al.* (1977) and results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde/kg muscle. Cook loss was expressed as percentages of the original sample weight.

Results & Discussion

The addition of rosemary to both hot and cold boned hams resulted in greater ($p < 0.01$) lipid stability over the entire 28 day storage period. Liquid smoke application also ($p < 0.01$) reduced oxidation after 7 days of storage. The combination of rosemary and liquid smoke resulted in lower ($p < 0.05$) TBAR values after 14, 21 and 28 days in modified atmosphere packs at 4°C. Lipid oxidation was significantly greater ($p < 0.001$) in HB and CB cured loins treated with liquid smoke after 1 and 14 days of storage. Schwanke *et al.* (1996) reported that liquid smoke at a rate of 0.2 to 0.5% in product formulation was found to have effective antioxidant properties. It contains phenol, syringol, guaiacol, catechol and eugenol, as well as acetic, propionic and other organic acids which lower pH and destroy bacterial cell walls (Pszczola, 1995). High levels (10%) have been shown to have a pro-oxidant effect (Maga, 1988), however. Rosemary extract was a successful antioxidant on loins up to 21 days of chilled storage ($p < 0.05$).

Contrary to other studies (Moeller and Jensen, 1993), Warner Bratzler shear force (WBSF) values concluded that boning time did not have a significant effect on tenderness values of hams or loins. Hams treated with rosemary extract prior to cooking had lower ($p < 0.01$) WBSF values after 1, 14 and 28 days of storage. Liquid smoke also had a positive effect ($p < 0.05$) on tenderness up to day 14 in a retail display unit.

Cook loss was significantly greater ($p < 0.05$) for CB hams which had been dipped in liquid smoke. The rosemary did not have any apparent effect on cook loss. There was a

significant difference noted between cook losses of hot and cold boned cured loins. The HB samples exhibited lower cook losses after 1 ($p<0.001$), 7 ($p<0.05$) and 21 ($p<0.05$) days of storage. The functional properties of HB meat, especially water holding and emulsifying capacities are superior to CB meat. This is due to the higher pH and level of ATP, dissociation of actomyosin and better solubility of myofibrillar proteins (Pisula and Tyburcy, 1996).

Rosemary addition had a significant effect ($p<0.05$) on lightness values of hams over the entire storage period. Cured loins treated with rosemary had greater 'L' values after 7 ($p<0.05$), 14 ($p<0.001$) and 21 ($p<0.001$) days of storage. The hams and loins treated with liquid smoke were significantly darker than the control after 14 ($p<0.001$) and 21 ($p<0.01$) days of retail storage.

The application of rosemary resulted in greater ham 'a' values after 14 ($p<0.001$) and 21 ($p<0.01$) storage days. Loins which had been dipped in liquid smoke had significantly lower 'a' values after 14 ($p<0.001$) and 21 ($p<0.01$) days of storage. CB and HB hams and loins which had been treated with rosemary extract had significantly lower 'b' values than the control group on all sampling days except on loins after 7 days of retail storage. The application of liquid smoke resulted in lower ($p<0.001$) 'b' values of hams and loins on all storage days except on hams after 7 days of retail storage at 4°C.

Conclusions

In conclusion, a combination of liquid smoke treatment and rosemary reduced oxidation in Wiltshire cured hams over the storage period. The colour of both hams and loins was enhanced by the antioxidant effect of the rosemary extract. TBAR analysis and colour evaluation of cured loins assessed over the storage period, would suggest that variation in processing treatment did not have a consistent impact on the meat and further modification to improve the final product was necessary.

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Tables and Figures

Table 1. Effect of liquid smoke and rosemary extract on Hunter lightness ('L'), Hunter redness ('a'), Hunter yellowness ('b'), TBARS and Warner Bratzler shear force (WBSF) (mean \pm standard deviation) values of Wiltshire cured hams stored in modified atmosphere packs (70% N₂, 30% CO₂) at 4°C.

	Carcass Side	Treatment Group	Storage time, days					
			1	7	14	21	28	
"L"	Hot-boned	Control	53.74 \pm 2.93	53.51 \pm 1.81	56.93 \pm 2.51			
		Smoke	50.57 \pm 1.47	50.61 \pm 0.95	53.87 \pm 1.25			
		Rosemary	57.86 \pm 0.72	56.31 \pm 1.97	56.73 \pm 1.44			
		Rosemary, Smoke	54.75 \pm 3.09	56.50 \pm 2.39	56.55 \pm 1.95			
	Cold-boned	Control	56.30 \pm 3.68	53.47 \pm 2.83	57.88 \pm 4.71			
		Smoke	57.95 \pm 1.72	50.12 \pm 1.06	53.66 \pm 0.86			
		Rosemary	56.52 \pm 3.33	55.27 \pm 0.21	55.79 \pm 3.36			
		Rosemary, Smoke	57.44 \pm 2.49	58.34 \pm 2.35	58.00 \pm 3.30			
	"a"	Hot-boned	Control	6.08 \pm 1.26	7.71 \pm 0.28	7.31 \pm 0.79		
			Smoke	8.05 \pm 1.02	6.98 \pm 0.65	7.68 \pm 0.53		
			Rosemary	6.98 \pm 0.90	6.99 \pm 0.42	7.26 \pm 1.13		
			Rosemary, Smoke	6.98 \pm 1.42	5.76 \pm 1.88	8.14 \pm 1.96		
Cold-boned		Control	7.26 \pm 0.52	7.23 \pm 1.48	7.40 \pm 1.15			
		Smoke	7.51 \pm 0.87	5.43 \pm 0.21	7.85 \pm 0.27			
		Rosemary	6.52 \pm 1.86	3.6 \pm 0.42	7.25 \pm 1.12			
		Rosemary, Smoke	5.62 \pm 0.73	5.87 \pm 1.35	6.62 \pm 2.45			
'b'		Hot-boned	Control	6.72 \pm 1.27	7.93 \pm 0.39	7.30 \pm 0.65		
			Smoke	6.01 \pm 0.58	5.21 \pm 0.63	6.59 \pm 0.61		
			Rosemary	7.52 \pm 0.66	7.68 \pm 0.18	6.83 \pm 1.57		
			Rosemary, Smoke	7.11 \pm 0.88	8.25 \pm 1.42	6.22 \pm 0.68		
	Cold-boned	Control	6.99 \pm 1.27	7.59 \pm 1.11	7.25 \pm 0.96			
		Smoke	7.34 \pm 0.62	6.01 \pm 1.02	5.55 \pm 0.49			
		Rosemary	8.29 \pm 0.82	8.44 \pm 0.03	7.72 \pm 1.22			
		Rosemary, Smoke	8.66 \pm 0.55	8.39 \pm 0.87	7.94 \pm 1.17			
	TBARS	Hot-boned	Control	0.66 \pm 0.65	0.38 \pm 0.15	0.31 \pm 0.13	0.38 \pm 0.17	0.34 \pm 0.19
			Smoke	0.45 \pm 0.13	0.27 \pm 0.06	0.28 \pm 0.11	0.23 \pm 0.05	0.44 \pm 0.34

		Rosemary	0.15 ± 0.05	0.20 ± 0.05	0.28 ± 0.09	0.22 ± 0.08	0.43 ± 0.26
		Rosemary, Smoke	0.34 ± 0.13	0.16 ± 0.08	0.26 ± 0.09	0.21 ± 0.04	0.13 ± 0.02
	Cold-boned	Control	0.41 ± 0.14	0.30 ± 0.08	0.30 ± 0.17	0.41 ± 0.21	0.38 ± 0.09
		Smoke	0.46 ± 0.27	0.30 ± 0.08	0.57 ± 0.15	0.27 ± 0.09	0.58 ± 0.26
		Rosemary	0.16 ± 0.07	0.23 ± 0.06	0.27 ± 0.08	0.18 ± 0.06	0.31 ± 0.15
		Rosemary, Smoke	0.35 ± 0.15	0.19 ± 0.06	0.23 ± 0.04	0.23 ± 0.04	0.18 ± 0.05
WBSF	Hot-boned	Control	5.15 ± 1.13	5.85 ± 1.81	8.40 ± 2.79	6.56 ± 0.91	4.56 ± 0.75
		Smoke	7.11 ± 2.08	5.90 ± 1.56	4.90 ± 0.82	5.76 ± 1.66	6.59 ± 1.47
		Rosemary	4.42 ± 1.11	6.61 ± 0.83	4.80 ± 0.72	5.51 ± 1.05	4.61 ± 0.79
		Rosemary, Smoke	6.07 ± 1.30	5.78 ± 1.34	4.65 ± 1.03	6.63 ± 2.54	5.47 ± 1.19
	Cold-boned	Control	7.07 ± 1.70	7.35 ± 3.28	9.53 ± 3.20	8.41 ± 2.82	5.78 ± 1.48
		Smoke	7.13 ± 1.30	5.96 ± 0.81	5.69 ± 0.98	6.23 ± 1.45	6.55 ± 1.68
		Rosemary	4.48 ± 0.92	7.24 ± 1.24	4.17 ± 1.12	5.19 ± 0.52	4.58 ± 1.01
		Rosemary, Smoke	5.55 ± 2.04	5.24 ± 1.60	4.10 ± 0.79	6.78 ± 0.95	5.66 ± 1.34

Table 2. Effect of liquid smoke and rosemary extract on Hunter lightness ('L'), Hunter redness ('a'), Hunter yellowness ('b'), TBARS, cook loss and Warner Bratzler shear force (mean ± standard deviation) values of Wiltshire cured loins stored in modified atmosphere packs (70% N₂, 30% CO₂) at 4°C.

	Carcass Side	Treatment Group	Storage time, days				
			1	7	14	21	28
'L'	Hot-boned	Control		40.57 ± 0.38	40.65 ± 0.89	42.92 ± 1.27	
		Smoke		42.47 ± 1.61	43.56 ± 0.25	45.17 ± 0.33	
		Rosemary		40.75 ± 1.37	39.43 ± 0.25	41.46 ± 1.45	
		Rosemary, Smoke		41.00 ± 0.95	41.06 ± 0.91	41.33 ± 1.84	
	Cold-boned	Control		43.27 ± 1.26	43.13 ± 1.66	43.64 ± 0.35	
		Smoke		43.75 ± 2.46	46.98 ± 0.55	41.52 ± 0.69	
		Rosemary		42.48 ± 1.29	43.27 ± 0.49	43.39 ± 1.07	
		Rosemary, Smoke		41.29 ± 1.99	41.94 ± 1.07	41.40 ± 2.34	
'a'	Hot-boned	Control		1.75 ± 0.61	1.31 ± 0.67	1.48 ± 0.38	
		Smoke		2.01 ± 0.49	0.99 ± 0.69	1.13 ± 0.53	
		Rosemary		1.64 ± 0.64	0.88 ± 0.48	2.47 ± 0.76	
		Rosemary, Smoke		1.66 ± 0.73	2.26 ± 0.36	2.53 ± 1.80	
	Cold-boned	Control		1.71 ± 0.91	1.80 ± 0.97	1.76 ± 0.99	
		Smoke		2.91 ± 1.03	1.39 ± 0.42	1.42 ± 0.32	
		Rosemary		1.47 ± 0.56	1.25 ± 0.29	1.91 ± 1.04	
		Rosemary, Smoke		2.15 ± 0.71	2.10 ± 0.40	3.28 ± 1.44	
'b'	Hot-boned	Control		2.85 ± 0.43	2.94 ± 0.45	3.02 ± 0.13	
		Smoke		3.63 ± 0.45	4.89 ± 0.59	4.77 ± 0.64	
		Rosemary		7.52 ± 0.66	7.68 ± 0.18	6.83 ± 1.57	
		Rosemary, Smoke		3.64 ± 0.60	3.84 ± 0.16	3.22 ± 1.05	
	Cold-boned	Control		3.09 ± 0.60	3.58 ± 0.55	3.16 ± 0.62	
		Smoke		4.30 ± 0.19	4.72 ± 0.69	5.25 ± 0.89	
		Rosemary		8.29 ± 0.82	8.44 ± 0.03	7.72 ± 1.22	
		Rosemary, Smoke		4.17 ± 0.89	4.29 ± 0.28	3.72 ± 1.09	
TBARS	Hot-boned	Control	0.21 ± 0.05	0.21 ± 0.04	0.26 ± 0.09	0.31 ± 0.17	0.32 ± 0.20
		Smoke	0.45 ± 0.12	0.30 ± 0.07	0.58 ± 0.17	0.27 ± 0.05	0.42 ± 0.22
		Rosemary	0.08 ± 0.02	0.20 ± 0.04	0.26 ± 0.12	0.21 ± 0.09	0.39 ± 0.12
		Rosemary, Smoke	0.32 ± 0.13	0.15 ± 0.07	0.36 ± 0.11	0.25 ± 0.09	0.16 ± 0.02
	Cold-boned	Control	0.23 ± 0.07	0.21 ± 0.07	0.26 ± 0.15	0.32 ± 0.19	0.24 ± 0.17
		Smoke	0.31 ± 0.05	0.25 ± 0.11	0.49 ± 0.09	0.30 ± 0.04	0.28 ± 0.07

		Rosemary	0.05 ± 0.01	0.18 ± 0.02	0.29 ± 0.13	0.17 ± 0.08	0.35 ± 0.10
		Rosemary, Smoke	0.42 ± 0.18	0.23 ± 0.12	0.25 ± 0.06	0.23 ± 0.05	0.19 ± 0.07
Cook Loss	Hot-boned	Control	22.18 ± 1.52	19.71 ± 2.70	20.82 ± 3.48	17.40 ± 3.09	20.65 ± 5.99
		Smoke	23.41 ± 3.18	22.40 ± 3.76	18.44 ± 3.60	25.40 ± 3.51	21.31 ± 4.12
		Rosemary	22.66 ± 2.16	17.32 ± 2.69	17.55 ± 2.92	17.64 ± 4.48	18.48 ± 2.94
	Cold-boned	Rosemary, Smoke	21.92 ± 4.60	20.95 ± 2.73	20.28 ± 3.63	22.56 ± 2.67	21.89 ± 4.24
		Control	25.61 ± 2.92	22.39 ± 2.79	23.17 ± 2.89	20.16 ± 3.19	20.52 ± 4.97
		Smoke	26.83 ± 3.40	24.66 ± 3.15	21.49 ± 4.65	26.30 ± 2.44	22.57 ± 3.69
WBSF	Hot-boned	Rosemary	25.25 ± 2.31	19.80 ± 4.09	19.83 ± 2.47	18.01 ± 3.65	22.48 ± 2.82
		Rosemary, Smoke	25.69 ± 3.55	21.97 ± 3.32	21.35 ± 4.25	27.57 ± 1.56	22.79 ± 3.84
		Control	5.20 ± 0.72	5.05 ± 0.88	6.66 ± 0.80	6.13 ± 1.42	4.49 ± 0.53
	Cold-boned	Smoke	6.07 ± 1.08	5.23 ± 0.51	6.01 ± 0.97	5.26 ± 0.97	5.90 ± 1.28
		Rosemary	5.84 ± 1.30	5.10 ± 0.91	5.25 ± 0.84	5.84 ± 1.79	5.62 ± 1.36
		Rosemary, Smoke	5.56 ± 1.23	4.73 ± 0.59	5.28 ± 0.89	3.88 ± 0.39	3.97 ± 0.66
		Control	5.68 ± 1.00	5.94 ± 1.08	7.27 ± 1.34	6.46 ± 2.06	5.37 ± 0.94
		Smoke	6.33 ± 1.43	5.34 ± 0.88	5.96 ± 0.77	4.62 ± 0.49	4.85 ± 0.83
		Rosemary	5.49 ± 0.70	5.79 ± 1.04	4.35 ± 0.68	4.88 ± 0.79	4.66 ± 0.76
		Rosemary, Smoke	5.05 ± 0.58	4.55 ± 0.54	4.87 ± 0.52	3.71 ± 0.62	3.86 ± 0.79

THE EFFECT OF PHOSPHATE LEVEL AND TUMBLING TIME ON HOT AND COLD BONED CURED PIGMEAT PRODUCTS

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Key Words: Phosphate, tumbling, TBARS, hot boning

Introduction

Hot boning, the removal of muscles from the still hot carcass, was developed in response to commercial demands for lower energy use and chilling space requirements (Taylor *et al.*, 1995). Accelerated boning of meat carcasses has been conducted for a number of years. Accelerated boning enables production costs to be reduced due to lower labour requirements, a reduction in chiller space, energy input and increased product turnover (Cross and Seideman, 1985). Wide-scale accelerated boning of pork has yet to be employed, largely due to the reluctance to employ new systems and the potential detrimental impact on tenderness resulting from the rapid drop in muscle temperature that occurs when muscles are removed pre-rigor (Rees *et al.*, 2002). There is much emphasis now on healthier products and ingredients within the food sector. Reduced phosphate levels would meet the demand for healthier brine formulations. A reduced tumbling time would have economical benefits and increased product turnover for the processor. The present study was designed to determine the optimal combination of methods in order to improve overall pork quality and maximize tenderness following accelerated boning.

Objectives

The main objective of this study was to investigate the effect of two phosphate levels (1.25 and 2.50%) and two tumbling rates (4 and 16 hours) on hot and cold boned hams and shoulders.

Methodology

The right hand sides of 24 Large White x Landrace pork carcasses were hot boned (HB) at a local abattoir immediately after slaughter. The left sides were chilled at 1°C for 24 hours *postmortem* before cold boning (CB). The HB and CB legs and shoulders were then cured using 2 levels of phosphate (1.25 or 2.50%) and 2 intermittent tumbling rates (short [4 hours] or long [16 hours]). The meat was vacuum packed in cook in-shrink bags (Cryovac BB4L) and placed in ham moulds. The legs and shoulders were cooked in dry heat, to an internal temperature of 80°C for 8-9 hours (h). Samples of hams and shoulders

were stored under MAP (70%N₂, 30%CO₂) conditions in visual display units (4°C, 616 lux) for up to 28 days.

Colour measurements were made using a Cr-300 Chromameter (Minolta Co. Ltd, Japan) set on the Hunter colour scale and reported as the 'L' lightness, 'a' redness and 'b' yellowness values (after 7, 14 and 21 days of retail storage). Warner Bratzler shear force (tenderness) values were measured on a texture analyzer (Stable Micro Systems, UK) and results expressed in kg (after 1, 7, 14, 21 and 28 days of retail storage). Lipid oxidation was measured by the distillation method of Tarladgis *et al.* (1960) as modified by Ke *et al.* (1977) and results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde/kg muscle (after 1, 7, 14, 21 and 28 days of retail storage).

Results & Discussion

Phosphate added at the lower, 1.25% level, resulted in greater lipid stability of cooked hams and shoulders during retail storage at 4°C. Ham TBAR values were lower after 1 (p<0.001), 7 (p<0.001) and 28 (p<0.01) and cooked shoulders after 1 (p<0.001) and 21 (p<0.01) days of storage. Tumbling for 4 h resulted in lower oxidation values of hams and shoulders after 7 (p<0.001), 14 (p<0.001) and 28 (p<0.01) storage days. A combination of lower phosphate levels and shorter tumbling time significantly reduced lipid oxidation during retail storage.

Warner Bratzler shear force (WBSF) values were lower in hams in the higher, 2.5% phosphate, treatment group after 1 (p<0.01), 7 (p<0.001), 14 (p<0.05) and 28 (p<0.01) days of storage. A slight (p<0.05) difference in tenderness was observed between hot and cold boned hams after 21 days of retail storage only.

Overall lower yield values were reported for HB hams (87.75%) and shoulders (84.22%) compared to their CB counterparts, 90.45% and 91.23%, respectively. It has been suggested that increased water holding capacity of HB meat may be diminished by cold shortening brought on by injection of deep chilled brine (Pisula and Tyburcy, 1996). In this study tumbling time did not have a significant effect on water holding capacity of hams or shoulders (results not shown).

Statistical analysis showed that CB cooked shoulders had higher (p<0.05) 'L' lightness values than HB samples. Tumbling for 4 h improved the 'a' redness values of shoulders after 7 (p<0.05) and 21 (p<0.01) days in a retail display unit.

Conclusions

A combination of lower phosphate levels and shorter tumbling time significantly reduced lipid oxidation during retail storage. Warner Bratzler shear force (WBSF) values were lower in hams in the higher, 2.5% phosphate, treatment group during retail storage. Overall lower yield values were reported for hot boned hams and shoulders compared to cold boned samples.

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Tables and Figures

Table 1. Effect of phosphate level and tumbling time on Hunter lightness (“L”), Hunter redness (“a”), TBARS, cook loss and Warner Bratzler shear force (mean ± standard deviation) values of cooked hams stored in modified atmosphere packs (70% N₂, 30% CO₂) at 4°C.

	Carcass Side	Treatment Group	Storage time, days					
			1	7	14	21	28	
“L”	Hot-boned	Control	55.30 ± 2.66	56.32 ± 2.19	56.58 ± 2.20			
		Short T	55.96 ± 3.57	54.10 ± 2.48	57.05 ± 2.82			
		Low P	52.81 ± 6.93	57.15 ± 4.72	55.42 ± 3.24			
		Low P, short T	55.79 ± 3.77	55.34 ± 5.18	55.99 ± 3.05			
	Cold-boned	Control	58.20 ± 3.96	59.49 ± 2.80	58.18 ± 3.48			
		Short T	56.64 ± 2.68	53.88 ± 3.41	57.24 ± 3.55			
		Low P	58.42 ± 1.97	56.42 ± 4.62	56.06 ± 4.79			
		Low P, short T	57.48 ± 2.99	55.29 ± 4.35	57.93 ± 3.51			
	“a”	Hot-boned	Control	7.23 ± 0.83	6.77 ± 0.90	7.12 ± 1.08		
			Short T	7.96 ± 0.92	7.78 ± 0.93	8.54 ± 0.60		
			Low P	8.73 ± 1.52	7.61 ± 1.11	8.11 ± 0.65		
			Low P, short T	8.12 ± 1.33	8.12 ± 1.03	4.76 ± 1.15		
Cold-boned		Control	6.82 ± 1.04	6.76 ± 0.72	7.21 ± 0.26			
		Short T	8.14 ± 0.80	7.66 ± 1.33	8.53 ± 0.64			
		Low P	8.17 ± 1.42	8.10 ± 1.39	7.88 ± 1.04			
		Low P, short T	7.95 ± 1.69	7.38 ± 1.33	7.52 ± 1.92			

	'b'	Hot-boned	Control		7.37 ± 0.75	7.14 ± 1.07	7.56 ± 0.92	
			Short T		7.11 ± 0.77	7.19 ± 0.71	8.07 ± 0.61	
			Low P		6.97 ± 1.41	7.85 ± 1.06	7.34 ± 0.77	
			Low P, short T		7.67 ± 0.98	8.08 ± 0.93	8.06 ± 0.64	
		Cold-boned	Control		7.50 ± 0.98	7.13 ± 0.91	7.64 ± 0.92	
			Short T		7.28 ± 1.00	7.08 ± 0.85	7.59 ± 0.93	
			Low P		8.50 ± 1.17	8.19 ± 0.85	7.87 ± 1.10	
			Low P, short T		7.64 ± 1.11	7.34 ± 0.97	7.90 ± 1.63	
	TBARS	Hot-boned	Control	0.44 ± 0.14	0.52 ± 0.12	0.49 ± 0.12	0.39 ± 0.11	0.49 ± 0.27
			Short T	0.57 ± 0.17	0.50 ± 0.20	0.41 ± 0.11	0.51 ± 0.15	0.47 ± 0.10
			Low P	0.40 ± 0.03	0.37 ± 0.10	0.31 ± 0.12	0.44 ± 0.08	0.30 ± 0.09
			Low P, short T	0.26 ± 0.07	0.39 ± 0.06	0.39 ± 0.06	0.32 ± 0.06	0.32 ± 0.07
Cold-boned		Control	0.49 ± 0.14	0.45 ± 0.06	0.46 ± 0.11	0.34 ± 0.05	0.42 ± 0.07	
		Short T	0.47 ± 0.17	0.35 ± 0.12	0.38 ± 0.14	0.38 ± 0.17	0.39 ± 0.10	
		Low P	0.38 ± 0.11	0.28 ± 0.18	0.28 ± 0.25	0.35 ± 0.09	0.26 ± 0.07	
		Low P, short T	0.40 ± 0.17	0.38 ± 0.12	0.36 ± 0.04	0.31 ± 0.07	0.29 ± 0.08	
WBSF		Hot-boned	Control	3.81 ± 0.60	3.78 ± 1.02	3.07 ± 0.95	3.28 ± 0.52	2.90 ± 0.55
			Short T	2.87 ± 0.41	2.95 ± 0.98	2.51 ± 0.36	2.99 ± 0.55	2.77 ± 0.63
			Low P	3.81 ± 0.60	3.78 ± 1.02	3.07 ± 0.95	3.28 ± 0.52	2.90 ± 0.55
			Low P, short T	4.06 ± 1.24	3.52 ± 0.74	3.78 ± 1.94	3.14 ± 1.25	3.67 ± 1.63
	Cold-boned	Control	2.83 ± 0.49	2.57 ± 0.66	2.77 ± 0.73	3.58 ± 0.76	2.03 ± 0.39	
		Short T	3.48 ± 0.74	2.43 ± 0.67	3.04 ± 0.53	3.64 ± 0.72	2.96 ± 0.93	
		Low P	3.73 ± 0.78	5.04 ± 0.77	3.65 ± 0.95	4.65 ± 0.66	3.26 ± 0.75	
		Low P, short T	2.51 ± 0.74	3.01 ± 0.92	4.26 ± 1.41	4.26 ± 0.98	4.37 ± 2.02	

Table 2. Effect of phosphate (P) level and tumbling (T) time on Hunter lightness (“L”), Hunter redness (“a”), TBARS, cook loss and Warner Bratzler shear force (mean ± standard deviation) values of cooked shoulders stored in modified atmosphere packs (70% N₂, 30% CO₂) at 4°C.

Carcass Side	Treatment Group	Storage time, days					
		1	7	14	21	28	
'L'	Hot-boned	Control	48.72 ± 3.23	51.52 ± 1.78	50.59 ± 4.16		
		Short T	50.69 ± 3.18	49.21 ± 2.49	50.39 ± 2.60		
		Low P	50.79 ± 4.80	50.87 ± 3.48	48.15 ± 2.65		
		Low P, short T	50.62 ± 2.18	49.90 ± 4.24	49.61 ± 1.06		
	Cold-boned	Control	57.78 ± 3.14	54.88 ± 2.91	55.73 ± 1.80		
		Short T	55.77 ± 5.57	51.52 ± 4.64	53.19 ± 6.01		
		Low P	50.76 ± 0.37	52.26 ± 5.06	52.14 ± 2.51		
		Low P, short T	52.04 ± 2.99	52.61 ± 2.43	52.93 ± 3.38		
'a'	Hot-boned	Control	7.011 ± 0.43	8.25 ± 1.07	7.32 ± 1.26		
		Short T	8.98 ± 1.92	8.53 ± 1.10	9.26 ± 1.51		
		Low P	8.86 ± 1.40	8.00 ± 1.41	8.70 ± 0.76		
		Low P, short T	9.14 ± 1.01	9.18 ± 1.06	9.83 ± 1.36		
	Cold-boned	Control	7.18 ± 1.00	8.50 ± 1.62	7.88 ± 0.75		
		Short T	9.00 ± 1.09	8.98 ± 2.20	9.17 ± 1.78		
		Low P	8.95 ± 0.44	7.24 ± 0.89	7.60 ± 0.87		
		Low P, short T	8.82 ± 1.47	8.54 ± 1.41	8.70 ± 1.30		
'b'	Hot-boned	Control	5.84 ± 0.72	6.61 ± 0.76	6.42 ± 0.82		
		Short T	6.03 ± 0.53	6.45 ± 0.63	6.47 ± 0.67		
		Low P	6.68 ± 1.26	6.60 ± 1.25	6.06 ± 0.63		
		Low P, short T	6.87 ± 0.51	7.30 ± 0.93	7.55 ± 0.28		
	Cold-boned	Control	7.31 ± 1.08	6.78 ± 0.78	7.31 ± 0.62		
		Short T	7.10 ± 0.76	7.03 ± 1.05	6.99 ± 1.14		
		Low P	6.20 ± 0.34	6.21 ± 0.61	6.01 ± 0.58		

TBARS		Low P, short T		6.72 ± 0.71	7.04 ± 0.62	7.38 ± 0.95			
		Control	0.52 ± 0.18	0.55 ± 0.10	0.62 ± 0.20	0.49 ± 0.21	0.44 ± 0.15		
		Short T	0.57 ± 0.16	0.47 ± 0.22	0.42 ± 0.05	0.60 ± 0.29	0.42 ± 0.16		
		Low P	0.45 ± 0.03	0.34 ± 0.31	0.28 ± 0.39	0.45 ± 0.09	0.32 ± 0.09		
	Cold-boned		Low P, short T	0.39 ± 0.28	0.42 ± 0.06	0.47 ± 0.15	0.34 ± 0.10	0.34 ± 0.11	
			Control	0.43 ± 0.25	0.57 ± 0.15	0.54 ± 0.11	0.37 ± 0.10	0.44 ± 0.13	
			Short T	0.52 ± 0.15	0.48 ± 0.10	0.38 ± 0.12	0.41 ± 0.18	0.39 ± 0.09	
	WBSF	Hot-boned		Low P	0.38 ± 0.04	0.33 ± 0.27	0.29 ± 0.38	0.36 ± 0.13	0.30 ± 0.09
				Low P, short T	0.30 ± 0.09	0.42 ± 0.12	0.44 ± 0.06	0.32 ± 0.06	0.35 ± 0.13
			Control	3.01 ± 0.69	2.93 ± 0.42	2.86 ± 0.60	3.28 ± 0.38	3.55 ± 0.61	
Cold-boned			Short T	3.24 ± 0.97	3.32 ± 0.95	2.97 ± 0.80	2.78 ± 0.51	3.30 ± 0.39	
			Low P	2.99 ± 0.51	2.99 ± 0.82	3.77 ± 0.42	2.95 ± 0.53	2.58 ± 0.47	
			Low P, short T	3.39 ± 0.31	3.19 ± 0.63	3.52 ± 0.60	3.40 ± 0.50	3.87 ± 0.80	
			Control	2.79 ± 0.39	2.51 ± 0.45	2.07 ± 0.33	2.35 ± 0.88	2.46 ± 0.40	
			Short T	3.19 ± 0.78	3.13 ± 0.60	2.64 ± 0.24	2.45 ± 0.58	2.53 ± 0.57	
			Low P	2.62 ± 0.48	2.54 ± 0.39	3.05 ± 0.14	2.95 ± 0.73	2.27 ± 0.53	
	Low P, short T	3.37 ± 0.45	3.27 ± 0.96	3.31 ± 0.76	3.16 ± 0.69	3.17 ± 1.30			

CONSUMER EVALUATIONS OF BACON PRODUCED WITH OR WITHOUT NITRITE AND WITH OR WITHOUT AMMONIUM HYDROXIDE/CARBON OXIDE ENHANCEMENT

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Key Words: Bacon, Nitrite-free, Consumer, Preference, Carbon Oxide

Introduction

The safe production of quality bacon without the use of sodium nitrite has been of interest for many years and was greatly debated in the United States in the 1970's (Wasserman et al., 1977). However, to obtain traditional bacon taste and color, the addition of nitrite is important. Carbon oxide addition to meat results in the formation of a stable red color that will persist during packaging and cooking at low temperatures (Jayasingh, et al., 2001). To our knowledge, carbon oxide has not been used in bacon production.

Objectives

To evaluate consumer preference of appearance and taste of bacon produced with and without sodium nitrite and with and without pH and carbon oxide enhancement (PHE) via the addition of ammonium hydroxide and dissolved carbon oxide to the brine.¹

Methodology

Bacon Treatments. The experimental design consisted of four treatments in a 2 × 2 factorial arrangement, which included pH enhanced product (PHE) (control vs. PHE), and the addition of sodium nitrite (with nitrite vs. nitrite free). Some bellies served as controls while others were pH enhanced using a patent pending process of Freezing Machines, Inc. (FMI) at a Beef Products Inc. (BPI) production facility. Bellies were injected (ingredients expressed on percent finished product) using a pH enhanced solution also consisting of salt (1.68%); Sucrose (1.45%); sodium erythorbate (0.05%); and cure salt (0.18% - in the “with nitrite” treatments only). After the bellies were injected, they were transported to the South Dakota State University Meat Laboratory for smoking. The smoked bellies were then transported back to BPI, where they were sliced. After slicing, sample slices representing each treatment were vacuum packaged and transported to SDSU for consumer testing. One belly was used for each of the control treatments (with nitrite and nitrite free). Four bellies were used for the “PHE with nitrite” treatment and six bellies were used for the “PHE nitrite free” treatment.

¹ Patent pending, processing aid with validated pathogen reduction properties.

Panels. Consumer panels were conducted according to standards set by the American Meat Science Association. Panelists were recruited from the Brookings, SD area using fliers and newspaper advertising. One hundred eighteen consumers participated in the study over six different panel times.

Appearance Evaluation. Six representative bacon slices from each treatment were vacuum packaged in a shingle arrangement to simulate typical retail packaging. Packages were identified with random two-letter codes, placed in ice-filled trays, and evaluated by consumers under cool florescent lighting. Each panelist scored the four bacon samples (representing the four treatments) for “Overall Like of Appearance” on a 10-point scale, “Like of Color” on a 10-point scale, and “Like of the Lean to Fat Ratio” on a 10-point scale. Each panelist was also asked, “Would you be likely to purchase this bacon? (Yes or No)” based on appearance only, and each panelist was given the opportunity to write additional comments about each sample.

Taste Evaluation. Bacon was cooked in a microwave according to a predetermined length of time based on weight. These cooking times were predetermined to result in an approximate 37.5% cooking yield. Immediately following cooking, bacon strips were cut into four pieces each, placed into Styrofoam bowls with holes punched in the bottom to allow juices to drain, covered with aluminum foil, and held in a 140°F warming oven until served. Panels were conducted in booths preventing panelist interaction. Prior to the start of the panel, panelists were given brief instructions about panel procedure and were asked to sign a notice of informed consent. All samples were served under red lights to limit differences in visual appearance. One sample of each treatment was served in a random order to the panel. The first sample served was always a bacon sample obtained from the SDSU Meat Lab and was used as a warm-up sample to prevent errors associated with order of sample; this data was not included in the analysis of data nor were any conclusions drawn from that sample. Samples were coded with a random code to blind consumers to treatment combinations. Consumers rated each sample for "Overall Like", "Like of Flavor", "Crispiness", and "Tenderness" on a 10-point scale.

Results & Discussion

Figure 1 shows consumer ratings for bacon based on appearance only. Based on appearance only, consumers preferred ($P < 0.05$) PHE bacon over control bacon, regardless of whether or not it contained nitrite. In fact, consumers preferred the appearance of PHE nitrite free bacon over control bacon with nitrite. Consumers preferred ($P < 0.05$) the appearance of PHE bacon with nitrite over the PHE nitrite free bacon. Consumers liked the color of the bacon in the following order from best to worst: 1. PHE with nitrite, 2. PHE nitrite free, 3. control with nitrite, 4. control nitrite free. The control with nitrite bacon rated lower ($P < 0.05$) for “like of lean to fat ratio” than the other three treatments. Overall, PHE had twice as large of an effect at improving bacon appearance when compared to the effect of sodium nitrite.

Consumer taste ratings are shown in Figure 2. The “Overall Like” ratings almost exactly matched the “Like of Flavor” ratings, meaning that flavor is probably the primary driver in determining consumer overall satisfaction with a bacon eating experience. Of the four primary treatments, control nitrite free bacon rated the highest ($P < 0.05$) and

control with nitrite rated the lowest ($P < 0.05$), with the PHE bacons rated intermediate to the two control bacons.

The consumer rating for crispiness and tenderness are shown in Figure 3. The control nitrite free bacon rated crispier ($P < 0.05$) than the other three bacon types. Both PHE bacons were rated as more tender ($P < 0.05$) than both control bacons. Therefore, the PHE process is resulting in more tender bacon, a desirable characteristic when bacon is used as a condiment on sandwiches.

Based on appearance only, consumers said they were most likely to purchase the PHE bacon with nitrite (Figure 4). Based on appearance only, the percentage of consumers who said they would be likely to purchase the PHE bacon with nitrite was 87%, followed by PHE nitrite free bacon at 62%, control with nitrite bacon at 48%, and control nitrite free bacon at 38%. Based on taste only, the differences among the four treatments in consumers' "willingness-to-purchase" was not statistically significant.

Conclusions

In summary, consumers preferred the appearance of PHE bacon over control bacon, but they had no preference in taste for either PHE or control bacon.

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Tables and Figures

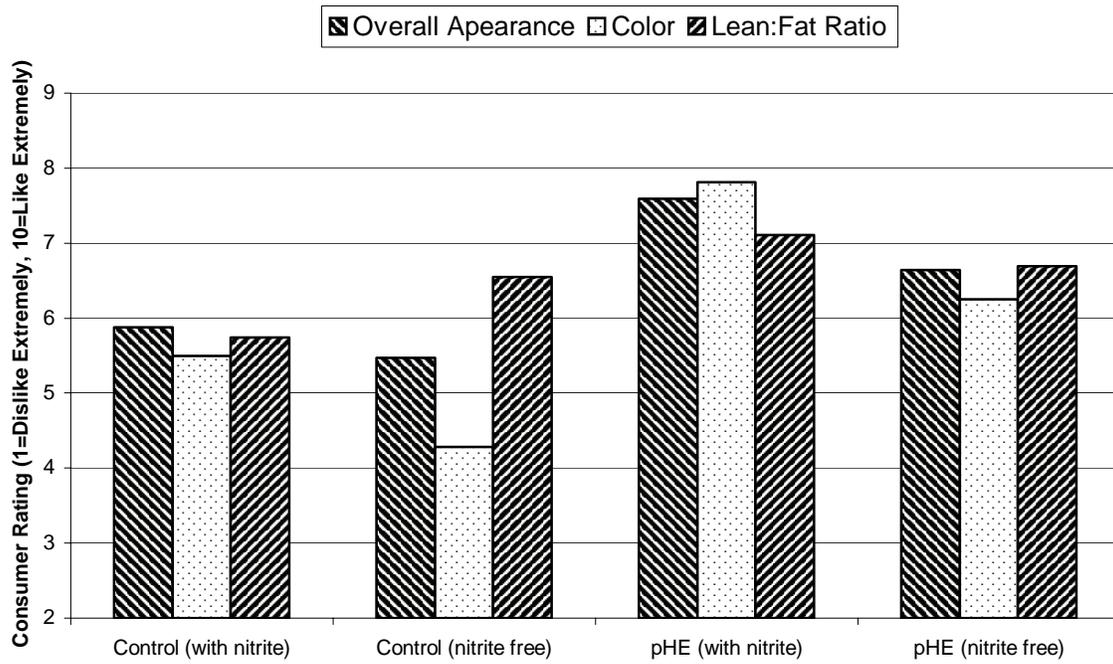


Figure 1. Consumer panel ratings of like of overall appearance, like of color, and like of lean:fat for control and pH enhanced (PHE) bacon.

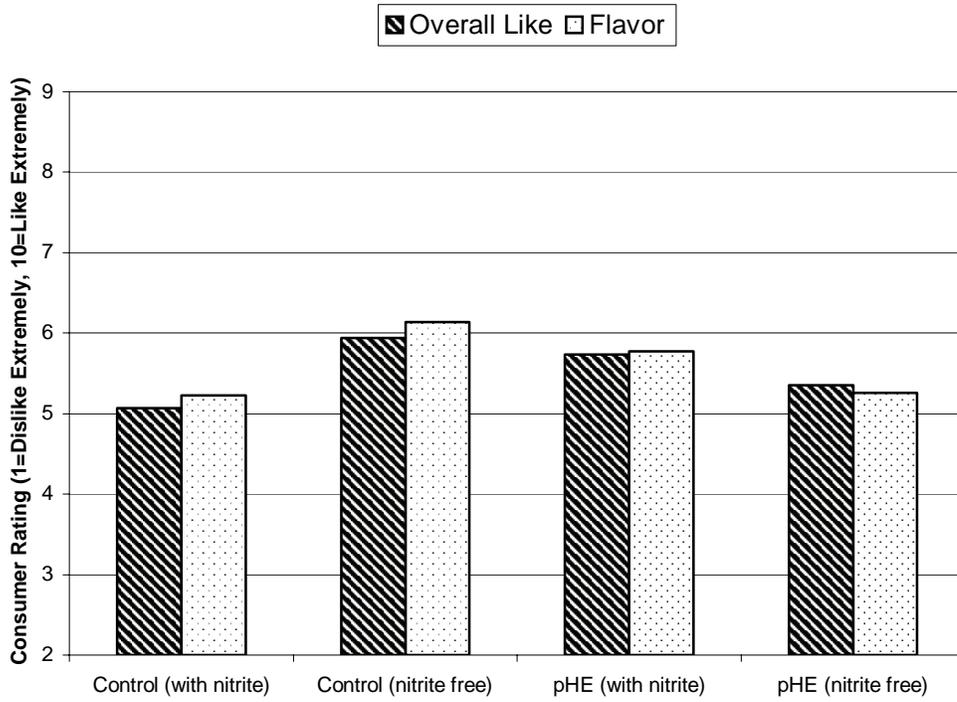


Figure 2. Consumer panel ratings for overall like and like of flavor, for control and pH enhanced (PHE) bacon.

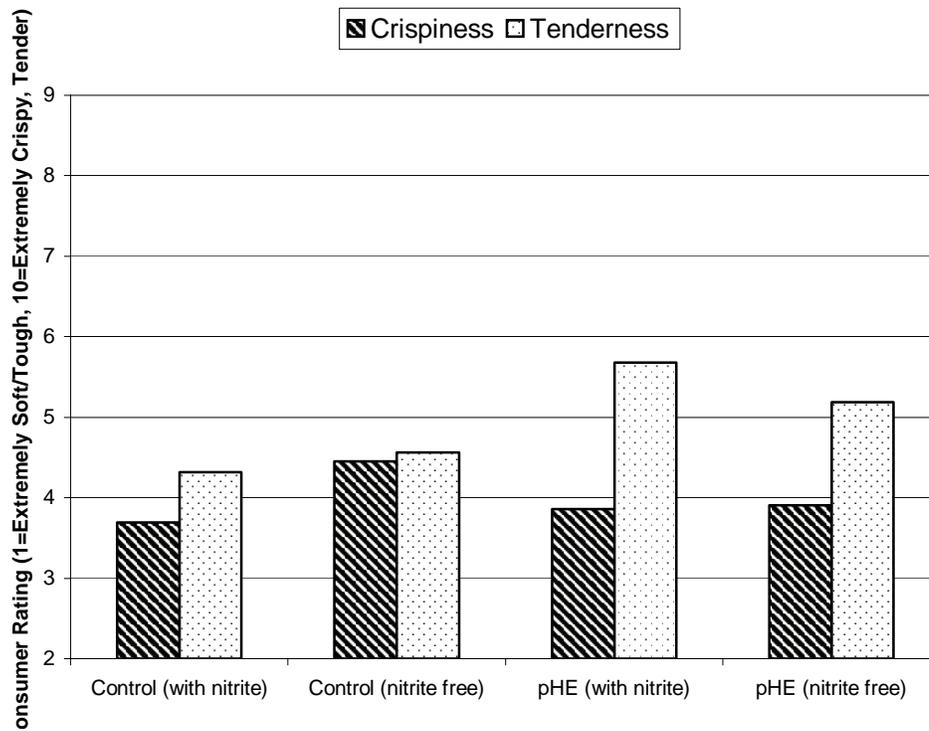


Figure 3. Consumer panel ratings for crispiness and tenderness, for control and pH enhanced (PHE) bacon.

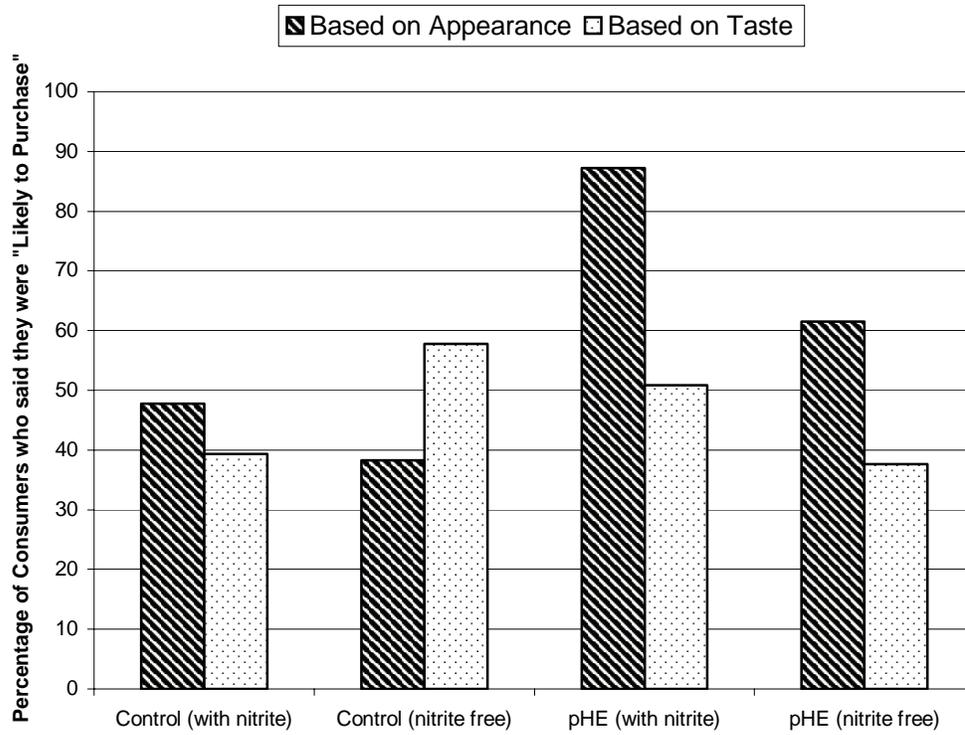


Figure 4. Consumer likelihood of purchase, for control and pH enhanced (PHE) bacon based on appearance or taste.

CHANGES OF FREE AMINO ACID CONCENTRATION AND OF ALANYL AMINOPEPTIDASE ACTIVITY IN *BICEPS FEMORIS* DURING PROCESSING OF JINHUA HAM

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Key Words: Jinhua ham; aminopeptidase; alanyl aminopeptidase; free amino acid; response surface methodology

Introduction

Jinhua ham is appreciated for its characteristic flavor in most Asian countries. The development of desired aging flavor requires a long processing time. Intense proteolysis has been observed during dry-curing process (Martín, Córdoba, Antequera, Timón, & Ventanas, 1998; Molina, & Toldrá, 1992), giving rise to an important collection of free amino acids (Buscailhon, Monin, Cornet, & Bousset, 1994; Cordoba, Antequera, Garcia, Ventanas, Lopez, & Asensio, 1994; Sforza, Pigazzani, Motti, Porta, Virgili, Galaverna, Dossena, & Marchelli, 2001) that can directly contribute to flavor such as sweet, sour or bitter tastes, or indirectly contribute as precursors of volatile flavor compounds.

The free amino acids in dry-cured hams are mainly generated from muscle proteins and peptides by the actions of aminopeptidases (Toldrá, Flores, & Sanz, 1997). Muscle aminopeptidase activities have been reported in both raw and dry-cured ham with good stability even after 8 months of curing (Toldrá, Aristoy, Part, Cerveró, Rico, Motillva, & Flores, 1992). Alanyl aminopeptidase (AAP) accounts for 83% of the total porcine skeletal muscle aminopeptidase activities and demonstrates broader substrate specificity (Flores, Aristoy, & Toldrá, 1996). Therefore, it may be the aminopeptidase that takes the most important role in the generation of free amino acids during dry-cured ham processing.

The action of aminopeptides and free amino acid generation are very important for dry-cured hams because they are involved in the development of characteristic ham flavor. However, relevant information on Jinhua ham processing has not been well documented up till now.

Objectives

The main objective of the present work was tracing the changes of AAP activity and free amino acid contents in *biceps femoris* during the processing of Jinhua ham and evaluating the contributions of AAP and amino acids to the formation of Jinhua ham flavor.

Methodology

Materials

Sixty trimmed green hams weighting 6.2-6.9 kg from local cross swine (5-6 months, 90-100 kg) of Lanxi (Zhejiang province of China) were used to produce Jinhua hams. 7-amido-4-methyl-coumarin (AMC), Ala-AMC was purchased from Bachem Feinchemikalien (Bubendorf, Switzerland) and other chemical agents, purchased from Sigma (St. Louis, MO).

Jinhua ham processing

Jinhua ham processing was carried out under natural condition as prescribed by traditional processing technology in Zhejiang Provincial Food Company, China. The process involves 6 stages, i.e. natural cooling, salting, soaking and washing, sun-drying, loft-aging and post-aging. After natural cooling for about 24 h, green hams were piled and salted for 30 d during which salt was added 5 times (50 mg nitrate per kg green ham was mixed in the salt used at the 2nd time) and ham piles were turned over for 7 times. After soaking and washing for 24 h and sun-drying for 20 d (including 11 days of cloudy or rainy or snowy weather), hams were transferred to aging-loft and aged for five months. Then the hams were post-aged at room temperature for two months.

Sampling

Biceps femoris was fully sampled for analysis from 5 hams randomly taken after each processing stages, i.e. prior-salting (about 24 h), end of salting (31 d), end of sun-drying (51 d), middle loft-aging (124 d), end of loft-aging (203 d) and end of post-aging (264 d). Samples were packed and stored under -40 °C before analyzing.

Amino acid analysis

About 5 g chopped sample was homogenized in 20 ml deionized water with a polytron (three stokes, 10 s each at 22000 r.p.m. with cooling in ice) homogenizer (IKA T18 basic, Made in IKA, German). The homogenate was deproteinized by mixing it with 20 ml of 10% sulfosalicylic acid. The mixture was maintained at 4 °C for 17 h and then filtered through filter paper. The filtrate was adjusted to pH 6.0 with 4N NaOH and diluted to 50ml with a buffer (100 mM sodium citrate buffer, pH 6.00). The diluted solution was ultra-filtrated through a 10 kDa ultrafiltration membrane with a Stirred Ultrafiltration Cell Model 8200 (Millipore Co., USA) before amino acid derivatization was carried out with AccQ-Fluor Reagent Kit (P/N WAT052880, Waters Co., USA) on the direction for use attached to the Kit. Amino acid derivates were detected on a Waters High Performance Liquid Chromatograph equipped with two pumps (Waters 515 HPLC pump) and a UV detector (Waters 2487 Dual λ Absorbance Detector, detecting at 254 nm). The column was an AccQ-Tag Column (Nava-PakTM C18, 3.9×150 mm, interior diameter 4 μ m) and column temperature was controlled to 37 °C. The eluents used were: (A) AccQ-TagTM Eluent A (Borate buffer): water at 1: 10, and (B) acetonitrile: water: methanol at 45: 40: 15. The flow rate was 1 ml·min⁻¹ and the flowing solvent gradient

was performed on the following procedure: initial 0% B, linear change to 1.0% B in 0.5 min, linear change to 7.0% B in 16.5 min, linear change to 10.0% B in 4 min, linear change to 33.0% B in 9 min and maintained 3min at 33.0% B, then linear change to 100% B in 1 min and maintained at 100% B for 3 min, at last linear change to 0% B in 1 min and maintained for 12 min.

Preparation of enzyme extract

The extraction was performed according to Toldrá et al. (1992) and Rosell, & Toldrá (1998) with slight modifications. About 5 g chopped sample was homogenized in 35 ml extraction buffer (50 mM sodium phosphate buffer, pH 7.5, containing 5 mM EGTA) with a polytron (three stokes, 10 s each at 23000 r.p.m. with cooling in ice) homogenizer (IKA T18 basic, Made in IKA, German). The homogenate was stirred for 60 min under 4 and then centrifuged at 22000 g for 20 min. The supernatant was filtered through fine silk cloth and finally diluted to 50 ml with extraction buffer for enzyme activity determination after shaking.

Enzyme assays

The enzyme activity was determined according to Toldrá et al. (1992) and Rosell et al. (1998), with some modifications. To 2.5 ml substrate solution (100 mM sodium phosphate buffer, pH 7.00, containing 0.33% of 30% Brij 35, 5.0 mM CaCl₂, 1.0 mM DTT and 0.25 mM Ala-AMC), 0.5 ml enzyme extract was added. The mixture was incubated in a water bath at 37 for 30 min and the reaction was terminated by immediate addition of 6 ml of ethanol. The generated fluorescence was determined with a Cary Eclipse Fluorescence Spectrophotometer (VARIAN, Australia) at excitation and emission wavelengths of 380 nm and 440 nm, respectively. One unit of enzyme activity (U) was defined as the amount of enzyme required to produce 1 nmol AMC per min at 37, and called potential enzyme activity. Muscle AAP activity was calculated on the basis of *biceps femoris* dry matter subtracting fat and sodium chloride, i.e. (DM-Fat-Salt), and simply expressed as U·g⁻¹.

2.6. Response surface experimental design and method

Response surface methodology (RSM) based on Box-Behnken design (BBD) was adopted to investigate the effects of processing factors on AAP activity. Four factors, i.e. temperature (Temp.), sodium chloride content (Salt), sodium nitrate content (Nitrate) and pH value (pH) (Table 1), were considered in the design and their value ranges were set according to the determining results from Jinhua ham processing (refer to Zhao, Zhou, Xu, Peng, Huan, Jing, & Chen, 2005). With Design-Expert software (version 6.0.5, Stat-Ease Inc., Minneapolis, USA), a four-factor and one-response (AAP activity) experimental design was performed and totally 29 sets of experiments were generated. A prior-salting sample was used for AAP activity determination for the convenience of control of salt and nitrate content in reaction mixture. Preparation of enzyme extracts was similar to the above mentioned procedure, but before the supernatants were diluted to 50 ml with extraction buffer, definite amount of NaCl and NaNO₃ were added and pH was adjusted to definite values as designed. Enzyme assay was similar to the above mentioned

procedure, except for that the buffers used in substrate solutions were different in pH value, NaCl and NaNO₃ content. In preparation of different substrate solutions in pH value, 200 mM sodium acetate buffer of pH 5.40, 200 mM sodium citrate buffer of pH 5.75 and 200 mM sodium phosphate buffer of pH 6.50 were used. One unit of enzyme activity (U) was defined as the amount of enzyme required to produce 1 nmol AMC per min at the designed reaction temperature. Muscle enzyme activity was calculated on the basis of *biceps femoris* dry matter subtracting fat and sodium chloride (DM-Fat-Salt), and expressed as U·g⁻¹.

2.7. Statistics

Free amino acid and AAP activity data were assessed by analysis of variance using one-way ANOVA procedure of SPSS 10.0 (SPSS Inc.). The data from response surface experiments were analyzed through analysis of variance and quadratic regression, and rotatable response surface figures were drawn by 3-D surface procedures inside Design-Expert software (version 6.0.5, Stat-Ease Inc., Minneapolis, USA). The actual activity of muscle AAP (the ability of hydrolyzing proteins and peptides under actual processing condition of dry-cured ham) was estimated by the regression equation.

Results & Discussion

Changes of free amino acid contents in muscle during Jinhua ham processing

All the free amino acids detected in prior-salting hams increased significantly ($P < 0.05$) during processing except arginine and cystine (Table 2). Most free amino acids increased by 5-20 folds after processing compared with those in prior-salting hams, with the exception of arginine and cystine which concentration didn't change very much during processing ($P > 0.05$). Arginine, glutamic acid, leucine, lysine and alanine were found to be the free amino acids present in higher concentration in the final products, while cystine was the lowest. The highest increase was lysine that reached a final concentration of more than 80 times of that found in prior-salting hams, followed by aspartic acid, serine, tyrosine and isoleucine. With regard to the lengths of different processing stages, the fastest increase in the concentrations of total free amino acids took place in the second half of loft-aging and the sun-drying processes, followed by the first half of loft-aging and post-aging stages.

It has been well documented that the concentrations of all free amino acids except for arginine and histidine, increased to some extents during dry-cured ham processing (Buscailhon et al., 1994; Cordoba et al., 1994; Flores, Aristoy, Spanier, & Toldrá, 1997; Sforza et al., 2001; Ventanas, Cordoba, Antequera, Garcia, Lopez-Bote, & Asensio, 1992; Zhu, & Hu, 1993). The most abundant free amino acids were arginine, glutamic acid, leucine, lysine and alanine (Cordoba et al., 1994; Sforza et al., 2001; Zarkadas, Karatzas, Khalili, Khanizadeh, & Morin, 1988; Zhu et al., 1993). These reports generally accorded with our research on Jinhua ham. We also found that the concentration of cystine was very low and didn't change very much during processing. Cystine represents the oxidation form of cysteine that is very active and easily changes into other compounds, which may explain our result.

It is reported (Cordoba et al., 1994; Zhu et al., 1993) that the contents of most free amino acids in dry-cured ham products were 40-60 folds of those in fresh pork, which was much higher than our results. This is because our initial samples were from prior-salting hams rather than fresh hams. Prior-salting hams had been cooled under natural temperature (3-12 °C with daily average of 7.8 °C) for about 24 h. Considering the relatively high temperature and strong activities of proteases in muscle with no restraining effect from salt, muscle proteins experienced considerable hydrolysis before samples were taken.

3.2. Changes of potential AAP activity during processing

As shown in Table 3, porcine muscle possessed very strong potential AAP activity, but it decreased gradually during processing from 201635.43 U·g⁻¹ at prior-salting to 6147.11 U·g⁻¹ at the end of post-aging. Salting, sun-drying and the second half of loft-aging processes greatly reduced AAP activity ($P < 0.05$) and about 27.11%, 35.88% and 20.27% of AAP activity losses were respectively observed during each stage. After processing, about 3.05% of prior-salting activity was remained.

Past work has revealed that the activities of all the aminopeptidases detected decreased gradually along the processing of Spain dry-cured ham and AAP showed the highest exopeptidase activity along the full process (Toldrá, Aristoy, & Flores, 2000). Our work proved the decline tendency of AAP activity during Jinhua ham processing, but the activity loss rate was much higher and the activity remains much lower than those demonstrated in Spain ham. The differences may result from different processing technologies adopted in manufacturing the two hams. Jinhua ham is salted and aged under completely natural conditions and only salt or salt and nitrate is used at salting stage, instead of salting agents mixture including glucose and vitamin C used in Spain ham salting. In addition, Jinhua ham aging temperature is relatively high (highest to 40 °C). High temperature accelerates denaturation of enzyme proteins and intensifies chemical and biochemical reactions such as oxidation processes, resulting in fast losing of enzyme activity, while glucose activates AAP hydrolyzing activity (Toldrá, Cerveró, & Part, 1993).

Effects of main processing factors on AAP activity

Statistical results showed that temperature, pH value and salt content had significant effects on AAP activity ($P < 0.001$). Both temperature and salt content interacted with pH value on AAP activity ($P < 0.01$). However, 0-50 mg·L⁻¹ of sodium nitrate in the reaction mixtures didn't evidently affect AAP activity ($P > 0.05$). By stepwise regression analysis, an optimal quadratic regression equation ($P < 0.001$) was generated (Eq.1). Related statistics ($R^2 = 0.99$, $Adj R^2 = 0.98$, $Pred R^2 = 0.95$, $Adeq Precision = 49.02$) indicated that the equation was well fit and could accurately predict the actual activity of AAP under practical condition.

$$\begin{aligned} \text{Ln(AAP activity)} = & 32.31334 - 0.17946 \times \text{Temp} + 0.10028 \times \text{Salt} - 9.55187 \times \text{pH} - \\ & 0.00053 \times \text{Temp}^2 + 0.02052 \times \text{Salt}^2 + 0.94804 \times \text{pH}^2 - 0.04506 \times \\ & \text{Temp} \times \text{pH} - 0.09083 \times \text{Salt} \times \text{pH} \dots\dots\dots(\text{Eq.1}) \end{aligned}$$

As shown in Fig.1, muscle AAP activity increased in exponential curve along with the increase of temperature from 4 to 40 and pH value from 5.00 to 6.50. The effect of temperature on AAP activity was enhanced by the increase of pH value. Increase of temperature also strengthened the effect of pH on AAP activity. Fig.2 displayed that AAP activity was activated by increase of pH value but restrained by salt, but the increase of salt content inhibited the activating effect of pH rise on AAP activity.

It is reported (Toldrá et al., 1992; Toldrá et al., 1993; Toldrá et al., 1997) that muscle AAP displayed its maximum activity at 40 and pH 7.0, salt intensely inhibited AAP activity, but 0 mg·L⁻¹ and 50 mg·L⁻¹ of nitrate didn't display different effects on AAP activity (Toldrá et al., 1993). These were in agreement with our result. In addition, we found that pH value interacted with temperature and salt content on AAP activity, which has not documented before.

Changes of actual activities of muscle AAP during Jinhua ham processing

AAP activity was observably affected by temperature, salt content and pH value that changed continuously during processing of Jinhua ham (Zhao et al., 2005). Therefore, it is normally very difficult to determine the actual activity of muscle AAP during processing. Using data of processing factors determined from Jinhua ham processing (refer to Zhao et al., 2005), changes of actual activity of muscle AAP during Jinhua ham processing were predicted with Eq.1 (Table 4). Table 4 revealed that muscle AAP could always keep actual activity during Jinhua ham processing and 143.05 U·g⁻¹ actual AAP activities were retained even at the end of post-aging. Considering the extensive substrate specificity of AAP (Flores et al., 1996), it must have taken some effects in generating free amino acids during whole process of Jinhua ham processing. However, it is obvious that the spectrum of AAP activity against terminal amino acids does not accord with the observed release of free amino acids in Jinhua hams, indicating that other muscle aminopeptidases may also play important roles in the generation of free amino acids during processing.

Conclusions

Muscle AAP possessed very strong potential activity that decreased gradually during Jinhua ham processing and about 3.05% of prior-salting activity was left in the products. Temperature, salt content and pH value significantly affected AAP hydrolyzing activity, which made AAP actual activity accounting for less than 5% of its corresponding potential activity at each stage of Jinhua ham processing. Even so, AAP could always show considerable actual activity. All the free amino acids but arginine and cystine increased to some extents during processing and most of the free amino acids detected in the final products were 5-20 times of those found in prior-salting hams, which might be results of the coactions of muscle AAP and other aminopeptidases.

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Tables and Figures

Table 1. Factors and levels for Box-Behnken response surface experimental design

Levels	Temperature ()	Salt (%)	Sodium nitrate (mg·L ⁻¹)	pH value
-1	4.0	0.0	0.0	5.00
0	22.0	5.0	25.0	5.75
+1	40.0	10.0	50.0	6.50

Table 2. Results of 17 free amino acid contents in biceps femoris after each stage of Jinhua ham processing

FAA	Prior-salting	End of salting	End of sun-drying	Middle of loft-aging	End of loft-aging	End of post-aging	Prob.
Alanine	215.44±27.23 ^d	239.76±8.47 ^d	243.30±17.69 ^d	353.29±45.25 ^c	820.79±5.13 ^b	964.96±36.81 ^a	***
Arginine	1843.60±185.71	1321.84±98.92	1701.66±132.61	1646.52±198.30	1833.80±224.21	1871.83±111.06	ns
Aspartic acid	20.16±9.43 ^c	12.46±3.40 ^c	24.20±11.24 ^c	43.24±17.95 ^c	449.99±52.84 ^b	563.93±57.25 ^a	***
Cystine	14.99±1.72	19.26±2.14	33.33±10.97	41.01±5.86	39.89±5.20	31.65±5.81	ns
Glutamic acid	170.94±26.92 ^d	242.56±10.39 ^d	293.49±48.58 ^d	477.01±60.94 ^c	1033.36±51.52 ^b	1212.52±51.54 ^a	***
Glycine	80.35±7.56 ^c	105.72±3.19 ^c	138.32±17.45 ^c	281.25±31.26 ^b	407.79±39.52 ^a	426.83±32.25 ^a	***
Histidine	183.35±20.80 ^c	194.40±18.19 ^c	286.22±10.82 ^b	343.12±37.27 ^{ab}	387.54±41.19 ^a	365.38±37.33 ^{ab}	***
Isoleucine	42.72±9.88 ^d	110.78±8.41 ^{cd}	155.73±16.44 ^c	319.26±38.96 ^b	609.47±50.74 ^a	612.51±40.45 ^a	***
Leucine	87.97±15.50 ^d	197.05±16.53 ^{cd}	268.72±26.10 ^c	542.88±57.73 ^b	1096.62±88.41 ^a	1095.39±63.62 ^a	***
Lysine	12.53±3.06 ^c	32.92±7.04 ^c	79.16±28.60 ^c	43.79±9.81 ^c	469.83±60.03 ^b	1006.72±119.26 ^a	***
Methionine	44.30±6.35 ^c	85.60±5.80 ^c	117.29±12.34 ^c	241.52±30.51 ^b	420.65±40.91 ^a	394.33±29.45 ^a	***
Phenylalanine	61.56±8.88 ^c	124.26±12.50 ^{bc}	220.71±19.01 ^b	524.84±51.57 ^a	655.22±80.39 ^a	584.25±51.91 ^a	***
Proline	86.97±14.15 ^d	99.24±11.99 ^d	141.84±10.24 ^d	280.42±37.31 ^c	463.65±44.17 ^b	577.89±46.47 ^a	***
Serine	27.05±3.15 ^c	21.97±3.00 ^c	115.13±52.09 ^c	84.39±38.56 ^c	423.72±69.63 ^b	576.11±46.18 ^a	***
Threonine	127.54±14.76 ^c	174.09±6.43 ^c	221.83±27.61 ^c	358.06±35.56 ^b	696.01±26.32 ^a	644.98±49.78 ^a	***
Tyrosine	29.72±0.39 ^b	51.86±15.31 ^b	113.27±39.83 ^b	86.21±30.22 ^b	432.37±33.61 ^a	454.80±38.11 ^a	***
Valine	67.16±10.38 ^d	160.07±10.12 ^{cd}	197.79±26.47 ^c	401.52±47.31 ^b	753.69±62.45 ^a	762.28±51.89 ^a	***
Total	3116.33±316.28 ^c	3193.85±106.73 ^c	4351.99±302.56 ^c	6068.32±596.03 ^b	10994.40±612.85 ^a	12146.36±584.60 ^a	***

Note:

- Contents of free amino acids were in mg·100g⁻¹ on the basis of *biceps femoris* dry matter subtracting fat and sodium chloride, i.e. (DM – Fat – Salt) and expressed as mean ± standard error.
- Means within the same row without common superscripts differed significantly, $P < 0.05$.
- Prob.: ANOVA results; ns: not significant; ***: significant, $P < 0.001$

Table 3. Results of potential AAP activity after each processing stage of Jinhua ham

	Prior-salting	End of salting	End of sun-drying	Middle of loft-aging	End of loft-aging	End of post-aging
AAP Activity (U·g ⁻¹) ^a	201635.43±8378.37 ^a	146964.89±15841.88 ^b	74616.12±12171.14 ^c	48897.60±8522.33 ^c	8030.60±2464.40 ^d	6147.11±1841.59 ^d
Residual (%) ^b	100.00	72.89	37.01	24.25	3.98	3.05

Note:

- a. Means within the same row without common superscripts differed significantly, $P < 0.05$.
- b. Residual (%) indicated the percentage of AAP activity after each stage accounting for that of prior-salting.

Table 4. Results of predicted actual AAP activity after each processing stage of Jinhua ham

	Prior-salting	End of salting	End of sun-drying	Middle of loft-aging	End of loft-aging	End of post-aging
Predicted Actual AAP Activity (U·g ⁻¹)	8333.27	2467.56	991.45	623.37	454.07	143.05
Predicted / Potential (%) [*]	4.13	1.68	1.33	1.27	5.65	2.33

Note:

* Predicted / potential (%) was the percentage of predicted actual AAP activity accounting for the potential AAP activity at the same processing stage.

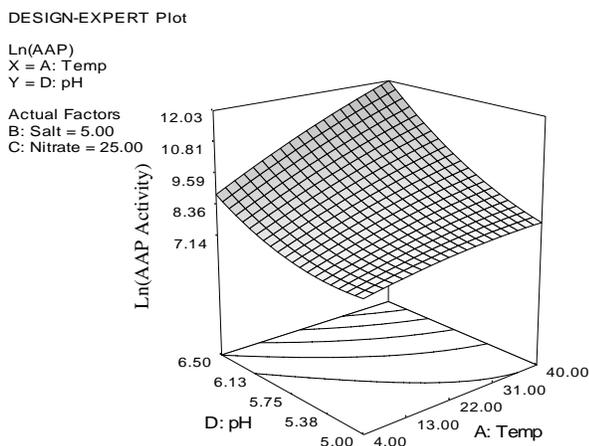


Fig.1 Effects of temperature and pH value on AAP

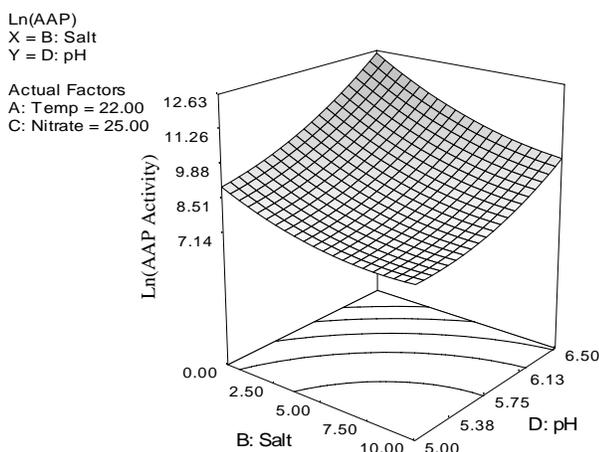


Fig.2 Effects of salt content and pH value on AAP activity

CHANGES IN CATHEPSIN B AND L ACTIVITIES DURING JINHUA HAM PROCESSING

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Key Words: dry-cured ham; Jinhua ham; cathepsin B; cathepsin L; response surface methodology

Introduction

Up till now, about 20 proteases have been found in skeletal muscle, of which cathepsins and calpains are the possibly crucial endopeptidases playing main roles in muscle proteolysis during dry-cured ham processing. However, calpains lost their activities after salting (Sárraga, Gil, & Garcia-Regueiro, 1993) and among cathepsins, cathepsin D lost its activity the most quickly during processing (Sárraga et al., 1993; Toldra, & Etherington, 1988; Toldrá, Rico, & Flores, 1993) and may play some role only in the first several months of processing (Toldrá et al., 1993). On the other hand, cathepsin B, L and H always kept some activities during dry-cured ham processing (Sárraga et al., 1993; Toldrá et al., 1993). It is reported that cathepsin H hardly hydrolyzes any fibrillin (Ouali, Garrel, Obléd, Deval, Valin, & Penny, 1987), while cathepsin B and L possess extensive hydrolyzing activities on myofibril proteins (Parreno, Cusso, Gil, & Sarraga, 1994; Sárraga et al., 1993; Toldrá, Rico, & Flores, 1992; Toldrá et al., 1993). In addition, cathepsin B and L are rather stable during dry-cured ham processing (Parreno et al., 1994; Sárraga et al., 1993; Toldrá et al., 1992; Toldrá et al., 1993) and so they are considered to be the main endopeptidases responsible for muscle proteolysis and flavor formation in dry-cured ham.

Jinhua ham is one of the famous dry-cured hams of the world. However, little work was done on this ham. There has been no report on changes of cathepsin B and L activities during Jinhua ham processing.

Objectives

The objective of this investigation was to follow the changes of *biceps femoris* cathepsin B and L activities and their influencing factors during Jinhua ham processing using Response Surface Methodology (RSM).

Methodology

Materials

Sixty trimmed green hams weighing 6.2-6.9 kg from local crossbred swine (5-6 months, 90-100 kg) from Lanxi (Zhejiang province of P. R. China) were used to produce Jinhua ham. 7-amido-4-methyl-coumarin (AMC), Z-Arg-Arg-AMC and Z-Phe-Arg-AMC used for enzyme activity determination were purchased from Bachem Feinchemikalien (Bubendorf, Switzerland) and other chemical agents, purchased from Sigma (St. Louis, MO).

Jinhua ham processing

Jinhua hams were processed under natural condition as prescribed by traditional processing technology in Zhejiang Provincial Food Company, P. R. China. The process mainly involves 6 stages, i.e. natural cooling, dry salting, soaking and washing, sun-drying, loft-aging and post-aging. After natural cooling for about 24 h, green hams were piled and salted for 30 d during which salt was added 5 times (50 mg nitrate per kg green ham was mixed in the salt used at the 2nd time) and ham piles were turned over for 7 times. After soaking and washing for 24 h and sun-drying for 20 d (including 11 days of cloudy or rainy or snowy weather), hams were transferred to aging-loft and aged for five months. Then the hams were post-aged at room temperature for two months.

Sampling

Biceps femoris was fully sampled for analysis from 5 hams randomly taken after each processing stages, i.e. prior-salting (about 24 h), end of salting (31d), end of sun-drying (51d), middle loft-aging (124 d), end of loft-aging (203 d) and end of post-aging (264 d). Samples were packed and stored under -40 °C before analyzing.

Potential enzyme activity determination

Preparation of enzyme extract

The extraction was performed refer to Koohmaraie & Kretchmar (1990), García-Garrido et al. (2000) and Rosell & Toldrá (1998), with slight modifications. Samples were thawed at 4 °C, trimmed off visible fat or connective tissue and finely chopped. About 5 g chopped sample was homogenized in 35 ml extraction buffer (50 mM sodium acetate buffer, pH 5.00, containing 100 mM sodium chloride, 1 mM EDTA and 2 ml·L⁻¹ Triton X-100) with a polytron (three strokes, 10 s each at 23000 r.p.m. with cooling in ice) homogenizer (IKA T18 basic, Made in IKA, German). The homogenate was stirred for 60 min under 4 °C and then centrifuged at 22000 g for 20 min. The supernatant was filtered through fine silk cloth and finally diluted to 50 ml with extraction buffer for enzyme activity determination after shaking.

Enzyme assays

Enzyme activity was determined according to Parolari, Virgili, & Schivazappa, (1994), Blanchard & Mantle (1996) and García-Garrido, Quiles-Zafra, Tapiador, &

Luque-de-Castro (2000) with some modifications. To 2.5 ml substrate solution (50 mM sodium phosphate buffer, pH 6.00, containing 0.3125 mM Z-Arg-Arg-AMC, 4 mM EDTA, 2 mM DTT and 3.4 ml·L⁻¹ Brij for cathepsin B assay and 50 mM sodium phosphate buffer, pH 6.00, containing 0.3125 mM Z-Phe-Arg-AMC, 4 mM EDTA, 2 mM DTT and 3.4 ml·L⁻¹ Brij for cathepsin B+L assay), 0.5 ml enzyme extract was added. The mixture was incubated in a water bath at 37 °C for 20 min and the reaction was terminated by immediate addition of 6 ml of ethanol. The generated fluorescence was determined with a Cary Eclipse Fluorescence Spectrophotometer (VARIAN, Australia) at excitation and emission wavelengths of 380 nm and 440 nm, respectively. Calibration curves were drawn with AMC under similar condition. Cathepsin L activity was calculated by cathepsin B+L activity subtracting cathepsin B activity. One unit of enzyme activity (U) was defined as the amount of enzyme required to produce 1 nmol AMC per min at 37 °C. Potential muscle enzyme activity (the ability of hydrolyzing proteins under the defined condition of dry-cured ham) was calculated on the basis of *biceps femoris* dry matter subtracting fat and sodium chloride, i.e. (DM-Fat-Salt), and simply expressed as U·g⁻¹.

Response surface experimental design and method

In the present study, RSM based on Box-Behnken design (BBD) was adopted. Four factors, i.e. temperature (Temp.), salt content (Salt), sodium nitrate content (Nitrate) and pH value (Table 1), were considered in the design and their value ranges were set according to the determining results from Jinhua ham processing (Zhao, Zhou, Xu, Peng, Huan, Jing, & Chen, 2005). With Design-Expert software (version 6.0.5, Stat-Ease Inc., Minneapolis, USA), a four-factor and two-response (cathepsin B and L activities) experimental design was performed and 29 sets of experiments for each response were generated. For the convenience of controlling salt and nitrate content in reaction mixture, prior-salting samples were used in the experiments. Preparation of enzyme extracts was similar to the above mentioned procedure, but before the supernatants were diluted to 50 ml with extraction buffer, definite amount of NaCl and sodium nitrate were added and pH was adjusted to definite values as designed. Enzyme assay was similar to the above mentioned procedure, except for that the buffers used in substrate solutions were different in pH value, salt content and sodium nitrate content. In preparation of different substrate solutions in pH value, 200 mM sodium acetate buffer of pH 5.40, 200 mM sodium citrate buffer of pH 5.80 and 200 mM sodium phosphate buffer of pH 6.20 were used. One unit of enzyme activity (U) was defined as the amount of enzyme required to produce 1 nmol AMC per min at the designed reaction temperature. Muscle enzyme activity was calculated on the basis of *biceps femoris* dry matter subtracting fat and sodium chloride (DM-Fat-Salt), and simply expressed as U·g⁻¹.

Statistics

Enzyme activities were assessed by analysis of variance using one-way ANOVA procedure of SPSS 10.0 (SPSS Inc.). The data from response surface experiments were analyzed through analysis of variance and stepwise regression, and rotatable response surface figures were drawn by 3-D surface procedures inside Design-Expert software (version 6.0.5, Stat-Ease Inc., Minneapolis, USA). The actual activities of cathepsins (the

ability of hydrolyzing proteins under actual processing condition of Jinhua ham) were estimated by gained regression equations.

Results & Discussion

Changes of potential activities of cathepsin B and L

Potential activity of cathepsin B decreased gradually during processing, from 11332.05 U·g⁻¹ prior salting to 1055.32 U·g⁻¹ at the end of post-aging (Table 2). The potential activity of cathepsin L changed in the similar way to that of cathepsin B with the exception that the former showed a clear rise at the sun-drying stage. At the end of post-aging, 9.31% and 13.66% of the prior-salting activities were left for cathepsin B and cathepsin L, respectively.

By far, no information on change of muscle cathepsin activity during Jinhua ham processing can be referred. Related documents revealed that cathepsin B and L lost their activities (potential activities) gradually during dry-cured ham processing and 5% to 15% of the original activities of cathepsin B and B+L remained at the end of processing (Toldrá, Rico, & Flores, 1993). These statements were generally consistent with our results on Jinhua ham. In addition, an obvious increase of cathepsin L activity during sun-drying was recorded in this study. This may be out of the specific Jinhua ham processing technology. Since Jinhua ham is dehydrated directly in sunshine after salting and soaking, chemical and biochemical reactions may take place in muscle, which may produce compounds that boost the activity of cathepsin L.

3.2. Response surface of main influencing factors affected cathepsin B activity

Response surface experiments revealed that temperature, pH value and salt content had significant effects on cathepsin B and L activities ($P < 0.01$) (Table 3 and Table 4). In addition, temperature interacted with salt content ($P < 0.001$) on cathepsin B activity and interacted with pH value as well as with salt content ($P < 0.01$) on cathepsin L activity. However, sodium nitrate content in the range of 0-50 mg·kg⁻¹ didn't obviously affect cathepsin B or L activity ($P > 0.05$). Stepwise regression analysis produced two optimal quadratic regression equations for predicting cathepsin B and L activities individually (Eq.1 and Eq.2). Regression diagnostic analysis indicated that the regression equations were effective and very high R^2 , $Adj. R^2$, $Pred. R^2$ and $Adeq. Precision$ proved the effectiveness of the equations.

$$\begin{aligned} \text{Cathepsin B activity} = & 106866.43 + 222.28 \times \text{Temp} - 37348.27 \times \text{pH} - 187.54 \times \text{Salt} + \\ & 3.78 \times \text{Temp}^2 + 3280.59 \times \text{pH}^2 + 18.21 \times \text{Salt}^2 - 35.77 \times \text{Temp} \\ & \times \text{pH} - 9.16 \times \text{Temp} \times \text{Salt} \dots \dots \dots \text{Eq.1} \end{aligned}$$

$$\begin{aligned} \text{Cathepsin L activity} = & 1300.20 + 585.72 \times \text{Temp} - 220.27 \times \text{pH} - 1440.96 \times \text{Salt} + 6.90 \times \\ & \text{Temp}^2 - 107.58 \times \text{Temp} \times \text{pH} - 14.47 \times \text{Temp} \times \text{Salt} + 265.74 \times \\ & \text{pH} \times \text{Salt} \times \text{pH} \dots \dots \dots \text{Eq.2} \end{aligned}$$

Similar effects of temperature and pH value on cathepsin B and L activities were observed (Fig.1 and Fig.3). Cathepsin B and L activities increased in a quadratic curve

way along with the increase of temperature from 4 to 40 °C. On the other hand, the increase of pH value from 5.40 to 6.20 restrained cathepsin B and L activities to some degree. Temperature showed stronger effect at relatively lower pH value, while pH value could only influence cathepsin B and L activity at high temperature and no obvious pH effect could be observed below 10 °C on cathepsin B activity or below 20 °C on cathepsin L activity.

The effects of temperature and salt content on cathepsin B and L activities were also similar as shown in Fig.2 and Fig.4. Salt inhibited cathepsin B and L activities remarkably. The increase of salt content also weakened the effect of temperature on cathepsin B and L activities, but the increase of temperature strengthened the inhibiting effect of salt. However, when temperature was below 10 °C for cathepsin B or below 20 °C, the inhibiting effects of salt on cathepsin B and L activities were very weak.

According to our knowledge, no record of research on factors influencing cathepsin activity using RSM has been published up to now. Related studies by single-factor experiments, control experiments or simulating experiments revealed that salt notably inhibited the activities of cathepsin B and L (Rico, Toldrá, & Flores, 1991), increase of salt content reducing cathepsin B activity (Rico et al., 1991), but nitrate or nitrite showed little effect on cathepsins (Jaarsveld, Naude, & Oelofsen, 1998). The optimal pH value and temperature of cathepsin B and L were respectively 3.0 to 6.0 and 40 °C to 45 °C (Jaarsveld et al., 1998). Their activities weakened when pH value rose up and strengthened as pH dropped down (Arnau, Guerrero, & Sarraga, 1998). These results were in agreement with our studies by RSM. We also found that factors interacted with each other impacting the activities of cathepsin B and L, which should be helpful in interpreting changes of cathepsin B and L activities during dry-cured ham processing.

3.3. Changes of actual activities of cathepsin B and L during Jinhua ham processing

As a result of continuous changes of processing temperature, muscle salt content and pH value during dry-cured ham processing, muscle enzymes may seldom fully express their potential activities. Toldrá et al. (1992) simulated dry-cured ham processing conditions and studied the activities of muscle proteases, which was very useful in understanding the role of proteases in dry-cured ham processing. However, systematical investigation on changes of actual activities of cathepsin B and L along processing of dry-cured ham has not been documented. Through Eq.1 and Eq.2, changes of actual activities of *biceps femoris* cathepsin B and L during Jinhua ham processing were estimated with the factor data (refer to Zhao et al., 2005) and the results are shown in table 5. Table 5 revealed that less than 5% of the corresponding potential activity of cathepsin B and L could be displayed most of the time before half loft-aging, except for prior-salting when 8.31% of potential cathepsin B activity was exhibited. However, about 15% and 20% of cathepsin B and L potential activity, separately, showed as actual activities during the second half of loft-aging and post-aging stages. Relatively, the actual activity of cathepsin B was always higher than that of cathepsin L before half of loft-aging, but cathepsin L showed stronger actual activity than that of cathepsin B at the last two stages because a sharp increase of cathepsin L activity during the second half of loft-aging.

Conclusions

The activities of cathepsin B and L were significantly influenced by temperature, salt content, pH value and their interactions, but were not obviously affected by sodium nitrate. Cathepsin B and L could always hold high potential activities that fell gradually during Jinhua ham processing. At the end of processing, 9.31% original potential activity of cathepsin B and 13.66% original potential activity of cathepsin L were left. During most of the processing periods, only small part of cathepsin B and L potential activities were expressed as actual activities. Anyway, cathepsin B and L retained actual activities throughout processing, especially during loft-aging and post-aging periods.

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Tables and Figures

Table 1. Factors and levels for Box-Behnken response surface experimental design

Levels	Temperature (°)	Salt (%)	Sodium nitrate (mg·kg ⁻¹)	pH value
-1	4.0	0.0	0.0	5.40
0	22.0	5.0	25.0	5.80
+1	40.0	10.0	50.0	6.20

Table 2. Results of potential activities of cathepsin B and L after each processing stage of Jinhua ham

Time	Cathepsin B Activity		Cathepsin L Activity	
	M ± S.E. (U·g ⁻¹)	Remains (%)	M ± S.E. (U·g ⁻¹)	Remains (%)
Prior-salting	11332.05±717.67 ^a	100.00	9955.50±468.30 ^{ab}	100.00
End of salting	9444.73±1167.78 ^{ab}	83.35	8577.98±916.44 ^{bc}	86.16
End of sun-drying	7727.67±318.11 ^{bc}	68.19	11649.70±667.22 ^a	117.02
Middle of loft-aging	7314.73±822.02 ^c	64.55	6815.09±799.34 ^c	68.46
End of loft-aging	1988.61±459.57 ^d	17.55	2281.95±365.71 ^d	22.92
End of post-aging	1055.32±138.67 ^d	9.31	1359.90±212.87 ^d	13.66

Note:

- Means within the same row without common superscripts differed significantly, $P < 0.05$.
- M ± S.E. stands for means ± standard error.
- Remains (%) indicate the percentage of cathepsin activity after each stage accounting for that of prior-salting.

Table 3. ANOVA results of quadratic regression model for cathepsin B response surface

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	98439892.20	8	12304986.53	149.33	< 0.001
A (Temp.)	71404453.42	1	71404453.42	866.54	< 0.001
B (pH)	12376.72	1	12376.72	0.15	0.702
C (salt)	12850797.92	1	12850797.92	155.95	< 0.001
A ²	10112216.43	10	10112216.43	122.72	< 0.001
B ²	1853463.98	1	1853463.98	22.49	0.001
C ²	1394246.52	1	1394246.52	16.92	0.005
AB	265299.27	1	265299.27	3.22	0.088
AC	2718890.42	1	2718890.42	33.00	< 0.001
Residual	1648036.46	20	82401.82		
Error	58023.40	4	14505.85		
Total	100087928.67	28			

$R^2=0.98$ $Adj. R^2=0.98$ $Pred. R^2=0.96$ $Adeq. Precision=44.98$

Table 4. ANOVA results of quadratic regression model for cathepsin L response surface

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	207758656.76	7	29679808.11	82.77	< 0.001
A (Temp.)	144972262.04	1	144972262.04	404.27	< 0.001
B (pH)	3040410.65	1	3040410.65	8.48	0.008
C (Salt)	14251914.95	1	14251914.95	39.74	< 0.001
A ²	35181814.72	1	35181814.72	98.11	< 0.001
AB	2400014.17	1	2400014.17	6.69	0.017
AC	6782345.16	1	6782345.16	18.91	0.003
BC	1129895.07	1	1129895.07	3.15	0.090
Residual	7530656.77	21	358602.70		
Error	87330.54	40	21832.63		
Total	215289313.53	28			

$R^2=0.97$ $Adj. R^2=0.95$ $Pred. R^2=0.91$ $Adeq. Precision=30.57$

Table 5. Predicted actual activities of cathepsin B and L after each processing stage

Cathepsin	Statistics	Prior-salting	End of salting	End of sun-drying	Middle of loft-aging	End of loft-aging	End of post-aging
Cathepsin B	Predicted activity (U·g ⁻¹)	941.71	237.31	198.18	302.37	300.58	164.70
	Activity present (%)	8.31	2.51	2.56	4.13	15.12	15.61
Cathepsin L	Predicted activity (U·g ⁻¹)	335.20	101.27	194.86	186.15	467.39	274.89
	Activity present (%)	3.37	1.18	1.67	2.73	20.48	20.21

Note: Activity present % = Predicted actual ÷ Potential activity × 100

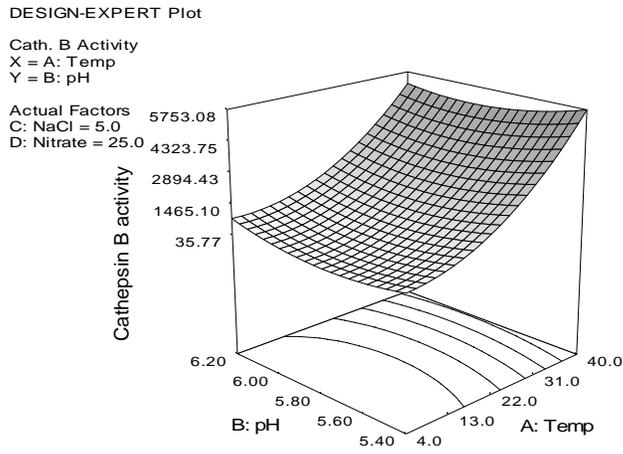


Fig.1 Effects of temperature and pH value on cathepsin B activity

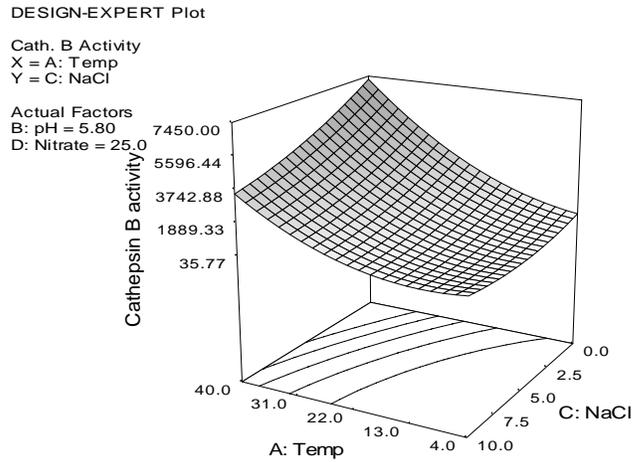


Fig.2 Effects of temperature and salt content on cathepsin B activity

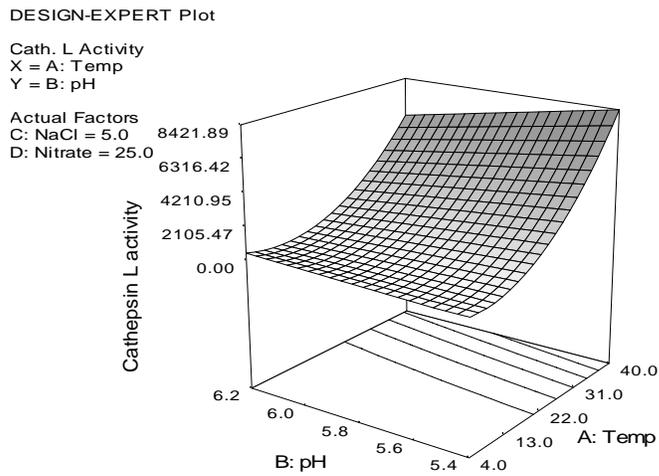


Fig.3 Effects of temperature and pH value on cathepsin L activity

DESIGN-EXPERT Plot

Cath. L Activity
X = A: Temp
Y = C: NaCl

Actual Factors
B: pH = 5.8
D: Nitrate = 25.0

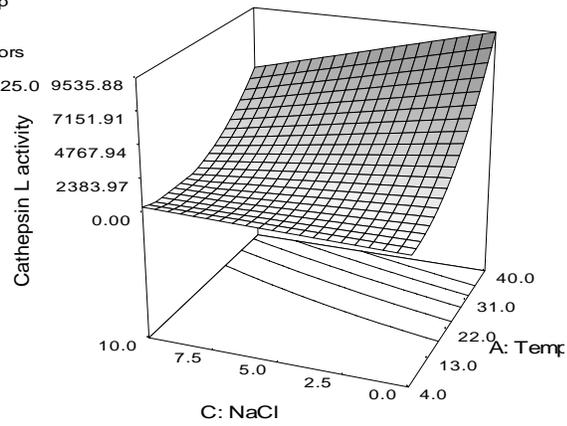


Fig.4 Effects of temperature and salt content on cathepsin L activity

EFFECT OF HYDRODYNAMIC PRESSURE TREATMENT BEFORE PROCESSING ON PORK HAM QUALITY

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Key Words: ham, hydrodynamic pressure process, further processed

Introduction

The tenderizing effect of the hydrodynamic pressure process (HDP) has been studied in a variety of fresh and frozen/thawed meats. A small amount of explosive, suspended above the meat, is detonated to create a shock wave that tenderizes meat as a result of microscopic tears in the myofibrillar structure (Zuckerman and Solomon, 1998). Very little research has evaluated the tenderization and quality effects of HDP in further processed meat products (Senecal et al., 2001; Schilling et al., 2002). When HDP was applied to beef semitendinosus, which was then subjected to freeze-drying and stored 60 days (37.8°C), HDP treatment reduced shear stress and increased the percent rehydration (9.9%) of freeze-dried meat compared to the control (Senecal et al., 2001). Schilling et al. (2002) HDP treated beef biceps femoris before processing into frankfurters and found no difference between control and HDP treated samples for color, cook yield, protein solubility or textural properties. The hams used for this preliminary experiment were part of a larger study involving transgenic and dietary conjugated linoleic acid (CLA) supplemented pigs. Eastridge et al. (2001) reported that neither gene (IGF-I transgene) or diet (CLA) affected pork loin quality parameters (ultimate muscle pH, amount of purge during storage, TBARS malonaldehyde formation during storage, cook yield, and shear force).

Objectives

The objective of this study was to determine the effect of HDP treatment (before processing) on the processing, water holding capacity, color, and textural properties of smoke-cooked ham.

Methodology

Hams for this study were obtained from IGF-I transgenic and control pigs fed a diet supplemented with 2% corn oil (control) or with 1% corn oil and 1% CLA-60 until 120 kg target weight. The pigs were slaughtered on site under humane conditions (USDA Abattoir #68, Beltsville, MD). The carcasses were chilled at 3°C for 48 hours. A 15 cm section of ham was removed from the bone at the largest area of the leg. The green hams were packaged in 3 mil high performance vacuum packaging bags (Model 030044, Koch, Kansas City, MO), frozen and stored at -24°C. A total of sixteen hams were chosen such

that transgene and diet factors of the study were balanced. For each genetic/diet group, HDP treatment was assigned to two hams and the remaining two hams served as controls. Due to the size of the ham and the multiple muscles it contained, it was not possible to have a paired control for each treated ham.

For each of two processing days (replications), eight frozen hams were thawed for 5 days at 2°C. The external skin and fat were removed from all meat samples. Two hams designated for HDP treatment were vacuum packaged into a single bone guard bag (Model B650TBGW, Cryovac®/Sealed Air Corp., Duncan, SC) and briefly heat shrunk (91°C). A single package was placed onto a 1.3 cm thick flat metal reflector plate inside a water filled 98-L plastic explosive container (Rubbermaid Inc., Wooster, OH) for each of two HDP treatments. A 100 g binary explosive (cylinder shape) was suspended 31 cm above the meat and detonated to create the shock wave treatment. Control hams were kept at 2°C during HDP treatment.

After HDP treatment, control and HDP hams were injected using a three prong needle with brown sugar cure (Palmer House, Waterloo, IA) brine to 115% target weight. All hams were placed in the vacuum tumbler (Model ET-3, Sipromac, Quebec, Canada) and tumbled 15 minutes on and 15 minutes off (9 RPM) for a total of 2 hours. Brined hams were kept at 2°C for 12-14 hours and then placed into heavy weight poly smoking nets (Model 260700455, Koch Supplies, North Kansas City, MO). The hams were natural smoke-cooked to 68.3°C in the smokehouse (Model 700 HP, Alkar, Lodi, WI) using the Alkar schedule: boneless hams, netted, heavy smoke, conditioning step (Alkar Operations and Maintenance Manual, Process Schedules, Section 4, page 3). Immediately after cooking, the hams were placed in the 2°C cooler overnight. The hams were vacuum packaged in 3 mil bags and stored (2°C) for 5 days. After storage, the hams were cut into three 2.5 cm thick slices. Two slices were packaged individually in 3 mil vacuum packaging bags and stored (2°C) until needed for textural evaluation. The third slice was immediately analyzed for color, water holding capacity (WHC) and fat and moisture analysis.

During processing, weights were recorded for the following processing parameters. Percent brine uptake was determined by the difference between after and before tumbling weights. Smoke-cook loss was the weight lost between tumbling and smoking. Processing yield was determined by the weight difference between after 18 hr chilling and original ham weight. 18 hr chilled purge was determined between the weights after 18 hr storage and the smoke-cook process. The 5 days storage purge was determined by the difference between the weights of 5 days storage and the 18 hr storage.

The ham slice designated for color, WHC and moisture and fat analysis was placed on a plastic tray and covered with All-Purpose Food Wrap (Polyvinyl Films, Inc., Sutton, MA) to allow blooming of the hams at room temperature (25°C) for 1 hour. A minimum of six color measurements were taken on each slice with a Chroma Meter (Model CR-200, Minolta Camera Co., Ltd, Osaka, Japan). WHC was measured on a minimum of three 2.5 cm diameter, 10 g ham cores as described by Desmond et al. (2000) with modifications. For centrifugation, two absorbent pieces of paper (Kimwipes, Kimberly-Clark, Neenah, WI) were placed in the bottom of the centrifuge tube and covered with a Whatman #1 filter paper disk (2.6 cm diameter). Soxhlet fat and moisture analysis was determined with the remaining sample.

Ham slices were randomly assigned to instrumental analysis using the Warner-Bratzler Shear Test (WBS, AMSA, 1995) or Texture Profile Analysis (TPA, Bourne, 1978). A minimum of six cores were removed using a 1.3 cm diameter corer parallel to the direction of the muscle fibers for WBS and measured on the Universal Instron Testing Machine (Model 1122, Canton, MA) using a 100 kg load cell with a crosshead speed of 250 mm/min. Ham cores (2.5 cm diameter, 2.0 cm thick) were compressed to 50% of original height two times for TPA using the Universal Instron Testing Machine (crosshead speed 50 mm/min, 7.5 cm diameter compression platen). TPA parameters calculated were hardness, cohesiveness, springiness, chewiness, and gumminess.

Ham processing parameters, color, and textural instrumental analysis were analyzed using SAS® (Version 9.1, SAS Institute Inc., Cary, NC, 2002-2003) PROC MIXED with a model that included animal as a random effect and fixed effects were HDP treatment, gene, and diet. Least square means for HDP treatment and control were separated using pairwise t-tests (LSMEANS/DIFFS option).

Results & Discussion

Since testing parameters were not affected with any practical significance by genetic background or diet, only HDP treated vs. control effects are presented. HDP treated hams were not found to be different ($P>0.05$) than the controls for processing parameters (Table 1). Senecal et al. (2001) reported that the rehydration rate was increased by 9% on semitendinosus muscle treated with HDP, freeze dehydrated and rehydrated on day 0 compared to controls. The percent rehydration increased to 10% on freeze dehydrated meat stored 60 days (37.8°C) and rehydrated. For this ham study, the water holding capacity was slightly but not significantly higher for HDP treated hams than controls. It also was noted in Senecal and coworkers' research that percent cook loss was reduced 2 to 4% for HDP treated samples. In the present study, the smoke-cook loss was slightly but not significantly higher for HDP treated hams than from controls.

The surface color of the ham slices were not significantly different for L, a*, and b* due to HDP treatment (Table 2). These results are similar to previous studies evaluating color parameters (Schilling et al., 2002; Berry et al., 1999). No differences in finished product color were found for beef treated with HDP prior to processing into frankfurters (Schilling et al., 2002). Berry et al. (1999) found that color properties of ground beef patties were not affected when the lean and fat materials were HDP treated. No significant differences were found for fat and moisture content for treated and control hams (Table 2).

WBS force values and TPA parameters are presented in Table 3. No significant differences were found for WBS values. TPA springiness was higher ($P<0.05$) for the control hams in comparison to the hams treated with HDP. Springiness is the ability for the sample to recover its height from the first compression to the second (Bourne, 1978). A possible explanation for the inability of the HDP treated sample to return to its original form could be due to the microscopic tearing of the myofibrillar structure that occurs during HDP (Zuckerman and Solomon, 1998). No other TPA parameters, hardness, cohesiveness, chewiness and gumminess, were significantly affected by the HDP treatment. Initially these ham samples were tender before HDP treatment with WBS values less than 2.0 kgf. Thus, any HDP treatment improvements for tenderness/texture

may be difficult to detect. These textural results are in agreement with Schilling et al. (2002) who did not find any differences for TPA parameters between frankfurters made with either HDP treated biceps femoris or controls. In contrast, Berry et al. (2000) reported $\geq 40\%$ tenderness improvement for HDP treated freeze dried beef and beef and chicken sticks which improved in tenderness as a result of HDP treatment.

Conclusions

Hams processed from HDP treated muscles were not different from the controls for any of the processing parameters that are of interest to ham manufacturers. No detectable differences were found for water holding capacity, color or chemical analysis. HDP treatment did not affect textural properties with the exception of TPA springiness. These results indicate that use of HDP before processing hams does not adversely or favorably affect ham quality.

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† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Tables and Figures

Table 1. Mean values^a (standard errors) for the effects of hydrodynamic pressure processing (HDP) for processing parameters^b and water holding capacity

Treatment	Percent	Smoke-	Processing	18 hr	5 Day	Water
	Brine Uptake	cook Loss		Chilled Purge	Storage Purge	Holding Capacity
Control	11.61 (0.84)	24.57 (1.50)	82.02 (1.90)	3.37 (0.18)	0.93 (0.09)	87.97 (0.57)
HDP	11.01 (0.84)	26.15 (1.50)	80.44 (1.90)	3.47 (0.18)	0.95 (0.09)	88.33 (0.55)

^aMeans without any superscripts within a column are not significantly different at $\alpha=0.05$.

^bHam processing parameters were percent brine uptake, processing yield, smoke-cook loss, processing yield, 18 hr chilled purge, 5 day storage purge.

Table 2. Mean values^a (standard errors) for the effects of hydrodynamic pressure processing (HDP) for smoke-cooked ham color and chemical analysis

Treatment	Color			Chemical Analysis	
	L	a*	b*	% Moisture	% Fat
Control	61.65 (1.54)	11.26 (0.34)	12.02 (0.13)	64.07 (0.97)	9.19 (1.01)
HDP	61.06 (1.53)	11.52 (0.34)	11.79 (0.13)	65.80 (0.97)	6.67 (1.01)

^aMeans without any superscripts within a column are not significantly different at $\alpha=0.05$.

Table 3. Mean values (standard errors) for the effects of hydrodynamic pressure processing (HDP) for Warner-Bratzler shear force (WBS) and Texture Profile Analysis

Treatment	WBS (kgf)	Texture Profile Analysis				
		Hardness (kgf)	Cohesiveness	Springiness (mm)	Chewiness (kgf mm)	Gumminess
Control	1.77 (0.04)	10.87 (0.66)	0.45 (0.01)	0.61 ^a (0.01)	2.96 (0.17)	4.95 (0.29)
HDP	1.84 (0.04)	12.21 (0.60)	0.44 (0.01)	0.57 ^b (0.01)	3.07 (0.19)	5.41 (0.32)

^{ab}Means with different letters within a column are significantly different at $\alpha=0.05$.

**ASSESSMENT OF CARCASS HANGING TECHNIQUE AND CONDITIONING
TEMPERATURE ON QUALITY PARAMETERS OF CURED PIGMEAT
PRODUCTS**

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Key Words: pigmeat, cured, hanging, temperature conditioning.

Introduction

A number of post-slaughter treatments can be used to influence meat tenderness, such as electrical stimulation, pelvic suspension and conditioning. To improve tenderness of meat, by preventing some muscles from shortening during rigor mortis, carcasses may be suspended from the aitch bone (pelvic suspension) shortly after slaughter instead of using the Achilles tendon (Hostetler *et al.*, 1975).

As is the case for electrical stimulation, pelvic suspension has been applied primarily to increase beef tenderness. However, pelvic suspension has also been shown to improve tenderness in pork (Moller and Vestergaard, 1986; Taylor *et al.*, 1995), as well as reduce drip loss (Dransfield *et al.*, 1991) and the practice of pelvic suspension has been adopted by both beef and pork industries to improve tenderness.

The post mortem chilling procedure of blast-chilling shortens the carcasses' chilling time and reduces the occurrence of PSE meat (Milligan *et al.*, 1998). Blast-chilling is thus attractive to the pork industry and is now commonly used. However, this more efficient chilling has been reported to produce tougher pork (Dransfield and Lockyer, 1985; Moller & Vestergaard, 1986). Tougher meat can be related to the phenomenon of cold-shortening, which occurs in meat that is chilled below 10°C pre-rigor, when the adenosine triphosphate (ATP) level is still high (pH above 6.0) (Bendall, 1973; Bendall, 1975). A milder chilling regime would therefore be preferable when tenderness is the quality aspect of highest priority (Josell *et al.*, 2004).

Objectives

The effects of temperature conditioning and hanging method on the quality of cured meat products are not well documented. Therefore, the objective of this study was to improve processed pork quality by a combination of alternative hanging methods and higher temperature conditioning.

Methodology

Large White × Landrace cross pigs (n=32) of approx 95kg live weight were slaughtered by exsanguination after CO₂ stunning. Following evisceration, pigs were centrally split and the left sides of all carcasses were hung from the Achilles Tendon (AT) and the right sides were suspended from the Pelvic Bone (PB). The groups were then halved and subjected to either (i) conditioning in air at 17°C for ~3 hours (hrs) followed by air at 1°C for 21 hrs (n=16), or (ii) no conditioning and were chilled at 1°C for 24 hrs (n=16). Ham, shoulder and loin primal cuts were then removed from each carcass. Excess fat, connective tissue, bone and rind were removed from the primal cuts prior to injection (15% weight increase) with brine (12% salt, 0.15% sodium nitrite, 0.15% sodium nitrate, 0.5% sodium ascorbate, 2.5% potassium phosphate and 3.5% sugar). Raw hams and shoulders were subsequently vacuum tumbled at 10 rpm for 1 hr (0°C). Hams were stuffed into elasticated nettings (Micromesh Net, 8 inch) and vacuum packed in heat-shrinkable cooking bags (Cryovac BB4L) while the shoulders were vacuum packed into aluminium moulds using heat-shrinkable cooking bags (Cryovac BB4L) and cooked to an internal temperature of 72°C and at a relative humidity of 99%. Ham, shoulder and loin slices were flushed with 70% N₂ : 30% CO₂ for storage under modified atmosphere conditions. All samples were stored for up to 18 days in a refrigerated display cabinet (4°C, 616 lux fluorescent lighting).

The extent of lipid oxidation was assessed by measuring thiobarbituric acid reacting substances (TBARS) using the method of Ke *et al.* (1977) and expressed as mg malonaldehyde/kg sample. CIE L* and a* values were recorded using a Minolta chromameter CR-300 (Minolta Camera Co., Chou-Ku, Osaka 541, Japan). Warner Bratzler shear force (WBSF) was measured using the blade and guillotine attachment of the SMS TA.XT2i Texture Analyser (Stable Micro Systems, UK). Cook loss of loin samples was assessed gravimetrically.

A full-repeated measures ANOVA was conducted to investigate the effects of hanging, temperature and day on meat quality using SPSS 11.0 software package for Windows (SPSS, Chicago, IL, USA).

Results & Discussion

Hanging methods had no significant effects on WBSF determined for any of the cured products examined (Table 1). This finding is at odds with the generally accepted principle that pelvic bone suspension can aid in improving meat tenderness (Desmond and Kenny, 2005). In general, higher temperature conditioning of pork products did not improve tenderness although hams hung by both AT and PB were significantly ($p < 0.001$) more tender at the end of the 18 day storage period. This effect may have been due to the combined effects of prevention of early *post-mortem* cold shortening, promotion of proteolysis and the tenderising effects of ageing. Rees *et al.* (2002) reported a temperature of 14 °C as suitable for conditioning of pork in order to enhance tenderness without detrimentally affecting other quality attributes and such a recommendation is supported, in the case of hams, by the present study.

The levels of lipid oxidation in the cured pork products examined (Table 2) remained low throughout storage and below that (0.5mg malonaldehyde/kg sample) detectable by

trained sensory panelists (Lanari *et al.*, 1995). Such low values are the result of the presence of known antioxidants (nitrites, phosphates and ascorbates) in the brine solution and anoxic packaging conditions. Hanging method did not significantly affect shelf life stability. Trends observed for the effects of temperature were not consistent for the three primal cuts. In the case of hams, TBARs values were, in general, lower for samples conditioned at 17°C. The opposite effect was found for shoulders and loins. No pertinent literature was available for comparison of such effects.

Colour stability during refrigerated storage was not influenced by hanging method (Table 3). Higher conditioning temperature did, however, significantly affect L* values of ham samples, which were lower (paler) than corresponding samples conditioned at 1°C. This result contrasts with the findings of Rees *et al.* (2002) who reported a linear relationship between L* values and conditioning temperature. In general, L* values of shoulders and loins were not affected by conditioning temperature. In the case of a* (redness) values, higher conditioning temperature resulted in a decreased values during storage of all three primal cuts.

Hanging method had no significant effect on cook loss of cured products during storage (Table 4). Overall, pork conditioned at 17°C had lower cook loss and this effect decreased with storage time. Higher temperature conditioning may have caused a decrease in the extent of muscle contraction early *post mortem* and a subsequent decrease in moisture losses upon cooking.

Conclusions

Hanging method did not influence the quality attributes of cured hams, shoulders and loins. Conditioning temperature did affect the pork quality although these effects were not consistent between the cuts examined. The results suggest that the effects of temperature conditioning may be muscle dependent and that the use of such a processing technique holds promise as a means of controlling the quality of pork products.

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Tables and Figures

Table 1. Effect of hanging method and conditioning temperature on Warner Bratzler shear force of cured pork hams, shoulder and loins.

Primal Cut			Hanging method				Significance	
			<i>AT</i>		<i>PB</i>		<i>H</i>	<i>T</i>
(Time, Days)			Temperature (T)					
			<i>1°C</i>	<i>17°C</i>	<i>1°C</i>	<i>17°C</i>		
WBSF (kg)	Ham	1	2.56	2.69	2.15	2.22	ns	ns
		4	2.38	2.18	2.58	1.84	ns	ns
		8	2.70	2.33	2.45	1.95	ns	ns
		12	3.00	2.17	3.12	2.86	ns	ns
		18	4.92	2.00	3.91	2.65	ns	***
	Shoulder	1	2.60	3.02	2.91	3.59	ns	ns
		4	2.79	2.82	3.61	3.74	ns	*
		8	3.39	2.72	3.36	2.95	ns	ns
		12	2.68	2.56	2.65	2.77	ns	ns
		18	3.47	4.00	2.54	2.95	ns	*
	Loin	1	4.14	3.83	3.85	3.55	ns	ns
		4	3.51	3.26	3.60	3.27	ns	ns
		8	3.92	3.73	3.25	3.12	ns	**
		12	3.55	3.52	3.63	3.25	ns	ns
		18	3.16	3.09	3.54	3.45	ns	ns

Significance: * = $P < 0.05$, ** = $P < 0.01$, P = $P < 0.001$, ns = non-significant; $P > 0.05$.

Table 2. Effect of hanging method and conditioning temperature on lipid oxidation (TBARS) of cured pork hams, shoulder and loins held during storage at 4 °C.

	Primal Cut	(Time, Days)	Hanging method				Significance	
			<i>AT</i>		<i>PB</i>		<i>H</i>	<i>T</i>
			Temperature (T)					
			1°C	17°C	1°C	17°C		
TBARS (mg MDA/kg)	Ham	1	0.27	0.08	0.15	0.09	ns	**
		4	0.13	0.09	0.10	0.10	ns	ns
		8	0.13	0.08	0.11	0.13	ns	ns
		12	0.18	0.09	0.11	0.08	ns	**
		18	0.14	0.13	0.19	0.12	ns	*
	Shoulder	1	0.10	0.18	0.12	0.17	ns	ns
		4	0.09	0.16	0.11	0.14	ns	*
		8	0.10	0.13	0.08	0.15	ns	*
		12	0.18	0.10	0.10	0.08	*	*
		18	0.09	0.13	0.06	0.13	ns	**
	Loin	1	0.10	0.13	0.08	0.13	ns	*
		4	0.05	0.08	0.05	0.10	ns	*
		8	0.07	0.12	0.05	0.08	ns	***
		12	0.08	0.08	0.07	0.08	ns	ns
		18	0.04	0.11	0.04	0.10	ns	***

Significance: * = $P < 0.05$, ** = $P < 0.01$, P = $P < 0.001$, ns = non-significant; $P > 0.05$.

Table 3. Effect of hanging method and conditioning temperature on colour stability of cured pork hams, shoulder and loins held during storage at 4 °C.

Test	Primal Cut		Hanging method				Significance	
			<i>AT</i>		<i>PB</i>		<i>H</i>	<i>T</i>
			Temperature (T)					
(Time, Days)		<i>1°C</i>	<i>17°C</i>	<i>1°C</i>	<i>17°C</i>			
L* Values	Ham	1	62.80	58.25	62.90	57.20	ns	**
		4	63.70	56.31	64.61	55.89	ns	***
		8	63.66	57.36	65.41	56.33	ns	***
		12	61.35	57.17	62.54	53.09	ns	***
		18	59.36	56.19	60.67	52.07	ns	***
	Shoulder	1	57.14	54.15	58.03	54.56	ns	*
		4	53.01	55.44	54.51	54.97	ns	ns
		8	52.41	52.56	53.57	52.90	ns	ns
		12	56.56	55.93	58.10	54.64	ns	ns
		18	52.08	52.99	53.66	51.64	ns	ns
	Loin	1	41.62	43.78	40.28	44.02	ns	ns
		4	42.08	42.26	41.13	44.00	ns	ns
		8	41.08	43.99	40.05	41.30	ns	ns
		12	44.14	39.65	43.87	41.29	ns	**
		18	40.14	38.55	41.30	42.47	*	ns
a* values	Ham	1	7.06	6.54	7.2	6.71	ns	ns
		4	8.38	6.83	8.41	6.93	ns	*
		8	7.73	6.42	8.25	6.24	ns	**
		12	5.02	6.51	7.12	6.93	ns	ns
		18	3.20	5.22	5.38	5.33	ns	ns
	Shoulder	1	6.41	7.65	7.15	6.69	ns	ns
		4	5.87	7.86	6.39	8.63	ns	**
		8	7.5	8.61	6.42	7.75	ns	ns
		12	4.61	7.65	5.9	7.6	ns	**
		18	5.88	6.42	5.65	7.49	ns	ns
	Loin	1	1.81	3.14	2.51	2.55	ns	ns
		4	1.16	3.72	2.01	3.85	ns	***
		8	1.41	2.84	1.4	2.53	ns	**
		12	0.89	2.95	1.43	2.41	ns	**
		18	1.5	2.75	1.92	2.16	ns	ns

Significance: * = P < 0.05, ** = P < 0.01, P = P < 0.001, ns = non-significant; P > 0.05.

Table 4. Effect of hanging method and conditioning temperature on cook loss of cured loins held during storage at 4 °C.

		Primal Cut	Hanging method				Significance	
			<i>AT</i>		<i>PB</i>		<i>H</i>	<i>T</i>
Cook loss (%)	Loin	(Time, Days)	Temperature (T)					
			<i>1°C</i>	<i>17°C</i>	<i>1°C</i>	<i>17°C</i>		
		1	30.41	24.38	30.56	24.76	ns	***
		4	29.83	25.25	29.57	24.94	ns	*
		8	25.21	27.01	27.94	26.44	ns	ns
		12	24.43	25.42	26.06	24.45	ns	ns
		18	24.47	22.62	25.76	22.38	ns	ns

Significance: * = $P < 0.05$, ** = $P < 0.01$, P = $P < 0.001$, ns = non-significant; $P > 0.05$.

PROTEOLYSIS IN NORWEGIAN DRY-CURED HAM; PRELIMINARY RESULTS

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Key Words: Dry-cured ham, proteolysis, proteome, 2DE

Introduction

The processing of dry-cured hams is very complex and involves numerous biochemical reactions. One of the most important biochemical reactions in the development and processing of dry-cured ham is proteolysis. Along the dry-curing ripening process, numerous free amino acids are generated from peptides and protein fragments. It is speculated that the presence of some of these free amino acids and peptides in dry-cured hams make an important contribution to taste or even interact with volatile compounds to affect the whole flavor (Sentandreu and Toldra, 2000). Moreover, the combined effect of different proteolytic enzymes (cathepsin B, D, H, L, dipeptidases etc.) stimulate proteolysis, leading to flavor development and in reducing the hardness of dry-cured hams produced by a long ripening process.

Proteomics is a tool to study protein degradation in dry-cured hams and may be used to compare degradation patterns between different samples (Luccia *et al.*, 2005).

Objectives

1. Characterization of Norwegian dry-cured hams using chemical analysis.
2. To investigate and compare proteolytic degradation among different dry-cured hams produced in Norway using proteomics.

Methodology

A total number of 10 samples from Norwegian dry-cured hams from 6-month-old pigs from three different producers and four samples from non-processed fresh Norwegian hams were used in this study (Table 1). The dry-curing process consist of the traditional stage of salting between 0.8 and 1.0 days/kg ham, followed by post salting between 10 and 12 weeks. Finally, hams were allowed for ripening-drying period of 11-14 months, depending upon variable traditional production process and type of companies. Samples were analyzed for proteolytic index, protein content, salt content

and water activity (a_w) and compared for proteolytic degradation using proteomics. Salt content and a_w were measured in the dry-cured hams only.

Crude protein was determined by the Kjeldahl method and proteolytic index was calculated as percent ratio between nitrogen soluble in 5% trichloroacetic acid, determined by the Kjeldahl method after protein precipitation with trichloroacetic acid, and total nitrogen, as recommended by the Nordic Committee on Food Analysis standards (NMKL 6:2003). Salt (chloride) content in the dry-cured hams was measured according to the International Dairy Federation standards (IDF 179:1997). The water activity in the dry-cured hams was detected using the official AOAC method (AOAC 978.18). Extraction of muscle proteins were performed as described in Lametsch and Bendixen, (2001). 100 μ g proteins were separated on 18 cm IPG pH 4 -7 in the first dimension and a 10% SDS-PAGE in the second dimension. Gels are silver stained according to the protocol in Blum *et al.* (1987) and aligned and quantitated using Image Master 2D Platinum v5.0 (Amersham Biosciences).

Multivariate statistical methods such as Principal Component Analysis (PCA) was used to interpret the variations in the data set obtained by 2DE using Unscrambler 9.0 (Camo, Norway).

Results & Discussion

Proteomics using two-dimensional gel-electrophoresis (2DE) is a powerful tool to study degradation of muscle proteins. However, to our knowledge only one study are published describing the use of proteomics to investigate protein degradation in dry-cured hams (Luccia *et al.*, 2005). In this study, we describe the preliminary results of 2DE on Norwegian dry-cured hams and non-processed fresh ham. The results shown in Figure 1 showed that the protein patterns are generally changed between fresh non-processed and finished dry-cured ham during ripening. In the dry-cured hams, the main protein spots observed in Figure 1A are degraded as shown in figure 1B. In addition, a lot of proteins are observed in the upper part of the gel. This may be degradation products resulting from cleavage of the myofibrillar proteins. Furthermore, several protein spots appear in the lower part of the gels. These are also probably peptides generated from degradation of the structural muscle proteins in dry-cured hams during the ripening period. However, further analysis is needed to identify these proteins or peptides. The mechanism of protein degradation and effect of proteolytic enzymes were not studied. Previous investigators have demonstrated the release of free amino acids, peptides in the dry-cured hams are due to proteolytic enzymatic processes (Sentandreu and Toldra, 2000). The effect of proteolytic enzyme could be of great interest in stimulating proteolysis, in flavor development and in reducing the hardness of dry fermented sausages produced by a long ripening process.

Muscle samples from seven dry-cured hams were studied by 2DE (Samples S3, S4, S5, S7, S8, S9, and S10 in Table 1). Comparison of the protein patterns from the different samples showed a great variation in the degradation pattern between the different hams. Representative images of some of the 2DE protein patterns are shown in Figure 2. Multivariate statistical tools are useful in the analysis of complex data like 2DE protein patterns. A Principal Component Analysis (PCA) plot of the protein patterns are shown in Figure 3. The analysis demonstrated a great variation in the protein pattern observed in

the 2DE gels in the hams, and five principal components were needed to explain 88% of the variation in the data. However, a slight covariance is observed within the hams from the different production plants marked by circles in the PCA score plot. Further investigations are needed to identify the proteins and peptides that are related to the degree of proteolysis and end product quality of dry-cured hams.

Results from chemical investigation revealed that salt content among dry-cured hams are variable, ranging between 3.6 % and 7.8 % (Table 1). Theoretically, a lower salt content and less dehydration should result in dry-cured ham with more aroma and protein degradation. Generally, salt content and salt penetration the dry-cured hams are related to initial weight of the hams. Small hams have a large surface to mass ratio to receive more salt leading to salty product. Generally, our results suggest good agreement when salt content in dry-cured hams was calculated with initial weight of each ham. The results in the present study indicate that salt content is mainly related to the a_w , showing that high salt concentration enhances ham drying process (Figure 4). Protein content for the tested samples ranged between 20.8 % and 39.8 %. Proteolytic index among dry-cured hams varied between 0.20 and 0.36 (Table 1). Some of the dry-cured hams showed higher proteolytic index, indicating accelerated protein degradation. It is well known that proteolysis contribute positively to flavor and to the nutritional quality of matured ham, but also results in undesirable traits and disadvantages such as softness and color changes. The enzymes activity levels of the muscles, significantly depend on the properties of pre-slaughter meat properties (raw ham), such as age, and crossbreeding as well as the process conditions such as temperature, time, water activity, salt content (Sentandreu and Toldra, 2000). Thus, the control of the muscle enzyme systems plays important role for the standardization of the processing and/or enhancement of flavor quality of dry-cured ham.

Conclusions

The preliminary results showed that salt distribution, proteolytic index and protein degradation is variable among Norwegian dry-cured hams. However, the results are preliminary and based on few samples from different producers. Protein degradation giving rise to an important collection of free amino acids and small peptides that may directly contribute to flavor or indirectly contribute as precursors of other flavor compounds. Further investigation is needed to study the relation between protein degradation, and quality parameters such as sensory analyses.

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Table 1. Collection of samples from Norwegian dry-cured hams and preprocessed hams from three different companies.

Dry-cured hams	Type	Analysis	
		Protein	Proteolytic index
<i>Company 1</i>			
Sample S1	Dry-cured ham	27,9	0,29
Sample S2	Dry-cured ham	30,8	0,20
Sample S3	Dry-cured ham	38,0	0,25
Sample S4	Dry-cured ham	39,8	0,25
Sample S5	Dry-cured ham	34,0	0,28
Sample S6	Dry-cured ham	35,2	0,27
Sample R12	Preprocessed hams	21,7	0,14
Sample R13	Preprocessed hams	19,0	0,13
Sample R14	Preprocessed hams	22,3	0,13
Sample R15	Preprocessed hams	23,6	0,14
<i>Company 2</i>			
Sample S7	Dry-cured ham	26,3	0,27
Sample S8	Dry-cured ham	27,6	0,24
<i>Company 3</i>			
Sample S9	Dry-cured ham	26,3	0,20
Sample S10	Dry-cured ham	27,0	0,22

Figure 1. Representative 2DE maps of porcine muscle proteins. A. Non-processed muscle. B. Finished dry-cured ham. Proteins are separated on IPG 4-7 and 10% SDS-PAGE.

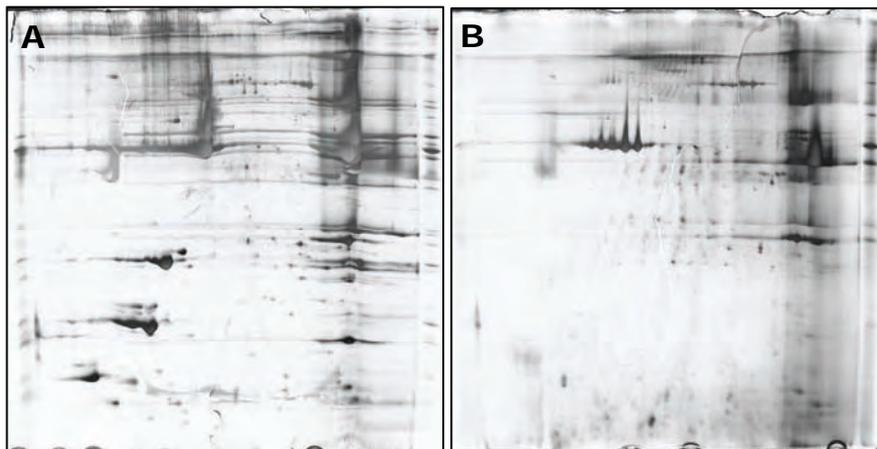


Figure 2. Representative 2DE images of dry-cured hams from 3 different companies. A. Sample S4, B. Sample S5, C. Sample S8 and D. Sample S9. Proteins are separated on IPG 4-7 and 10% SDS-PAGE.

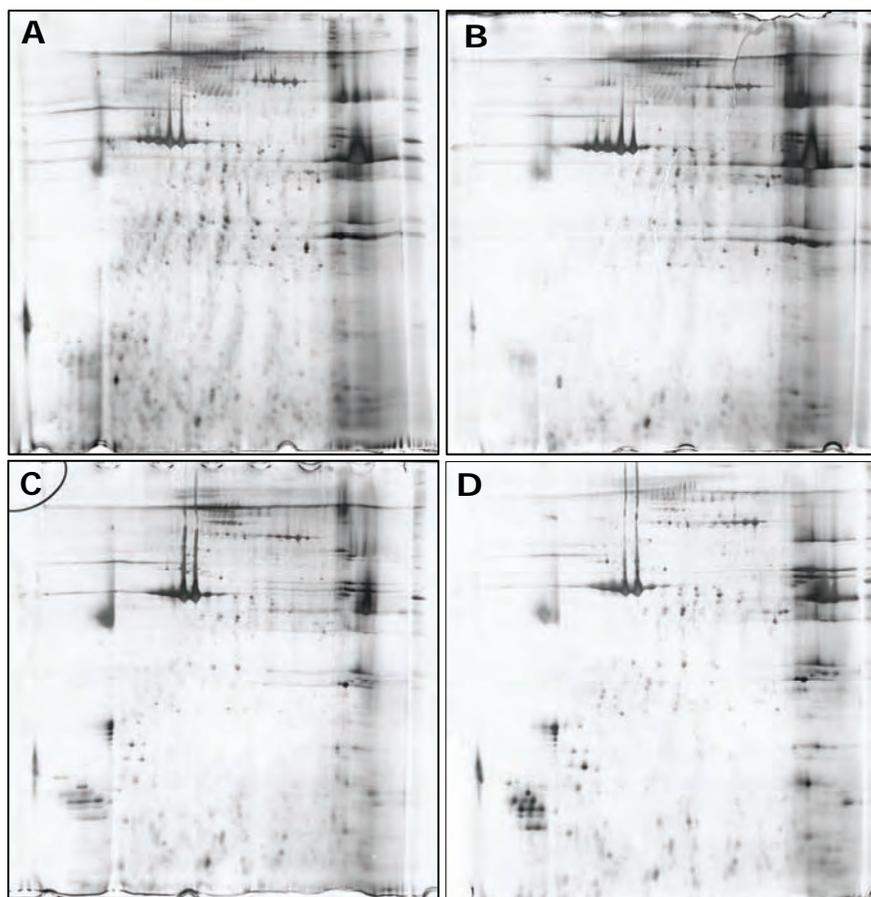


Figure 3. A principal component analysis (PCA) of the protein patterns in Norwegian dry-cured hams. The figure shows the variations in PC1 and PC2. Hams from the three different companies are circled.

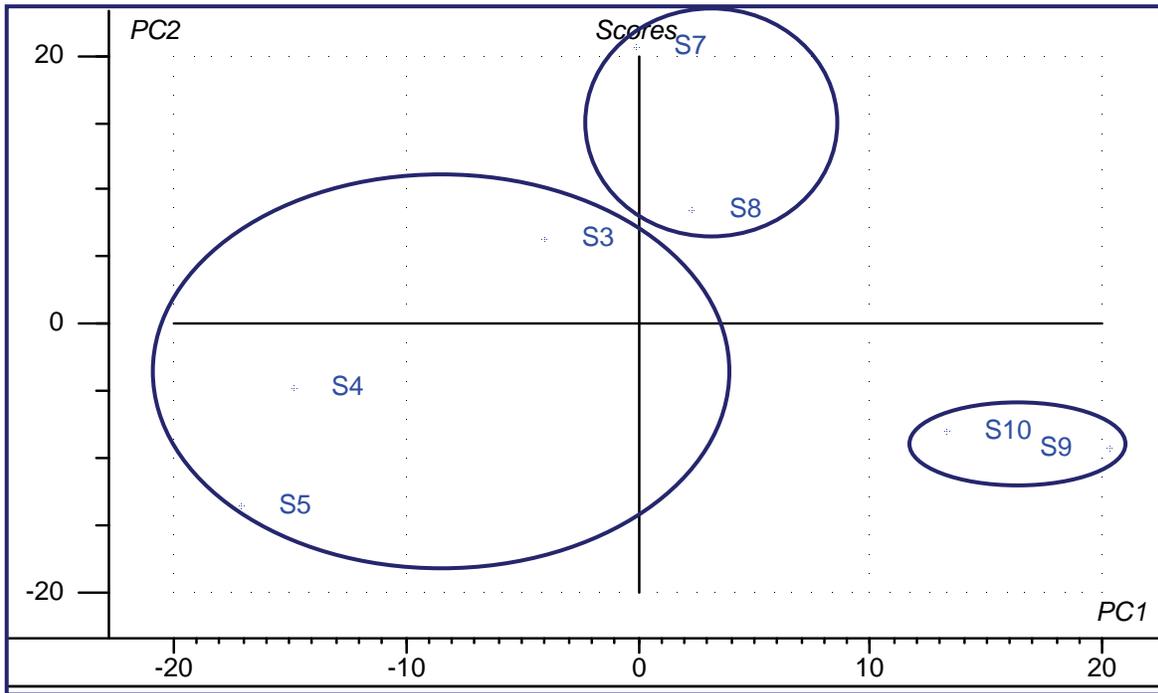
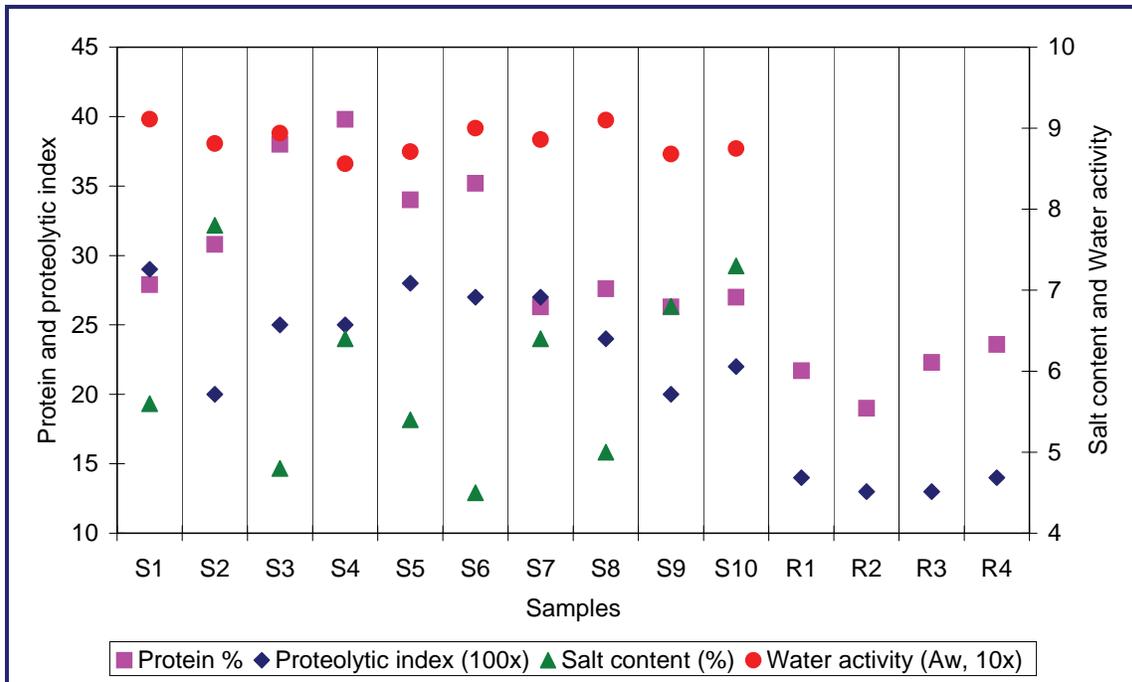


Figure 4. Protein content, proteolytic index, salt and water activity (A_w) analysis for dry-cured hams and non-processed Norwegian hams.



EFFECTS OF *MONASCUS PURPUREUS* AND *MONASCUS PILOSUS* ON THE QUALITY OF LOW-NITRITE CHINESE STYLE SAUSAGES

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Key Words: *Monascus purpureus*, *Monascus pilosus*, low-nitrite, Chinese-style sausage

Introduction

In the orient, *Monascus* is usually used in meat or fish as a preservative or coloring agent to increase flavor. Many researches also indicated that *Monascus* can be used as a starter in fermentative foods such as sake, soy sauce, soy cheese and miso owing to some metabolites as alcohol, ester, organic acids, enzyme, pigment, antihypertension, anti-hycholesteremia, and antimicrobial agent (Wong and Bau, 1977; Yasuda, 1983; Tsuji et al., 1992, Chen and Tseng, 1989). Nitrite is usually used as an ingredient in the curing solution as an antibacterial, coloring and antioxidant. However, a class of carcinogenic compounds known as nitrosamine can be formed in meat products by reactions between nitrite and amines (Judge et al., 1989). A low nitrite meat product with *Monascus*, therefore, is expected for the health concern in this study.

Objectives

The purpose of this study was to understand effects of different levels (0, k1=0.5, k2=1 and k3=1.5%) of *Monascus purpureus* (CCRC No. 31499) and 1% *Monascus pilosus* (CCRC No. 31527) on the quality of low-nitrite Chinese-style sausages during storage at 4° for 56 days.

Methodology

Monascus purpureus (CCRC No. 31499) and *Monascus pilosus* (CCRC No. 31527) will be incubated as the following procedure was described by Food Industry Research and Development Institute in Taiwan. The Chinese style sausage and curing formula were manufactured according to the description of Tseng (1999). A 100ppm sodium nitrite was used as a commercial control (A) and a only 25 sodium nitrite was used as low nitrite (C). K1 is a low-nitrite sausage with 0.5% *Monascus purpureus* and 1% *Monascus pilosus*. K2 is a low-nitrite sausage with 1% *Monascus purpureus* and 1% *Monascus pilosus*. K3 is a low-nitrite sausage with 1.5% *Monascus purpureus* and 1% *Monascus pilosus*.

The chemical composition, water activity, pH value, TBA value, VBN value, total plate count, mold count, anaerobic bacteria count, and color of the products with vacuum package were to determine at the 0, 7th, 14th, 28th and 56th day during cold storage at 4°.

The sensory panels (color, flavor, texture and overall acceptance) of all cooked sausages were also performed at the 0, 28th and 56th day. A 7 scores hedonic system was used in this experiment to evaluate which one is accepted (score 4). All data were analyzed by SAS system (2002).

Results & Discussion

The results showed that the chemical compositions of low-nitrite Chinese-style sausages were not affected by addition of different levels of *Monascus purpureus* and 1% *Monascus pilosus*. The water activity of all treatments were not significantly different with storage time and the value was from 0.919 to 0.935. A slower decline rate of pH for all treatment of low-nitrite Chinese-style sausages with *Monascus* can be found. Additionally, with addition of *Monascus*, the degrading rate of nitrite can be slowed down in this study. In the aspect of microorganism, the total plate count, mold count and anaerobic bacteria count of Chinese-style sausage with different levels of *Monascus purpureus*(K1:0.5%, K2:1% and K3:1.5%) and 1% *Monascus pilosus* were significantly higher than that of A and C, and the values were from 7.07 to 7.91 log CFU/g, 6.87 to 8.16 log CFU/g and 7.09 to 7.99 log CFU/g, individually, during cold storage (4°).

TBA value of all treatments with different levels of *Monascus purpureus* (K1:0.5%, K2:1% and K3:1.5%) and 1% *Monascus pilosus* were higher than that of the A and C at the 7th day during storage, and TBA value increased with *Monascus* levels. The products with 0.5% *Monascus* and 1% *Monascus pilosus* (K1) had the similar VBN values as the A and C. The a value of all sausages with *Monascus* were higher than that of A and C. Moreover, a value of K1 was almost the same as the C treatment. The L value of A and C were higher than that of all sausages with *Monascus*, therefore darker red color were showed in sausages with *Monascus*. The overall acceptance of Chinese style sausage with 0.5% *Monascus purpureus* and 1% *Monascus pilosus*(K1) can be accepted when these products stored at 4 for 28 days.

Conclusions

In conclusion, 0.5% *Monascus purpureus* and 1% *Monascus pilosus*(K1) was an optimal addition for Chinese style sausage owing to it has better sensory results and shelflife also can be extended up to 28 days at 4°.

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Tables and Figures

Table 1. Changes of pH value of Chinese-style sausages with different levels of *Monascus purpureus* and 1% *Monascus pilosus* during cold storage (4°)

Storage (days)	Treatments ¹				
	A	C	0.5%	1%	1.5%
0	6.40±0.10 _{a,x}	6.35±0.05 _{a,x}	6.32±0.04 _{a,x}	6.32±0.02 _{a,x}	6.32±0.03 _{a,x}
7	6.22±0.23 _{ab,x}	6.23±0.07 _{a,x}	6.14±0.23 _{a,x}	6.19±0.13 _{a,x}	6.34±0.04 _{a,x}
14	5.77±0.18 _{b,x}	5.87±0.19 _{a,x}	5.80±0.29 _{a,x}	5.81±0.25 _{a,x}	6.12±0.15 _{a,x}
28	4.68±0.23 _{c,x}	4.92±0.12 _{b,x}	5.10±0.24 _{b,x}	5.05±0.38 _{b,x}	4.97±0.23 _{b,x}
56	4.52±0.13 _{c,x}	4.66±0.13 _{b,x}	4.73±0.14 _{b,x}	4.75±0.14 _{b,x}	4.75±0.18 _{b,x}

^{a-c} Means within the same column with different superscripts are significantly different (p<0.05).

^{x,y} Means within the same row with different superscripts are significantly different (p<0.05). A: 100ppm sodium nitrite, C:25 ppm sodium nitrite.

Table 2. The panel scores₁ of the overall acceptance of Chinese-style sausages with addition of various levels of *Monascus purpureus* and 1% *Monascus pilosus* during cold storage (4°)

Treatments	Storage time (days)		
	0	28	56
A	*5.0 ± 1.05 _{a,x}	3.3 ± 1.15 _{bx}	3.1 ± 1.37 _{a,y}
C	5.6 ± 1.26 _{a,x}	3.4 ± 0.84 _{b,y}	3.1 ± 0.99 _{a,y}
0.5%	5.2 ± 0.91 _a	4.5 ± 0.84 _{ab}	3.6 ± 1.17 _a
1%	5.0 ± 1.05 _a	3.5 ± 0.52 _b	3.6 ± 0.69 _a
1.5%	4.4 ± 0.84 _a	3.0 ± 1.05 _b	3.8 ± 1.60 _a

Footnote is the same as Table 1

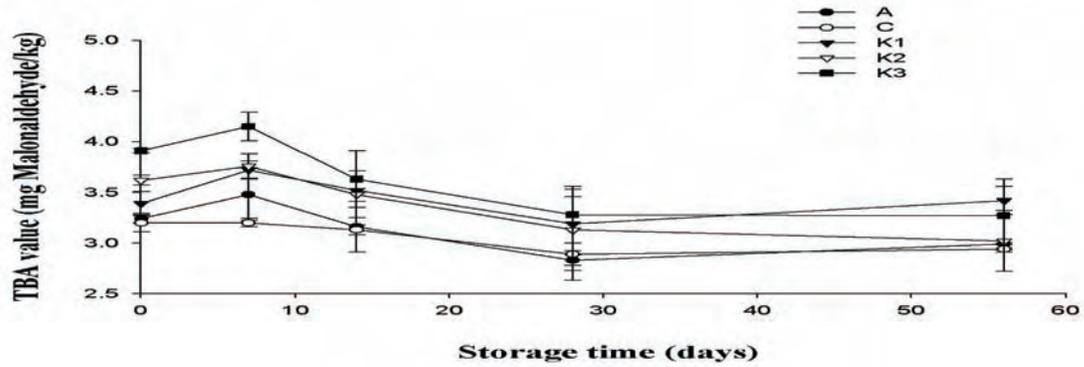


Fig. 1. Changes in TBA value of Chinese-style sausages with different levels of *Monascus purpureus* and 1% *Monascus pilosus* during cold storage (4°).

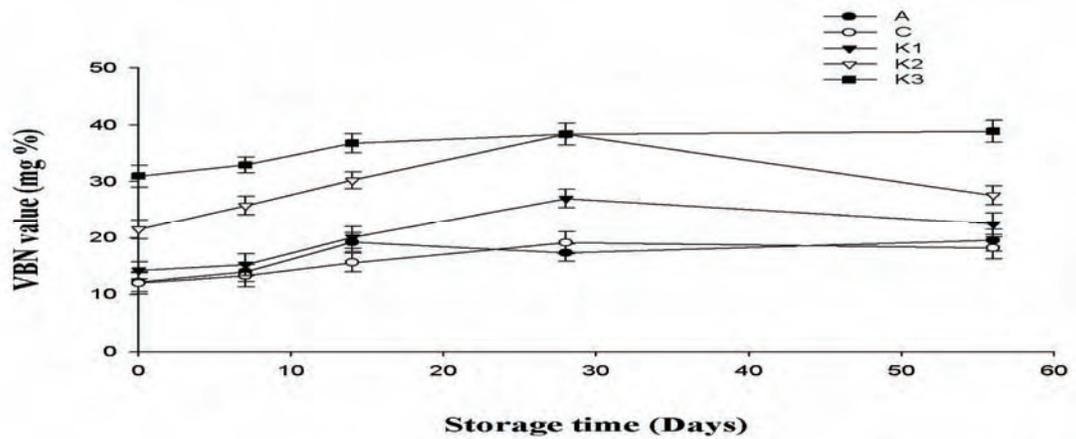


Fig. 2. Changes in VBN value of Chinese-style sausages with different levels of *Monascus purpureus* and 1% *Monascus pilosus* during cold storage (4°).

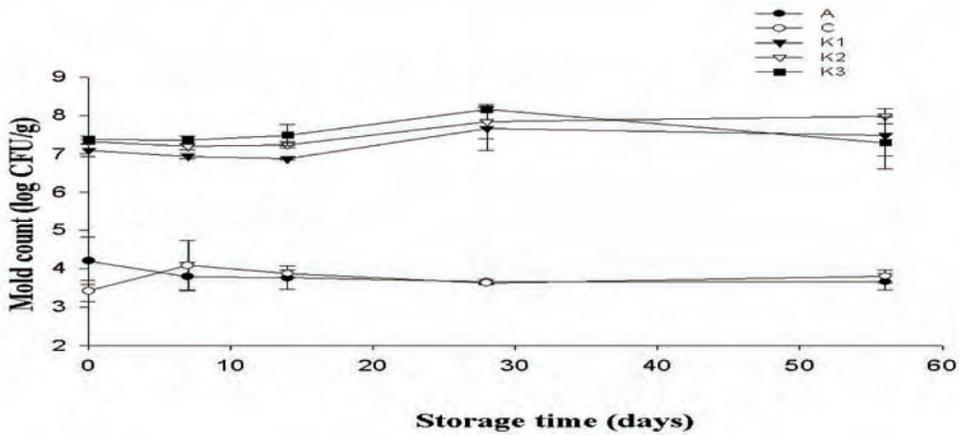


Fig. 3. Changes in mold count of Chinese-style sausages with different levels of *Monascus purpureus* and 1% *Monascus pilosus* during cold storage (4°).

AN ALTERNATIVE SURFACE CULTURE FOR SALAMI

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Key Words: yeast, mould, salami, sausage, starter culture, surface, flavor, sensory, texture

Introduction

In meat processing, surface cultures are mostly applied for firm raw sausages and some raw cured meat products as dried beef (e.g. Buendnerfleisch). Their use facilitates flavor formation, microbial competitiveness, peeling of the skin and protection against too intense drying and oxidation processes (Sunesen and Stahnke, 2002). To get a more unique color on the surface, rice meal or marble powder are sometimes supplemented additionally. *Penicillium* strains (mainly *Penicillium nalgiovense*) are the principal components of such cultures, whereas yeast strains can be introduced to improve skin adhesion (Sugimoto, 2004).

Objectives

Based on the long-term experiences of ALP in developing cheese surface cultures (Bachmann et al., 2005), a yeast culture originally dedicated to cheese was tested for its application to raw sausages as salami.

Methodology

The salamis were produced according to a traditional recipe with pork meat and beef, bacon, salt, herbs and some additives (including starter culture Scheid LMP, nr. 7527) at the ABZ Spiez. Meats and bacon were minced, mixed with the other components and stuffed into natural skins for both treatments together. One group of the salamis was then dipped into a suspension of surface culture Scheid nr. 7615 with *Penicillium nalgiovense* (control salami, CS), the other group was treated with a suspension of an ALP culture with *Geotrichum candidum* (experimental salami, ES). To avoid cross contamination between the two groups, seven salamis per treatment were air-dried in two separated climate chambers for five weeks by allowing a reddening period of five days at the beginning of the drying process.

The sausages were analyzed for nutrient contents and microbial counts by the usual chemical, enzymatic and microbial methods.

Aroma components were characterized by solid-phase microextraction (SPME) gas chromatography - mass spectrometry (GC-MS) in combination with GC-olfactometry (GC-O) by a panel of six trained internal panelists. Identification was based on the linear retention index (RI), mass spectrum and odor quality of the compounds.

The salamis were also assessed for their sensory profile (14 different odor, flavor and texture criteria on a 10-point intensity scale) by a panel of ten trained internal panelists.

In three salamis per treatment, culture adhesion on the surface (as relative skin friction) and skin peeling were determined by inhouse-methods (Guggisberg, 2005). Warner Bratzler shear force was measured to characterize salami firmness.

Results & Discussion

Nutrient and lactate contents were similar for ES and CS (table 1). Dry matter content was slightly higher in ES, which could be due to minor condition differences in the two chambers. Nitrate reduction seemed to be elevated in ES, which is indicated by a higher nitrite and a lower nitrate concentration. It may be partly explained by the also higher pH value (+ 0.34 units).

Table 1: Nutrient content, lactate content and pH in salami (per kg salami)

Parameter	Control salami (CS)	Experimental salami (ES)
Dry matter (g)	640	624
Crude protein (g)	265	247
Crude fat (g)	325	317
Crude ash (g)	55	48
Nitrate (mg)	2.22	2.02
Nitrite (mg)	0.70	1.47
L-lactate (g)	3.23	2.95
D-lactate (g)	2.14	2.26
Total lactate (g)	5.37	5.21
Percentage of L-lactate (%)	60.1	56.6
pH	6.39	6.73

Table 2: Microbial counts in salami (CFU/ml)

Type of microorganism	Control salami (CS)	Experimental salami (ES)
Moulds	7.6×10^3	7.1×10^3
Yeasts	< 10	< 10
Lactic acid bacteria	2.2×10^8	2.2×10^7
Salt-tolerant bacteria	5.1×10^5	9.9×10^5
Enterococcaceae	6.6×10^3	4.1×10^3
Bacillus cereus	< 10	50
Aerobic psychrotrophic bacteria	1.8×10^8	1.1×10^8
Coagulase-positive Staphylococcaceae	< 10	< 10
Enterobacteriaceae	< 10	< 10
Coagulase-negative Staphylococcaceae	5.5×10^5	1.1×10^6
Pseudomonadeae	< 10	< 10
Pediococcaceae	7.5×10^5	1.8×10^6

Microbial counts were comparable (table 2) and fulfilled Swiss regulations. *Bacillus cereus* was only detected in ES, whereas lactobacilli were 10 times higher in CS. In both treatments, no yeasts but similar counts for moulds were found. This may be due to the fact that the salami samples were peeled for the microbial analyses, according to the eating process. It may be concluded for future trials, that microbial analyses should also be performed for intact salami (including the skin).

Sensory panel tests showed no significant differences for none of the odor, flavor and texture characteristics evaluated (figure 1). This is in accordance to Selgas et al. (2003), who also found no significant differences in organoleptic characteristics between control and yeast-inoculated dry fermented sausages, in spite of the enzymatic activity of the yeasts. In general, the salamis were characterized by a quite high tenderness, medium intense salty and spicy notes as well as by slightly pungent and roasted notes.

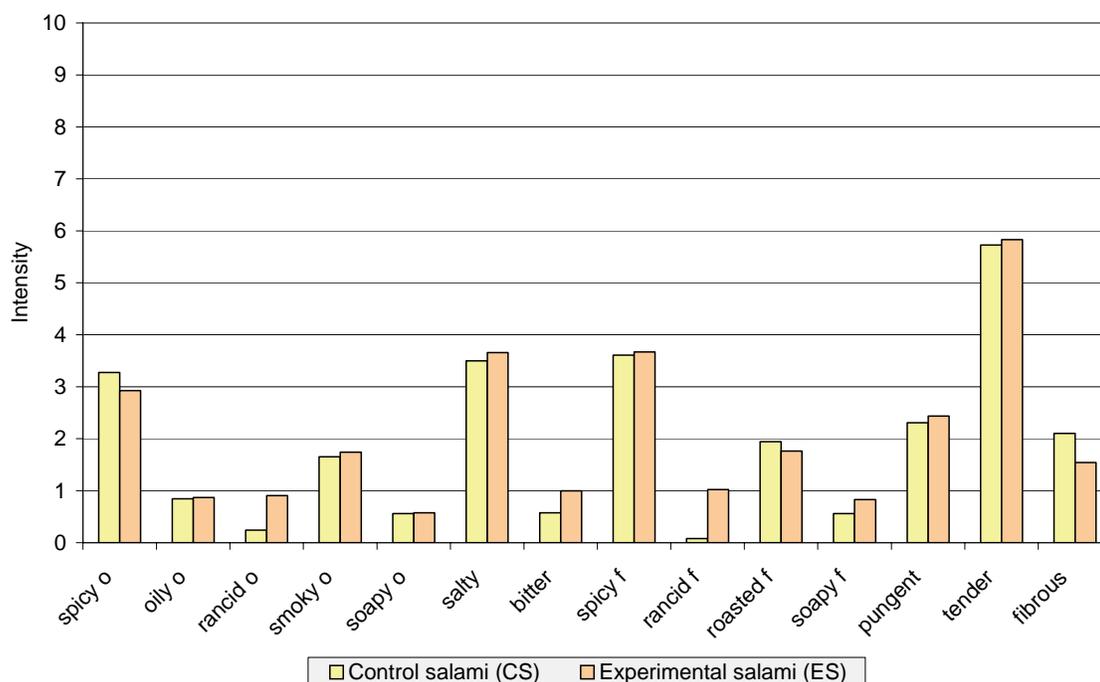


Fig. 1: Sensory profile of the two salami types (o = odor, f = flavor)

Preliminary results for aroma analysis obtained by GC-O in combination with GC-MS revealed an intense roasty popcorn-like odor note, identified as 2-acetyl-1-pyrroline present in both samples. 2-Acetyl-1-pyrroline was first described in salami by Stahnke (2000) and also found by Blank et al. (2001) in Italian-type salami. Both samples revealed the presence of terpenes and sulfur aroma compounds which are mainly caused by spices or feed of plant origin, e.g. allyl sulfide, diallyl sulfide, α - and β -pinene, α -phellandrene, limonene and linalool. Typical lipid oxidation products such as the mushroom-like smelling 1-octen-3-ol, aldehydes such as hexanal (green odor note), heptanal and nonanal (both having a fatty, soapy aroma) were detected in both types of salami. 2-Heptanone and 2-nonanone, further lipid oxidation products, were also found in both salami types, however, the peak heights differed between the two types of salami. 4-

Heptanone was only present in the ES sample. The higher signals for the ketones originating from lipid oxidation in the ES sample, might be at least partly responsible for the slightly more pronounced rancid note perceived for ES by the sensory panel. CS alone revealed the presence of the roasty smelling 2,5-dimethyl pyrazine and showed 10 times higher peak intensities for 2,6-dimethyl pyrazine which was described as roasty by the panelists, too (table 3). Only in ES, 2,3-butandione, which exhibits a buttery-creamy aroma note, was detected by GC-O. These results indicate some shift in metabolic pathway due to the type of surface culture as it was also stated by Sunesen et al. (2004).

Table 3: Prominent aroma differences detected in the two types of salami

Aroma compound	Linear retention index (RI)	Control salami (CS) <i>Peak height/1000</i>	Experimental salami (ES) <i>Peak height/1000</i>
1-Octen-3-ol	978	51	74
Hexanal	793	303	441
Heptanal	896	81	94
Nonanal	1'099	166	189
2-Heptanone	886	27	115
4-Heptanone	868	n.d.	96
2-Nonanone	1'087	67	309
2,3 Butandione	575	n.d.	detected by GC-O only
2,5-Dimethyl pyrazine	908	22	n.d.
2,6-Dimethyl pyrazine	904	226	23

n.d. = not detected

No treatment differences could be seen in firmness by WBSF (table 4). Culture surface adhesion was significantly lowered and peeling was easier ($p < 0.10$) in ES.

Table 4: Skin adhesion and firmness of salami

Parameter (n = 3)	Control salami (CS)	Experimental salami (ES)	p-value
Relative skin friction (% of salami weight)	0.468 ± 0.084	0.804 ± 0.173	0.01
Skin peeling			
- average of force (50-150 mm) [N]	1.680 ± 0.169	1.141 ± 0.340	0.07
- median of force (50-150 mm) [N]	1.674 ± 0.187	1.144 ± 0.360	0.09
Firmness			
- Maximum of force [N]	75.35 ± 22.02	82.39 ± 28.93	0.75
- Total work [mJ]	2'277 ± 195	2'351 ± 576	0.83

Conclusions

In comparison to CS, similar nutrient and lactate contents, a higher pH-value and an increased nitrate reduction could be seen for ES. Microbial counts were also comparable and in the regulation limits. Similar characteristics were observed in sensory profiles in CS and ES, whereas remarkable differences were determined for some single aroma components. Firmness was comparable between the two salami treatments, whereas the easier peeling of the skin in ES could be in relation with the decreased adhesion on the skin.

It was concluded that the tested cheese yeast surface culture can be a valuable alternative to the known mould applications for salami. Further analyses should elucidate whether the yeasts are also competitive to unfavorable moulds (Vallone et al., 1995), which may occasionally appear in some processing plants.

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CHANGES ON BIOGENIC AMINE PROFILES IN “PAINHO DE PORTALEGRE” DRY FERMENTED SAUSAGE FROM STARTER CULTURES ADDITION

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Key Words: Dry fermented sausage, Biogenic amines, Microbiological counts, Starter culture.

Introduction

Protein hydrolysis carried on during the processing stages and along the storage period of dry fermented sausages is the source of non-proteic material production, including small peptides (<1000 Da m.w.) and free amino acids (Toldrá *et al.*, 1992; Ordóñez *et al.*, 1999). These compounds contribute to the taste and, as precursor agents, to the formation of relevant volatile and non volatile flavouring components in the final product. Within the last group, biogenic amines may appear with increased concentrations in traditional dry fermented sausages (Roseiro *et al.*, 2005) since, among other reasons, the curing salts and starter cultures are usually absent in the formulation. Then, a greater variety of bacteria species and strains find out environmental conditions to grow up longer, namely those GRAM⁻ having pronounced carboxilase activity. Biogenic amines are considered undesirable components due to their possible toxic and allergic impact in consumers. For histamine, the most studied amine, limits of 8-40mg kg⁻¹, 40-100 mg kg⁻¹ and over 100mg kg⁻¹ have been associated to slight, intermediate and intensive poisoning outbreaks (Maijala & Eerola, 1993), while the toxicity threshold for tyramine is not so precise, ranging from 100 to 800 mg kg⁻¹. In relation to putrescine and cadaverine, both known as not producing “per se” adverse health effects but increasing the effect of others by inhibiting the amino oxidase enzymes (Bardócz, 1995; Eerola *et al.*, 1997), no limits were established so far.

Objectives

The present study aimed to clear out in what extent starter cultures addition, from the natural flora, may influence quantitative and qualitatively the biogenic amines contents and profiles in “Painho de Portalegre” a Portuguese traditional IGP dry fermented sausage.

Methodology

Preparation of sausages – Meat from the shoulder and ham and belly pieces, obtained from 24h *post-mortem* carcasses of “Alentejano” pigs, raised according the handling system described in “IGP” production requirements, were used on formulation. Minced raw materials (plate holes diameter-3 cm) and seasoning ingredients were mixed together (atmospheric pressure during about 5 minutes) to give the final proximate composition: lean pork (73%); fatty belly (8%); NaCl (2%); paprika paste (5%); garlic paste (0.5%); tap water (11.5%). Batches with approximately 10 kg were prepared as follows: Batch C (control with no starter inoculation); Batch Ls1, same as batch C but inoculated with *Lactobacillus sakei*-strain 1; Batch Ls2, inoculated with *Lactobacillus sakei* - strain 2; batch Sx1, inoculated with *Staphylococcus xylosus*-strain 1; batch Sx2 inoculated with *Staphylococcus xylosus* - strain 2; batch LS1; batch LS2, followed of two minutes of additional mixing. Between batches preparation, the mixer bowl was cleaned up, sanitized and washed with abundant water flow. Batches were held afterwards in a chilling room at +2° C for two days (seasoning up-take purposes) before stuffing into natural casings (pig rectum) and put at the drying/smoking house. Environmental conditions observed during the early phase of this processing stage reflected the typical traditional manufacturing process. Sampling included raw meat/fat mixture before salt/seasoning and starters addition, the end of the seasoning uptake period and stuffed product ripened up to day 15, 30, 40 (finished product) and 70 (corresponding to 30 days of storage). At storage, sausages were packaged under vacuum and held at room temperature, which was not controlled but ranged between 12-17°C.

Biogenic amine analysis - Biogenic amines were determined by HPLC according to Eerola et al. (1993). Eight grams of the samples were homogenized in 40 mL 0.4M perchloric acid with a Polytron homogenizer. The samples were centrifuged for 10 min. at 3000 r. p. m. and the supernatant rinsed into 25 mL bottle through filter paper. The extraction was repeated with 40 mL 0.4M perchloric acid. The supernatants were combined and adjust to 25 mL with 0.4M perchloric acid. A volume of 1 mL of the sample extract was derivatisated with dansyl chloride by incubation for 40 min. in alkaline media. The samples were finally dissolved in acetonitrile and filtrated through a membrane Acrodisc 25 mm GHP, GF 0.45 m (Gelman Sciences, Inc.). As internal standard, 1,7-diaminopentane was used.

Biogenic amines (tryptamine, -phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) were separated using a HPLC system Waters consisted of the quaternary pump (Waters model 510 HPLC pump), automatic sampler (Waters 717Plus autosampler), diode array detector (Waters 2996). Separation was performed on the reverse phase Spherisorb ODS2 cartridge, 5 m, 4.0x125 mm. A gradient elution program with mixture of 0,1M ammonium acetate as solvent A and acetonitrile as solvent B was used. Samples were analysed in duplicate. The biogenic amines content was expressed as mg.kg⁻¹ dry matter of muscle.

Inoculum preparation – Strains used in this study were collected and selected from the natural microbiota of portuguese traditional dry fermented sausages produced in Portalegre district. Brain heart infusion (BHI) and deMan, Rogosa, Sharpe (MRS) broths were used for Micrococcaceae and Lactobacilli growth, respectively.

Results & Discussion

Data obtained from starters addition on BA formation during “Painho de Portalegre” processing stages and a further short storage period is shown on Table 1. The observed trends varied with BA considered and depended on the starter composition. Regardless spermidine and spermine, which concentrations did not expressively differ from control batches, the other evaluated biogenic amines were, in general, strongly inhibited on their formation by both individual and combined starters. Exceptionally, batches inoculated with Sx1 presented significantly higher cadaverine and histamine contents than in controls, for samples picked up at day 30 and 40 of the drying stage and over the entire ripening phase, respectively. This specific ability for Histamine formation exhibited by certain microorganisms must be taken into account when selected active starter cultures are to be used instead of wild fermentations. In our case, in Sx1 inoculated samples at day 30 of the drying/smoking phase, which correspond in practice to a product ready for consumption, the histamine concentration reached a level 2 fold higher than that detected in the controls (95.79 vs 29.07 mg.kg⁻¹, on dry matter), a level close to the upper limit of acceptability recommended in food (TenBrink *et al.*, 1990). Among the microflora commonly found in meat products, different potentials for producing and degrading BA have been detected (Masson *et al.*, 1996; Martuscelli *et al.*, 2000).

The effect of storage time, run under vacuum at room temperature on the evolution of BA concentrations in “Painho de Portalegre” depended on the microbial composition of the installed flora and, in agreement with this, it varied with the BA considered. Apart the phenylethylamine in Ls1 samples, the other BA mean concentrations decreased during the storage period in batches with strains 1. This main trend diverged in batches with strains 2 for putrescine, cadaverine and tyramine. In agreement with the explanation of Eerola *et al.*, (1997), the anaerobic conditions associated to the vacuum packaging, by favouring the activity of amine producing bacteria, could effectively be the main reason involved in the increased levels detected.

Strains 2 were more effective in reducing BA contents all over the ripening periods than their counterparts, with differences being particularly relevant on cadaverine for both “genus” and on histamine in relation to *Staphylococcus*. Regardless the selected microorganism strains, *Lactobacillaceae* showed, in most situations, superiority in controlling the BA level, mainly for those compounds detected in greater concentrations. However this condition was not confirmed for putrescine in batches inoculated with Sx2, which showed lower values than those with Ls2. These different bacterial potentials in influencing BA formation were confirmed when the impact of starters addition on the sum of vasoactive compounds (tryptamine+phenylethylamine +histamine+tyramine) and histamine potentiators (putrescine+cadaverine) (Chu and Bjeldanes, 1981) were considered. In fact, the level of both indexes in controls at day 30 of the drying/smoking phase (345.99 and 1287.02 mg.kg⁻¹ on dry matter, respectively) decreased about the double in batches with strains 2 (64.05 and 202.16 mg.kg⁻¹ – Ls2; 88.84 and 234.47 mg.kg⁻¹ – Sx2; 50.37 and 147.07 mg.kg⁻¹ – LS2) comparatively to those inoculated with strains 1 (153.5 and 562.91 mg.kg⁻¹ – Ls1; 269.26 and 917.8 mg.kg⁻¹ – Sx1; 105.98 and 223.15 mg.kg⁻¹ – LS1). Data also clearly shows that combined starters were more effective in their action than pure cultures and that, within those, Ls1 and Ls2 decreased more those indexes than Sx1 and Sx2.

If the safety of the traditional production is clearly enhanced through starters addition, the influence of BA reduction may be adverse on the sensorial properties. Eerola *et al.*, (1997), referred a positive relationship between sensorial evaluation and the putrescine concentration in samples stored up to 30 days in vacuum, but this results were not confirmed in all tested situations. Otherwise, this trend is contradictory to the undesirable sensorial properties of diamines.

Conclusions

The addition of selected starter cultures in the manufacture of traditional dry fermented sausages may deeply promote the safety of the final products by decreasing the biogenic amines formation.

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Tables and Figures

Biogenic amine	Time	Starter						
		Control	Ls1	Sx1	LS1	Ls2	Sx2	LS2
Tryptamine	0	1.59	1.59	1.59	1.59	1.59	1.59	1.59
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		13.39	12.06	11.30	7.71	3.90	0.00	4.16
		0.00	4.45	0.00	0.00	0.00	0.16	0.20
		27.07	0.00	2.40	0.70	1.42	2.55	2.25
		7.86	1.49	1.36	0.35	0.00	1.36	0.00
		3.96	3.96	3.96	3.96	3.96	3.96	3.96
	2	7.07	9.41	10.84	5.83	11.75	7.22	10.05
		4.31	7.28	5.48	3.91	0.64	0.00	0.87
		16.33	3.73	3.19	1.14	0.21	0.78	3.65
		34.14	2.84	9.89	6.66	7.58	7.31	4.98
		53.79	6.63	2.75	4.87	0.89	2.75	0.03
		0.35	0.35	0.35	0.35	0.35	0.35	0.35
	2	0.56	1.71	0.61	0.60	0.35	0.12	2.48
		401.73	45.61	105.39	57.15	60.84	12.11	28.94
		662.46	110.66	215.12	66.76	153.47	71.67	102.59
		511.34	139.93	163.71	61.09	55.74	17.95	55.65
		466.13	63.85	112.59	13.00	112.26	n.d.	72.98
		0.60	0.60	0.60	0.60	0.60	0.60	0.60
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		509.59	250.16	492.48	132.12	104.89	63.06	110.63
		959.33	540.71	1159.07	223.86	283.04	378.85	195.84
		775.73	422.98	808.13	162.06	146.42	216.52	91.42
		720.95	233.55	644.22	74.69	237.93	n.d.	146.06
		1.75	1.75	1.75	1.75	1.75	1.75	1.75
	2	1.11	1.87	1.58	2.88	0.71	0.33	0.64
		14.15	5.20	23.78	6.48	2.40	3.08	2.18
		29.07	7.18	95.79	12.63	4.62	9.38	2.70
		18.97	4.26	61.56	6.54	1.49	7.53	1.78
		24.48	0.87	39.08	1.88	0.32	n.d.	0.62
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		191.53	69.36	218.74	93.88	67.71	19.32	40.98
		345.26	118.88	270.53	121.53	112.20	108.09	72.22
		265.81	146.20	195.41	92.08	53.56	71.45	41.36
		252.90	55.98	137.60	48.34	56.07	n.d.	42.99
		3.49	3.49	3.49	3.49	3.49	3.49	3.49
	2	4.14	4.65	4.72	2.82	3.67	5.57	5.13
		3.52	3.71	3.41	3.64	3.84	0.77	4.21
		3.60	3.47	3.73	3.16	3.37	5.49	5.50
		2.92	5.33	4.24	3.33	2.61	3.31	3.61
		2.88	2.17	2.85	2.59	1.50	n.d.	2.65
		38.04	38.04	38.04	38.04	38.04	38.04	38.04
	2	49.53	54.80	57.10	34.93	36.91	45.28	66.57
		34.10	57.44	45.43	38.37	33.12	8.88	46.39
		37.04	41.64	37.23	33.97	34.35	56.75	58.59
		34.24	61.18	50.08	38.06	33.04	41.79	42.57
		32.16	30.95	33.09	34.12	24.96	n.d.	29.92

Ls1 – *Lactobacillus sakei* (strain 1); Sx1 – *Staphylococcus xilosus* (strain 1); LS1 - *Lactobacillus sakei* (strain 1) e *Staphylococcus xilosus* (strain 1); Ls2 – *Lactobacillus sakei* (strain 2); Sx2 – *Staphylococcus xilosus* (strain 2); LS2 - *Lactobacillus sakei* (strain 2) e *Staphylococcus xilosus* (strain 2).

“UZICE BEEF PRSHUTA”: INFLUENCE OF DIFFERENT SALTING PROCESSES ON SENSORY PROPERTIES

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Key Words: “Uzice Beef Prshuta”; salt penetration dynamics; dry-salting; vacuum tumbling; selected sensory characteristics.

Introduction

Traditional dry-cured meat products constitute diverse group of food products. Originating from distinct geographic regions they bear characteristic sensorial properties gathered in superb quality meat products. Traditionally processed in a remote area of mountain Zlatibor (the south-western part of Serbia) “Uzice Beef Prshuta” remains the product that largely contributes to the local economy and gastronomic heritages. Being made of the most valuable parts of beef carcass (round muscles, loin muscles and tenderloin) originating from well feed, 3 – 5 years old cattle this product conforms fully with its PDO mark (Protected Designation of Origin). Detailed processing was reported earlier (Radovanovic et al., 1990-a, 1990-b, 1993).

Objectives

Objective of meat salting process is salt diffusion into a muscle tissue, where salt, as a humectant, reduces water activity (a_w) providing preservation effect. Additionally salt contributes to appropriate salty taste, juiciness, softness, as well as to the development in a specific red colour. Salting process in production of dry-cured meat products is specific and differs from salting of other meat products. Generally, meat pieces are treated with dry salt and whole process is conducted under refrigeration temperatures (0-4 °C). In these conditions the rate of salt diffusion from the product surface to the center is relatively slow. Application of mechanical treatments (tumbling and massaging) enhances salt penetration throughout the muscle by folding and crumpling them, which in return results in incoherent structure. In addition, vacuum applied along with massaging improves salt penetration through induced under-pressure.

The aim of this study was to determine salt penetration dynamics during the muscle salting process with and without vacuum tumbling, as well as to investigate influence of these two different salting treatments on sensory properties of “Uzice Beef Prshuta”.

Methodology

These investigations were conducted during the processing of “Uzice Beef Prshuta” according to the traditional technology described by Radovanovic et al., 1990-a. Twenty four samples of two different muscles obtained from beef carcasses were analyzed: 12 m. semitendinosus (MS) and 12 m. longissimus lumborum et thoracis (from cranial surface of 8. thoracic vertebra to caudal surface of 6. lumbal vertebra; ML). After deboning, cleaning of superficial fat and connective tissue, shaping of muscles, pieces were rubbed only with a 3.5% (w/w) NaCl. Six MS and six ML were immediately left in the salting chamber at the temperature 0-4 oC (traditional); other six MS and six ML were at first submitted to vacuum tumbling process for 20 hours (2 rpm with 70% of vacuum at 0-4 oC; 10 minutes tumbling and 50 minutes resting) and then left in the same salting room separately from the previous batch. In that way four experimental groups were obtained. After salting process was completed, muscles were washed and submerged into water for 12 hours. Later on, muscles were hanged on sticks in the chamber where smoking and drying processes were performed. Ripening was completed in 10 days.

Determination of chloride content was conducted according to Volhard method (ISO 1841-1:1996) in three layers (layer A: 1 cm in depth from external surface; layer B: 1 cm in depth from layer A; layer C: central part) of shaped muscles during whole salting process. Cross section cuts were taken up to 1 cm in thickness.

Sensory evaluation of ripened product “Uzice Beef Prshuta” was carried out by six experts. Five selected sensory characteristics were evaluated (appearance; cross section structure and appearance; colour; taste and smell; texture and juiciness) using the five level scale (from 1 – unacceptable to 5 excellent) – Radovanovic and Jovanka Popov-Raljić, 2001. Each of selected sensory characteristics were corrected by corresponding coefficient of importance (CI); sum of corrected scores gave the “percentage of total sensory quality” (Joksimovic, 1977).

Influence of different salting method on sensory quality of “Uzice Beef Prshuta” was statistically analyzed by analysis of variance.

Results & Discussion

Results obtained (table 1. and figures 1., 2., 3. and 4.) show similar trend of salt penetration for each of formed combinations (muscle – salting process). During salting period, salt content in layer A has a decreasing, while in layers B and C increasing trend. Changes in salt content in all of three layers were more intensive in first 7 and 9 days of salting for vacuum tumbled meat and non-vacuum tumbled meat, respectively, than in the rest of the salting period. Necessary preservation effect by the salt content of about 2.5 % in layer C, was reached on 3rd day for vacuum tumbled ML, on 5th day for vacuum tumbled MS and non-vacuum tumbled ML, as well as on 7th day for non-vacuum tumbled MS. Critical concentration of NaCl in the muscle center are obtained in a faster rate by the vacuum-tumbling, which further induces shortening of a salting period. The effect of diameter difference between MS and ML expectedly led to faster salt diffusion into central part in ML than of MS. Salt content in muscle center (layer C) obtained at the of the salting process, in all four experimental groups, were approximately the same and ranged from 3.30% to 3.50 %. The end of salting process is traditionally determined by

cross section colour observation conducted by trained and experienced person. Salting period for vacuum tumbled muscles is 4 days shorter than for traditional one and had decreased from 16 to 12 days.

Table 1. Changes of salt content in different layers of muscles during the salting process for each experimental group

Muscle type	Process Salting days	Dry salted and vacuum tumbled muscle						Dry salted muscle					
		layer - A		layer - B		layer - C		layer - A		layer - B		layer - C	
		NaCl (%)	Sd	NaCl (%)	Sd	NaCl (%)	Sd	NaCl (%)	Sd	NaCl (%)	Sd	NaCl (%)	Sd
MS	1.	6.01	1.13	1.81	0.05	0.89	0.08	6.92	0.98	1.62	0.03	0.48	0.04
	2.	4.13	0.07	2.13	0.06	1.54	0.09	4.50	0.87	1.92	0.38	0.63	0.07
	5.	3.62	0.07	3.08	0.25	2.47	0.04	4.44	0.77	3.36	0.41	1.97	0.03
	7.	4.19	0.05	3.60	0.00	3.04	0.29	4.54	0.79	3.84	0.38	2.51	0.06
	9.	4.15	0.19	3.53	0.03	3.15	0.17	5.01	0.58	4.06	0.30	3.02	0.10
	12.	4.27	0.23	3.89	0.08	3.30	0.11	4.93	0.10	4.07	0.19	3.30	0.22
	14.	-	-	-	-	-	-	4.93	0.17	4.24	0.12	3.27	0.16
	16.	-	-	-	-	-	-	4.82	0.50	4.33	0.10	3.38	0.06
ML	1.	7.05	0.81	3.21	0.17	1.26	0.10	5.66	0.16	2.46	0.29	0.78	0.06
	2.	5.55	1.10	3.41	0.01	2.24	0.02	5.79	0.48	3.00	0.28	1.96	0.05
	5.	4.41	0.19	3.81	0.07	3.32	0.11	5.02	0.14	3.81	0.15	2.69	0.02
	7.	4.24	0.30	3.88	0.30	3.44	0.19	4.80	0.23	3.78	0.09	3.15	0.10
	9.	4.38	0.08	3.94	0.18	3.28	0.07	4.47	0.14	3.96	0.14	3.34	0.29
	12.	4.09	0.08	3.79	0.16	3.41	0.25	4.38	0.12	4.00	0.23	3.45	0.14
	14.	-	-	-	-	-	-	4.45	0.29	4.04	0.41	3.51	0.06
	16.	-	-	-	-	-	-	4.36	0.21	4.09	0.19	3.49	0.19

Figure 1. Changes of salt content in different layers of MS during salting process with vacuum tumbling

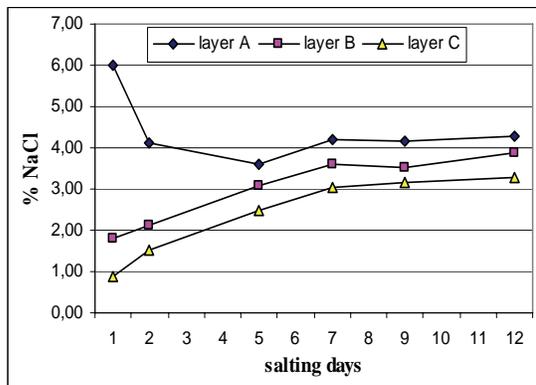


Figure 2. Changes of salt content in different layers of MS during salting process without vacuum tumbling

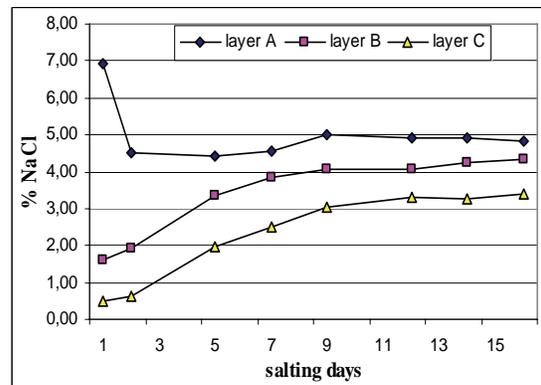


Figure 3. Changes of salt content in different layers of ML during salting process with vacuum tumbling

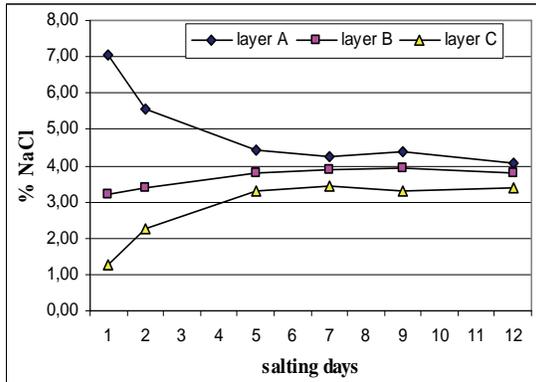
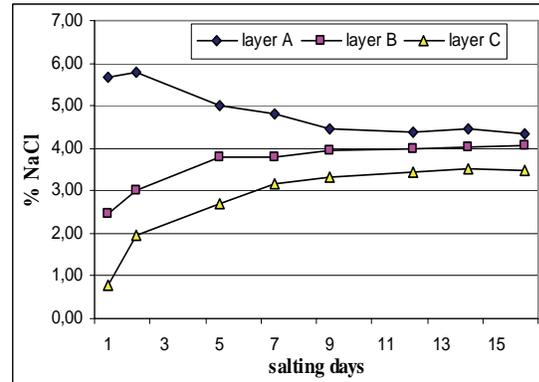


Figure 4. Changes of salt content in different layers of ML during salting process without vacuum tumbling



Furthermore, the influence of changes in the salting process on selected sensory properties of “Uzice Beef Prshuta” have been investigated. Sensory evaluation showed approximately uniform score values for corresponding sensory characteristics, as well as for total score value (table 2.). Analysis of variance has showed that there are no significant differences ($p < 0.01$) in total sensory quality between examined four groups of samples, leading to conclusion that vacuum tumbling will have no impact on sensory quality of “Uzice Beef Prshuta”, but will shorten salting process and the total production time.

Table 2. Sensory evaluation scores of the investigated samples of “Uzice Beef Prshuta”

Muscle type and selected sensory characteristics		CI	Dry salted and vacuum tumbled muscle		Dry salted muscle	
			“% of total sensory quality”	Sd	“% of total sensory quality”	Sd
MS	appearance	3	12.42	0.88	12.00	0.25
	cross section structure and appearance	3	12.58	0.52	12.25	0.87
	colour	3	12.42	0.29	12.25	0.43
	taste and smell	7	23.92	1.17	25.28	1.21
	texture and juiciness	4	14.11	0.69	14.67	1.33
	total score value	20	75.44	1.27	76.44	3.63
ML	appearance	3	11.58	0.29	9.67	1.01
	cross section structure and appearance	3	11.58	0.95	10.17	1.01
	colour	3	12.50	0.25	9.92	1.04
	taste and smell	7	27.03	1.68	27.03	2.05
	texture and juiciness	4	15.89	0.77	14.89	1.71
	total score value	20	78.58	2.05	71.67	4.84

CI – Coefficient of importance; Sd – Standard deviation

Conclusions

According to the results presented following should be emphasized:

- Each of four experimental groups has a similar trend of salt penetration during the salting period. Salt content in layer A has a decreasing, while in layers B and C increasing trend.
- Salt content of about 2.5% in central part of muscle (layer C) is reached on day three for vacuum tumbled ML, on day five for vacuum tumbled MS and non-vacuum tumbled ML, as well as on day seven for non-vacuum tumbled MS.
- Salt content in muscle center (layer C) obtained at the end of the salting process, in all four experimental groups, were approximately the same and ranged from 3.30% to 3.50 %.
- A vacuum tumbling process shortens a period of salting in the production of "Uzice Beef Prshuta" for 4 days.
- Analysis of variance showed no significant differences ($p < 0.01$) in total sensory quality between examined four groups of samples (dry-salted MS; dry-salted and tumbled MS; dry-salted ML; and dry-salted and tumbled ML).
- It is justified to include vacuum tumbling into traditional way of "Uzice Beef Prshuta" production.

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QUALITY CHARACTERISTICS OF EMULSION TYPE SAUSAGES MANUFACTURED WITH BAMBOO SALTS

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Key Words: bamboo salt; pH; emulsion stability; sensory properties; TBARS

Introduction

Sodium chloride is added in the processing to extract salt soluble protein and thus increase the binding, yield and juiciness of meat products. Also salt is involved in texture, taste, and flavor, safety as well as the shelf life of the products (Lin et al., 1991; Schwartz & Mandigo, 1976). In emulsion type products, the salt contributes to the formation of a stable emulsion which, upon heating, results in a product of acceptable quality characteristics (Schmidt et al., 1981). However in recent years high salt consumption has been associated with hypertension and other disorders in sensitive individuals and the current trend is to reduce the salt in processed foods (IFT, 1980). In order to reach these aims, many scientists have studied the possibility of salt reduction in meat products and a number of binding agents have been used to overcome property problems in low-salt products, including problems associated with water and fat binding properties and texture.

In Korea, the bamboo salts are mainly ingested for the health of the human body because bamboo salts that are produced through the several processes give the decrease in toxicity and the conversion of acidity into strong alkalinity compared to sun-dried salts. It is also known to have various therapeutic effects on diseases such as inflammations, viral disease, diabetes, circulation organ disorder and cancer (Shin et al., 2004). The production process of bamboo salts is ordinarily as follows: stamping inside bamboo hardens sun-dried salt and plugging with yellow mud; it is baked with pine wood and pine resin at about 1,000-1,500°C for 8-10h in a kiln, resulting in composing a lump of salt through bamboo's resin soaking into sun-dried salt; the lump is powdered; repeatedly stamped inside bamboo and baked, and lastly, it is baked at about 1,300-2,000°C of higher temperature. Compared with crude salt, the contents of iron, silicon, potassium, and phosphate in the bamboo salt were higher whereas the sulfate content was lower (Kim et al., 1998). No reports have been found on the emulsion stability, texture, and sensory characteristics of meat batter and cooked sausage with added bamboo salt as a sodium chloride substitute.

Objectives

The objective of this study was to evaluate bamboo salt as a substitute of NaCl in emulsion type sausages.

Methodology

Sample preparation: Fresh pork hams and pork backfats were purchased from a retail meat market in Seoul, Korea. Baking frequency two times (BS-2) and nine times (BS-9) bamboo salts were used as a NaCl replacer. The sausages used in this study were made from lean pork (60%), ice (20%), backfat (20%), and salts. The studied variable was salt types (NaCl, BS-2, and BS-9) and the added salt content was 1.5%. Phosphate and other spices were not added. Pork meat was trimmed of visible fat and connective tissue. Meat and pork fat were passed through a grinder with an 8 mm plate. Ground meat and fat were frozen and stored at -20°C until use. Partially thawed ground meat was cut for 1min in a cutter at low speed and mixed with salt. The mixed meat was dry chopped for 1min at high speed. After dry cutting the pork fat and part of the ice were added and the batter was cut at high speed to 8°C . At that point the remaining ice was added and the batter was cut at high speed to 14°C . Immediately after cutting, the batter was stuffed by hand stuffer, in 24mm diameter cellulose casings. Sausages were hand-linked at 15 cm intervals, weighed and cooked at 75°C to achieve an endpoint product temperature of 72°C (30min) and cooled in an ice water bath for 30 min. The sausages were stored in a cooler at 4°C overnight. After chilling the sausages were weighed, then peeled and vacuum packed with a vacuum packaging machine in film pouches and stored in the dark at 4°C until subsequent analysis.

Proximate analysis: Moisture, fat (ether-extractable), protein and ash contents of cooked samples were determined according to standard AOAC (1990) procedure.

pH: The pH values of the meat batters and cooked sausages were determined by blending 5 g of samples with 20 mL distilled water for 30 s. Readings were taken with a digital pH meter and combination electrode.

Emulsion stability: Emulsion stability of the batters was determined according to the procedure by Ensor et al. (1987).

Color determination: The CIE $L^*a^*b^*$ values of samples were determined as indicators of lightness (L^*), redness (a^*) and yellowness (b^*) using a Minolta Chromameter (Model CR200, Minolta, Japan).

Cooking yield: Cooking yield was determined by weighing the sausages before cooking and after cooling.

Sensory evaluation: Samples were assessed for color, flavor, texture, juiciness, and overall acceptability by an eight-member trained panel. The characteristics were evaluated using 10-point hedonic scale. A score of 1 indicated very low desirability and a score of 10 indicated very high desirability. Samples were heated in boiling water for 6 min and sliced into 3 cm lengths and three random samples were immediately served to panelists.

TBARS value: The thiobarbituric acid reactive substances (TBARS) content of the samples was determined according to Tarladgis et al. (1960).

Statistical analysis: Statistical analyses were by Duncan's multiple range test using the statistical analysis system.

Results & Discussion

The effects of bamboo salts on pH and emulsion stability of meat batters are shown in Table 1. The bamboo salts used in this study increased the pH value of meat batters. The mean pH values of the meat batter made with NaCl was pH5.82 while those of meat batters containing BS-2 and BS-9 were pH6.04 and pH6.48, respectively.

Water binding and gelation characteristics are important in determining the stability of comminuted meat products. In this study, the emulsion stability of meat batters was affected by bamboo salts. Water loss was highest in control sample and lowest in BS-9 treatment ($p < 0.05$). As in water loss, the fat loss was also lowest in BS-9 sample. Substitution of NaCl with bamboo salts significantly decreased the water and fat release from meat batter and showed good fat and water-binding properties.

The effects of bamboo salts on proximate analysis, pH, cooking yield and color of cooked sausages are shown in Table 2. The moisture content of the products ranged between 58.30-62.59%. The protein content of cooked sausages ranged between 13.38% and 15.14% and the fat content between 24.52-25.80%. Protein and fat contents were inversely proportional to the moisture content of the product. Bamboo salts significantly affected the moisture and protein contents of cooked sausages. The BS-9 treatment had the highest moisture content (62.59%) and the lowest protein content ($p < 0.05$). However, fat and ash contents were not significantly different among the treatments. The pH value of the control cooked sausage was pH 5.87 while these of BS-2 and BS-9 treatments were pH6.07 and pH6.55, respectively. The bamboo salts used in the present study increased the pH value of the product by about 0.2-0.68 pH-units. The cooking yields of BS-9 and BS-2 treatments were significantly higher than the control (NaCl) and cooking yield of BS-9 treatment was highest. The higher cooking yield with addition of bamboo salts was thought to be due to an increasing pH effect, resulting in a higher water binding and gelling capacity during heat treatment.

Ruusunen et al. (2003) reported that when frankfurters were made without phosphate, non-meat ingredients were needed at salt concentrations of less than 1.5%. While our results suggested that bamboo salts could increase emulsion stability and hydration properties of emulsion type sausages without phosphate.

The BS-2 and BS-9 treatments had significantly lower L*- and b*-values than the control ($p < 0.05$). a*-values were highest in BS-9 sample ($p < 0.05$) but a*-values of control and BS-2 samples were not significantly different ($p > 0.05$). This difference of color values probably resulted from the different color of salts used.

The effects of bamboo salts on sensory properties are given in Table 3. The bamboo salts had a significant effect on sensory characteristics. Color, flavor, texture, juiciness and overall acceptability of BS-2 and BS-9 treatments were evaluated higher than control treatment. As expected, BS-9 treatment was more effective than the SB-2 treatment in sensory properties.

A major cause of meat product deterioration is oxidative rancidity. Oxidation of lipids in meat and meat products is responsible for changes in its nutritional quality, color, flavor, odor and texture. Several authors consider that sodium chloride acts as a

prooxidant in meat and meat products. The TBARS contents increase with increasing salt concentration and storage time (Hernández et al., 2002). It has also been demonstrated many times that spices inhibit rancidity, often showing synergism (Madsen & Bertelsen, 1995).

Changes in TBARS values of cooked sausages during refrigerated storage are shown in Table 4. Initial TBARS values did not differ among all treatments, but TBARS values of BS-9 treatment were lower than those of control since two weeks. These differences were significant from two weeks to the end of storage time. Especially, TBARS values of BS-9 treatment did not increase since one week, demonstrating the antioxidant effect of bamboo salt. It appears that bamboo salts inhibited lipid oxidation.

Conclusions

In conclusion, the results obtained in the present study provide evidence that bamboo salts improve emulsion stability, sensory properties, and antioxidation activity in cooked sausage. These suggest a possible use of bamboo salt in meat products, but further studies about the function of bamboo salt are needed.

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Tables and Figures

Table 1: Effect of bamboo salts of pH and emulsion stability of meat batters

		Treatment ¹⁾		
		Control	BS-2	BS-9
pH		5.82±0.04 ^c	6.04±0.01 ^b	6.48±0.04 ^a
Emulsion	Water loss (%)	19.67±0.33 ^a	13.17±2.16 ^b	8.33±1.66 ^c
Stability	Fat loss (%)	5.33±0.66 ^a	4.50±0.83 ^b	2.67±0.35 ^c

¹⁾ Control: NaCl, BS-2: Bamboo salt that was baked two times, BS-9: Bamboo salt that was baked nine times.

^{a-c} Means in the same row with different letters are significantly different (p<0.05).

Table 2: Effects of bamboo salts on proximate analysis, pH, cooking yield and CIE L*a*b*-values of cooked sausages

		Treatment ¹⁾		
		Control	BS-2	BS-9
Moisture (%)		58.30±0.59 ^b	58.45±0.95 ^b	62.59±2.33 ^a
Crude protein (%)		15.14±1.01 ^a	13.96±0.49 ^{ab}	13.38±0.80 ^b
Crude fat (%)		25.80±1.34	24.74±1.09	24.52±2.12
Crude ash (%)		1.59±0.19	1.41±0.21	1.53±0.16
Sausage pH		5.87±0.03 ^c	6.07±0.04 ^b	6.55±0.03 ^a
Cooking Yield (%)		84.97±0.70 ^a	91.06±0.96 ^b	92.51±0.85 ^c
CIE L*-value		69.15±0.74 ^a	66.82±0.09 ^b	67.56±0.07 ^c
CIE a*-value		8.60±0.20 ^b	8.79±0.38 ^b	9.45±0.08 ^a
CIE b*-value		9.91±0.03 ^a	9.57±0.05 ^b	9.32±0.05 ^c

¹⁾ Control: NaCl, BS-2: Bamboo salt that was baked two times, BS-9: Bamboo salt that was baked nine times.

^{a-c} Means in the same row with different letters are significantly different (p<0.05).

Table 3: Effects of bamboo salts on sensory properties of cooked sausages

Sensory properties ¹⁾	Treatment ²⁾		
	Control	BS-2	BS-9
Color	6.88±0.35 ^b	7.25±0.46 ^{ab}	7.75±0.71 ^a
Flavor	6.38±0.74 ^b	7.25±1.04 ^{ab}	8.00±1.41 ^a
Texture	6.88±0.35 ^b	7.25±0.89 ^{ab}	8.00±1.20 ^a
Juiciness	6.38±0.74 ^b	6.50±1.31 ^{ab}	7.63±1.30 ^a
Overall acceptability	6.13±0.99 ^b	7.25±1.04 ^{ab}	8.25±1.04 ^a

¹⁾ Sensory scores were assessed on 10 point hedonic scale where 1 = extremely bad or poor, 10 = extremely good or much.

²⁾ Control: NaCl, BS-2: Bamboo salt that was baked two times, BS-9: Bamboo salt that

^{a,b} Means in the same row with different letters are significantly different(p<0.05).

Table 4: Changes of TBARS-values of cooked sausages manufactured with bamboo salts during storage at 4 °C

Treatment ¹⁾	Storage period (weeks)					
	0	1	2	3	4	5
Control	0.54±0.07 ^d	1.23±0.03 ^c	1.66±0.02 ^{bx}	1.61±0.13 ^{bx}	1.77±0.03 ^{ax}	1.78±0.03 ^{ax}
BS-2	0.59±0.02 ^d	1.26±0.12 ^c	1.59±0.08 ^{abx}	1.47±0.06 ^{bxy}	1.65±0.10 ^{ay}	1.63±0.15 ^{ay}
BS-9	0.53±0.06 ^b	1.22±0.99 ^a	1.26±0.10 ^{ay}	1.34±0.07 ^{ay}	1.29±0.10 ^{az}	1.31±0.12 ^{az}

¹⁾ Control: NaCl, BS-2: Bamboo salt that was baked two times, BS-9: Bamboo salt that was baked nine times.

^{a-d} Means in the same row with different letters are significantly different(p<0.05).

^{x-z} Means in the same column with different letters are significantly different(p<0.05).

EFFECT OF *RHUS VERNICIFLUA* STOKES AND ITS EXTRACT ON THE QUALITY OF EMULSION-TYPE SAUSAGE DURING REFRIGERATED STORAGE

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Key Words: *Rhus verniciflua* Stokes, emulsion-type sausage, pH, color, VBN, TBARS

Introduction

Rhus verniciflua Stokes (RVS) has been used as a traditional medicine and as a food additive in Korea (Hong et al. 1999). It is also used to treat gastritis, stomach cancer, and arteriosclerosis (Jung 1998). Recently, various biological activities of RVS have been reported. In model linoleic emulsion experiments, antioxidant activity of RVS has been shown to correspond to well-known enzymatic and non-enzymatic antioxidants (Lim et al. 1997). Recently we studied the effect of dietary RVS supplementation on the quality of Hanwoo (Korean cattle) steer beef (Lee et al. 2004). We found that RVS-supplemented Hanwoo (Korean cattle) beef was effective in increasing color stability, water holding capacity, unsaturated fatty acid and retarding lipid oxidation than the control during refrigerated storage.

Objective

The objective of this study was to determine whether addition of RVS extract to pork from the finishing pigs fed dietary RVS can improve the qualities of emulsion-type sausages during refrigerated storage.

Methodology

Raw materials and sausage processing

Fresh hams from pigs (gilts, live weight 110 kg) fed with 0 and 4% RVS in diet for 4 months prior to slaughter were utilized. The hams so collected were further treated with RVS extract. Thus the treatments (n = 3/treatment) include hams from pigs with 0 % RVS (Control), 4 % RVS (T1), 0 % RVS + RVS extract (T2), and 4% RVS + RVS extract (T3). The RVS extract was prepared from 100 g of RVS sawdust and 1 L of deionized water that was heated for 48 hours using Red Ginseng Master (HS-777, Hans Science, South Korea). 20.8% of frozen RVS extracts were added to T3 and T4. Emulsion-type sausages were prepared using lean meat (54.1%), back fat (30%), starch (0.5%), ISP (0.5%), FOS/ENR (0.14%), NaCl (1.5%), and ice (20.8%). Ground lean meat and back fat, starch, ISP, FOS/ENR, NaCl, ice were mixed using a silent cutter and vacuum-mixer for 15 min. The mixtures were stuffed into a cellulose casing, and cooked in a water bath at 80°C for 60 min.

The cooked sausages were cooled in ice water for 15 min, dried at room temperature (10°C) for 30 min, and vacuum-packaged in polyethylene bags. All samples were stored at 4°C for 4 weeks.

Analytical methods

The proximate composition was analyzed according to AOAC (1995). The pH value was determined on a 10 g sample with 100 ml deionized water for 1 min. Sample color was measured by a Minolta Chroma meter (CR-301, Minolta Co., Japan) that recorded the lightness (L^*), redness (a^*), and yellowness (b^*). The chroma (C^*) was obtained from a^* and b^* by formula: $C^* = (a^{*2} + b^{*2})^{1/2}$ (Commission Internationale de l'Eclairage. 1986). The TBARS (2-thiobarbituric acid reactive substances) value was determined as described by Sinnhuber and Yu (1977) and reported as mg malonaldehyde (MA)/kg sample. The VBN (volatile basic nitrogen) value was measured as described by Kohsaka (1975). Data was analyzed using the General Linear Model procedure of SAS (1999) program. Differences between means at the 5% level were determined by the Duncan's multiple range tests.

Results & Discussion

The proximate composition and pH value of emulsion-type sausages are presented in Table 1. Moisture, crude protein, and crude ash were significantly higher in T1 than in control and T2 ($P < 0.05$). The crude fat and pH value were significantly lower in T1 and T3 than control ($P < 0.05$). This showed that feeding of RVS in diet had a significant impact on the sausage quality. As shown in Fig. 1-4, the lightness (L^*) and redness (a^*) were significantly lower in T2 and T3 than in control and T1 during refrigerated storage ($P < 0.05$), and the yellowness (b^*) and chroma (C^*) were significantly higher in T2 and T3 than control and T1 ($P < 0.05$). The lightness (L^*) and yellowness (b^*) were significantly lower in T1 than in control ($P < 0.05$), and the redness (a^*) was higher in T1 than the other treatments ($P < 0.05$). This showed that the sausage color values are affected by feeding RVS to pigs and adding RVS extract to hams. The VBN (Fig. 5) and TBARS value (Fig. 6) were significantly lower in T2 and T3 treatments than in control and T1 during refrigerated storage ($P < 0.05$), and those were lower in T3 at all measurements as compared to its counterparts ($P < 0.05$).

Conclusions

The sausage (T1) prepared from pigs fed 4% RVS was more effective in increasing the redness (a^*) and delaying the protein deterioration, lipid oxidation than that without RVS in diet.

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Tables and Figures

Table 1: Effect of RVS and its extract on the proximate composition and pH value of emulsion-type sausage.

Items	Treatments*			
	Control	T1	T2	T3
Moisture	57.11 ^B	58.23 ^A	55.33 ^C	58.63 ^A
Crude fat	28.03 ^A	24.03 ^B	28.44 ^A	23.73 ^B
Crude protein	13.96 ^C	16.47 ^A	15.51 ^B	15.59 ^B
Crude ash	0.71 ^B	0.79 ^A	0.66 ^C	0.78 ^A
pH	6.08 ^A	5.98 ^B	6.01 ^B	5.87 ^C

^{ABC}Means in the same rows with different superscripts are significantly different ($P < 0.05$).

*Control: 0% RVS-fed pork, T1: 4% RVS-fed pork, T2: 0% RVS-fed pork + RVS extract, T3: 4% RVS-fed pork + RVS extract.

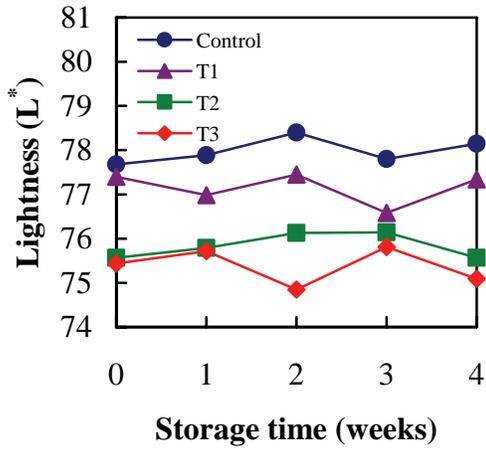


Fig. 1: Effect of RVS and its extract on the lightness (L^*) of emulsion-type sausage during refrigerated storage.

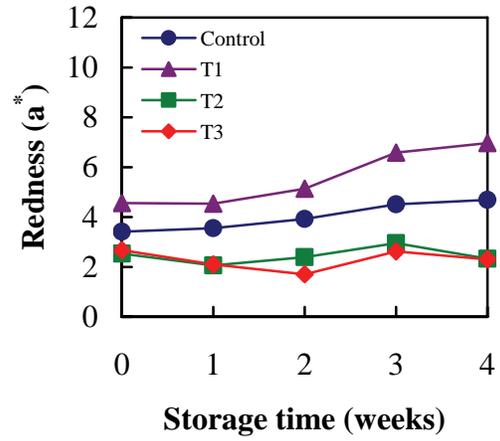


Fig. 2: Effect of RVS and its extract on the redness (a^*) of emulsion-type sausage during refrigerated storage.

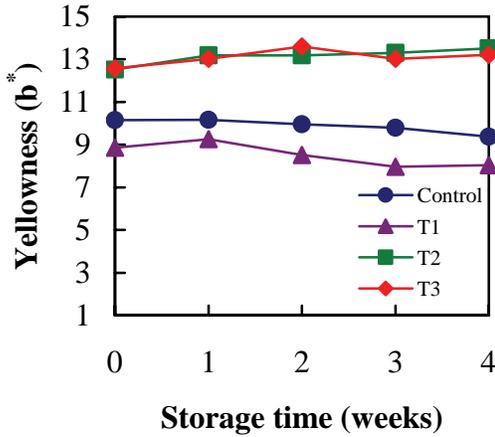


Fig. 3: Effect of RVS and its extract on the yellowness (b^*) of emulsion-type sausage during refrigerated storage.

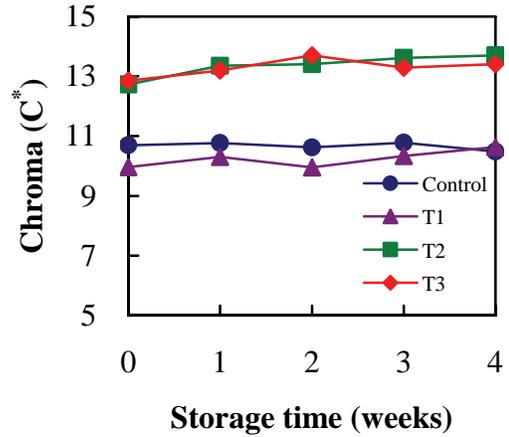


Fig. 4: Effect of RVS and its extract on the chroma (C^*) of emulsion-type sausage during refrigerated storage.

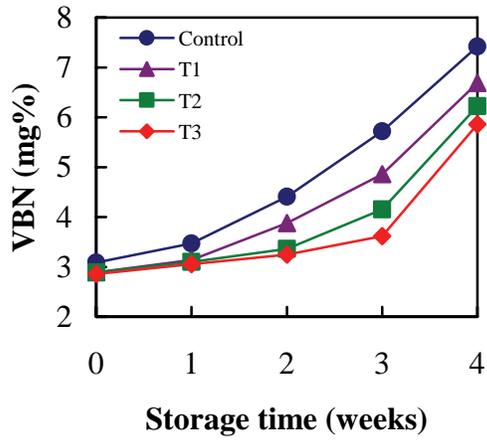


Fig. 5: Effect of RVS and its extract on the VBN value of emulsion-type sausage during refrigerated storage.

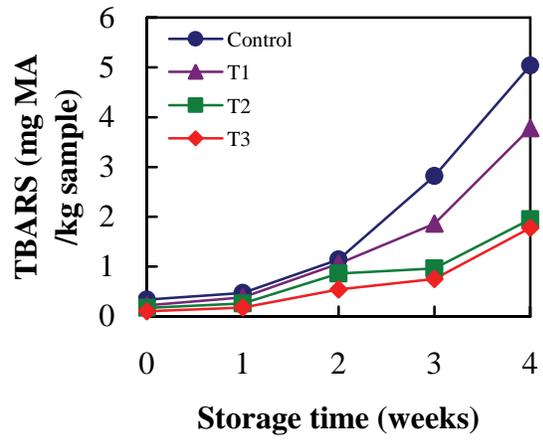


Fig. 6: Effect of RVS and its extract on the TBARS value of emulsion-type sausage during refrigerated storage.

THE IMPROVEMENT OF GEL STRENGTH OF SAUSAGES PREPARED FROM THREE TYPES OF MEAT WITH MICROBIAL TRANSGLUTAMINASE

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Key words: Chicken, Beef, Pork, Texture, Transglutaminase

Abstract

An attempt to improve the texture of chicken, beef, and pork sausages by using the microbial transglutaminase (MTG) has been investigated. In regards to the proteins, actin and myosin are the uppermost capable myofibril proteins for meat gelation, and pursuant to the affinity of MTG to these proteins; that promotes the improvement of the texture of the meat. Sausages were treated for breaking strength at two different temperatures, 40°C and 80°C, for 30 min. From the data we saw that the breaking score increased by adding MTG. That data gave the concept that the type of meat that reacted best was beef. The sausages were incubated at 40°C, so that shows the content of ϵ -(γ -glutamyl) lysine content (G-L) of chicken, beef, and pork after adding MTG has been significantly increased and the values were mostly the same in pork as well as beef. On the other hands, the G-L content of chicken was lower than beef and pork. To shore up the tasks of MTG, the characteristics of protein solubility of chicken, beef, and pork were investigated using Guba-Straub ATP solution. The extractability of myosin heavy chain (MHC) was significantly decreased in beef and pork, that leads to the high interaction of MHC with MTG; there was a good reaction binding ability between MTG and myofibrillar proteins, especially, MHC. Lastly, Cross-linking of proteins by using MTG may be useful in texturization in beef and pork better than chicken.

Background: Eating healthy food is an important initial process to the maintenance of life itself. Recently, many institutions have been involved in achieving a lot of new food with high quality and nutritional value to satisfy the appetite of people; especially in the 21st century, the sense of people has arisen. The factories producing foods came to rely somewhat on the MTG productions, for good reason-the cost to produce MTG itself is not expensive compared to the original TG, and it has proven its competence as a food texture additive ¹).

Objectives:

In our quest for completeness we have attempted to provide information on sausages of chicken, beef, and pork. The aim of our research was focused on investigating the differences and abilities of sausage texture improvement by using the MTG. This study

has involved determining G-L content and evaluating the reactivity of MHC as well as the extractability of the myofibrillar proteins.

Methods and materials:

Meat and sausage preparations: Chicken, beef, and pork meat was purchased from local markets in Japan; the meat, before using it, was kept in the refrigerator after slaughtering. The skeletal muscle blocks were minced in a meat grinder; also the fat was degreased and the temperature was maintained at 4°C. The sausages were prepared by adding distilled water, 1M imidazole-HCl (pH6.0), NaCl, and sodium pyrophosphate, and then MTG²⁾. The MTG in meat was 0.005%. All the additives and the minced meat were homogenized and the paste was put in a cloth rain rest (funnel-shaped bag) and ended up in a clear plastic casing. The paste was stuffed into casing until the sausage was a length of 12-14 cm, and the ends of the casing were fastened well with string. The sausage was divided into two groups. Each group contained -MTG and +MTG. The first group was incubated at 40°C for 30 min; and the second group was incubated at 80°C for 30 min.

Protein extraction: Since we were expecting that the muscle proteins became a G-L complex by MTG reaction; that gives us a concept to extract the proteins by low ionic strength solution [A-solution: 50mM Imidazole-HCl (pH6.0) 2mM EDTA], as well as high ionic strength solution [Guba-Straub-ATP solution: 0.09M KH₂PO₄, 0.06M K₂HPO₄, 0.3M KCl, 1mM ATP]. The purpose of adding ATP was to increase the MHC extractability, in general cases ATP in the postmortem state reduces. It might be that ATP broke the bonds between MHC and actin, especially the tight bond. Consequently, 28 ml of both solutions were respectively added to 2g of the sausage which had been divided into two groups as mentioned previously. Thereafter, the melange was and then centrifuged at 15,000rpm for 20min. Eventually the supernatant was taken out and filtrated with a filter paper; thus the final solution was used as an extracted protein solution. The protein concentration was determined by the biuret method³⁾.

Analysis of SDS-polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was carried out on gradient slab gel (7.5-17.5% acrylamide) with 2-mercaptoethanol at 20 mA/gel employing the discontinuous buffer system of Laemmli⁴⁾.

Assay of ϵ -(γ -glutamyl) lysine content: Determination of G-L content proteolytic digestion was carried out by the method of Kumazawa et al.⁵⁾. Briefly, 100g of sausage was homogenized and then kept in a freeze-dryer for drying. That was followed by dissolving it in a buffer (0.1M borate pH8.0). Pronase (0.2 unite/mg proteins) was mixed with the melange that was incubated for 24 hr. In this step the leucine aminopeptidase (0.4 units) and prolidase (0.45 units) were added after inactivating the pronase by boiling it for 10 min. After incubation for 24 hr, leucine aminopeptidase (0.4 units) was added with further incubation for 24 hr. The mixture was finalized by adding carboxypeptidase's A (0.4 units), and then the mixture was incubated another 24 hr. All this was performed at 37°C. Before lyophilized and dried digests were filled up to a volume of 7.5ml with distilled water, digested samples were heated at 100°C for 10 min. A part of the samples were subjected to reverse-phase HPLC, sent after collecting the fractions which contained G-L. Then the G-L fraction was evaporated to enhance the concentration. Afterward, we mixed in *o*-phthalaldehyde (OPA); lastly, the rest of the samples were subjected to reverse-phase HPLC.

Results and Discussions

The chemical and physical properties of muscle tissue and the interpenetrated connective tissue are of uppermost importance in the usefulness of meat as a food. The following discussion is intended only to point out the differences of improvement in sausage and how some factors can facilitate the methods to improve meat textures. Changes in breaking strength and deformation of meat gels (Fig.1) shows the breaking strength of sausages were treated at two different temperatures, 40°C and 80°C for 30 min; the breaking score increased by adding MTG as well as increasing the temperature. The sausage was subjected to the puncture test, which was measured by a knife of creep meter (Rheoner II, Yamaden Co. Ltd., Tokyo) at room temperature. The values presented in Fig.1 give the concept that the type of meat that reacted best was beef, and the deformation increased for beef from 39% in the case of -MTG to 51% in the case of +MTG. For chicken it was slightly decreased in the case of -MTG by 34% (data not shown). So from the categorized data we illustrated that the temperature was affected significantly on the breaking strength score, even though pork reacted formidably at 40°C; but we must consider the sausages that were treated at 80°C. A particularly interesting approach was proposed by the cooking temperature to prepare well-cooked food which is around 80°C. That was the reason for concern about this temperature itself. Therefore, this leads to the fact that the best meat type that can be improved as far as texture is concerned, is beef.

When we compared the extractability of the proteins in two different solutions, A-solution and GS-ATP solution, a difference was realized, and the changes of the extractions were significantly increased between the solutions. Values which are provided in Fig. 2 clarify that the protein concentration of the GS-ATP solution was higher than the protein concentration of A-solution. It was clear that chicken in the A-solution was less than beef and pork, but in the GS-ATP case was higher than the others. That might lead to considerations of the mechanisms and the high affinity reaction between MTG and MHC of beef and pork. So probably ATP played an important rule in the extractability, which might release some proteins and make some cleft in all the structures.

SDS-PAGE analysis of sausage's proteins extracted in A-solution and GS-ATP solution and their reaction with the MTG was shown in Fig. 3. The analysis of extracted protein solutions illustrated the density of MHC; the band reduced significantly, which clearly showed what the differences were between truancy and presence of MTG itself. The pattern shows the values which were obtained from sausage treated at 40°C for 30 min. This suggested that the binding ability of myofibrillar proteins with MTG might be formed and fabricated predominantly by MHC. It is known that MHC is the most important capable protein for meat gelation, and even myosin constitutes approximately 50-55% percent of the myofibrillar protein, and is characterized by a high proportion of basic and acidic amino acid, making it a highly charged molecule (Fig. 3). As shown in the lanes of chicken the MTG did not react well with MHC. At the same time the reaction in beef and pork significantly increased by adding MTG and that is seen clearly from the disappearance of the label in the MHC. It must however be recognized that there are other influential proteins; similarly they can react as the influencing factors of the cross linking battlefield such as actin.

G-L content (Fig. 4) contributed a great value of intelligibility of the reactions in the presence of MTG itself. This leads us to realize how different the reaction was between these types of meat sausages with the MTG. The sausages were incubated at 40°C, so the content of G-L of sausages after adding MTG was significantly increased and the values were mostly the same in pork as well as beef. The glutamine residues are the acyl donor and the MTG catalyzed an acyl transfer reaction in the carboxamide group of proteins, and the difference refers to the glutamine residues reacting at different rates depending on their location in the protein and obviously resultants from the shape of the muscle.

Conclusion

A few concepts will be discussed. To sum up all the values which were provided in our data. All the figures gave very clear differences between the three types of meat, although the meat proteins are same of the compositions. For instance, MTG was a good additive for beef and pork to improve their texture as well as gelation, and that was different to chicken. Cross-linking of proteins by using MTG may be useful in texturization and in modification of solubility and emulsifying properties in beef and pork better than chicken. Finally, we recommend improving upon our methods in experiments to get more clarification in the future.

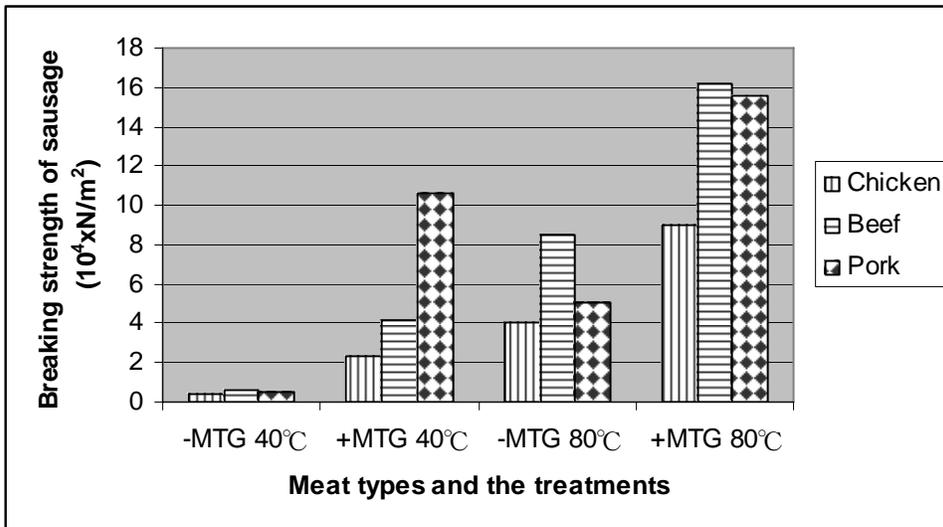


Fig. 1 Changes in breaking strength and deformation of chicken, beef, and pork sausage as the functions of MTG and temperature. The sausages were set at 40°C and 80°C for 30 min.

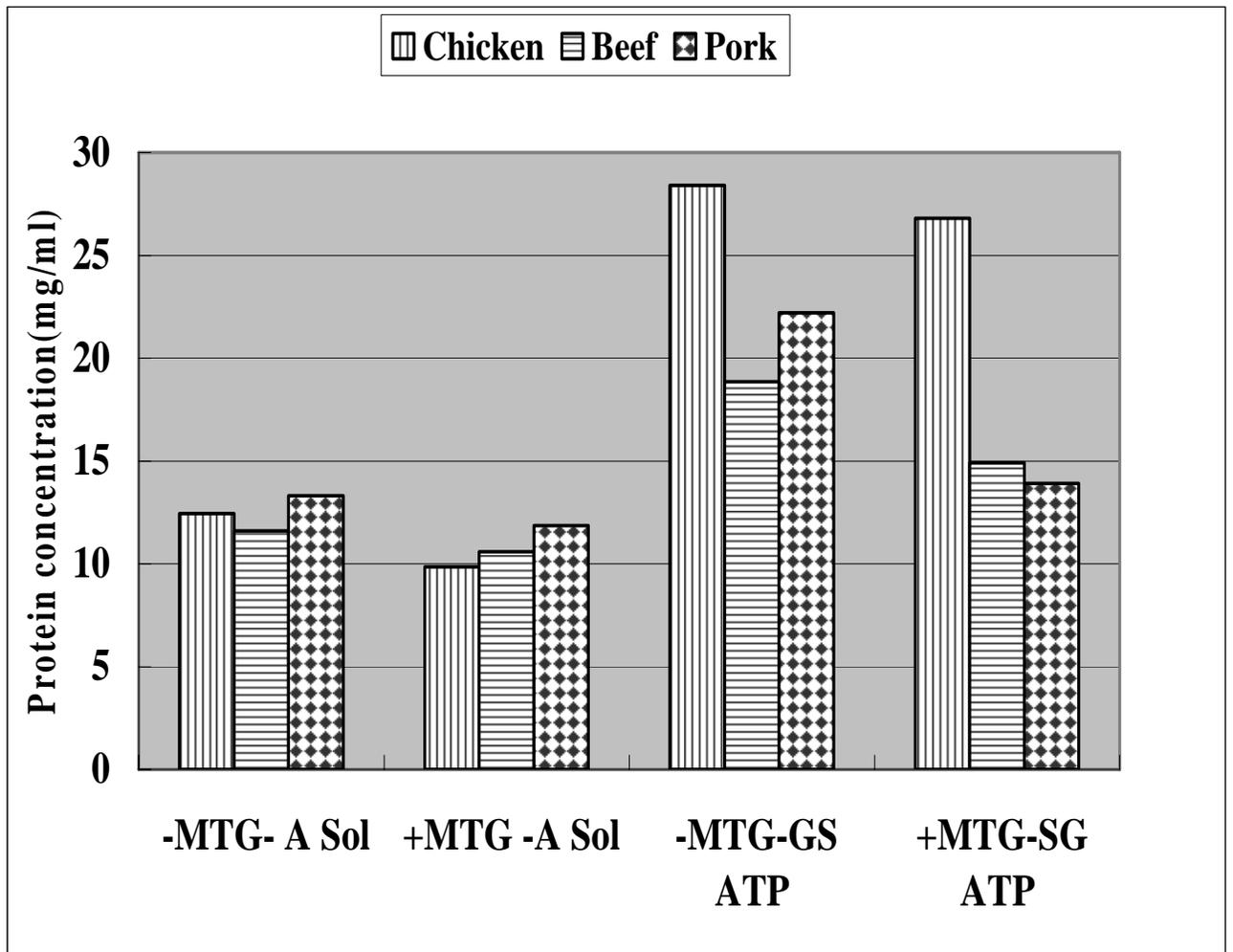


Fig. 2 Extracted protein concentration of chicken, beef, and pork in A-solution (50mM Imidazole-HCl (1M pH6.0), 2mM EDTA and GS-ATP solution (0.09M KH_2PO_4 , 0.06M K_2HPO_4 , 0.3M KCL, 1mM ATP). The sausages were set at 40°C for 30 min.

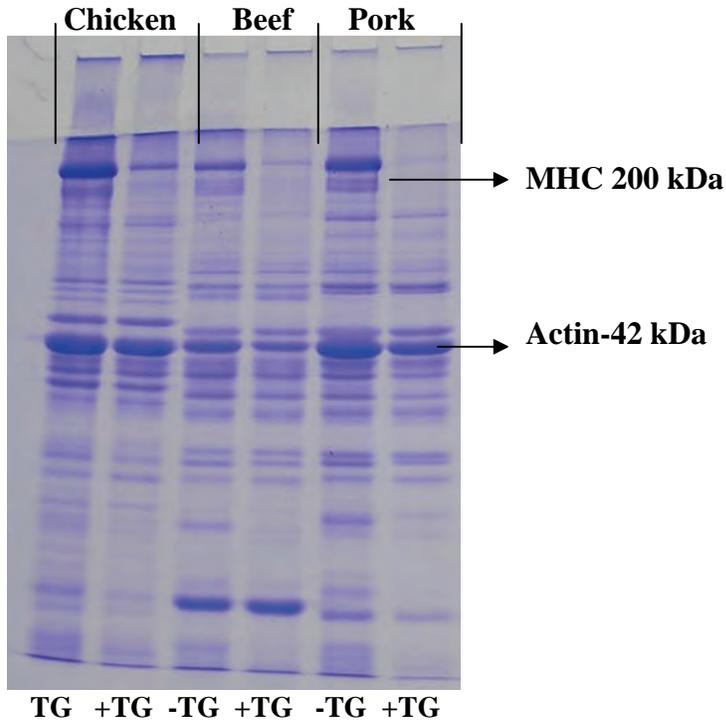


Fig. 3 Changes in SDS-page pattern of chicken, beef, and pork sausage proteins. Illustration of the influence of MTG on MHC of the proteins which were extracted from sausage samples which were incubated at 40°C for 30 min, and dissolved in GS-ATP solution.

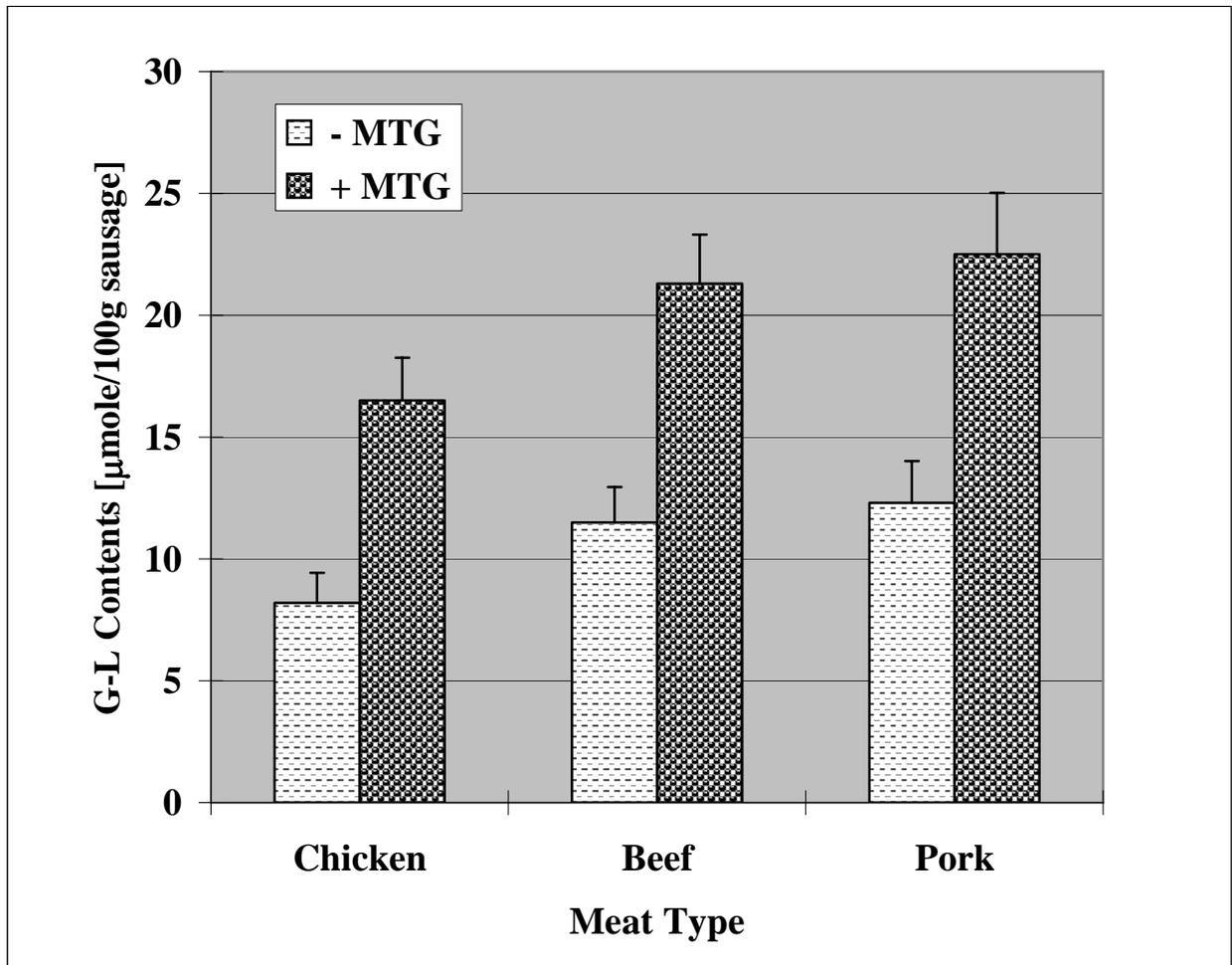


Fig. 4 Changes in ϵ -(γ -glutamyl) lysine content ($\mu\text{mole}/100\text{gm}$ of sample). The sausage samples were treated at 40°C for 30 min.

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COOKED SAUSAGE BATTER – PHYSICAL PARAMETERS

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Federal Research Center for Nutrition and Food

Key Words: cooked sausage batter, viscosity, electric power consumption, temperature, strength of batter

Introduction

Much about the technology of successful production of cooked sausage batter is known. Some became published in the “Kulmbacher Reihe” in the volume "Technologie der Brühwurst" (Technologie der Brühwurst, 1984). Additionally, factors, which cause the ability of the salted meat to stabilize the heated sausage, are identified (Hamm, 1972). On combining of these areas of knowledge and regarding general principles of chemistry, reasons for strong water and fat- separation of the sausage, for unsatisfactory bite and in principle for all product properties and/or their lack can be found.

Our institute cooperates with 12 other research institutes within the European Union on the research- project REDALL. Within the frame of this project the allergenicity of food of animal origin shall be reduced. It is likely that by each treatment of food, and then of meat, its allergenicity can be affected. The comminution during chopping of meat, for instance, for the production of cooked sausage batter, surely is an intensive treatment. During the cutting in a bowl chopper energy is applied to the batter. This application of energy can contribute to a change of the allergenicity of the meat/ the cooked sausage. The following has to be considered.

The chopper is electrically propelled. The electricity is converted into energy of movement of the knives. Thereby some losses develop before the knives rotate. These losses can be quantified only very heavily or not at all.

So we are observing transformation of electricity into kinetic energy of the chopper-knives. The energy consists of several components, which are not easily separable from each other. We assume that a part of the energy is used for the size reduction of the raw materials. Further the raw materials are moved. That means that another part of the energy is used for the transportation of the batter. During cutting and local movement friction develops. The friction between batter and knives leads to a transformation of mechanical energy into heat- energy. So the energy, which is consumed during chopping, has to be differentiated:

1. Some part of the kinetic energy is transformed into heat energy. It determines the temperature- history of the batter during the process of chopping. The developing heat can be explained by friction between knives and batter. That is, viscosity- effects arise. Therefore it is of importance to follow the viscosity of the batter.

2. The second area of investigation concerns the electricity consumed by the chopping- knives and/or the shaft of the chopper.

Objectives

The following questions arise:

Does the energy, consumed by the knives, change with increasing destruction of the meat and – connected with that - with rising temperature of the batter?

Or, the other way:

Which effect has the transfer of energy into the batter, in how far do its physically measurable characteristics change?

Which physical methods are suitable for a description of the batter changes?

Methodology

We measured the viscosity with a modern equipment (Rheometer AR2000 from TI, see fig. 1), which permitted the measurement of the tested material at different temperatures. The opinion, the temperature rise of the batter during the chopping results from an increasing viscosity of the batter during chopping, is frequently expressed - it should be examined however.

We proceeded as follows: Batters were chopped up to 0°C or up to 20°C. In the rheometer the measurements were made at either 0 or 10 or 20°C. That is, a batter for example chopped to a temperature of 0°C was measured three times, at 0, at 10 and at 20°C. Measuring was made in each case with a new sample. The investigations were repeated 4 times.

It is known from direct observation of the chopping process and examination of the batter with the hand (hand- feeling), that the batter changes during the chopping process. It appears to become tougher and tougher, as long as the temperature doesn't rise over about 12°C. For the sensitivity of sensory perceptions of the hand there is so far no other method. It appeared however not senseless to fasten a geometry, which is immersed into the batter, to a scale and measure the strength, with which the batter in the chopping process flows against that geometry (fig. 2). These results should explain at least a part of the hand impression. It was interesting whether there is a relationship between the results of the strength measurement and those of the viscosity measurement.

Further, in the past due to the measurement of the electrical power, which is consumed by the chopper, criteria were developed at which time the chopping process can be stopped (Grabein and Raeuber, 1988). During the chopping process the consumed power shows a typical curve, which exhibits a maximum. Briefly after this maximum the chopping should be terminated. Whether this consumption of power is correlated with the viscosity and the strength, which the batter causes by flowing against a geometry, was a further question which should be clarified.

Results & Discussion

First we regard the viscosity of the batter, as affected by the measuring temperature, the temperature of the rheometer- geometry (fig 3). Clearly the rheometer temperature

affected the viscosity of the batters. At rheometer temperatures of 20°C the batters were low viscous. On measuring at 10°C, they behaved more viscous. The highest viscous, most tough, the batters were, when they were measured at a temperature of 0°C. The connection between viscosity and temperature of measurement existed as well for batters chopped to only 0°C as for very intensively, to 20°C, chopped batters. Due to these results it was completely clear that the temperature of measurement had a substantial influence on the viscosity results.

After recognizing these effects of the measurement temperature, it was clear that for pursuing the change of viscosity during the chopping, a constant rheometer- temperature could not be used. Namely the knives move a batter, which exhibits different temperatures at different times. The viscosity at the respective chopping temperature must therefore be of interest.

If the temperatures of the batters and their measuring temperatures were the same, another picture resulted (fig 3). It is obvious, that batters chopped to higher temperatures were less viscous than those, chopped to a lower temperature. With rise of the chopping temperature, the knives have to transport a less viscose, a less tough mass.

There is by viscosity measurements no hint about the formation of a batter structure, a so-called secondary structure, on chopping.

As it is well known, the batter temperature drops first under 0°C (fig. 4), if ice and not water is added. On further chopping it stays for a short time at these temperature and rises then monotonously, but not linearly up to the desired chopping end-temperature.

The electric power taken up by the chopper is transferred without large losses to the batter. At the beginning of the chopping only the tissue particles are cut by the knives, later they are primarily mixed and rubbed. These changes corresponded with the power and strength measurements. Three sequential phases of consumed electrical power could be recognized (fig. 4): An initial rise of the electrical power to a maximum, afterwards a nearly constant phase and a following flatter decrease of the electrical power.

The strength of the batter – measured with a geometry, which was immersed into the batter during chopping, corresponded, however temporally retarded, approximately to the electrical power consumption of the chopper (fig. 5). With increasing gluing of the chopped components and the increase of their specific surface, probably also with the increase of protein swelling, it was more and more difficult for the batter to flow around the geometry. The batter had to develop an increasing strength. In the second phase the strength decreased, probably because with rising temperature viscosity effects dominated increasingly.

Up to the maximum of the strength and up to the plateau of the power consumption the batter became chopped more and more intensively and became more and more sticky. At later times, and after the temperature curve bent, effects of viscosity dominated. The exact time of bending of the temperature curve depended on a couple of effects. We found so far an influence of shaft revolution, batch size and recipe. The phenomenon arose at approximately 5 to 9°C.

A connection between the shear viscosity and the results of the strength and power measurements did not exist. Conditions in the shear gap of the rheometer and in the chopping bowl are not comparable.

Concerning the transformation of kinetic energy of the knives into friction, there were two cases to differentiate: Sliding friction between batter and knives and the inner or

viscose friction between the batter particles. Only influences of the viscose friction can be identified by viscosity measurements with the rheometer.

The heat development resulting from friction of the knives in the batter was measured with a high-speed IR camera. It turned out that the knives do not warm up very much. The knife edges became at most about 5°C warmer than the batter (fig 6).

Conclusions

On chopping cooked sausage batters, the viscosity decreases with a rising temperature during comminution.

The strength of the batter increases during chopping and until a temperature of about 6 to 10 °C is achieved. At higher temperatures the strength decreases.

The consumed electrical power shows three phases. After starting the chopping process it rises (phase 1). After continuing the chopping a plateau develops (phase 2). After that the consumption of electrical power decreases.

The temperature of the chopper knives is maximally 5°C higher than the temperature of the batter.

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Tables and Figures

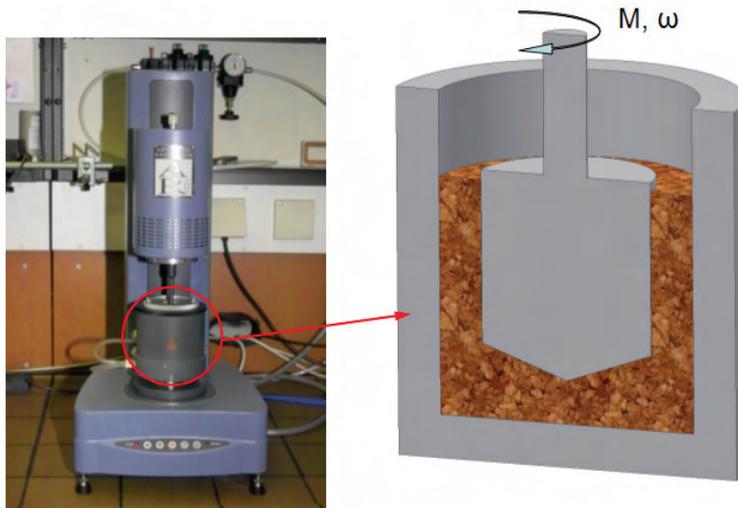


Fig. 1: Rheometer for measurement of viscosity. Searl- geometry (see right side) attached.

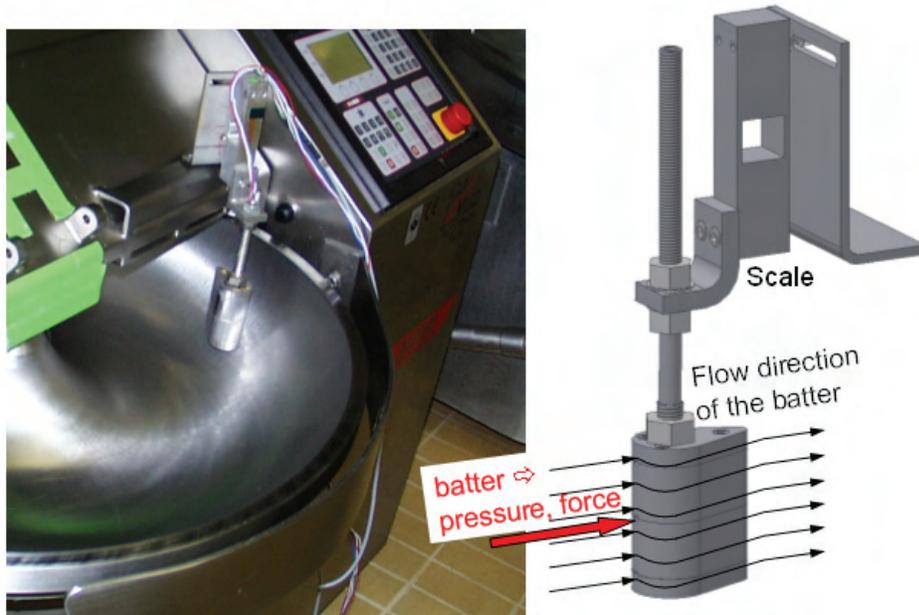


Fig. 2: Geometry in the batter for strength measurement

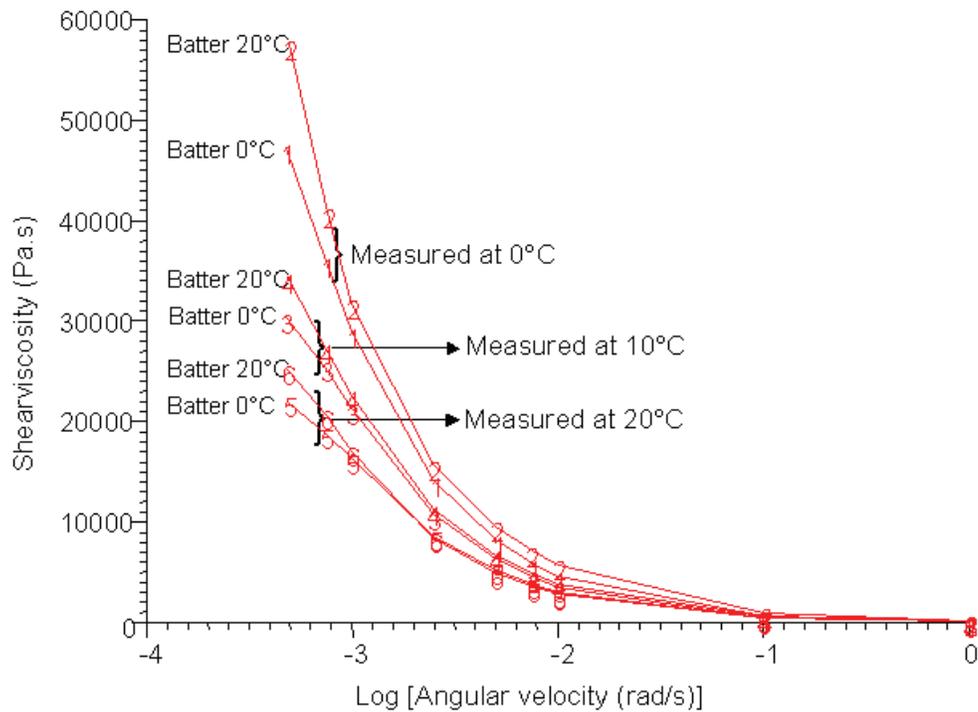


Fig. 3: Measurement of the shear viscosity of batters at different chopping temperatures and different measurement temperatures

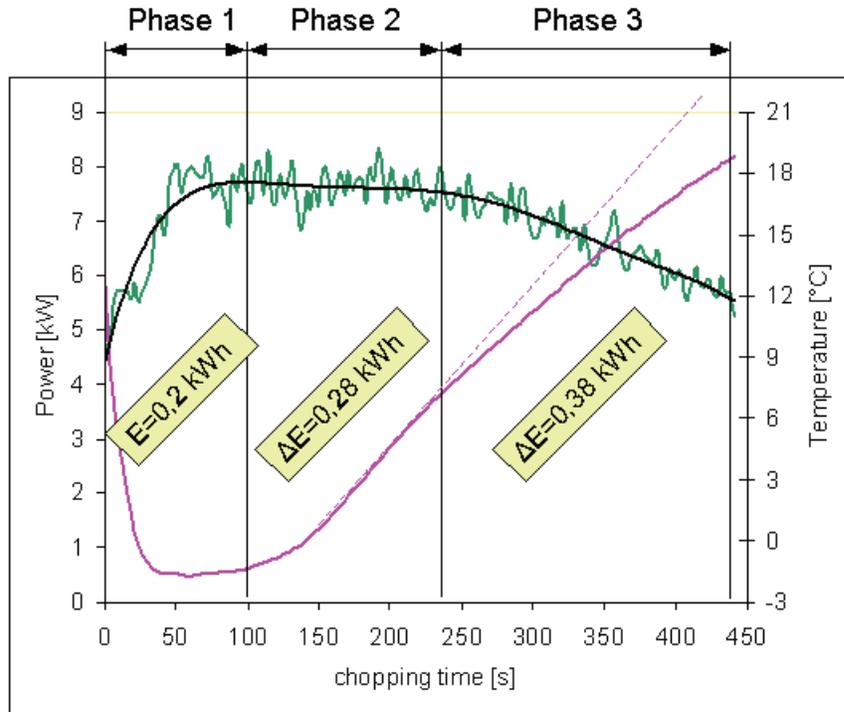


Fig. 4: Measurement of the electrical power consumption of a bowl-chopper and the temperature during the chopping process

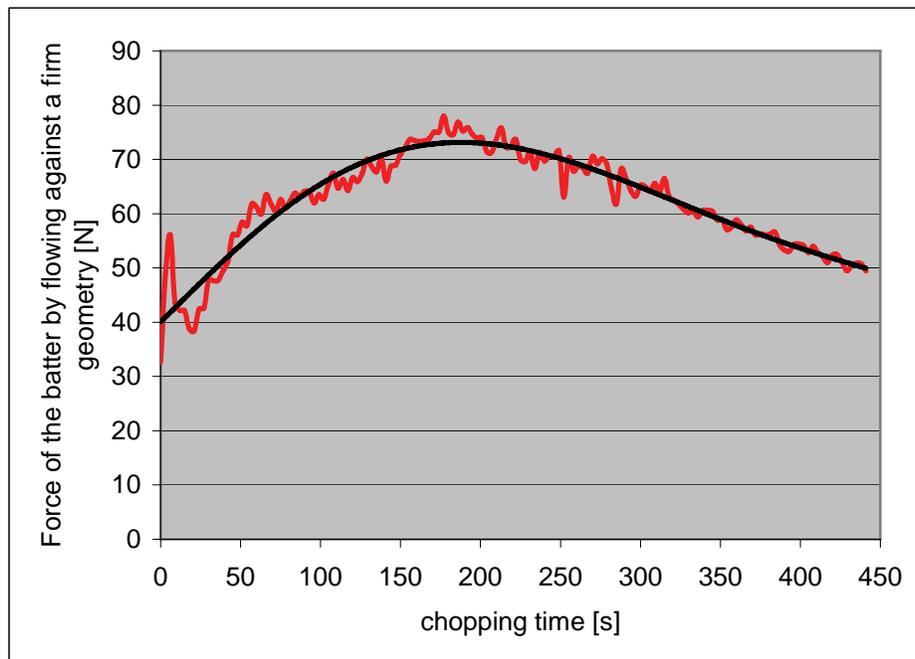


Fig. 5: Force of the batter by flowing against a firm geometry during the chopping process

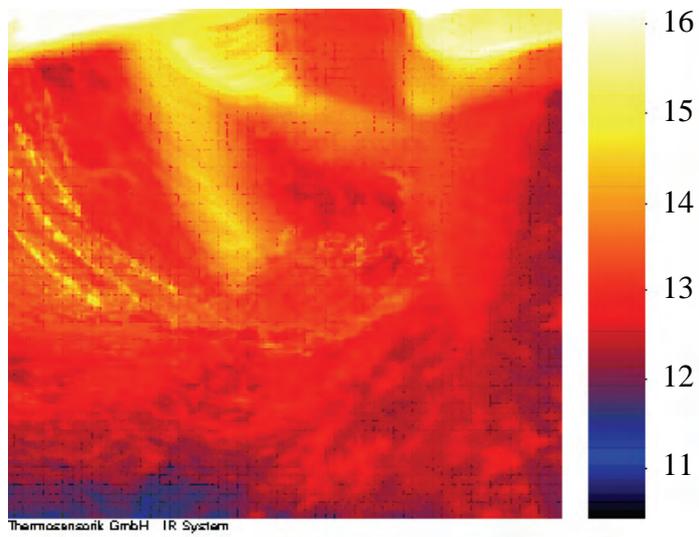


Fig. 6: Thermographic picture of the chopping process: traces of heat in the batter

IMPACT OF SOLUBILIZED BEEF PROTEIN ON THE TEXTURE PROPERTIES OF FRANKFURTERS

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Key Words: Frankfurter, solubilized protein, salt, pH

Introduction

The undesirable tastes, colors and odors characteristic of meat byproducts, has resulted in a significant amount of research to improve palatability and functionality (James and Mireles Dewitt, 2003). A method designed to retain myofibrillar protein functionality is the acid solubilization isoelectric precipitation (SIP) process (Kelleher and Hultin, 1999). Acid-SIP was first patented in 1999, and subsequent research led to the finding that the myofibrillar proteins recovered retained their functionality and could still be used to create good quality meat gels. (James and Mireles Dewitt, 2003). In addition, research has shown that the Acid-SIP treatment decreases fat and produces strong gels without the addition of salt (James and Mireles DeWitt, 2003). Theoretically, this process of protein recovery will help meat processors recover valuable protein with enhanced protein functionality

Objectives

The objective of this research was to evaluate how a solubilized protein affects the textural properties of a processed meat product.

Methodology

Preparation of Beef Round

A highly trimmed beef inside round was obtained 3 days postmortem and cut into approximately 2.54 cm square cubes and ground through a General MC-100 meat grinder (Red Goat Disposers, Mufreesboro, TN, USA) using a 5 mm extrusion die at refrigeration temperature.

Preparation of Solubilized Protein

The solubilized protein samples were prepared according to DeWitt and others (2002). A 1:9 (w/v) mixture of beef round to 2mM citrate buffer was blended in a vacuum chopper (UMC 5 Electronic, Stephan Machinery Corp., Columbus, OH, USA)

for 3 min. at 5°C. The pH was lowered to 2.5 with 2N HCl and centrifuged at an average 9,000-10,000 x g, and the supernatant was collected. The pH of the supernatant was raised to 5.5 with 2N NaOH to precipitate and ultimately recover the myofibrillar proteins. Precipitated protein was recovered with cheese cloth and excess water was removed by centrifuging the recovered precipitated protein at 3,000 x g at 4°C for 20 min. Cryoprotectants (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate) were added according to Kelleher and Hultin (2000). Initial moistures were determined (AOAC, 1995) and the samples were blast-frozen overnight in vacuum-sealed Cryovac bags (Sealed Air Corp., Saddle Brooks, NJ).

Preparation of the Treatments

A basic frankfurter formulation was used as a control (CL, 2% NaCl and 0% solubilized protein: Table 1). Beef in the control formula was substituted with solubilized protein at pH 5.5 or pH 7. Salt was varied in the substituted formulas at 1 or 2%. The four modifications of the substituted formulas were:

- A = 2% NaCl and 2% solubilized protein
- B = 2% NaCl and 20% solubilized protein
- Y = 1% NaCl and 2% solubilized protein
- Z = 2% NaCl and 20% solubilized protein

The quantity of sodium tripolyphosphate was adjusted according to the amount present in the solubilized protein with added cryoprotectants. Each sample was blended in a vacuum chopper (UMC 5 electronic, Stephan Machinery Corp., Columbus, OH, USA) for 4 min at 5°C. The resulting batters were then stuffed (American Harvest Jerky Works Kit, 12.7 mm horn, The Metal Ware Corp., Two Rivers, WI) into two-21 mm cellulose casings (Viskase, E-Z Peel® Nojax, Willowbrook, IL), around 70 cm long, and rolled into links about 10 cm long. Liquid smoke was applied to the links and they were cooked under normal conditions in an Alkar smokehouse (Lodi, WI). The cooked links were then chilled overnight (4°C), peeled, and vacuum packaged (Cryovac bags, Sealed Air Corp., Saddle Brooks, NJ, USA).

Table 1. Control Formula

Control Formula	Weight	% Raw Composition
Beef	906 grams	70.5
Water	272 grams	21.2
Salt	25.78 grams	2.0
Spice	21.6 grams	1.7
Puracal	26.3 grams	2.0
Corn Syrup	18.14 grams	1.4
Dextrose	13.15 grams	1.0
Cure	2.26 grams	0.2

Analysis of Cooked Frankfurters

Texture was evaluated with a Stable Micro Systems Texture Analyzer (Model TA-XT2i, Texture Technologies, Inc., Scarsdale, NY, USA) with 2 cm skinned portion tempered to room temperature. The Texture analyzer measured the tertiary texture attributes as (www.texturetechnologies.com):

- Hardness = Peak force of the 1st Compression
- Cohesivness = Area 2nd Compression / Area 1st Compression
- Gumminess = Hardness x Cohesivness
- Springiness = Length of 2nd Compression / Length of 1st Compression
- Chewiness = Gumminess x Springiness
- Resilience = Area withdrawal of 1st Compression / Area 1st Compression

All tests were performed in at least triplicate.

Statistical Analysis

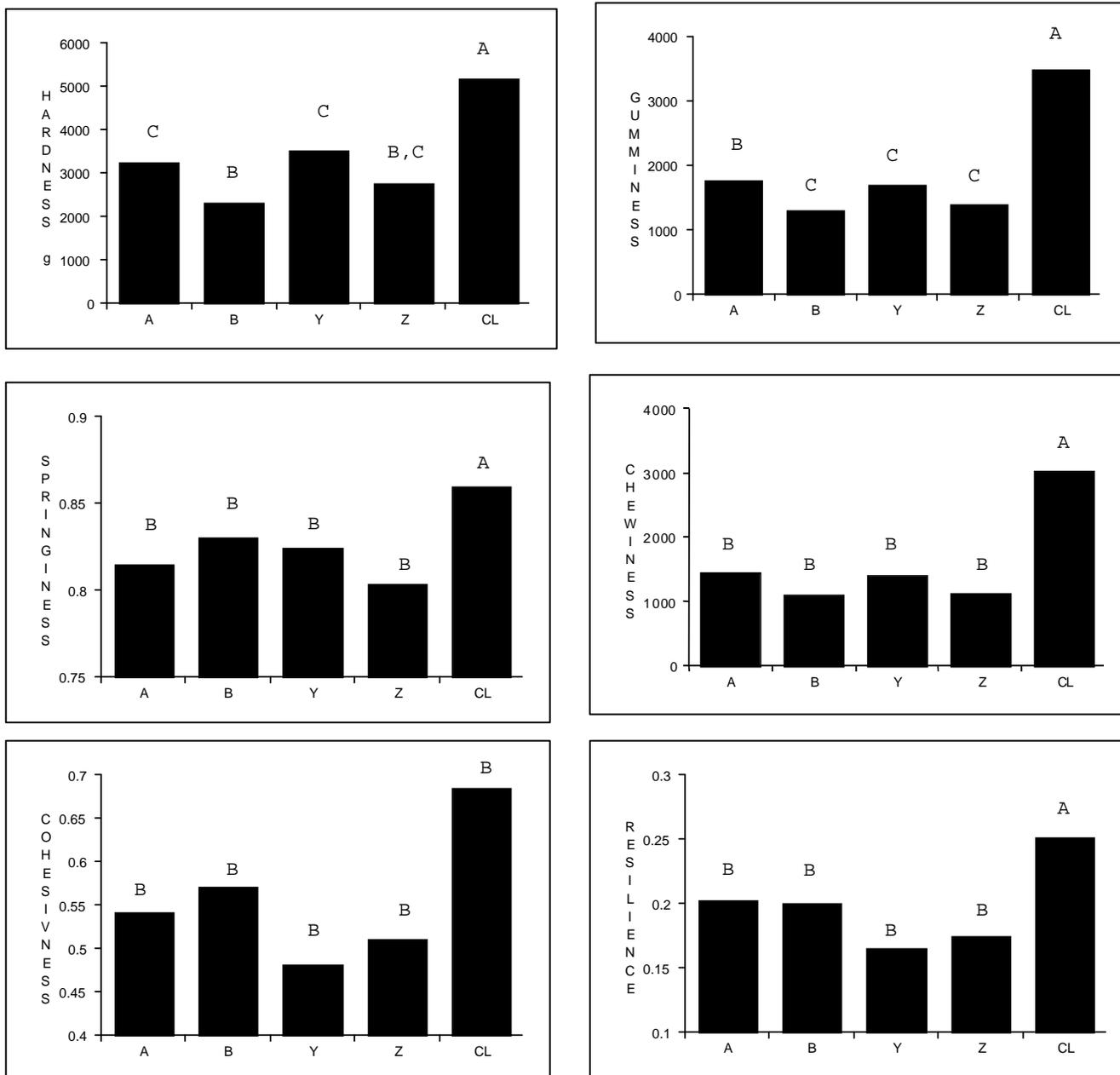
The data was analyzed using 2-way ANOVA (Sigma Stat 8.0, Rockware, Inc. Golden, CO). The model included treatment and NaCl levels as main effects. All interactions among treatment and NaCl were included in the model. Mean separation was accomplished using Least Significant Difference.

Results and Discussion

Solubilized Protein, pH 5.5

In each of the texture analyses (Figure 1), the Control performed statistically better than any of the treatments containing solubilized protein. For springiness, chewiness, cohesivness, and resilience, there was no difference between the treatments. However, in the case of cohesivness and resilience, substituted formulas with 1% NaCl performed poorer. For chewiness and hardness, treatments containing 20% solubilized protein were less chewy than those containing 2% solubilized protein.

**Figure 1. Solubilized Protein at pH 5.5
Texture Profile Analyses of Different Treatments**



Texture Profile Analysis (TPA) of cooked links containing Acid-SIP BR protein. a,b,c means lacking a common superscript differ ($P < 0.05$).

1084
A = 2% Salt, 2% Acid-SIP
Z = 1% Salt, 20% Acid-SIP

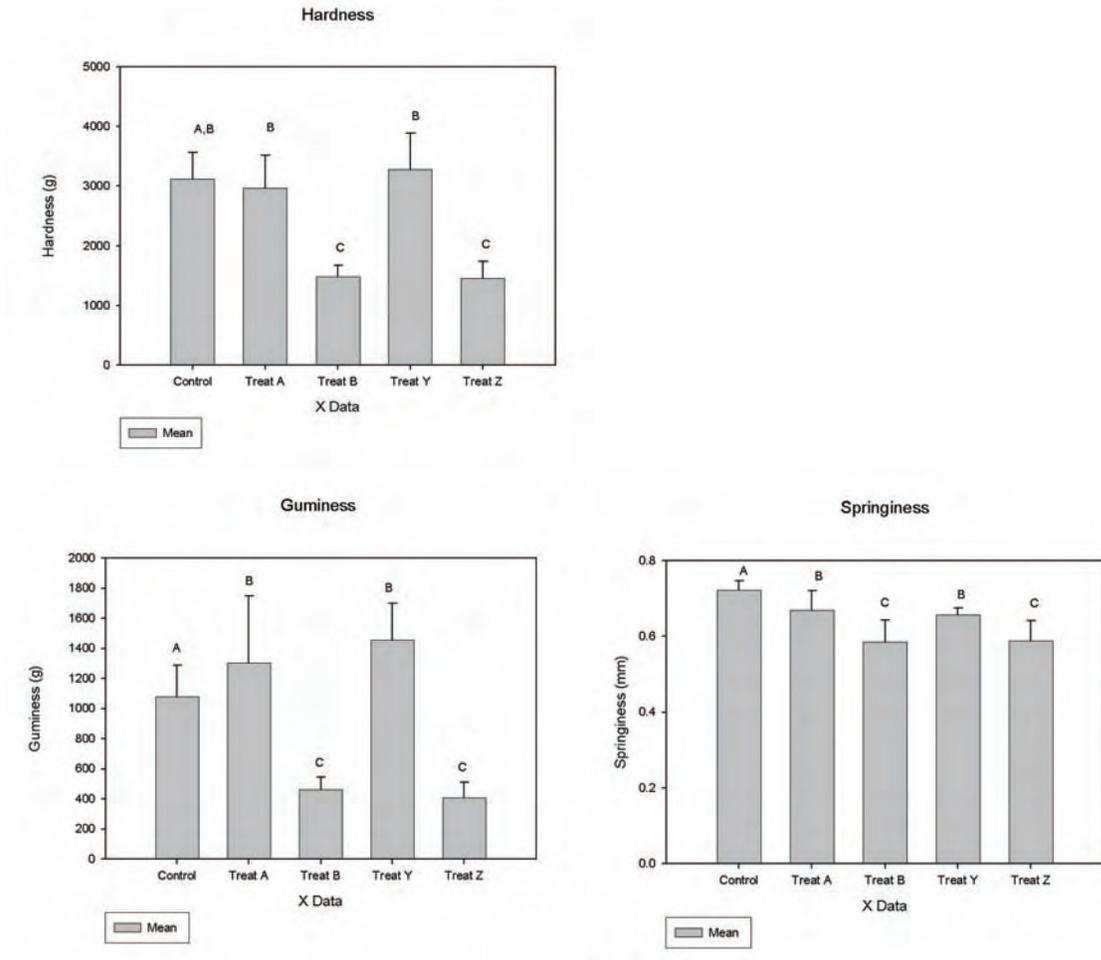
B = 2% Salt, 20% Acid-SIP
CL = 2% Salt, 0% Acid-SIP

Y = 1% Salt, 2% Acid-SIP

Solubilized Protein, pH 7

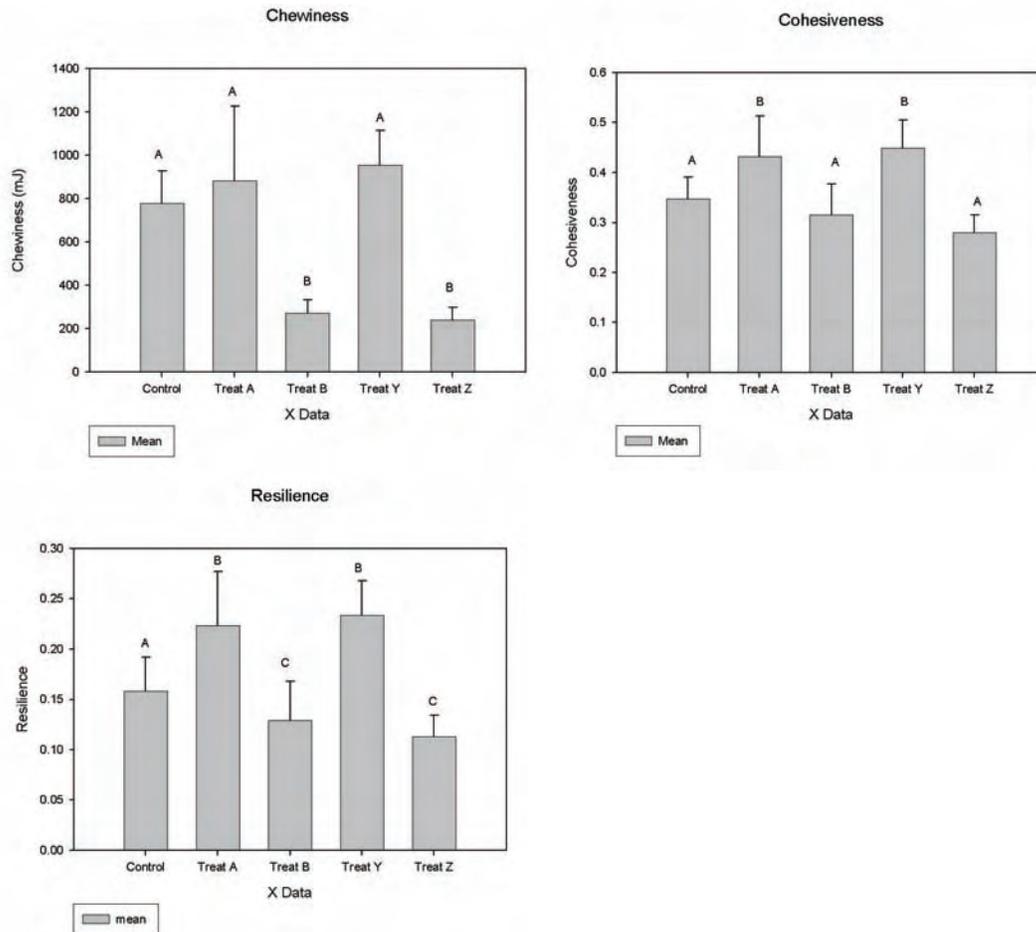
Frankfurters were formulated with solubilized protein and adjusted to pH 7 using sodium bicarbonate. Texture analysis was conducted on the five different treatments (Figure 2). Overall salt did not have an affect on texture analysis. For hardness the frankfurters with 20% solubilized protein were significantly softer than the control and 2% solubilized protein formulas. Springiness decreased significantly as the amount of solubilized protein present in the frankfurter increased. For cohesiveness the 2% solubilized protein increased overall cohesiveness and the 20% solubilized protein showed no improvement over the control formula. Gumminess increased significantly with the addition of 2% solubilized protein but decreased significantly with the addition of 20% solubilized protein as compared to the control formula. Chewiness was not significantly affected by the addition of 2% solubilized protein but was significantly lower in the 20% protein formulas. It was also shown that resilience values in the 2% solubilized protein formulas were significantly higher then the control while 20% solubilized protein were significantly lower.

**Figure 2. Solubilized Protein at pH 7
Texture Profile Analyses of Different Treatments**



Texture Profile Analysis (TPA) of cooked links containing solubilized protein. Mean lacking a common subscript differ ($P < 0.05$).

Control= 2% Salt, 0% Solubilized Protein Treat A = 2% Salt 2% Solubilized Protein Treat B= 2% Salt 2% Solubilized Protein Treat Y= 1% Salt 2% Solubilized Protein Treat Z= 1% Salt 20% Solubilized Protein



Conclusion

Frankfurters made with solubilized protein at pH 7 performed closer to the control than frankfurters made with solubilized protein at pH 5.5. Formulations with solubilized proteins at pH 7 the 2% solubilized protein formulations performed as well or better than the control. However, 20% protein formulations performed poorly and were generally softer than the control.

Purposed future work for this project includes using a formulation to make the frankfurters with beef from the inside round or clod as opposed to using ground beef. In addition, it is suggested that lower levels of protein such as 2, 5, and 10% be evaluated for texture properties using texture profile analysis and sensory evaluation in order to optimize solubilized protein substitution.

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ASSESSMENT OF VARIOUS FAT COMPONENT OF SAUCES FORMULATED FOR USE IN MUSCLE BASED CONVENIENT FOODS WITH RESPECT TO PRODUCT QUALITY AND STABILITY

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Key Words: Convenience foods, Sauces, lipid oxidation

Introduction

There are general trends in the food industry, which indicate that the sector is in transition, with shifts in consumer expenditure from fresh to frozen products and from basic products to more prepared convenient products such as ready meals¹. Total consumption of all ready meal products grew from 1.9 million tonnes in 1996 to over 2.1 million tonnes in 2001. Average per person consumption for all ready meals within the EU stands at 5.8 kilos, having grown from 5.4 kilos in 1996, with forecasts suggesting an increase to over 6 kilos per person by 2006². Ready meals usually consist of a portion of carbohydrates such as potatoes, a portion of vegetables and a portion of meat. The meat forms the core of the meal and ultimately its presentation and more importantly its quality, will result in the consumer repurchasing the product. Sauce, whether it is a tomato sauce, white sauce, gravy or marinade is used to complement a meal, and serves to improve the presentation of the meat. Sauce can be used to mask the off flavours of lower quality meat, prevent product dehydration, tenderise the meat and can also be used for adding desired flavours³. In the production of convenience foods, the quality of the raw materials, particularly that of the sauce and meat components, will ultimately determine the quality of the final product. The task for the food technologist is to determine the degree of interactions, which occur between these raw materials.

Objectives

The objective of this study was to evaluate the stability of a tomato-based sauce, manufactured from a range of commercial oils, for application in muscle-based convenience-style food products.

Methodology

The commercial oils tested were: corn oil (CO), grape seed oil (GSO), olive oil (OO), sunflower oil (SFO) soya bean oil (SBO) rape seed oil (RSO), vegetable oil (VO), linseed oil (LO) and tallow (T). Tomato sauces (water (56.25%), oil (15%), tomato paste (13.5%), sun-dried tomatoes (1.5%), skim milk powder (5%), cheese (5%), starch (2.5%),

salt (1%), pepper (0.25%)) were processed (80°C x 15 min) in a Limitech mixer (Limitech, Denmark), modified atmosphere packaged (65% CO₂: 35% N₂) and held in refrigerated display, under light, for 30 days. Oxidative stability was measured using four methods, anisidine value, peroxide value, totox value and 2-thiobarbituric acid method. The peroxide value (PV) was measured (meq O₂ kg⁻¹ oil) according to a modification of the method of the American Oil Chemist's Society⁴. The anisidine value (AV) was determined according to the British Standard method⁵. The Totox value (TV) was calculated from PV and AV values (Totox= 2PV+AV)⁶. The extent of lipid oxidation was also assessed by measuring thiobarbituric acid reacting substances (TBARS)⁷ and expressed as mg malonaldehyde per kg sample. CIE 'L', 'a', 'b' colour analysis (Minolta chromameter CR-300), and microbiological analysis (standard plate counts) were assessed throughout storage. Syneresis was assessed by measuring graduated change over the storage period.

Results & Discussion

Peroxide, anisidine, totox and TBARS analysis of all sauces revealed that in general, sauces made with OO, CO, SBO, and RSO were less susceptible to lipid oxidation in comparison to sauces made with LO, VO and SFO (Table 1). This is most likely due to the degree of unsaturation of these oils. LO, VO and SFO have higher levels of unsaturated fatty acids in comparison to the other test oils, which would make them more vulnerable to lipid oxidation. SFO has a high level of linoleic acid (approx 68%) and LSO has a high level of linolenic acid (approx 58%), which may be responsible for the higher degree of lipid oxidation in these sauces. The fatty acid profile of the VO used was unknown, however, results of lipid oxidation indicate that it may contain high levels of unsaturated fatty acids. Tallow fat contains a low level of unsaturated fatty acids, and as expected lipid oxidation results indicated that the sauce manufactured using tallow, remained stable during storage. TBARS analysis indicated that all sauces except LO sauce, (TBARS value greater than 1mg malonaldehyde per kg sample) were sensorially acceptable following 30 days of storage (Table 1). CIE 'L' value of all sauces ranged between 62 and 69, the CIE 'a' values, an indicator of redness, ranged between 19 and 24 and the CIE 'b' values ranged between 47 and 58. Results showed that all sauces prepared using the selected oils were microbiologically safe and free from syneresis over a 30-day storage period.

Conclusions

Oxidative stability of sauces formulated using different oil sources decreased over a 30-day storage period in the general order of: SBO sauce > CO sauce > RSO sauce > OO sauce > T sauce > GSO sauce > SFO sauce > VO sauce > LO sauce. Results showed all sauces prepared using the test oils were microbiologically safe and free from syneresis over the assessment period. Analysis showed that OO, CO, RSO and SBO were most acceptable for tomato sauce manufacture based on the test parameters assessed.

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Tables and Figure

Table 1: Lipid Oxidation of sauce using method: (a) (TBARS) Thiobarbituric acid, (b) PV (Peroxide value), (c) Anisidine value, (d) Totox value

Test	Oil	Time					
		1	4	7	10	20	30
TBARS	CO	0.218 ^a	0.274 ^a	0.346 ^a	0.56 ^{ab}	0.491 ^{ab}	0.237 ^{ab}
	GSO	0.296 ^a	0.213 ^a	0.345 ^a	0.277 ^a	0.308 ^{ab}	0.238 ^{ab}
	OO	0.301 ^a	0.398 ^{ab}	0.33 ^a	0.384 ^a	0.426 ^{ab}	0.357 ^{ab}
	SFO	0.448 ^{ab}	0.562 ^{ab}	0.42 ^a	0.337 ^a	0.439 ^{ab}	0.387 ^{ab}
	VO	0.759 ^b	0.552 ^{ab}	0.485 ^a	0.569 ^{ab}	0.648 ^b	0.642 ^{ab}
	SBO	0.35 ^a	0.249 ^a	0.286 ^a	0.481 ^{ab}	0.338 ^{ab}	0.168 ^a
	RSO	0.342 ^a	0.474 ^{ab}	0.326 ^a	0.502 ^{ab}	0.259 ^a	0.483 ^{ab}
	LO	0.569 ^{ab}	0.931 ^b	0.73 ^a	0.735 ^b	0.54 ^{ab}	1.133 ^c
	T	0.306 ^a	0.407 ^{ab}	0.292 ^a	0.332 ^a	0.474 ^{ab}	0.306 ^{ab}
PV	CO	0.65 ^a	2.07 ^b	0.83 ^a	3.93 ^{bcd}	1.78 ^{ab}	1.33 ^a
	GSO	0.850 ^a	1.100 ^a	3.070 ^a	5.670 ^d	3.110 ^c	2.210 ^a
	OO	0.85 ^a	2.2 ^b	7.33 ^{ab}	3.43 ^{abcd}	2.67 ^{bc}	2.21 ^a
	SFO	3.17 ^b	5 ^{cd}	6.04 ^{ab}	2.17 ^{abc}	1 ^a	2.67 ^a
	VO	4 ^b	3.857 ^{bcd}	11.5 ^b	1.551 ^{ab}	0.944 ^a	1.556 ^a
	SBO	0.789 ^a	1.667 ^b	0.722 ^a	2.273 ^{abc}	1.17 ^a	1.435 ^a
	RSO	0.944 ^a	3.125 ^{abcd}	3.333 ^a	1.068 ^a	1.846 ^{ab}	0.659 ^a
	LO	13.00 ^c	5.36 ^d	7.83 ^{ab}	10.67 ^e	6.22 ^d	5.08 ^b
	T	3.00 ^b	4.06 ^{abc}	2.93 ^a	5.44 ^{cd}	6.83 ^d	2.67 ^a
AV	CO	7.06 ^b	9.13 ^b	8.54 ^b	7.86 ^b	10.23 ^d	8.77 ^{cd}
	GSO	23.280 ^c	22.680 ^d	25.480 ^d	21.580 ^d	nd	10.960 ^{de}
	OO	5.89 ^b	5.5 ^{ab}	8.72 ^b	5.17 ^{ab}	nd	nd
	SFO	52.08 ^d	22.3 ^{cd}	19.36 ^{cd}	16.06 ^{cd}	17.9 ^f	15.82 ^{ef}
	VO	53.23 ^d	21.222 ^{cd}	23.038 ^{cd}	17.355 ^d	16.705 ^{ef}	18.182 ^{ef}
	SBO	nd	1.437 ^a	nd	nd	1.49 ^c	5.545 ^{bcd}
	RSO	nd	2.648 ^a	7.003 ^b	8.182 ^b	nd	4.512 ^{bc}
	LO	nd	18.48 ^c	17.79 ^c	10.53 ^{bc}	11.19 ^{de}	20.52 ^f
	T	5.06 ^b	6.06 ^b	6.47 ^b	8.29 ^b	nd	0.92 ^{ab}
TV	CO	8.36 ^{bc}	13.27 ^b	10.2 ^{ab}	15.72 ^{bc}	13.79 ^{de}	11.43 ^{bc}
	GSO	24.98 ^d	24.88 ^c	31.62 ^{cd}	32.92 ^d	nd	15.38 ^{cd}
	OO	7.59 ^b	9.9 ^{ab}	23.38 ^{bc}	12.03 ^{abc}	nd	0.89 ^a
	SFO	58.42 ^e	32.3 ^d	31.44 ^{cd}	20.4 ^c	19.9 ^{ef}	21.16 ^d
	VO	61.23 ^e	28.936 ^{cd}	46.038 ^d	20.457 ^c	18.593 ^{ef}	21.294 ^d
	SBO	nd	4.771 ^a	nd	3.964 ^a	3.83 ^c	8.415 ^{bc}
	RSO	nd	8.898 ^{ab}	13.669 ^b	10.318 ^{ab}	2.289 ^c	5.83 ^{ab}
	LO	20.773 ^d	29.201 ^{cd}	33.459 ^{cd}	31.866 ^d	23.637 ^f	30.688 ^e
	T	11.06 ^c	14.167 ^b	12.317 ^b	19.173 ^{bc}	12.933 ^d	6.249 ^{ab}

^{a,b,c,d,e,f} refer to tests of significance within test parameter, between oil types and within days of storage.

Means bearing different superscripts are significantly different (P< 0.05).

Table 2: Results of Minolta CIE 'L', 'a' and 'b' for test tomato sauce prepared using selected oils as a function of time (days)

Test	Oil	Time					
		1	4	7	10	20	30
CIE L	CO	66.23 ^c	66.97 ^{ab}	66.85 ^b	67.20 ^{cd}	66.16 ^c	65.66 ^{ab}
	GSO	63.73 ^{abc}	65.81 ^{ab}	66.08 ^b	65.82 ^b	66.49 ^c	63.98 ^b
	OO	62.38 ^a	64.38 ^a	63.59 ^a	63.46 ^a	63.70 ^{ab}	63.18 ^a
	SFO	64.68 ^{bc}	65.32 ^b	65.35 ^b	64.61 ^{cd}	65.96 ^{bc}	64.55 ^b
	VO	64.79 ^{abc}	64.15 ^{ab}	64.72 ^b	65.25 ^{bc}	65.40 ^c	63.76 ^{ab}
	SBO	65.42 ^{bc}	64.71 ^{ab}	65.61 ^b	65.58 ^{bc}	64.98 ^{bc}	65.65 ^{ab}
	RSO	63.29 ^{ab}	64.31 ^a	64.80 ^{ab}	64.72 ^{ab}	63.87 ^{bc}	64.69 ^a
	LO	69.65 ^d	68.52 ^c	68.23 ^c	68.17 ^{de}	68.53 ^d	68.08 ^c
	T	60.06 ^d	64.68 ^c	65.59 ^c	66.00 ^e	65.18 ^a	65.29 ^c
CIE a	CO	19.60 ^a	20.42 ^b	20.51 ^a	19.50 ^{ab}	20.41 ^{bcd}	22.93 ^{bc}
	GSO	21.20 ^a	21.24 ^{ab}	20.23 ^{ab}	22.39 ^{ab}	20.22 ^{abc}	20.27 ^{abc}
	OO	20.88 ^a	20.46 ^a	20.69 ^a	22.69 ^{ab}	20.72 ^{ab}	18.74 ^a
	SFO	20.11 ^a	19.14 ^{ab}	20.23 ^{ab}	22.23 ^a	20.42 ^{ab}	20.18 ^{ab}
	VO	21.93 ^a	21.88 ^a	22.56 ^a	21.81 ^a	21.53 ^a	22.81 ^{ab}
	SBO	20.53 ^a	19.38 ^b	20.68 ^a	19.62 ^b	20.59 ^d	20.19 ^{abc}
	RSO	19.85 ^a	19.78 ^b	20.49 ^b	20.28 ^b	20.39 ^{cd}	21.89 ^c
	LO	22.33 ^a	21.64 ^a	22.50 ^a	23.40 ^{ab}	22.64 ^{abcd}	21.95 ^a
	T	23.34 ^a	19.55 ^a	19.84 ^a	19.72 ^a	19.53 ^a	19.40 ^a
CIE b	CO	57.96 ^a	53.14 ^{bc}	53.76 ^{ab}	52.90 ^a	52.83 ^{bc}	50.17 ^a
	GSO	55.35 ^a	52.79 ^{ab}	52.63 ^c	47.65 ^a	50.35 ^{bc}	49.58 ^a
	OO	54.45 ^a	51.07 ^a	52.19 ^c	48.68 ^a	53.52 ^{bc}	57.06 ^a
	SFO	53.13 ^a	49.80 ^{abc}	52.42 ^{bc}	48.33 ^a	52.18 ^b	49.40 ^a
	VO	49.74 ^a	48.96 ^{abc}	47.53 ^{abc}	49.36 ^a	47.77 ^{bc}	49.30 ^a
	SBO	52.34 ^a	49.30 ^{bc}	51.70 ^a	50.74 ^a	51.94 ^b	51.61 ^a
	RSO	55.39 ^a	51.29 ^c	52.52 ^c	52.40 ^a	50.12 ^c	48.07 ^a
	LO	53.72 ^a	51.45 ^{bc}	52.44 ^c	53.24 ^a	52.12 ^c	49.49 ^a
	T	50.78 ^a	50.69 ^{abc}	49.89 ^c	48.89 ^a	46.30 ^a	47.53 ^a

^{a,b,c,d,e} refer to tests of significance within test parameter, between oil types and within days of storage. Means bearing different superscripts are significantly different (P < 0.05).

EFFECT OF COOKING TEMPERATURE AND OLIVE OIL PERCENTAGE ON THE QUALITY OF MEAT EMULSIONS

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Key Words: Meat Emulsion, Olive Oil, Finite Elements Method

Introduction

No meaty proteins, as soya protein, are used to fabricate meat products because of the technological advantages they provide, such as favouring water retention, the formation of the meat emulsion and improving the texture. In addition to decreasing fat percentage in the product, the use of olive oil instead of pork fat, provides important nutritious properties to the meat emulsions. It is widely accepted that the intake of olive oil lowers the cholesterol plasma content (Mattson and Grundy, 1985).

Objectives

The purpose of the present work was to study the effect of heating temperature and the olive oil percentage on the sensory quality of meat emulsions fabricated with turkey meat and soya fibre.

Methodology

Three meat emulsions were fabricated with different olive oil concentration (30, 15 and 5%) and 50% turkey meat, 1% soya fibre and water until completing 100%. There were also added salt (1,5% in 30%olive oil emulsions and 2% in the rest), sodium nitrite (150 ppm), sodium ascorbate (0,5 g/kg) and tripolyphosphate (0,3%). Cooking of the emulsions was performed in hot water (82°C) to internal endpoints of 60, 70 and 80° C, which were recorded by a probe. These probes were located in the middle of the 1 kg meat emulsion that was in a tin, and they were connected to a temperature data register PC-logger 2100 (Intab, Sweden). The following parameters were evaluated in the meat emulsions: cooking losses, chemical composition (water, protein, fat), color (Cie L*a*b*) and sensory analysis using a descriptive quantitative analysis (QDA) (Meilgaard et al., 1991). The trained panel assessed meat emulsions for initial and sustained juiciness, hardness, cohesiveness, granularity, fattiness and chewiness. The method of Finite Elements for simulation the heating conditions of meat emulsions with olive oil was used to describe thermal processing (ANSYS 5.7 program).

Results & Discussion

Figure 1 shows the values experimentally observed of the temperature evolution for the three meat emulsions. It can be observed that meat emulsion with 5% olive oil reached 60°C internal temperature after 16 minutes, approximately, while the other two emulsions needed 21 and 29 minutes, respectively. Regarding 70°C internal temperature, meat emulsions with 15 and 30% olive oil attained this temperature after approximately 48 minutes, while meat emulsions with 5% olive oil did it in 32 minutes. Time and temperature differences were lower at 80°C internal temperature. These results make in evidence that the composition of the meat emulsions modifies their thermal properties, as reported by Carballo et al. (1996) and Ngadi et al. (1998). Figure 2 shows simulation of heating process of 5% live oil meat emulsion with the time. The numerically calculated temperature values can be used to describe the thermal process of meat emulsions with respect to product quality. In this study also shows differences between meat emulsions fabricated with 30, 15 and 5% of olive oil where different heating degrees were observed experimentally for the studied meat emulsions.

Cooking yield was lower than 6% in all the studied meat emulsions, being that of 5% olive oil the one that showed higher weight losses when heating.

Fat percentage affected color coordinates (Cie L*a*b*), showing meat emulsions a lower Lightness (L*) with decreasing olive oil percentage. Table 1 shows that a* increased and b* decreased with increasing cooking temperature between 60 and 70°C, except for the meat emulsion fabricated with 30% olive oil, where no differences were observed between heating temperatures. Redness (a*) decreased and b* increased with increasing cooking temperature.

Figure 1, also shows the results from the sensory analysis by the trained panel that assessed meat emulsions with 5 and 15% olive oil as low in hardness, with soft flavour and low fattiness and juiciness was similar between 30 and 15% oil emulsions. Regarding cooking temperature, the texture profile at 70°C was better scored by the trained panel that the emulsions cooked at 60 and 80°C, showing the former higher juiciness, lower cohesiveness and hardness.

Conclusions

The time hended for meat emulsions to achieve 60 and 70°C internal temperature was lower for the meat emulsions fabricated with 5% olive oil, while no differences were observed at 80°C. The sensory quality of the meat emulsions fabricated with 5 and 15% olive oil were assessed as tender, soft in flavour and with low fattiness against those emulsions fabricated with 30% olive oil. Meat emulsions cooked until 70°C internal temperature had the best scores at the sensory analysis by the trained panel.

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Tables and Figures

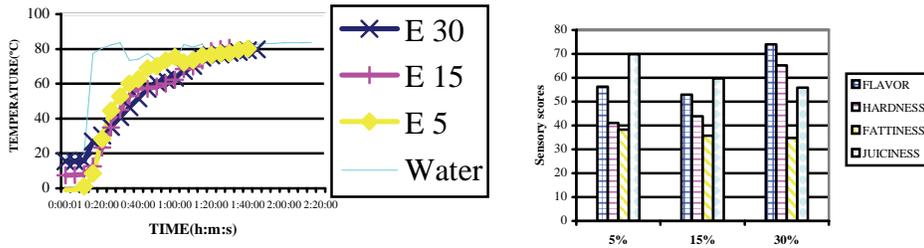


Figure 1. Experimental values of evolution of cooking temperature with the time and sensory evaluation of meat emulsions fabricated with 5, 15 and 30% olive oil.

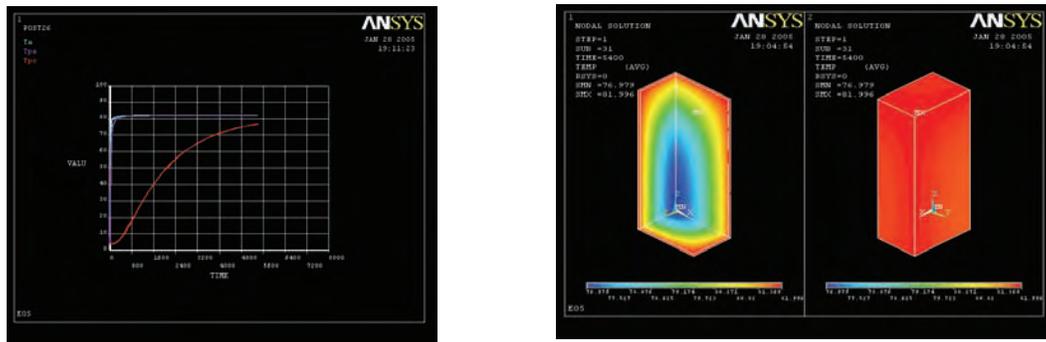


Figure 2. Simulation of heating process of 5% olive oil using the Finite Elements Method (FEM).

Table1. Effect of cooking temperature and olive oil percentage on Cie L*a*b* color coordinates (Letters compare columns, numbers compare raws; p<0.001 ***, p>0.05 ns).

	Fat %	60 °	70 °	80 °	Sig.
a *	5	5.29±(0.04) ^{b1}	5.59±(0.03) ^{b2}	5.70±(0.04) ^{c2}	***
	15	3.60±(0.03) ^{a1}	4.01±(0.04) ^{a2}	4.11±(0.02) ^{b2}	***
	30	4.01±(0.21) ^a	3.98±(0.03) ^a	3.93±(0.03) ^a	ns
	Sig.	***	***	***	
b*	5	12.34±(0.05) ^{b2}	10.06±(0.05) ^{a1}	10.25±(0.10) ¹	***
	15	11.33±(0.16) ^{a2}	10.07±(0.04) ^{a1}	10.33±(0.02) ¹	***
	30	11.09±(0.08) ^{a2}	10.59±(0.01) ^{b1}	10.50±(0.07) ¹	***
	Sig.	***	***	ns	

BURGER TEXTURE IN VIEW OF PHYSICAL CHARACTERISTICS OF MEAT DOUGH AND PATTY FORMING PROCESS

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Key Words: patty forming, burgers, rheology; thermal analysis; texture; meat dough

Introduction

Control of product characteristics and quality, and efficient adaptation to changes in desired quality through dedicated processing are important issues for the industry. Major aspects of consumer appreciation comprise visual appearance like colour and shape, textural aspects like bite and chewing properties, taste and smell. These characteristics depend on composition as well as on processing conditions. An often neglected aspect in process optimization is the interrelation between raw material properties and processing with regard to quality. An extensive overview was given by Mikkelsen (1993). However, most studies in this context have been published on the relation of texture, cooking loss and microbial safety with the heating process, e.g. Erdogu et.al. (2005) and Zorrilla et.al. (2000) The relation between patty forming process and product characteristics didn't receive much attention.

The present work relates, in more detail, to the relation between the physico-chemical properties of the meat dough, the thermal conditions during the patty forming process and rest after forming on final physical texture of poultry meat burgers after heating. The aim is to understand the different factors that dominate the quality aspects mentioned.

Objectives

Make manifest the interacting effects of process conditions during forming and meat characteristics on the final structure of burgers.

Methodology

Experimental work was based on meat doughs using simple basic formulations (table 1).

Meat preparation, storage and patty preparation

Chicken breast fillet and thigh meat were ground over 13 mm plates, chicken skin was ground over 2 mm plates (4°C). Salt and water (0°C) were added, while mixing in a cutter for 5 minutes. Next the skin and starch were added following another 10 minutes of mixing, while the temperature was kept at 4°C. The meat was divided and stored frozen

at temperatures between 0 and -8°C for 1 day till 4 days. Within each test series storage time and temperature were kept constant. A number of additional tests were performed with varying cutter time, from 1 till 13 minutes to examine free protein release in relation to dough elasticity.

Patties were formed at temperatures of 0, -2.5, -6 and -8.5 °C by use of a hydraulic press and a mould of 100 mm in diameter. Patties were formed with different heights depending on the subsequent tests. Patties prepared for heating and end product testing were 12 mm high. Those prepared for rheological testing of the meat dough were 5 mm high, whereas those intended for texture analysis of the doughs had a height of 15 mm. Effective pressure on the dough during forming was 4 bar during 5 seconds.

Heating

Each patty was treated outside with soy oil and packed in aluminium foil. Heating took place in a Fessman hot air oven, set at 90°C and an RH of 100% during 30 minutes. Under these conditions a core temperature of about 70°C was reached.

Physical testing

Physical characteristics of the dough were determined by differential scanning calorimetry (DSC), dynamic rheological testing and texture analysis.

DSC was performed with a TA Instruments 2920 calorimeter by scanning from -40°C up to +20°C with a scanning rate of 2°C/min. Samples were homogenised under cryogenic conditions and about 15 mg was taken to determine the heat flow during heating.

Dynamic rheological testing on meat dough was performed by using a Carri-Med CSL²-500 type stress controlled rheometer. This rheometer was equipped with serrated parallel flat plate geometry with a diameter of 40 mm and an ETM type temperature control unit using liquid nitrogen. The gap setting was 5 mm and an oscillatory shear was performed with a fixed amplitude of deformation of 10⁻³ (-) and a frequency of 1 Hz. The amplitude of deformation was selected within the linear region which extends up till about 2.10⁻². A temperature sweep was recorded at a rate of 1°C/min., during which the storage-(G') and loss-(G'') moduli were recorded. Samples were applied at a temperature of -10°C.

Free protein in meat dough was determined with a Technicon Infracalys 400 near infrared spectrometer using the calibration method analogous to Oh and Großklaus (1994) .

Heated burgers were tested using a Stable Micro Systems type TA-XT21 texture analyser with a flat disc steel probe (diameter 100 mm). A texture profile analysis (TPA) test was performed (Szczesniak (1973)), which comprises two subsequent compressions. Probe speed was 1 mm/sec and a maximum deformation of 40%. As relevant parameters we selected hardness and cohesiveness. At each process condition, TPA tests on heated samples were performed on 15 products in a first test series determining the effect of temperature of forming, and 8 products in the test series determining the effect of dough rest on texture.

Results & Discussion

Meat dough characteristics

Figure 1 shows the release of free protein in doughs, containing 15% fat, prepared with different cutter times as determined by NIR and the resulting elastic modulus (G') in relation to the free protein measured.

The results clearly show the effect of cutter time with protein release and its effect on the elasticity of the dough. Dough elasticity was found to vary by 40% depending on the cutter time as a result of protein release. The main effect found on final product quality with increasing free protein concentration was an increasing shrinkage. Shrinkage is expressed as the difference in diameter of the heated hamburger (d_{burger}) with the diameter of the patty (d_{patty}) direct after forming: $\Delta d = (d_{burger} - d_{patty}) / d_{patty} * 100$ (%). We found the following relationship between shrinkage and free protein concentration, as found by NIR: $\Delta d = -0.28 * C_{protein}$ (%). In the following tests the cutter time kept constant at 5 minutes, resulting in a free protein concentration of $11.2 \pm 2.0\%$.

The mechanical behaviour of the meat dough, as a function of temperature is presented in figure 2. This figure shows the storage (elastic) modulus and $\tan(\delta)$, the ratio of the loss (viscous) and storage modulus, of the dough during heating in the rheometer. The storage modulus is a measure of the elastic rigidity of the dough at very small deformation. The results of the dough stored at -2°C were not significantly different, indicating that storage temperature over the short storage time applied, did not affect the structure. The rheological differences (G' and $\tan(\delta)$) between the fat concentrations were small and not significant compared to changes in these parameters as a function of temperature during defrost. Within the range of -10 till about -7°C the structures were too rigid to determine the moduli.

From -6°C till -4°C the storage modulus weakly decreases, while the loss angle ($\tan(\delta) = G''/G'$) slowly increases from 0.1 up to 0.2. The loss angle is a measure of the ratio of elastic and viscous behaviour of the sample, the lower the value, the higher the elastic behaviour. Values below 1 indicate that elastic behaviour dominates over viscous loss. Below -4°C the dough behaves as an elastic solid with highly dominating elastic behaviour. Above -4°C the elastic modulus strongly decreases until a temperature of about 2°C is reached, Between -4°C and -3°C $\tan(\delta)$ strongly increases up to values of about 0.35. This sudden increase in $\tan(\delta)$ indicates a strong change in structural behaviour with more viscous loss. Above 2°C the elastic modulus decreases very slowly.

Figure 3 presents the heat flow during heating of a meat dough sample stored containing 5% fat in the temperature range from -15 till $+10^\circ\text{C}$. Included in this figure is the elasticity (G') of the same meat dough.

Dominating in the thermogram is the melting of ice. Frequently only the peak temperature of the melting is recorded, but the results show that the complete melting profile is relevant to the elasticity of the dough (see fig 3) and therefore for optimal processing. Note that melting already starts at very low temperatures and the absolute onset of melting is hard to detect since the deviation of the horizontal baseline is difficult to identify. This melting behaviour can be understood on the basis of the freezing process and the concept of freeze concentration. Integration of the melting peak results in the total heat of melting. Combining with the literature value for the heat of melting of ice,

334 J/g, provides the possibility to calculate the amount of frozen water in the sample. Comparison with the total water content in each sample delivers the amount of bound or unfreezable water. In two types of meat doughs, one containing 5 % and the other 15% fat the concentration of unfreezable water found in this way was respectively 13.2 and 13.4 %, while the amount of free (ice forming) water was respectively 54.8 and 59.6%. Freezing characteristics, as presented here, dominate product quality and are therefore considered relevant to other type of formulations.

Heated product texture

Hardness- and cohesiveness values obtained from the TPA tests of the heated burgers (15% fat) formed at different temperatures are presented in figure 4. Error bars indicate the (95%) standard error obtained from 15 repeats.

From the results it is obvious that the internal product structure is more weakened by forming at lower temperatures. At lower temperatures products contain more ice so deformation during forming therefore requires much more stress (energy) compared to forming at higher temperatures. Higher stress will cause more deterioration of internal bonds which apparently do not recover within the timeframe of dough rest between forming and heating.

Dough rest

In order to study the effect of dough rest in more detail, the rheology of patties formed at -8°C and at 0°C was compared within 15 minutes after the forming process. Figure 5 shows an example of the change in $\tan(\delta)$, i.e. ratio between viscous loss and elastic storage modulus, with time when measured at 12°C . A higher $\tan(\delta)$ indicates more viscous like behaviour, while a more elastic like behaviour is indicated by lower $\tan(\delta)$ values.

While the patty formed at 0°C hardly showed any change in the value of $\tan(\delta)$ with time, the patty formed at -8°C decreased strongly within the first 20 minutes of measurement and more slowly thereafter until it became equal in value to the dough formed at 0°C . This suggests a complete recovery of the structure of the dough formed at -8°C , at least comparable to that of the dough formed at 0°C after about 1 to 2 hours.

At several dough rest times after forming, patties were heated to study the effect of rest time on texture. Hardness and cohesiveness, determined with the TPA test, of heated products after different dough rest times is presented in figure 6. Error bars indicate the (95%) standard error of 8 repeats.

We note that the absolute values of hardness and cohesiveness differ from values obtained in an earlier experiment; this could be due to batch to batch variations. Nevertheless we observed lower hardness when patties were formed at lower temperatures, except after about 1000 minutes (about 17 hrs!) dough rest. Cohesiveness was also lower for the lower temperature at forming and showed to be independent of dough rest time. These results show that an irreversible structure breakdown occurs during forming at a low temperature of -8°C , which was unexpected when compared to the recovery of the mechanical behavior, $\tan(\delta)$ presented in figure 4. This is not yet fully understood.

Conclusions

The mechanical behaviour of the poultry meat doughs processed within the temperature range of -10°C till 5°C , and the texture of heated products was dominated by the melting profile. Three principal temperature ranges could be identified:

- 1 $T < -4^{\circ}\text{C}$, in which the dough behaves as a rigid mass which is mechanically difficult to deform.
- 2 $-4^{\circ}\text{C} < T < 0^{\circ}\text{C}$, in which the consistency of the dough is subject to a rapid change, due to a strong change in melting. This is a critical range since fluctuations in temperature will lead to strong variations in final product texture.
- 3 $T > 0^{\circ}\text{C}$ above which the mechanical dough characteristics vary not much.

Thermal analysis of the melting profile of the doughs tested showed to be in good relation to the mechanical dough properties and the forming process at the different temperature ranges. Variations in formulation will change the mechanical and thermal characteristics. We have shown that a variation from 5 to 15% fat lowered the consistency slightly but did not change the main behaviour.

Varying temperature during forming showed to have a significant effect on the final product texture after heating. This effect was most apparent in changes in cohesion in the product. It was shown that more breakdown of dough structure occurred at lower temperatures during forming. During dough rest the mechanical behaviour of the patty, formed at relatively low temperatures, recovered. However, cohesiveness of heated burgers after forming at relatively low temperatures was always significantly lower than products being formed at relatively higher temperatures.

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Tables and Figures

Table 1. Meat compositions

	"5% fat" (%)	"15% fat" (%)
Breast fillet	72.5	52.5
Thigh meat	17.8	19.8
Skin	0.0	20.0
Water	7.0	5.0
Starch	2.0	2.0
Salt	0.7	0.7

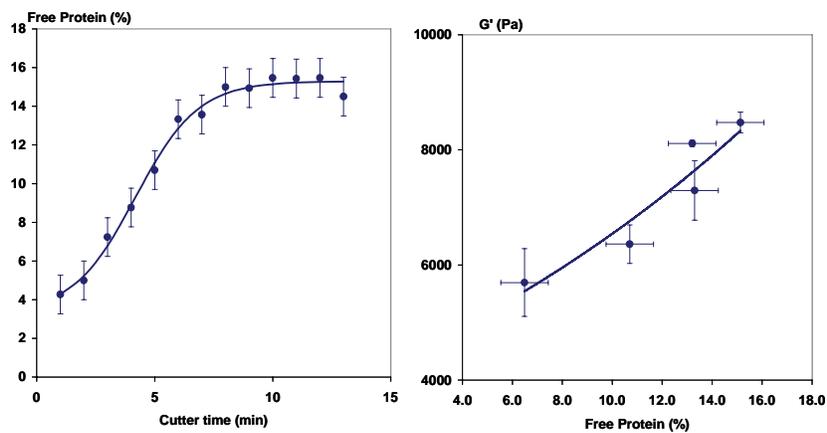


Figure 1. Free protein (NIR) as a function of cutter time (left) and resulting meat dough elasticity at 20°C (right).

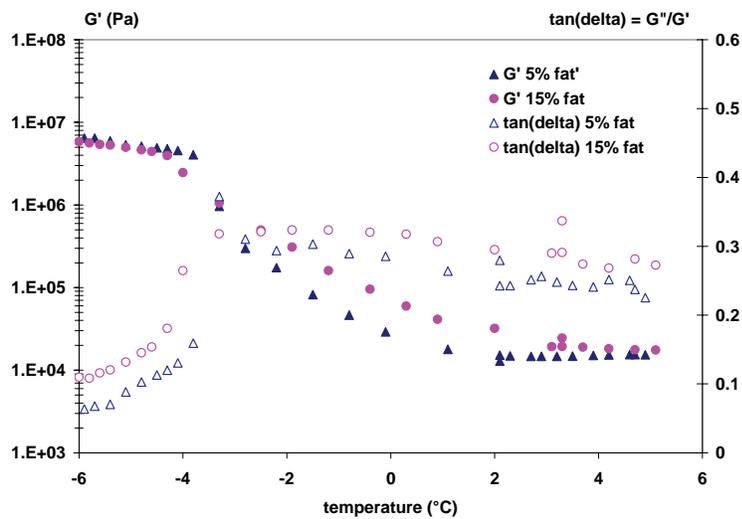


Figure 2. Storage moduli and loss angle of meat doughs, stored at -6°C.

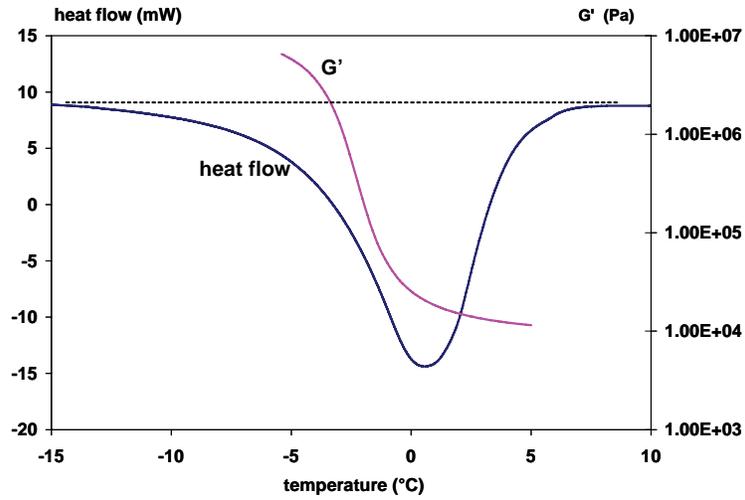


Figure 3. Heat flow determined by DSC during melting of a meat dough sample with 5% fat and the elasticity of the sample dough determined by dynamic rheology

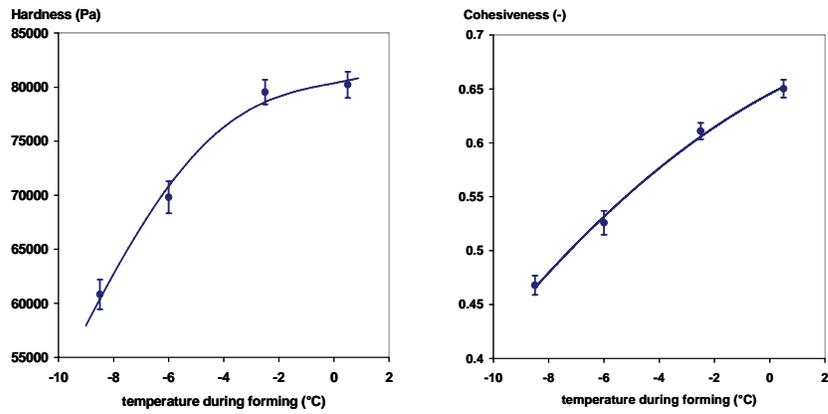


Figure 4. Hardness and cohesiveness of heated burgers.

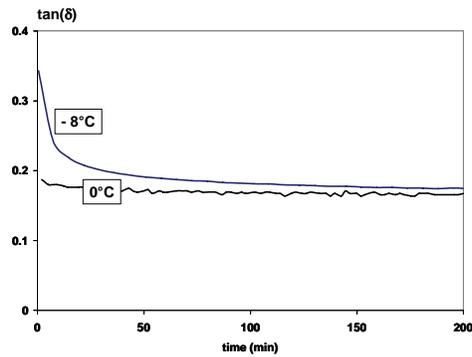


Figure 5. $\tan(\delta)$ vs. dough rest time of doughs formed at 0 and -8°C

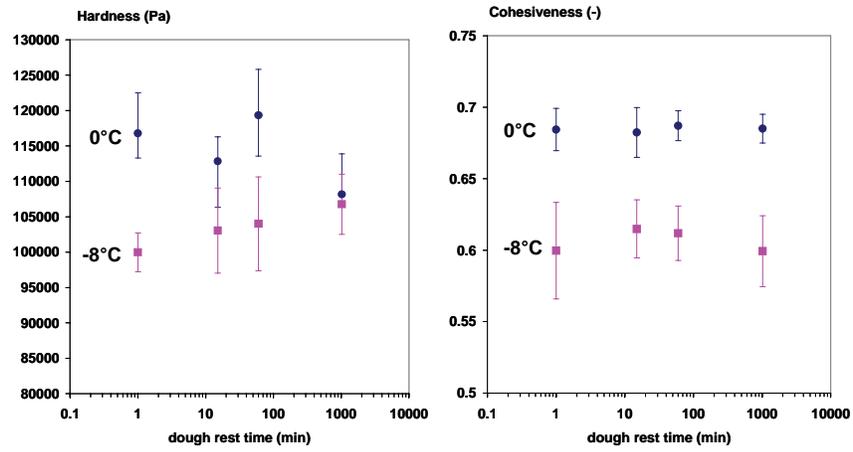


Figure 6. Hardness and Cohesiveness of heated patties.

DEVELOPMENT OF AN INJECTABLE “MODIFIED MARBLING” SOLUTION FOR WHOLE MUSCLE BEEF CUTS

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Key Words: intramuscular fat, "modified marbling", non-meat ingredients, injectable

Introduction

The amount of marbling or intramuscular fat has been shown to influence the palatability (juiciness, tenderness, flavor) of beef cuts. Tatum et al. (1982) showed that marbling has a low but positive relationship on all beef palatability traits and also found that 90% of the time steaks with Slight or higher degrees of marbling were more desirable in tenderness, flavor and overall palatability. Smith et al. (1984) also reported minute, but statistically significant differences in meat palatability as the degree of marbling decreased from Moderately Abundant (USDA Prime) to Practically Devoid (USDA Standard). Savell and Cross (1988) developed a “window of acceptability” for percent intramuscular fat or marbling of retail beef cuts. Beef cuts containing 3-7% intramuscular fat are perceived by consumers to be acceptable in tenderness, juiciness, flavor and overall palatability so it is important to have at least 3% intramuscular fat in whole muscle beef cuts.

Studies have been conducted to determine quality inconsistencies within the beef industry chain, from farm to retail. The results from the last National Beef Quality Audit (McKenna et al., 2002) indicated that the overall average scores for intramuscular fat or marbling and USDA beef carcass quality grades were below the expectations of the meat industry which can influence the consumer’s purchasing decisions. When consumers are not satisfied with the palatability of beef cuts, their intent to purchase beef may decrease and along with it is the opportunity for the beef industry to generate revenue.

The deposition of intramuscular fat or marbling is influenced by many factors such as breed, length of feeding, type of ration fed and management but it has been shown that there is plenty of room for improvement in the amount of marbling or intramuscular fat in whole muscle beef cuts in order to improve the palatability of the final beef product.

The palatability of whole muscle cuts fabricated from lower quality (less than USDA Choice) beef carcasses may be improved through innovative non-meat ingredient and processing technologies. Several different processing technologies have all ready been used to add value to lower quality meat products including whole muscle cuts. The development of a “modified marbling” from selective non-meat ingredients (sodium alginate, iota carrageenan, whey protein isolate and modified food starch) that can mimic the properties of intramuscular fat and can be directly injected into lower quality whole

muscle beef cuts may enhance its overall palatability by mimicking the organoleptic properties of fat and having an appearance similar to that of marbling.

Objectives

The overall goal was to develop an injectable “modified marbling” solution using selected non-meat ingredients that can mimic the properties of intramuscular fat and develop a processing system that can incorporate this “modified marbling” into lower quality, less marbled whole muscle beef cuts. To achieve this goal, there were two separate objectives. In the first objective, response surface methodology was utilized to determine the concentration of each ingredient (sodium alginate, iota carrageenan, whey protein isolate and modified food starch) to be used in the development of the “modified marbling” solution. The second objective was conducted to determine the processing system parameters used to inject the solution into whole muscle beef cuts and to verify the properties of the “modified marbling” solution in whole muscle beef cuts. Ribeye rolls were injected with the solution, cut into steaks and the sensory and chemical properties were compared to three separate controls.

Methodology

Experiment 1

Preliminary studies were conducted to determine the selection of ingredients to be used for the “modified marbling” solution and to determine the concentration ranges of the selected ingredients. From the results of the preliminary studies, twenty-five ingredient combinations (ranging from 0.25 to 0.50% addition) were formulated into 500g solutions using a 2⁴ central composite design. Solutions were mixed and the properties of the “modified marbling” solution were determined by measuring the solution viscosity (Brookfield viscometer, 30 °C at speed 100) and pH (Accumet pH Meter, at 22 °C) immediately after the solution was manufactured. The solutions were stored for 24 hr at 4 °C to allow the solutions to gel and objective color (Minolta Chromameter, L*a*b*), water holding capacity (centrifugation of 10 g of sample at 4 °C at 40,000 *x g* for 30 min), water holding capacity over time (exposing a 2.54 x 2.54 x 1.27 cm sample on filter paper at 22 °C for 2 h), and gel strength/hardness (TA-HDi texture analyzer with an acrylic probe penetrating the gel plug in the geometric center of the sample depressing the gel 2 cm) were determined.

Generated data were analyzed using the Proc GLM procedure of the Statistical Analysis System (SAS User’s Guide) to determine which factors were significant (P<0.05) within the total model. Response surface regression (Proc RSREG) equations were run on those factors that were significant (P<0.05). Least squares means tables and response surface graphs were generated and prediction equations were used to determine the optimal level of each ingredient.

Experiment 2

The solution was modified by adding beef tallow (3%) in order to minimize the amount of meat pigments absorbed into the meat matrix and beef flavoring (0.25%) was added to increase the intensity of the beef flavor. A continuous, multi-needle injector designed for low pump percentages (5-7%) was used to create the desired “modified marbling” pattern and the optimal injection parameters were determined. It was found that 200, 4mm needles at a belt speed of 39 strokes/min and pump pressure of 4.5 bar was optimal. The solution was manufactured using a Rotostat mixer, injected into USDA Select ribeye rolls (IMPS 112A), tumbled (1 min) and vacuum packaged. The controls, USDA Select, Low and Average Choice ribeye rolls were passed once through the injector without injecting solution to minimize bias when evaluating tenderness. Ribeye rolls were designated to 0, 14, 28, or 42 days of storage (1°C), weighed for ribeye purge and steaks (2.54 cm) were fabricated on each storage day (n=3 steaks). Warner Bratzler shear force, trained sensory evaluation and a 7-day retail shelf life study were conducted on fabricated steaks from each treatment and control after the designated storage period. TBARS values and percent purge were determined on day 0 and 7 and objective and subjective color were determined on days 0, 3, 5, and 7 of the retail shelf-life study.

The experimental design used was a two-way analysis of variance with four combinations, one treated (Injected USDA Select) and three controls (USDA Select Control, USDA Low Choice Control, and USDA Average Choice Control). Difference among attribute means was determined with a predetermined level of significance ($P < 0.05$) using Tukey’s Least Significant Difference procedure (SAS user’s guide).

Results & Discussion

Experiment 1

Solution viscosity tended to increase as the concentration of sodium alginate and iota carrageenan increased ($p < 0.05$). The pH was not influenced by any specific treatment combination and the L^* color values tended to increase as the concentration of sodium alginate increased to a color value comparable to beef fat L^* values (77.20 vs 83.58). A sodium alginate, iota carrageenan interaction increased values for water holding capacity ($p < 0.01$) and gel firmness ($p < 0.05$). Whey protein isolate and modified food starch were not significant for any of the attributes but were kept in the solution at 0.375% since they contributed to the color and water holding capacity of the gel even though they were not significant. Recommended levels of non-meat ingredients from analysis of the solution and gel were 0.4375% sodium alginate and iota carrageenan and 0.375% whey protein isolate and modified food starch.

Experiment 2

The average injection pick-up for the Injected Select was 9.75%, which was higher than the targeted injection pick-up of 5-7%. The Injected Select treatments had a higher ribeye purge (2.80%) ($p < 0.05$) than the USDA Average Choice control (1.26%), which

would be expected with the amount of solution injected into the ribeye halves. This was also demonstrated in a study by Milligan et al. (1997), where a solution of CaCl₂ was injected into USDA Standard beef inside rounds at 5%. They found that the purge loss was significantly greater the CaCl₂ injected roasts than for the control roasts.

There were no differences between the injected and control ribeyes for Warner Bratzler shear force, sensory tenderness, juiciness or steak purge. The similar tenderness and juiciness values between injected and non-injected ribeyes may be attributed to the cooking method (clamshell grill) and endpoint temperature (71 °C) used for analysis. A further study with two different cooking methods (clamshell grill and farberware grill) and two different endpoint temperatures (71 °C and 77 °C) were compared. There were not any differences seen between the cooking methods or the endpoint temperatures. The similar tenderness and juiciness values could also be from passing all control ribeye rolls through the injector (single pass without solution) to minimize bias when evaluating tenderness. The injected ribeyes were higher (p<0.05) compared to the USDA Select control in beef fat flavor (4.02 vs. 3.31). However, a slight off-flavor was found (p<0.05) in the injected ribeye (1.43), which corresponds to the higher TBARS values (0.85) (p<0.05), which may be due to the addition of beef tallow without an antioxidant. There were no significant differences in color scores.

Conclusions

The results indicate that more research is needed in this area. The combination of non-meat ingredients in this study was not affective but the amount and type of fat added to the solution may need to be studied to optimize the "modified marbling." Since the sensory panel was able to detect a difference in beef fat flavor after modifications were done to the "modified marbling," the combination of more fat, possibly in a different form could positively affect palatability attributes of whole muscle beef cuts. This study demonstrates that lower quality grades of beef can be improved using this technique.

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**EFFECT OF SORGHUM BRAN ADDITION ON LIPID OXIDATION AND
SENSORY PROPERTIES OF GROUND BEEF PATTIES DIFFERING IN FAT
LEVELS**

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Key Words: sorghum bran, ground beef, lipid oxidation, color, sensory

Introduction

The meat industry is faced with the challenge of supplying higher-quality meat products that have extended shelf-life. One of the major sources of changes that occurs during processing and storage of meat products is lipid oxidation. Oxidation of lipids influences the color and sensory qualities of meat products. Meat with a high fat content, such as ground meat, is susceptible to lipid oxidation that leads to the development of negative flavor and color changes. Antioxidants are used to control the effects of lipid oxidation. Common antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and extracts of rosemary, are used in meat products. Sources of antioxidants of plant origin that are naturally occurring recently have been examined. Awika (2000, 2003) found that sorghum bran is rich in phytochemicals that have high antioxidant properties. Jenschke et al. (2004) reported lower TBARS values in ground beef patties containing high levels of sorghum bran stored for up to fourteen days in a high oxygen environment at 4°C. We hypothesize that sorghum bran addition to ground beef patties will reduce lipid oxidation and stabilize color without affecting sensory characteristics during aerobic storage for up to 5 days in a retail display case at 4°C.

Objectives

The objective of this study was to evaluate ground beef patties that have been prepared with different treatments of synthetic and natural antioxidants. This study evaluated the effectiveness of sorghum bran antioxidant activity with varying levels of lipids. Comparative determinations were made by measuring oxidative rancidity, pH, color stability, and sensory characteristics of the ground beef patties. Knowledge from this study will enable future commercial application of sorghum bran varieties as targeted food ingredients to improve food quality and human health.

Methodology

Beef trimmings containing either 50% or 90% lean at 2 days post-fabrication were purchased on one of three processing days from two commercial beef processors. The beef trimmings were formulated into three meat blocks containing either 10, 20 or 30% chemical lipid (verified using the CEM Autoanalyzer). Within a processing day and fat content, ground beef was equally divided into one of six treatments: 1) negative control-no added ingredients; 2) BHA and BHT at .01% of the weight of the meat; 3) rosemary at 0.2% of the meat weight; 4) high level of sorghum at 1.0% of the meat weight; 5) medium level of sorghum at 0.5% of the meat weight; 6) and a low level of sorghum at .25% of the meat weight. Patties, 200 gm, were hand-formed and two patties were placed in a Styrofoam tray that was over-wrapped with PVC film. Six packages within a treatment and fat level were made. Four packages were randomly assigned to a storage day (0, 1, 3, and 5) and displayed at 4 °C in a retail coffin display case under standard fluorescent lighting for chemical and color analysis of pH, TBARS, sensory color, and Minolta color space values. Two packages were randomly assigned to either 1 or 5 storage days for trained meat descriptive sensory evaluation.

A Minolta Colorimeter (CR-300, Minolta Co., Ramsey, NJ) was calibrated each day using a white tile and PVC over-wrap and three random locations were evaluated on the exterior surface of one patty per package for CIE L*, a* and b* color space values. The average of the three readings was reported. A six-member selected and trained descriptive color sensory panel evaluated each package for lean color (1=very dark red ;8=light, grayish red), percentage discoloration (1=0%; 7=100%), and discoloration color (1=very dark red; 8=light, grayish red) as defined by AMSA (1991, 1995). pH was determined using a pH meter (HI 98240, Hanna Instruments, Italy). Three readings were obtained randomly. The pH meter was calibrated using 4.0 and 7.0 buffers. Two 60g samples were removed from a patty and ground. The amount of mg malonaldehyde/1000 g sample were determined using procedures described by Tarladgis et al (1960) as modified by Rhee et al (1978). Absorbance was measured at 530 nm using a DU-7 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). Purge was measured by weighing the packaged patties, removing the two patties and re-weighing the package materials with the remaining exudates. Weight of the package materials was determined and subtracted from both values to obtain the weight of the patties and the exudates.

Two patties per treatment were cooked to an internal temperature of 73°C on an electrical grill. Internal temperatures were monitored by a copper-constantan thermocouple (Omega Engineering, Stamford, CT) inserted into the geometric center of each patty. The patty was cut into eight equal wedges and served warm to the panelists, within 5 minutes post cooking. Each panelist received two wedges for evaluation. The panel consisted of a six member trained flavor and texture descriptive attribute sensory panel based on AMSA (1995) and Meilgaard et al (1999). Flavor, basic taste, mouth feel, after-taste, and texture attributes were determined during ballot development sessions. The lexicon for warmed over flavor in beef was used as a basis for descriptive attributes (Johnson and Civille, 1987). Panelists were provided samples of ground beef patties similar to treatments defined in the study for ballot development. After the attributes for the ballot were defined, training sessions were conducted. The study was conducted after panelists could consistently and accurately identify sensory attributes (AMSA, 1995). Each panelist was seated in individual booths equipped with red theater gel lights.

Samples were served in a random order and identified using three-digit codes. Unsalted saltine crackers and double distilled, deionized water were provided to the panelists between samples to cleanse their palates. The panelists evaluated each sample using a 15-point universal scale with 0 = none and 15 = extremely intense for attributes defined from ballot development sessions (AMSA, 1995). Three sessions were conducted with six treatment sample evaluations per session. A twenty minute break was given between sessions and samples were served a minimum of four minutes apart.

Data were analyzed as a factorial arrangement by Analysis of Variance using the general linear model (GLM) procedure of SAS (Version 6.12, Cary, NC, 1998) with a predetermined significance level of $P \leq 0.05$. For chemical data, processing day, treatment, fat level and storage day were defined as main effects. Two-way interactions for all main effects were examined and remained in the model if they were significant ($P < 0.05$). For sensory data, the data were analyzed to determine the effect of panel and panel interactions. These data then were averaged within panelists and analyzed as defined for the chemical data. Least squares means were calculated and when differences were defined by Analysis of Variance, least squares means were separated using the `stderr pdiff` function.

Results & Discussion

TBARS

Controls over time had increased TBAR values, indicating oxidation was occurring in the ground beef patties during storage (Figure 1). However, TBAR values were not greater than 1 after five days of storage, demonstrating oxidation was occurring in the patties, but not at a rapid rate. The patties from the treatments containing antioxidants had lower TBAR values after 1, 3, and 5 days of storage when compared to control patties. The TBAR values were slightly greater in the patties at the end of storage (day 5). Jenschke et al. (2004) showed a more pronounced effect in ground beef patties stored for up to fourteen days in an 80% oxygen /20% carbon dioxide environment.

Controls at different fat levels also had increased TBAR values (Figure 2). TBAR values were the highest in the 10% fat patties. As lean meat contains a high proportion of muscle fibers and the phospholipid component in muscle fiber membranes have greater susceptibility to oxidation, these results were expected. Patties containing antioxidant treatments had lower TBAR values when compared to control patties at all fat levels.

Purge, pH and color

Purge was not affected by treatment, fat level or storage day ($P < 0.05$). The addition of sorghum tended to increase pH and patties containing a high level of sorghum had the highest pH values (Table 1). Patties with higher fat levels were higher in pH and as storage time increased, pH increased slightly. The addition of rosemary or BHA/BHT resulted in lighter (higher L^* color space values and higher sensory lean color scores), were slightly more yellow, had slightly less discoloration and of the lean that was discolored, the discoloration was lighter than control patties. Sorghum bran addition impacted ground beef color (Table 1). Sorghum bran addition at the high level resulted in

darker ground beef patties that were less red and yellow. Patties containing sorghum bran has similar discoloration percentage as control patties and the color of the discoloration tended to be lighter than control patties. As fat level increased, ground beef patties were lighter, redder, and had more yellow. Increased storage days resulted in ground beef patties that were darker, less red and yellow, and had higher amounts of discoloration and the discoloration color became darker. Jenschke et al. (2004) found that the addition of sorghum bran at 2.0% of the meat weight resulted in lower raw color scores, greater amounts of discoloration, and darker discoloration. At the lower levels used in this study (high level = 1.0% meat weight), the sorghum bran patties were slightly darker, but differences were less pronounced than in Jenschke et al. (2004).

Sensory

The addition of rosemary and BHA/BHT to ground beef patties were similar in sensory flavor and basic taste attributes to control patties, except patties containing rosemary had less cooked beefy/brothy flavor aromatics than control patties (Tables 2 and 3). However, rosemary, BHA/BHT, and sorghum bran treated patties were harder and more springy than control patties. The addition of sorghum bran resulted in patties with lower levels of cooked beefy/brothy flavor aromatics. The patties containing the medium and high sorghum bran levels had less serummy flavor aromatics, and higher grainy and sorghum flavor aromatics when compared to control patties. Ground beef patties containing the high level of sorghum were slightly more bitter than control patties. Ground beef patties did not contain high levels of cardboardy, painty or fishy flavor aromatics (data not presented). Increasing levels of these aromatics are associated with increased lipid oxidation. As TBARS values were low, high levels of these aromatics would not be expected.

As fat level increased, patties had slightly higher levels of cooked beef fat, and serummy flavor aromatics, less bitter basic tastes, and the patties were softer, juicier and at the highest fat level, patties were more springy.

As storage day increased, patties had lower levels of cooked beefy/brothy flavor aromatics, and higher levels of cooked beef fat and browned flavor aromatics. Patties were harder and more springy with increased storage time.

Jenschke et al. (2004) found multiple off-flavor aromatics in ground beef patties containing 2.0% sorghum bran. By decreasing the level of sorghum bran addition to the patties, high levels of off-flavors were not detected.

Conclusions

Data from this study indicated that the addition of sorghum bran from low to high levels had comparable antioxidant properties to the commonly used food antioxidants, BHA/BHT and rosemary. However, as treated patties had low levels of oxidation, even with up to five days of storage, significant differences between treatments were not found. Except for the control patties, oxidation rates and levels were found to be very low within the patties at different fat levels and over storage time.

Although the addition of sorghum bran reduces TBARS values over time when compared to the controls, the addition of the high sorghum bran level resulted in lower raw color scores, greater amounts of discoloration, darker discoloration, and slightly

increased bitter basic taste. Moreover, the addition of sorghum bran reduced TBARS values over time and did not drastically affect color and sensory flavor attributes. Further research to isolate and extract the antioxidant components (anthocyanins and tannins) might reduce the negative effects on color and sensory characteristics of the higher sorghum level by removing unwanted or unnecessary compounds.

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Tables and Figures

Figure 1. Interaction of treatments and storage time on TBARS values for ground beef patties (P=0.04).

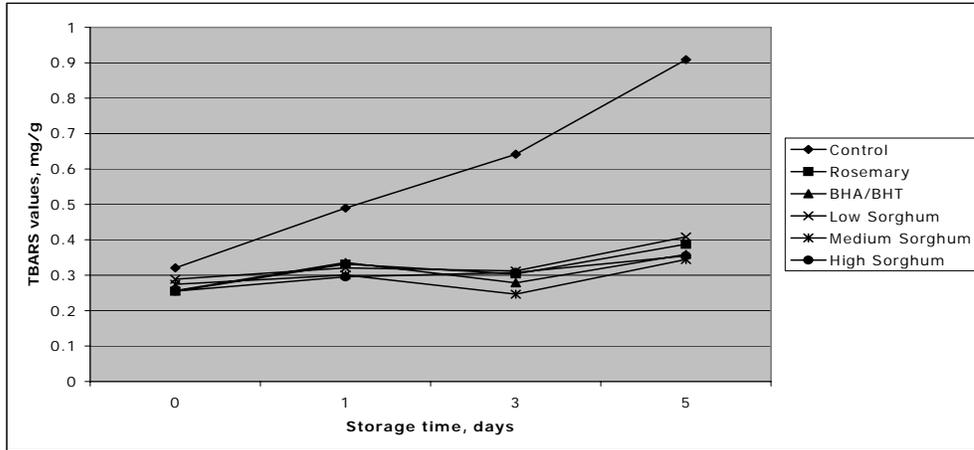


Figure 2. Effect of treatments and fat level on TBARS values of ground beef patties (P=0.02).

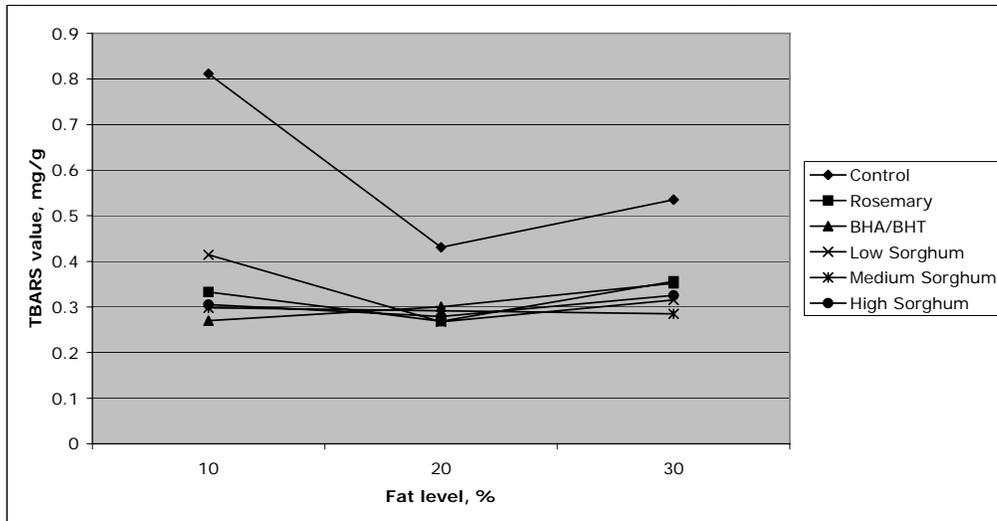


Table 1. Least squares means for main effects pH, Minolta color space values and trained descriptive sensory color attributes.

Effect	pH	Minolta color space values			Lean color ^a	Discolor- ation, % ^b	Color of Dis- coloration ^a
		L*	a*	b*			
<i>Treatment^c</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.005</i>	<i>0.0001</i>
Control	6.33 ^{de}	47.62 ^e	18.92 ^g	10.16 ^f	2.9 ^f	1.3 ^f	0.70 ^g
Rosemary	6.33 ^{de}	48.85 ^f	9.39 ^g	10.66 ^g	3.3 ^g	1.1 ^d	0.22 ^d
BHA/BHT	6.30 ^d	49.36 ^f	19.59 ^g	10.65 ^g	3.3 ^g	1.1 ^{de}	0.27 ^{de}
Low sorghum	6.35 ^e	47.79 ^e	17.85 ^c	10.01 ^f	2.9 ^f	1.3 ^{ef}	0.40 ^{ef}
Medium sorghum	6.36 ^e	47.35 ^{de}	16.39 ^e	9.58 ^e	2.5 ^e	1.4 ^f	0.52 ^{fg}
High sorghum	6.41 ^f	46.51 ^d	15.32 ^d	8.87 ^d	2.0 ^d	1.2 ^{def}	0.44 ^{ef}
<i>Fat Level, %^b</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.07</i>	<i>0.002</i>
10	6.29 ^d	44.88 ^d	17.00 ^d	8.73 ^d	2.2 ^d	1.1 ^d	0.30 ^d
20	6.38 ^e	47.95 ^e	17.86 ^e	10.05 ^e	2.8 ^e	1.3 ^e	0.44 ^e
30	6.37 ^e	50.91 ^f	18.87 ^f	11.19 ^f	3.6 ^f	1.2 ^{de}	0.54 ^e
<i>Storage Day^b</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>
0	6.24 ^d	49.62 ^f	21.56 ^g	11.16 ^g	3.5 ^g	1.0 ^d	0.08 ^d
1	6.47 ^f	47.94 ^e	18.74 ^f	10.16 ^f	3.0 ^f	1.0 ^d	0.09 ^d
3	6.26 ^d	46.87 ^d	17.24 ^e	9.52 ^e	2.6 ^e	1.2 ^e	0.49 ^e
5	6.41 ^e	47.23 ^{de}	14.10 ^d	9.14 ^d	2.4 ^d	1.7 ^f	1.04 ^f
<i>Root MSE</i>	<i>0.088</i>	<i>2.100</i>	<i>1.637</i>	<i>0.758</i>	<i>0.38</i>	<i>0.35</i>	<i>0.40</i>

^a1=very dark red; 8=light, grayish red.

^b1=none or 0%; 7=total discoloration or 100%.

^cP-value from analysis of variance tables.

^{def} Mean values within a column and a main effect followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Least squares means for main effects for trained sensory flavor aromatics descriptive attributes^a.

Effect	Cooked Beefy/Brothy	Cooked Beef Fat	Serumy	Grainy	Browned	Sorghum
<i>Treatment^b</i>	<i>0.0001</i>	<i>0.25</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.017</i>	<i>0.0002</i>
Control	5.00 ^f	3.28 ^c	1.86 ^d	0.58 ^c	1.27 ^c	0.24 ^c
Rosemary	4.68 ^{de}	3.45 ^{cd}	2.03 ^d	0.71 ^c	1.25 ^c	0.25 ^c
BHA/BHT	4.86 ^{ef}	3.32 ^{cd}	1.94 ^d	0.58 ^c	1.48 ^{cd}	0.24 ^c
Low sorghum	4.59 ^d	3.51 ^d	1.93 ^d	0.73 ^c	1.27 ^c	0.37 ^{cd}
Medium sorghum	4.63 ^d	3.40 ^{cd}	1.45 ^c	1.04 ^d	1.70 ^d	0.60 ^{de}
High sorghum	4.36 ^c	3.40 ^{cd}	1.40 ^c	1.12 ^d	1.55 ^{cd}	0.68 ^e
<i>Fat Level, %^b</i>	<i>0.029</i>	<i>0.0001</i>	<i>0.39</i>	<i>0.35</i>	<i>0.58</i>	<i>0.66</i>
10	4.61 ^c	3.16 ^c	1.71 ^c	0.85 ^c	1.42 ^c	0.37 ^c
20	4.79 ^d	3.44 ^d	1.82 ^c	0.75 ^c	1.48 ^c	0.37 ^c
30	4.62 ^c	3.58 ^d	1.78 ^c	0.72 ^c	1.36 ^c	0.44 ^c
<i>Storage Day^b</i>	<i>0.0005</i>	<i>0.026</i>	<i>0.22</i>	<i>0.15</i>	<i>0.034</i>	<i>0.25</i>
1	4.78 ^d	3.33 ^c	1.81 ^c	0.75 ^c	1.33 ^c	0.36 ^c
5	4.57 ^c	3.46 ^d	1.73 ^c	0.84 ^c	1.51 ^d	0.43 ^c
<i>Root MSE</i>	<i>0.309</i>	<i>0.308</i>	<i>0.438</i>	<i>0.318</i>	<i>0.455</i>	<i>0.355</i>

^aAromatics: 0=none; 15=extremely intense.

^bP-value from analysis of variance tables.

^{cdef}Mean values within a column and a main effect followed by the same letter are not significantly different ($P > 0.05$).

Table 3. Least squares means for main effects for trained sensory texture and bitter basic taste descriptive attributes.

Effect	Basic Tastes		Texture	
	Bitter ^a	Hardness ^b	Juiciness ^c	Springiness ^d
<i>Treatment^e</i>	<i>0.0069</i>	<i>0.0001</i>	<i>0.60</i>	<i>0.002</i>
Control	2.47 ^f	5.78 ^f	2.77 ^f	6.93 ^f
Rosemary	2.61 ^{fgh}	6.13 ^g	2.91 ^f	7.38 ^g
BHA/BHT	2.76 ^h	6.26 ^g	2.76 ^f	7.49 ^g
Low sorghum	2.49 ^{fg}	6.33 ^g	2.84 ^f	7.55 ^g
Medium sorghum	2.57 ^{fg}	6.33 ^g	2.79 ^f	7.41 ^g
High sorghum	2.65 ^{gh}	6.23 ^g	2.89 ^f	7.38 ^g
<i>Fat Level, %^e</i>	<i>0.048</i>	<i>0.0002</i>	<i>0.01</i>	<i>0.017</i>
10	2.66 ^g	6.35 ^h	2.70 ^f	7.48 ^g
20	2.59 ^{fg}	6.17 ^g	2.91 ^g	7.42 ^g
30	2.52 ^f	6.01 ^f	2.87 ^g	7.17 ^f
<i>Storage Day^e</i>	<i>0.19</i>	<i>0.0001</i>	<i>0.09</i>	<i>0.001</i>
1	2.56 ^f	5.98 ^f	2.77 ^f	7.11 ^f
5	2.62 ^f	6.37 ^g	2.88 ^f	7.61 ^g
<i>Root MSE</i>	<i>0.331</i>		<i>0.311</i>	<i>0.468 0.242</i>

^aBasic tastes: 0=none; 15=extremely intense.

^b1=very soft; 15=very hard.

^c1=none; 15=very juicy.

^d1=not springy; 15=very springy.

^eP-value from analysis of variance tables.

^{fgh}Mean values within a column and a main effect followed by the same letter are not significantly different ($P > 0.05$).

EFFECT OF PARTICLE SIZE AND FAT LEVELS ON THE PHYSICO-CHEMICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF LOW-FAT GROUND PORK PATTIES

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Key words: low-fat ground pork patties, low cholesterol meat product, particle size, fat levels

Introduction

Dietary fat and cholesterol content are considered to be controllable factors in multifactorial etiology of heart diseases and obesity. Meat and meat products are maligned with a fact of high-fat, high cholesterol and high calorie diet. This has led to increase in demand of low-fat meat products. Pork patties generally contain 30-40% fat and warrants reduction in fat contents. However rheological, structural, processing, nutritional and sensory characteristics of comminuted meat products are directed by the fat content in the formulation and particle size of ground meat system (Kregel et al, 1986; Manish Kumar and Sharma, 2003)

Objectives

The present study was envisaged with a twin objectives to standardize the level of particle size and added fat levels in the processing of low-fat ground pork patties(LFGPP) on the basis of physico-chemical, textural and sensory characteristics. The effect of varying fat levels on cholesterol and calorie content was also evaluated.

Methodology

The study involved two experiments each with three trials. Experiment I was carried out to investigate the influence of three particle sizes viz 3,4 and 6 mm at constant fat level of <10%. (4 added fat level). The experiment II was carried out to examine the influence of fat levels viz 3, 4 and 5% at a constant grind size of 3 mm on the quality of low-fat pork patties.

Formulation and Processing of pork patties

Market age crossbred (Landrace × Local) hogs (N=3) weighing 60-70kg were humanely slaughtered at Divisional Experimental Abattoir. Prerigor raw materials were obtained with in 1hr post mortem by fabricating each carcass into boston butt, picnic shoulder, loin and ham. All skin, external fascia, subcutaneous and seam fat and all adhering connective tissues were removed. Hand deboned lean meat and back fat free

from adhering skin were stored separately at $-18\pm 2^{\circ}\text{C}$ in low density polyethylene (LDPE) packs and were used after partial thawing at 5°C for 12-15hr. The spice mixture, condiments and other additives were purchased from local market. The refined wheat flour used as binder has composition 18.73 ± 2.11 moisture, 74.43 ± 0.85 carbohydrates percent.

Meat and back fat were cut into small cubes and minced separately through 3mm, 4mm and 6 mm grind plate in Electrolux meat mincer (Model 9512) as per requirements of Experiment I.

The formulation and processing of control and low-fat patties were standardized by preliminary trials (Manish Kumar and Sharma 2004). The formulation consist of added fat 4.0%, binder 4.0%, added water 15%, condiment mix 3%, table salt and spice mix 1.5%. All the ingredients and minced meat constituents were thoroughly mixed along with slowly adding chilled water by electrically operated meat mixer (Hobart Paddle Mixer, N-50) for 3min. Thereafter, 75g of each mix was moulded into patties with the help of a Petri dish of defined size (75mm \times 15mm). The moulded patties were cooked in preheated hot air oven at $190\pm 5^{\circ}\text{C}$ to an internal end point temperature of $75\pm 2^{\circ}\text{C}$ recorded at geometrical center of each patty using probe thermometer. The patties were turned upside down twice at 5min interval for better appearance, color and texture. Samples from each batch were analyzed on the same day.

The moisture, fat and protein content of buffalo meat and patties was determined by methods of AOAC (1995). Cooking yield was calculated from raw and cooked weights of 9 patties for each treatment. The dimensional parameters of cooked patties were recorded using vernier callipers minimum at three different positions respectively to obtain the mean values. The percent shrinkage, moisture and fat retention of patties were determined as per El-Magoli *et al.*, 1996.

The shear force value of 1cm^2 of the sample was recorded as per Berry and Stiffler (1981) using Warner- Bratzler Shear press (Model: 810310307 G.R Elect. Mfg. Co. USA) and expressed as kg/cm^2 . An experienced sensory panel consisting of seven scientists and post graduate students evaluated the sensory characteristics of warmed product viz., appearance and color, flavor, juiciness, texture and overall acceptability using 8- point objective scale (Keeton, 1983), where 8- denoted extremely desirable and 1 denoted extremely undesirable.

The textural profile was determined on Instron Universal Testing Machine (Model 4464) following the procedure described by Brady *et al.* (1985). The fat content of the samples were extracted adopting the method described by Folch *et al.*, (1957) and Total lipids were determined gravimetrically. The different components of lipids included total phospholipids, total cholesterol, glycolipids and free fatty acids were measured by standard procedures described by Marinetti (1962), Hanel and Dam (1955), Roughan and Batt (1968) and Koniecko (1979) respectively, whereas total glycerides were indirectly calculated by subtracting all these from total lipid values.

Gross energy of sample was determined by Gallenkamp and Ballistic Bomb Calorimeter (Haque and Murarilal, 1999) using Benzoic Acid as a standard and expressed as Kcal/100g. Total calorie estimates of raw and cooked sausages were calculated on the basis of 100g portion using Atwater values for fat (9.0 kcal/g), protein (4.02 kcal/g) and carbohydrates (4.0 kcal/g) calories. Since analysis of per cent carbohydrates in the meat samples was not performed, the calorie values were estimates and not actual values.

The data obtained from various trials under each experiment was pooled and processed at Institute's computer centre. The statistical design of the study was 4(treatment) × 3 (replication) randomized block design. All chemical and physical determinations were conducted in triplicate. There were seven sensory determinations (judges) for each treatment × replication combination. Data were subjected to one way analysis of variance. Duncan's Multiple Range test and critical difference were determined at 5% significance level (Snedecor and Cochran, 1989).

Result and Discussion

Particle Size

The effect of different particle size on physico-chemical properties of cooked low-fat ground pork patties are presented in table 1. Percent cooking yield, moisture and moisture retention were significantly ($P < 0.05$) better at lower particle size (3mm) than higher particle size (6mm). It could be due to more compact binding at lower particle sizes which do not allow the release of moisture. Lin and Keeton (1994) and Small et al. (1995) also reported the increase in cooking losses with the increase in particle size. The shear force value increased significantly ($P < 0.05$) with an increase in particle size. Mean sensory scores (table 2) were significantly ($P < 0.05$) better in juiciness, texture and overall acceptability for particle size 3mm than higher particle size. It appears that smaller particle size provided increased binding and compactness to low-fat ground pork patties. Our results are in conscience of Small et al. (1995) and Berry et al. (1999). Texture Profile showed an increase in hardness and springiness with the increase in particle size. Since cooking determinants and sensory attributes were better with 3mm particle size, hence it was adopted as optimum particle size for further studies.

Fat levels

The fat percent increased linearly with the increase in added fat levels (table 3). Cooking yield and moisture retention were recorded maximum at 4% added fat level. The percent gain in height increased significantly ($P < 0.05$) at 4 and 5% added fat levels. However shear force value decreased significantly ($P < 0.05$) with the increase in fat levels. These findings are in accordance with Trout et al. (1992). There was marginal decrease in the fat retention of the product with increasing fat levels. Mean sensory scores (table 4) are significantly ($P < 0.05$) better for 5% added fat level than 3% added fat level whereas patties with 4% added fat were comparable to patties with 5% added fat level. The juiciness scores of the product were marginally higher for increased fat levels.

Perusal of table 5 showed that in raw product total lipid content, phospholipids, cholesterol content and calorific value (wet weight basis) increased significantly ($P < 0.05$) with increasing added fat levels. However on dry weight basis, cholesterol content increased significantly ($P < 0.05$) only at 5% added fat level. As for calorific value, the relationship was linear with fat levels in raw patties. In cooked product the total lipid content increased significantly ($P < 0.05$) with increasing added fat levels, whereas phospholipids and cholesterol content showed only a marginal increase. The cholesterol

retention did not show any trend in the present study. Calorific value increased significantly ($P < 0.05$) with increasing added fat levels. It is obvious because fat contributes 9.2 kcal/g energy which is about 2.25 times more than carbohydrates and proteins.

Conclusions

Cooked patties with 5 % added fat levels have mean total fat level more than 10, which does not fit into the definition of low-fat meat products (Keeton, 1994). Since cooking determinants and sensory attributes are better for 3mm particle size and 4% added fat level. Therefore the optimum level of particle size 3mm and added fat level 4% are recommended for formulation of low-fat ground pork patties.

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Table 1. Effect of particle size on physico-chemical properties of cooked low-fat ground pork patties.

Parameters	Particle size (mm)		
	3	4	6
pH	6.14±0.007	6.15±0.009	6.15±0.009
Moisture (%)	61.63±0.24 ^a	60.44±0.27 ^b	57.53±0.55 ^c
Fat (%)	9.12±0.13	9.21±0.09	9.03±0.10
Protein (%)	18.57±0.04	18.51±0.09	18.51±0.1
Moisture Protein Ratio	3.32±0.01 ^a	3.27±0.04 ^a	3.10±0.04 ^b
Cooking Yield (%) ^A	75.86±0.21 ^a	73.95±0.38 ^b	67.82±0.30 ^c
Cooking Loss (%) ^B	24.47±0.25 ^c	26.05±0.38 ^b	32.18±0.30 ^a
Decrease in Diameter (%)	15.04±0.27 ^c	18.74±0.17 ^b	22.80±0.44 ^a
Gain in Height (%)	21.46±0.99	20.68±0.28	20.28±0.17
Shrinkage (%) ^C	10.79±0.24 ^c	12.10±0.15 ^b	15.55±0.44 ^a
Moisture Retention (%) ^D	46.76±0.27 ^a	44.09±0.24 ^b	39.02±0.35 ^c
Fat Retention (%) ^E	77.98±0.32 ^a	76.24±0.38 ^a	70.47±0.49 ^b
Shear Force Value (kg/cm ²)	0.37±0.007 ^c	0.40±0.006 ^b	0.47±0.005 ^a

*Mean± S.E with different superscripts in a row differ significantly (P<0.05)

N=6 for each treatment

A per cent yield= (raw weight-cooked weight/raw weight) ×100

B cook loss= per cent cook loss: 100-per cent yield

C Shrinkage %= (Raw thickness- cooked thickness) + (raw diameter- cooked diameter)/ (raw thickness+ raw diameter)

D % Moisture retention= % yield × % moisture in cooked patties/100

E % Fat Retention= (cooked weight × %fat in cooked patties/raw weight×% fat in raw patties) ×100

Table 2. Effect of particle size on sensory attributes of low- fat ground pork patties.
(Mean \pm S.E.)*

Attributes	Particle size (mm)		
	3	4	6
Appearance	6.98 ± 0.08	6.93 ± 0.12	6.81 ± 0.12
Flavor	7.00 ± 0.077	6.95 ± 0.048	6.91 ± 0.074
Texture	7.29 ^a ± 0.095	7.10 ^a ± 0.074	6.58 ^b ± 0.14
Juiciness	6.93 ^a ± 0.079	6.83 ^{ab} ± 0.072	6.62 ^b ± 0.097
Overall Acceptability	6.98 ^a ± 0.064	6.77 ^b ± 0.056	6.43 ^c ± 0.093

* Mean \pm S.E. with same superscript in a row do not differ significantly (P<0.05)
Means are scores given by sensory panelists on 8-point scale where
1: extremely poor and 8: extremely desirable
N=21 for each treatment

Table 3. Effect of added fat levels on physico-chemical properties of cooked low-fat ground pork patties.

Parameters	Added fat levels (%)		
	3	4	5
pH	6.14±0.008	6.16±0.004	6.16±0.015
Moisture (%)	61.27±0.18 ^a	61.73±0.27 ^a	60.32±0.22 ^b
Fat (%)	8.18±0.11 ^c	9.08±0.07 ^b	10.64±0.08 ^a
Protein (%)	19.04±0.04	18.87±0.09	18.64±0.04
Moisture Protein Ratio	3.22±0.009 ^b	3.27±0.015 ^a	3.24±0.01 ^b
Cooking Yield (%) ^A	74.14±0.22 ^b	75.34±0.28 ^a	74.87±0.38 ^{ab}
Cooking Loss (%) ^B	25.87±0.22 ^a	24.66±0.28 ^b	25.13±0.38 ^{ab}
Decrease in Diameter (%)	24.74±0.15	25.13±0.28	25.19±0.14
Gain in Height (%)	18.61±0.48 ^b	20.28±0.48 ^a	21.12±0.17 ^a
Shrinkage (%) ^C	17.51±0.14	17.56±0.29	17.40±0.11
Moisture Retention (%) ^D	45.42±0.25 ^b	46.51±0.21 ^a	45.62±0.32 ^{ab}
Fat Retention (%) ^E	79.46±0.50	79.22±0.38	79.01±0.39
Shear Force Value (kg/cm ²)	0.55±0.009 ^a	0.50±0.004 ^b	0.47±0.006 ^c

*Mean± S.E with different superscripts in a row differ significantly (P<0.05)

N=6 for each treatment

A per cent yield= (raw weight-cooked weight/raw weight) ×100

B cook loss= per cent cook loss: 100-per cent yield

C Shrinkage %= (Raw thickness- cooked thickness) + (raw diameter- cooked diameter)/ (raw thickness+ raw diameter)

D % Moisture retention= % yield × % moisture in cooked patties/100

E % Fat Retention= (cooked weight × %fat in cooked patties/raw weight×% fat in raw patties) ×100

Table 4. Effect of added fat levels on sensory attributes of low- fat ground pork patties.
(Mean \pm S.E.)*

Attributes	Added fat levels (%)		
	3	4	5
Appearance	6.88 ± 0.06	6.91 ± 0.04	6.98 ± 0.04
Flavor	6.74 ^b ± 0.09	6.93 ^{ab} ± 0.05	6.98 ^a ± 0.04
Texture	6.74 ^b ± 0.08	6.93 ^a ± 0.05	6.95 ^a ± 0.03
Juiciness	6.88 ± 0.06	6.95 ± 0.06	7.00 ± 0.05
Overall Acceptability	6.74 ^b ± 0.08	6.93 ^a ± 0.05	7.02 ^a ± 0.05

* Mean \pm S.E. with same superscript in a row do not differ significantly (P<0.05)
Means are scores given by sensory panelists on 8-point scale where 1: extremely poor and 8: extremely desirable
N=21 for each treatment

Table5. Effect of added fat levels on total lipids, phospholipids, cholesterol and calorific value of raw low-fat ground pork patties.

Parameters (mg/g)	Added fat levels (%)					
	3		4		5	
	Mean	SEM	Mean	SEM	Mean	SEM
Total Lipids	41.36 ^c	0.96	58.50 ^b	0.60	76.55 ^a	0.57
Phospholipids	13.75 ^c	0.13	15.02 ^b	0.19	17.42 ^a	0.15
Cholesterol (wet weight basis)	1.15 ^c	0.02	1.21 ^b	0.01	1.28 ^a	0.004
Cholesterol (dry weight basis)	362.82 ^b	7.23	371.55 ^{ab}	3.03	378.81 ^a	2.71
Calorific Value* (Kcal/100g)	128.60 ^c	0.35	162.82 ^b	0.64	178.98 ^a	0.81

N=6 for each treatment

*Calorific value measured by Gallenkamp and Ballistic Bomb calorimeter

Table 6. Effect of added fat levels on total lipids, phospholipids, cholesterol and calorific value of cooked low-fat ground pork patties.

Parameters (mg/g)	Added fat levels (%)					
	3		4		5	
	Mean	SEM	Mean	SEM	Mean	SEM
Total Lipids	42.97 ^c	0.34	61.55 ^b	1.02	71.28 ^a	1.09
Phospholipids	19.75 ^b	0.32	21.96 ^a	0.35	21.92 ^a	0.23
Cholesterol (wet weight basis)	1.34 ^b	0.18	1.45 ^a	0.15	1.49 ^a	0.01
Cholesterol (dry weight basis)	346.46 ^b	4.98	378.46 ^a	3.86	376.00 ^a	2.81
Cholesterol Retention (%)	86.38 ^b	1.41	90.43 ^a	0.93	87.35 ^b	0.25
Calorific Value* (Kcal/100g)	171.52 ^c	1.93	184.49 ^b	1.01	208.96 ^a	0.67

N=6 for each treatment

*Calorific value measured by Gallenkamp and Ballistic Bomb calorimeter

Table 7. Effect of refrigerated storage on physico-chemical, microbiological and sensory characteristics of aerobically packaged low-fat ground pork patties. (Mean ± S.E.)*

Treatments	Storage Period (Days)			
	0	7	14	21
Physico-Chemical Characteristics				
TBA Value (mg malonaldehyde/kg)				
Control	0.41±0.02 ^{d1}	0.52±0.01 ^{c1}	0.78±0.02 ^{b1}	0.98±0.01 ^{a1}
Low-Fat Patties	0.28±0.007 ^{d2}	0.39±0.01 ^{c2}	0.59±0.07 ^{b2}	0.67±0.01 ^{a2}
pH				
Control	6.13±0.009 ^c	6.20±0.007 ^{bc}	6.27±0.004 ^b	6.38±0.008 ^a
Low-Fat Patties	6.12±0.002 ^c	6.17±0.004 ^b	6.24±0.004 ^{ab}	6.33±0.012 ^a
Microbiological Characteristics				
Total Plate Count (log cfu/g)				
Control	1.65±0.03 ^{d2}	1.94±0.02 ^{c1}	2.29±0.05 ^b	2.86±0.04 ^a
Low-Fat Patties	1.73±0.11 ^{d1}	2.13±0.04 ^{c2}	2.24±0.08 ^b	2.74±0.07 ^a
Psychrophilic Count (log cfu/g)				
Control	ND	ND	1.15±0.02 ^b	1.40±0.09 ^a
Low-Fat Patties	ND	ND	1.19±0.01 ^b	1.42±0.05 ^a
Coliform Count (log cfu/g)				
Control	ND	ND	ND	ND
Low-Fat Patties	ND	ND	ND	ND
Sensory Characteristics**				
Appearance				
Control	7.12±0.11 ^a	6.86±0.10 ^{ab}	6.76±0.12 ^b	6.69±0.12 ^b
Low-Fat Patties	7.18±0.06 ^a	7.05±0.12 ^{ab}	6.89±0.08 ^b	6.72±0.14 ^b
Flavor				
Control	6.97±0.07 ^a	6.88±0.09 ^a	6.71±0.10 ^{ab}	6.63±0.11 ^b
Low-Fat Patties	6.94±0.10 ^a	6.91±0.11 ^a	6.78±0.09 ^{ab}	6.72±0.12 ^b
Juiciness				
Control	7.12±0.09 ^a	6.94±0.07 ^{ab}	6.71±0.10 ^{bc}	6.67±0.11 ^c
Low-Fat Patties	6.99±0.08 ^a	6.96±0.05 ^a	6.81±0.09 ^{ab}	6.70±0.13 ^b
Texture				
Control	7.07±0.09 ^a	7.00±0.09 ^a	6.86±0.09 ^{ab}	6.74±0.08 ^{b1}
Low-Fat Patties	7.00±0.09	6.96±0.08	6.89±0.09	6.81±0.11
Overall Acceptability				
Control	7.05±0.09 ^a	6.98±0.08 ^{ab}	6.76±0.09 ^{bc}	6.64±0.12 ^c
Low-Fat Patties	7.02±0.09 ^a	6.93±0.04 ^a	6.81±0.07 ^{ab}	6.69±0.07 ^b

*Mean±S.E. with different superscripts row wise (alphabet) and column wise (numeral) differ significantly (P<0.05)

ND= Not Detected

N=21 no. of observations for sensory parameters and N=6 for other parameters

** Means are scores given by sensory panelists on 8-point scale where

1: extremely poor and 8: extremely desirable

Table 8. Effect of refrigerated storage on physico-chemical, microbiological and sensory characteristics of vacuum packaged low-fat ground pork patties. (Mean ± S.E.) *

Treatments	Storage Period (Days)					
	0	7	14	21	28	35
Physico-Chemical Characteristics						
	TBA Value (mg malonaldehyde/kg)					
Control	0.41±0.018 ^{b1}	0.44±0.007 ^{b1}	0.47±0.08 ^{ab1}	0.49±0.012 ^{a1}	0.54±0.012 ^{a1}	0.56±0.004 ^{a1}
Low-Fat Patties	0.28±0.007 ²	0.32±0.04 ²	0.31±0.007 ^{d2}	0.34±0.019 ^{c2}	0.37±0.01 ^{b2}	0.37±0.009 ^{a2}
	pH					
Control	6.13±0.009 ^a	6.12±0.01 ^a	6.09±0.005 ^{a1}	6.00±0.01 ^{b1}	5.95±0.02 ^{bc1}	5.82±0.07 ^c
Low-Fat Patties	6.12±0.002 ^a	6.12±0.007 ^a	6.08±0.002 ^{ab}	6.00±0.007 ^b	5.89±0.011 ^{bc}	5.81±0.02 ^c
Microbiological characteristics						
	Total Plate Count (log cfu/g)					
Control	1.65±0.03 ^f	1.86±0.008 ^e	2.10±0.02 ^d	2.41±0.04 ^c	2.80±0.018 ^b	3.26±0.016 ^a
Low-Fat Patties	1.74±0.01 ^f	1.94±0.15 ^e	2.21±0.015 ^d	2.49±0.004 ^c	2.78±0.013 ^b	3.16±0.04 ^a
	Psychrophilic Count (log cfu/g)					
Control	ND	ND	ND	1.75±0.03 ^c	1.95±0.018 ^b	2.25±0.04 ^a
Low-Fat Patties	ND	ND	ND	1.84±0.02 ^b	2.02±0.03 ^{ab}	2.13±0.03 ^a
	Anaerobic Plate Count (log cfu/g)					
Control	ND	ND	ND	ND	1.26±0.013 ^b	1.48±0.05 ^a
Low-Fat Patties	ND	ND	ND	ND	1.22±0.012 ^b	1.43±0.03 ^a
	Lactic Acid Bacteria Count (log cfu/g)					
Control	ND	ND	ND	ND	1.05±0.024 ^b	1.18±0.007 ^a
Low-Fat Patties	ND	ND	ND	ND	1.15±0.007 ^b	1.22±0.008 ^a
	Coli form Count (log cfu/g)					
Control	ND	ND	ND	ND	ND	ND
Low-fat Patties	ND	ND	ND	ND	ND	ND
Sensory Characteristics						
	Appearance					
Control	7.12±0.11 ^a	7.00±0.08 ^{ab}	6.95±0.08 ^{ab}	6.81±0.11 ^{bc}	6.67±0.10 ^c	6.67±0.12 ^c
Low-Fat Patties	7.18±0.05 ^a	7.11±0.07 ^a	7.02±0.09 ^{ab}	6.93±0.09 ^{bc}	6.79±0.09 ^c	6.81±0.07 ^{bc}
	Flavor					
Control	7.02±0.07 ^{ab}	7.02±0.08 ^a	6.98±0.07 ^a	6.83±0.09 ^{abc}	6.71±0.07 ^{bc}	6.60±0.13 ^{c2}
Low-Fat Patties	6.95±0.10 ^{ab}	6.98±0.04 ^a	7.00±0.12 ^a	6.86±0.09 ^{ab}	6.81±0.06 ^{ab}	6.76±0.07 ^{b1}
	Juiciness					
Control	7.12±0.10 ^a	7.00±0.07 ^{ab}	6.90±0.09 ^{abc}	6.86±0.09 ^{abc}	6.76±0.06 ^c	6.53±0.12 ^d
Low-Fat Patties	6.99±0.08 ^a	7.00±0.05 ^a	6.98±0.09 ^a	6.88±0.08 ^b	6.71±0.07 ^b	6.65±0.13 ^b
	Texture					
Control	7.07±0.07 ^a	7.05±0.07 ^a	6.95±0.07 ^{ab}	6.79±0.08 ^{bc}	6.81±0.07 ^{bc}	6.67±0.11 ^c
Low-Fat Patties	7.00±0.08 ^a	7.00±0.05 ^a	6.93±0.09 ^{ab}	6.84±0.08 ^{ab}	6.78±0.06 ^b	6.71±0.10 ^b
	Overall Acceptability					
Control	7.05±0.07 ^a	7.02±0.07 ^a	6.93±0.08 ^{ab}	6.87±0.08 ^{ab}	6.71±0.07 ^b	6.59±0.12 ^b
Low-Fat Patties	7.02±0.09 ^a	7.00±0.08 ^a	7.00±0.05 ^a	6.83±0.09 ^{ab}	6.79±0.09 ^{ab}	6.72±0.09 ^b

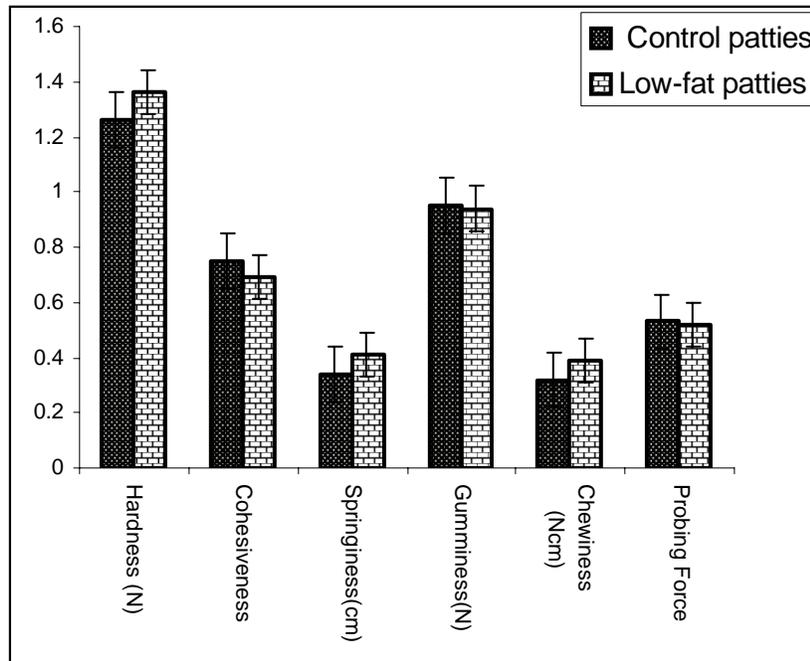
*Mean±S.E. with different superscripts row wise (alphabet) and column wise (numeral) differ significantly (P<0.05)

ND= Not Detected

N=21 no. of observations for sensory parameters and N=6 for other parameters

** Means are scores given by sensory panelists on 8-point scale where
1: extremely poor and 8: extremely desirable

Fig.1. Comparative instrumental Texture Profile of control and formulated low-fat patties.



EFFECTS OF MEAT TYPES AND FAT SUBSTITUTES ON COLOR AND TEXTURAL OF LOW FAT SAUSAGE

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Key Words: low fat sausage, meat types, fat substitutes, water binding capacity

Introduction

Due to concerns with obesity and related diseases, consumers are looking for no fat or low fat meat products. With excessive fat reduction, however, the low fat meat products have hard texture, lack of meat flavor and dark color, resulting in less acceptable to consumers. Fat substitutes based on proteins and carbohydrates have been widely used in meat industry to overcome the problems. The physical and structural properties of ingredients are strongly dependent on molecular interactions with water. Oat and soy protein have shown promise for increasing yield and juice retention in meat (Claus and Hunt, 1991; Lecomte et al., 1993). In addition, the constituents have been used in diets to control hypertension, diabetes, and health disease. However, there is little information on why the additions of oatmeal or tofu induce the change in textural attributes and/or water retention properties of low fat sausages.

Objectives

The objectives of this research were to evaluate the color and textural properties of low fat sausages by addition of oatmeal and tofu, to compare the effectiveness of incorporated oatmeal and tofu on sausages prepared with different types of meats such as beef, pork, and chicken, and to investigate the relationship between water binding properties of fat substitutes and changes in textural properties of low fat sausages.

Methodology

Sausage preparation : Beef, pork, and chicken were purchased from a local market and trimmed to reduce fat content before grinding through a 3mm plate. The moisture content meats, oatmeal, and, tofu were adjusted to 60%. This was done to ensure that any differences observed for added oatmeal and tofu would not be biased by differing moisture content. 10% hydrated oatmeal and tofu were added to each meat in a basis of total weight. The control sausages were also prepared without addition of oatmeal or tofu. For each batch, meat, oatmeal or tofu other ingredient were mixed thoroughly using mixer. After mixing, the mixture was stuffed into artificial cellulose casing with diameter of about 30mm using a stuffer. The sausages were then held for 24hrs at 4°C to allow for

ingredient equilibrium. The sausage samples were cooked for 30min in a steam chamber (SAA10, Absury, Germany) until the center temperature of the sausage reached of 70 °C.

Moisture absorption isotherm (%) : Meats, oatmeal, and tofu were dried for 3days using freezing dryer, after freezing them at -80 °C (clean vac 8, Biotron, Korea). Samples (approximately 1g each) were put into polystyrene weighting dishes (2× 2 inches, Fisher Scientific Co) and further dehydrated in a vacuum desiccator over P₂O₅ for 5-7 days until a content weight was attained. The dehydrated samples were equilibrated at 25 °C in sealed chambers over various saturated salt solutions with known relative vapor pressures (RVP): P₂O₅ (0), LiCl (0.11), KCH₃ (0.23), MgCl₂ (0.33), K₂CO₃ (0.43), Mg(NO₃)₂ (0.53), KI (0.69), (NH₄)₂SO₄ (0.81) and KNO₃ (0.93). Equilibrium moisture content (% db) was calculated from the weight gain after no further change in weight occurred. The triplicate samples from each treatment were measured.

Color : The color of the sausage samples was evaluated with CIE L* (lightness), a* (redness) and b* (yellowness) values using a Minolta chromameter (CR-310, Minolta Co. LTD. Japan). The measurement was done triplicated.

TPA (texture profile analysis) : Before analysis, cooked sausages were equilibrated at room temperature (20 °C) for 1 hr. Sausages were cored into a cylinder with 1 cm diameter and cut with 1.5 cm height using a sharp edged knife. TPA was performed by compressing the sample between parallel plates in a Universal Testing Machine (Model 3343) to 70% of the original height in two consecutive cycles at a crosshead speed 100mm/min. From the resulting force/deformation curves, the textural parameter of hardness, cohesiveness, springiness, brittleness, gummness, and chewiness were calculated. 10 specimens of each treatment were measured and analyzed statistically.

Statistical analysis : The data were analyzed using statistical analysis systems (SAS. 1999). To evaluate the differences among treatments, data were analyzed by analysis of variance (ANOVA) and Duncun's multiple range test.

Results & Discussion

The color of sausage samples with or without oatmeal and tofu is shown in table 1. Beef sausage showed higher lightness (L*) and lower redness (a*) values compared to chicken or pork sausage (P<0.05). However, as additions of oatmeal and tofu, the L* value increased but a* value decreased significantly (P<0.05). Data showed that addition of tofu was more effective to increase L* values and to decrease a* values of all meat sausages compared with addition of oatmeal. Table 2 shows TPA of the sausages with different types of meats and with/without oatmeal and tofu. As expected, beef sausage had a higher hardness compared to chicken and pork sausages, and the hardness of sausage samples decreased by additions of oatmeal and tofu. Results suggested that higher values in brittleness, hardness, gummness and chewiness of sausages from all meats could decrease significantly by additions of oatmeal and tofu. The sausage sample added with oatmeal gave softer (less brittleness, hardness) and higher springiness, compared to control and tofu added sausages. These results may be due to the difference in water binding properties among meats, oatmeal, and tofu.

Fig. 1 shows moisture absorption isotherms at 25 °C for freeze-dried powders from all meats, oatmeal and tofu of uncooked samples. All samples showed increase in moisture absorption as increase of a_w values from 0.11 to 0.93. The moisture absorption of oatmeal

was higher than those of others by a_w 0.53, however, oatmeal showed significantly lower moisture absorption above a_w 0.53. The moisture absorption of oatmeal was approximately 28% at a_w 0.93 whereas those of others were above 45%. However, the moisture absorption isotherms of cooked samples showed a considerable difference from uncooked samples (Fig. 2). Oatmeal showed higher moisture absorption % by a_w 0.69 compared to all meats and tofu. Moreover, at a_w 0.93, the moisture absorption of oatmeal was approximately 27% which was almost similar to uncooked sample, whereas that of tofu was approximately 22% which was almost half of uncooked sample. These results imply that the moisture absorption of tofu depends on condition of proteins that be denatured or not by heating. Therefore, it could be possible that the soft and tensile texture of sausage with addition of oatmeal compared to tofu (Table 2) was due to better water absorption capacity of oatmeal under cooking condition. In addition the result may imply that decreasing of hardness of sausage with addition of tofu might be due to soft textural property of tofu itself rather than increase in water binding capacity of tofu moisture absorption. Data suggested that addition of oatmeal would be more effective than tofu to improve texture of a low fat sausage because proteins in tofu could be denatured by cooking resulted in a poor water-binding capacity.

Conclusions

Color and textural properties of a low fat sausage could be improved by addition of oatmeal and tofu as a fat substitute. Addition of oatmeal and tofu increased lightness and tenderness of a low fat sausage. Moisture absorption of tofu was dependant on denaturation of proteins by heating while that of oatmeal was not changed by cooking. It was suggested that addition of oatmeal would be more effective than tofu to improve texture of a low fat sausage.

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Tables and Figures

Table 1. Color in low fat sausages with or without oatmeal and tofu

Treatments	L*	a*	b*
*Beef	49.86 ^H	19.19 ^A	9.56 ^C
*Pork	64.86 ^E	14.48 ^C	8.01 ^E
*Chicken	73.73 ^B	8.43 ^E	11.21 ^{AB}
Beef + 10% hydrated oatmeal	50.54 ^G	17.11 ^B	9.09 ^D
Pork + 10% hydrated oatmeal	66.68 ^D	12.57 ^D	9.90 ^C
Chicken + 10% hydrated oatmeal	74.91 ^A	6.54 ^F	11.51 ^A
Beef + 10% tofu	53.23 ^F	12.57 ^D	10.98 ^B
Pork + 10% tofu	70.66 ^C	11.53 ^D	7.59 ^E
Chicken + 10% tofu	73.99 ^B	7.81 ^{EF}	10.71 ^B

• A,B,C,D,E,F : Means in the same column with identical letters are significantly different ($p < 0.05$).

• * : Control; without oatmeal and tofu.

Table 2. TPA (texture profile analysis) in low fat sausages with or without oatmeal and tofu

Treatments	Brittleness (kgf)	Hardness (kgf)	Cohesivene ss (%)	Springiness (mm)	Gummness (kg)	Chewiness (kg*mm)
*Beef	0.47 ^A	0.63 ^A	56.79 ^B	14.00 ^C	35.64 ^A	498.66 ^A
*Pork	0.36 ^B	0.51 ^B	55.96 ^B	14.17 ^{BC}	28.59 ^B	406.77 ^B
*Chicken	0.36 ^B	0.40 ^C	49.69 ^C	13.99 ^C	20.09 ^C	281.09 ^C
Beef + 10% hydrated oatmeal	0.25 ^{DE}	0.31 ^{FG}	48.87 ^C	14.11 ^{BC}	15.33 ^D	219.29 ^E
Pork + 10% hydrated oatmeal	0.22 ^{EF}	0.28 ^{GH}	55.96 ^{AB}	14.17 ^{AB}	16.29 ^D	234.58 ^{DE}
Chicken + 10% hydrated oatmeal	0.18 ^F	0.27 ^H	61.77 ^A	14.52 ^A	16.05 ^D	233.04 ^{DE}
Beef + 10% tofu	0.31 ^C	0.39 ^{CD}	50.67 ^C	13.66 ^D	19.58 ^C	266.31 ^{DE}
Pork + 10% tofu	0.28 ^{CD}	0.36 ^{DE}	58.68 ^{AB}	12.99 ^E	20.82 ^C	270.18 ^C
Chicken + 10% tofu	0.26 ^{DE}	0.32 ^{EF}	48.35 ^C	14.26 ^{ABC}	15.52 ^D	220.93 ^E

• A,B,C,D,E,F,G,H : Means in the same column with identical letters are significantly different ($p < 0.05$).

• * : Control; without oatmeal and tofu.

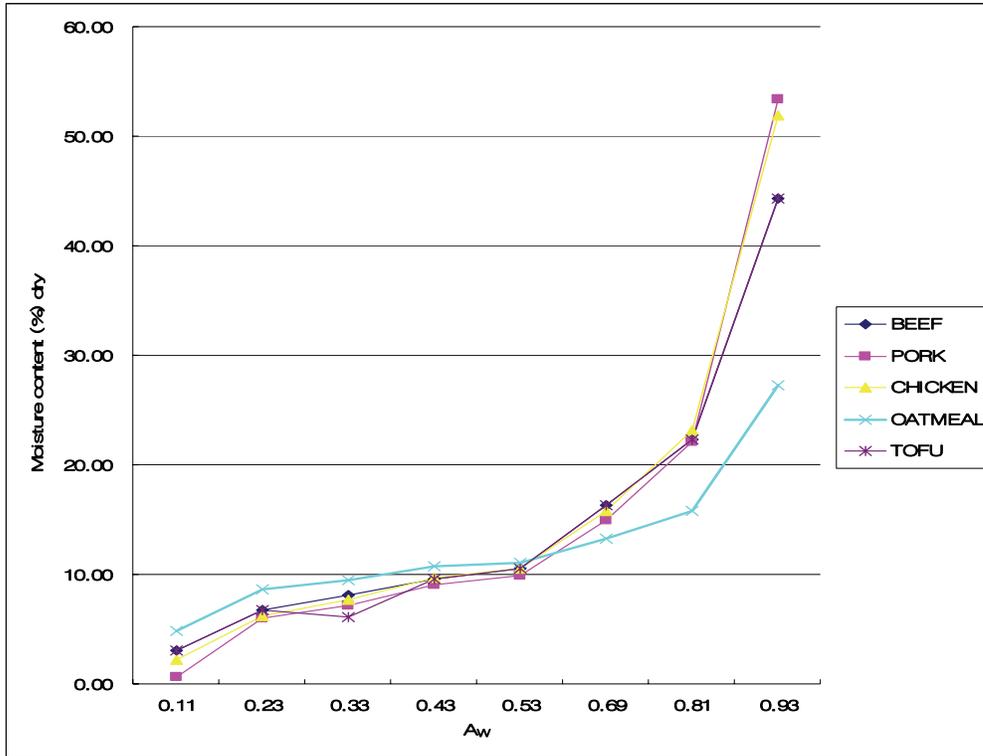


Figure 1. Moisture adsorption isotherm in meat types, oatmeal and tofu of raw powder samples.

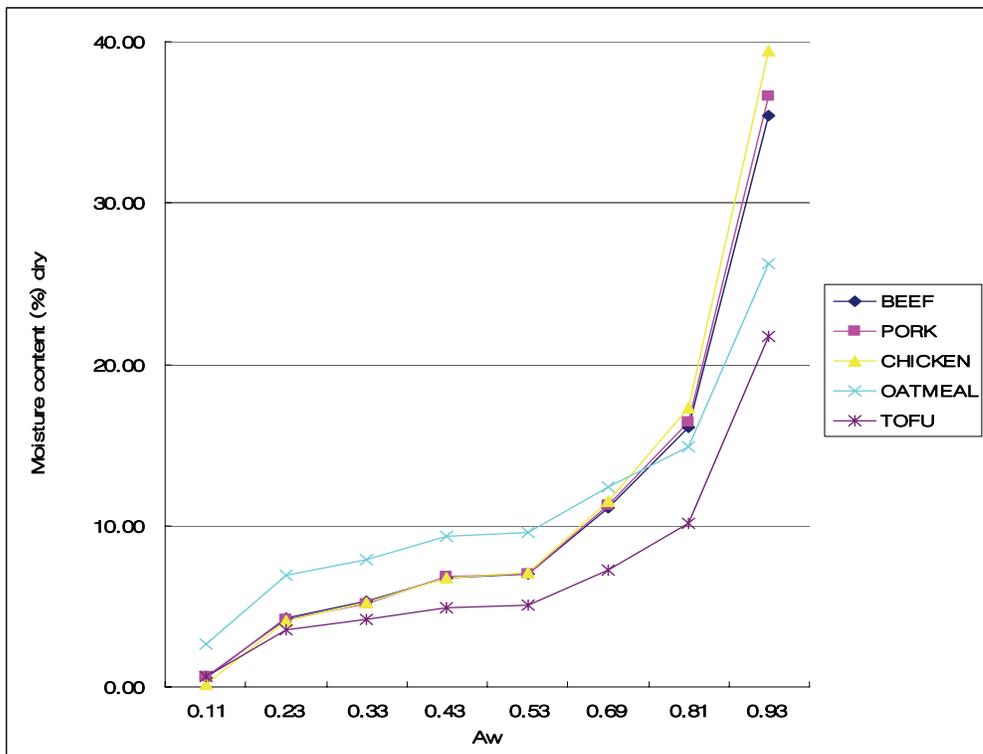


Figure 2. Moisture adsorption isotherm in meat types, oatmeal and tofu of cooked powder samples.

HIGH PRESSURE WITH OR WITHOUT ORGANIC ACIDS FOR TENDERIZING OF CHINESE HOG CASINGS

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Key Words: natural sausage casing, high pressure, organic acid, mechanical strength, collagen fiber

Introduction

In the meat industry, natural casing is preferred for the manufacture of sausage owing to its desirable texture. But such casing varies in quality, particularly with respect to strength and elasticity. Hog casing from China has far greater toughness compared to that from other countries (Sakata *et al.*, 1998, Nishiumi *et al.*, 1999). An appropriate method is being sought to tenderize this casing in consideration of the low price for its mass production.

Objectives

The previous study indicated that the thermal and structural stabilities of collagen fibers could be determined based on the mechanical strength of natural casing (Nishiumi *et al.*, 2001). Thus, in the present, attempt was made to soften collagen through application of high pressure and various organic acids. The authors thus sought to establish a method to achieve the best results by such application to provide tenderer Chinese hog casing.

Methodology

Materials: Analysis was conducted on hog casing (32-34 mm in diameter) from China after being washed and desalted in running water.

Mechanical strength measurement: Mechanical strength of the casing prior to and following high pressure and acid treatment was measured using a Rheometer (Fudoh NMR-2002J, Tokyo). The peak breaking point of casing strength was found using a cylindrical plunger 3-mm in diameter inserted into the casing.

High pressure treatment: Desalted casing was packed in a polyethylene bag, sealed with distilled water and pressurized using an isostatic press (Nikkiso KK, Tokyo) under the following conditions: pressure, 100-500 MPa; time, 5-60 min; temperature, 20-70°C.

Organic acid treatment: For acid application, desalted casing was immersed in lactic acid, acetic acid or citric acid solution at various concentrations ranging from 0.05 to 1.0 M for 10 min and then washed in running water for 30 min.

High pressure and organic acid application in conjunction: For this treatment, desalted casing was placed in a polyethylene bag, sealed with one of the three organic acid solutions mentioned above, pressurized with 200 MPa for 10 min and then washed in running water for 30 min.

Scanning electron microscopy (SEM): Structure and arrangement of collagen fibers on the surface of casing before and after treatment were observed with a scanning electron microscope (Hitachi S-430, Tokyo). All SEM specimens were prepared according to the cell-maceration method (Ohtani *et al.*, 1988).

Results & Discussion

Chinese hog casing ranged from 650-1,050 g, with mean value being 833 g. The casing is thus shown much tougher compared to other countries, being in the range, 600-700 g (Nishiumi *et al.*, 2001).

The effects of high pressure under various experimental conditions on hog casing strength are shown in Table 1. This treatment brought about significant tenderizing of Chinese hog casing under optimum conditions of 200 MPa, 10 min and 20-40°C.

Effects of three organic acid solutions at various concentrations on hog casing strength are also shown in Table 2. Acetic acid had no effect while 0.2 M lactic and citric acids both proved to be effective tenderizers.

Table 3 shows nearly all the treatments in this study to lessen hog casing strength. High pressure and organic acid treatment in conjunction was found to most effectively tenderize hog casing in consideration of the reduction in mean and standard error of the relative breaking strength.

The structure and arrangement of collagen fibers on the surface of the casing are presently being investigated by SEM and the relation of these parameters to casing tenderization by the present treatments will be taken up in a future study.

Conclusions

High pressure and organic acid treatment conducted individually or in conjunction was clearly shown to significantly tenderize tough Chinese hog casing, the latter proving most effective. High pressure effectively rendered uniformly tender casing.

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Tables and Figures

Table 1. Effects of high pressure on relative breaking strength of Chinese hog casing

Pressure (20°C, 10 min)		Time (200 MPa, 20°C)		Temp. (200 MPa, 10 min)	
0.1 MPa	100	0 min	100	20°C	87.1 ± 11.1*
100 MPa	94.5 ± 11.8	5 min	91.1 ± 13.5*	40°C	86.9 ± 11.3*
150 MPa	91.3 ± 11.3*	10 min	87.1 ± 11.1*	50°C	90.0 ± 12.1*
200 MPa	87.1 ± 11.1*	20 min	88.9 ± 11.4*	60°C	86.6 ± 12.4*
300 MPa	89.9 ± 12.7*	30 min	91.6 ± 11.0*	70°C	84.8 ± 11.9*
400 MPa	96.1 ± 10.8	45 min	94.4 ± 11.2		
500 MPa	94.7 ± 16.4	60 min	90.9 ± 11.2		

*, $P < 0.05$.

Table 2. Effects of organic acids within specified concentration range on relative breaking strength of hog casing

Lactic acid (20°C, 10 min)		Acetic acid (20°C, 10 min)		Citric acid (20°C, 10 min)	
0 M	100	0 M	100	0 M	100
0.05 M	98.6 ± 11.5	0.05 M	96.6 ± 14.9	0.05 M	99.1 ± 16.8
0.1 M	97.9 ± 13.3	0.1 M	98.4 ± 11.4	0.1 M	93.7 ± 14.2
0.2 M	82.8 ± 14.7*	0.2 M	98.2 ± 20.6	0.2 M	82.1 ± 15.2*
0.5 M	93.0 ± 13.9	0.5 M	98.5 ± 14.7	0.5 M	94.3 ± 14.8
1.0 M	95.5 ± 11.0	1.0 M	96.4 ± 12.5	1.0 M	93.0 ± 10.7

*, $P < 0.05$.

Table 3. Relative breaking strength for the treatment modes

Treatment	Relative BS
No treatment	100
HP (200 MPa, 20°C, 10 min)	87.1 ± 11.1*
LA (0.2 M, 20°C, 10 min)	82.8 ± 14.7*
AA (0.2 M, 20°C, 10 min)	98.2 ± 20.6
CA (0.2 M, 20°C, 10 min)	82.1 ± 15.2*
HP+LA (0.2 M, 200 MPa, 20°C, 10 min)	81.0 ± 13.8*
HP+CA (0.2 M, 200 MPa, 20°C, 10 min)	78.0 ± 13.8*

BS, breaking strength; HP, high pressure; LA, lactic acid; AA, acetic acid; CA, citric acid; *, $P < 0.05$.

EFFECTS OF OZONATION ON STRENGTH AND COLOR OF NATURAL HOG CASING

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Key Words: natural casings, pork, ozone

Introduction

Natural casings from lamb, pork, and beef are used throughout the U. S. sausage industry and are derived from the gastrointestinal tracts of the respective species above (Bakker, Houben, Koolmees, Bindrich, & Sprehe, 1999; Madhwaraj, Nair, Nair, Kadkol, & Baliga, 1980; Pearson & Gillett, 1999). After removal, tracts are washed, scraped, and treated to remove soluble components. Anatomical structures, such as the esophagus, stomach, small and large intestines, appendix, and rectum are separated, and the appropriate portions cleaned, salted, and graded according to size and condition (Pearson & Gillett, 1999).

Many factors influence the quality and properties of natural casings such as age of the animal, breed, type of fodder consumed, and other factors related to the animals themselves or the conditions in which they were raised. To be useful, natural casings must be sufficiently strong enough to withstand the pressure exerted on them during filling, stuffing and processing (Bakker *et al.*, 1999; Ockerman and Hansen, 2000).

Natural casings, by their nature, are contaminated with bacteria (10^4 to 10^7 CFU/gram) (Gabis and Silliker, 1974; Bakker *et al.*, 1999; Ockerman and Hansen, 2000) and may contain viruses within the interstitial portions of the casing. Ozone has been shown to be antimicrobial and antiviral in low ozone demand media, but its efficacy may be affected by the presence of readily available organic matter (Kim, Yousef, & Khadre, 2003). The United States Food and Drug Administration recently amended the food additive regulations to allow the use of gaseous and/or aqueous ozone as an antimicrobial treatment for foods including meat and poultry (CFR, 2003).

The purpose of this study was to initially determine the effects of ozonation on casing quality and ultimately evaluate its potential for inactivation of bacterial and viral pathogens while preserving the integrity of the casings.

Objective

The specific objective of this study was to: Determine the quality and integrity of natural hog casings exposed to ozonated water at ≈ 7 mg/L for up to four hours.

Methodology

Salted natural hog casings were obtained from a commercial supplier (DeWied International, Inc., San Antonio, TX) and held at $\approx 4^{\circ}\text{C}$. An Envirowash® Ozone Generator (Pure-O-Tech) was connected to a potable water supply line and used to produce up to 10 mg/L ozonated water. A stainless steel coil submerged in a 55-gallon plastic container filled with iced water served to chill recirculated, ozonated water to a constant temperature range. Previous, trials had shown that chilled water retained more ozone and that a constant ozone level could be achieved in a temperature range of 14.2° - 15.9°C .

After preliminary studies to establish consistent operating parameters, three consecutive sections, 20.3 cm in length, from 30 individual hog casings (38/42 mm dia) were removed and randomly assigned to 0, 2 or 4 h ozonation at ≈ 7 mg/L ozone and a water temperature of 14.2° – 15.9°C . Each casing section was assigned to a specific ozonation time and identified by attaching different colored plastic clips to the sample. A number was also attached to each sample to identify the casing strand (1 – 30) from which it was taken. The 2 and 4 h treatments were submerged under plastic grating in a 25 L plastic tub filled with recirculating ozonated water. Control samples (0 h ozonation), were placed in a container filled with distilled water and stored in a cooler ($\leq 4^{\circ}\text{C}$). At the end of each ozonation period, designated samples were removed from the tub, placed in a container of distilled water and stored at $\leq 4^{\circ}\text{C}$. pH, temperature and ozone level of the water were monitored at 0, 2 and 4 h. All samples were analyzed the following day for bursting strength, and $L^*a^*b^*$ color space values. Maximum rupture force values of casing segments from the same set were collected on three of five replications. The entire experiment was replicated 5 times.

pH Measurement

pH measurements were taken using an Orion™ (model 720A, Orion Research, Inc., Beverly, MA) pre-calibrated pH meter fitted with a combination electrode.

Actual (Spectrophotometric) Ozone Concentration

Actual ozone concentration was determined by the spectrophotometric method of Shechter, (1973). The method involved oxidation of a buffered iodide solution and measurement of the triiodide ion liberated by ozone. A stock iodine solution 0.01 N (1 mL = 240 μg of ozone) was diluted with a neutral potassium iodide reagent to obtain a 4.17×10^{-5} N (1 mL = 1 μg ozone) solution of iodine. This solution was serially diluted and used to prepare a standard curve with neutral potassium iodide reagent as the reference. Ozonated water samples (5 mL) were combined with 45 mL neutral potassium iodide buffered reagent held for 30 min in a cool and dark place and the absorbance was read at 352 nm against a standard curve.

Bursting Strength

Bursting strength was assessed using a modification of the method described by Bakker *et al.* (1999). Bursting strength was defined as the pressure required for rupturing

a casing as it filled with water at a constant flow rate of ≈ 1800 mL/min. Each casing segment (≈ 20.3 cm) was threaded onto a plastic pipe (15 cm length, 19.05 mm o.d. and 12.70 mm i.d.) with seven sets of six holes (3.18 mm dia.) or 42 water outlets equally spaced around the pipe ≈ 2 cm apart (Figure 1). After clamping each end of the casing to the pipe, one side of the pipe was connected to a distilled water source and the other side connected to a pressure transducer. The casing was then uniformly filled with water at a constant flow rate until the casing burst. The bursting strength data was collected at 20 points/second using a computer connected to the transducer.

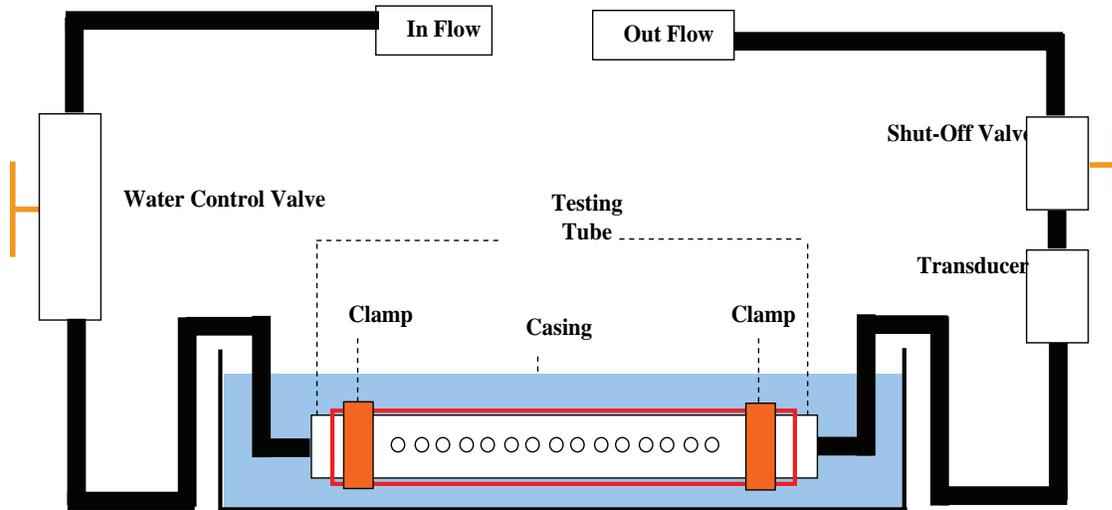


Figure 1: Bursting strength measuring device.

Maximum Rupture Force

A “pull – apart” testing device, similar to that described by Bakker *et al.*(1999) was developed to measure the maximum radial rupture force along a longitudinal section of casing. The device consisted of a solid aluminum bar (2.5 cm dia.; 15.5 cm length) split lengthwise (axially) into two equal halves. The ends of each bar half could then be attached to support brackets affixed to a Materials Testing System (MTS) instrument. For each measurement, a segment of casing (≈ 20.3 cm) was threaded onto the joined bar and the ends attached to upper and lower support brackets. The upper bracket was fixed while the lower bracket was attached to a 20 Kip Axial Torsion Load Frame set in the tension mode. A Sensotec 45.36 kg (100 lbs) load cell applied a 22.68 kg (50 lbs) full scale force to the lower bar at a cross-head speed of 20.32 mm/min. The force at rupture was recorded in kg.

*L*a*b* Color Space Values*

L*a*b* color space values were obtained by reflectance using a Minolta Colorimeter (model CR-200, Minolta Co., Ramsey, N.J., U.S.A) calibrated to a white plate standard surface ($C Y = 93.24$, $x = 0.3137$, $y = 0.3196$) at channel 00. Two readings were taken

from each end of a folded piece of casing (38/42 mm) exposed to ≈ 7 mg/L ozone for 0, 2 and 4 h. The results were expressed as positive or negative L* (lightness), a* (redness) and b* (yellowness) values.

Statistical Analysis

Mechanical characteristics and the color values of the casings were analyzed as a randomized block split-plot design using the Proc GLM procedure of SAS version 9.0 (SAS Institute, Cary, NC, 2002). The model was tested to determine if differences could be attributed to ozonation times or their interactions. Replications (5) were used as the random effect (blocking factor). When differences between treatment effects were significant ($p \leq 0.05$), a multiple comparison of means was performed using the least significant differences (LSD) method.

Results & Discussion

Preliminary Studies

From preliminary studies, it was determined that chilling the re-circulated water to $\approx 15.7^\circ\text{C}$ or lower was required to maintain a steady-state level of ozone (≈ 7 mg/L) over a 12 h period. After 4 h of ozonation (≈ 7 mg/L), it was observed that a significant decrease in the bursting strength of the hog casings (38/42 mm) occurred and that they were easily ruptured. Thus, the maximum ozonation time for 38/42 mm casings was determined to be limited to ≤ 4 h to prevent excessive weakening or deterioration. It was also observed that casings within a hank or even segments within a casing were quite variable in bursting strength due to the inherent nature of the raw material.

Process Conditions

Mean values of temperature, pH and ozone concentrations of ozonated water are presented in Table 1. Temperature values ranged from 14.2° to 15.9°C during processing. pH values of ozonated water remained relatively constant after 4 h ozonation. Ozonator readings ranged from 6.4–6.6 mg/L, while the actual (spectrophotometric) ozone concentration of ozonated water varied from 6.8–7.5 mg/L.

Table 1: Mean values of process conditions for ozonation of natural hog casings from 0 to 4 h.

Treatments (h)	Temp. $^\circ\text{C}$	pH	Ozone Concentration (Ozonator) mg/L	Actual (Spectrophotometric) Ozone Conc. mg/L
0	14.5	8.14	6.6	6.9
2	14.2	8.15	6.4	7.5
4	15.9	8.12	6.5	6.8

Bursting Strength

Analysis of variance p values for bursting strength and L*a*b* color space values of natural hog casings (38/42 mm) exposed to ≈ 7 mg/L ozone for 0, 2 and 4 h are presented in Table 2. There was no significant interaction effect between casing and ozone treatment nor were there significant differences among casing segments for these attributes.

Table 2: p-values of analysis of variance (ANOVA) for bursting strength, maximum rupture force and color space values of natural hog casings (38/42 mm) treated with ≈ 7 mg/L ozone for 0, 2 or 4 h.

Treatments	Bursting Strength	Maximum Rupture Force	L*	a*	b*
Casing segments	0.3640	0.0128	0.6677	0.7437	0.5927
Ozone Treatment (h)	<0.0001	0.0127	0.0009	<0.0001	<0.0001
Casing * Treatment	0.6425	0.5718	0.6220	0.6469	0.6855

Mean bursting strength values of hog casings for three ozone exposure times are shown in Table 3 and Figure 2. As ozone treatment time increased ($p \leq 0.05$), the strength of the casing decreased. After 2 and 4 h of ozonation, casings became progressively weaker with the 4 h treatment being more detrimental than the 2 h treatment. It can be concluded that casings exposed to ≈ 7 mg/L ozone for 2 or 4 h became progressively weaker, compared to non-ozonated casings. These results suggest that ozonation of hog casings (38/42 mm) be limited to not more than 2 h. Further testing by actually stuffing ozone treated casings with sausage will determine the extent of damage caused by 2 and 4 h ozonation times.

Table 3: Mean bursting strength values of natural hog casings (38/42 mm) treated with ≈ 7 mg/L ozone for 0, 2 or 4 h.

Ozone Treatments (h)	Bursting Strength (kPa)	Standard Deviation (s.d.)
0	16.55 ^a	± 4.00
2	14.48 ^b	± 3.86
4	13.51 ^c	± 4.41

^{a,b,c} Different superscript letters in the same column indicate that means are different ($p \leq 0.05$) (LSD).

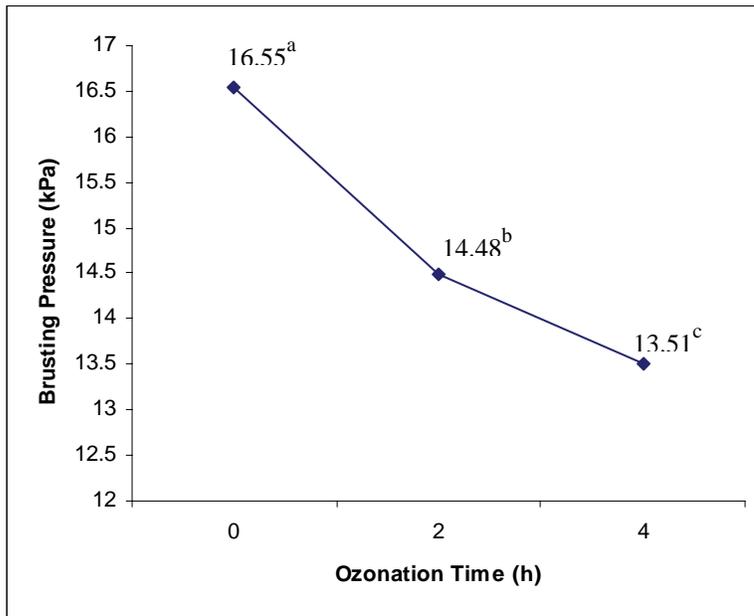


Figure 2: Mean bursting strength (kPa) values of natural hog casings (38/42 mm) treated with ≈ 7 mg/L ozone for 0, 2 and 4 h.

Maximum Rupture Force

Maximum rupture force was designed to give an indication of a casing's capacity to resist the internal forces applied during filling of a casing. Maximum rupture force values are presented in Table 4. There were no significant interaction effect between casing and ozone treatment. Casing and ozonation treatment effect were significant ($p \leq 0.05$) for maximum rupture force of the casings. Ozonation treatment caused a decline in maximum rupture force values. A significant decrease ($p \leq 0.05$) in maximum rupture force was noted between 0 and 4 h suggesting that ozonation time be limited to ≤ 2 h. This conclusion is supported by the observed decline in bursting strength as shown in Table 2 and Figure 2.

Table 4. Mean maximum rupture force values of natural hog casings (38/42 mm) treated with ≈ 7 mg/L ozone for 0, 2 or 4 h.

Ozone Treatments (h)	Maximum Rupture Force (kg)	Standard Deviation (s.d.)
0	9.18 ^a	± 1.94
2	8.95 ^{ab}	± 2.07
4	8.66 ^b	± 2.05

^{a,b,c} Different superscript letters in the same column indicate that means are different ($p \leq 0.05$) (LSD).

*L*a*b* Color Space Values*

Ozone treatment affected ($p \leq 0.05$) all $L^*a^*b^*$ color space values of the casings (Table 5). $L^*a^*b^*$ values decreased slightly due to ozone treatments, but the magnitude of these differences were small. A decrease in L^* value signifies a slight decrease in lightness of the casing or a slight darkening with exposure to ozone. a^* values for redness decreased (became negative) indicating that the casing became less red while declining b^* values indicated that casings became less yellow. Corresponding declines in redness and yellowness with ozonation make the casings actually appear lighter, because these components make up the actual hue or color (red, green, blue, etc.) of the casings. The ozone treated casings actually appeared less red and less yellow or in effect slightly lighter.

Table 5: Means and standard deviations of $L^*a^*b^*$ color space values of natural hog casings (38/42 mm) treated with ≈ 7 mg/L ozone for 0, 2 and 4 h.

Color Space Values (Minolta) ¹						
Ozone Treatment (h)	L^*	s.d. (L^*)	a^*	s.d. (a^*)	b^*	s.d. (b^*)
0	80.27 ^a	± 2.96	0.62 ^a	± 1.18	6.75 ^a	± 1.52
2	79.69 ^b	± 2.52	-0.38 ^b	± 0.84	5.71 ^b	± 1.49
4	79.83 ^b	± 2.90	-0.66 ^c	± 0.75	5.73 ^b	± 1.63

¹ L^* = Lightness (0 = black, 100 = white), a^* = redness, $-a^*$ = greenness, b^* = yellowness, $-b^*$ = blueness.

^{a,b,c} Different superscript letters in the same column indicate that means are different ($p \leq 0.05$) (LSD).

Conclusions

The bursting strength of natural hog casings ozonated for 0 to 4 h at ≈ 7 mg/L decreased over time and the casings became progressively weaker. In addition, 4 h of ozonation was noted to be more detrimental than the 2 h treatment. The maximum rupture force also decreased with ozonation time, however, the 2 h treatment was not different from the control. $L^*a^*b^*$ color space values of ozone treatments were slightly lower than the control, but the magnitude of these differences were small. Although there was a slight decrease in L^* value (slightly darker), ozonation made the casings actually appear lighter due to declines in the a^* and b^* values.

Results from the mechanical tests performed suggests that ozonation of hog casings (38/42 mm) weakens the casing and that treatment to be limited to less than 2 h at ≈ 7 mg/L in chilled ($< 18^\circ\text{C}$) ozonated water. Further confirmatory pilot plant filling tests

must be conducted to determine if the 2 and 4 h ozone treatments are sufficiently detrimental to cause premature rupture of hog casings.

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Meat Safety

**HYGIENIC ASSESSMENT OF SHEEP CARCASSES AT
SLAUGHTERHOUSES BY DESTRUCTIVE AND NON-DESTRUCTIVE
SAMPLING METHODS**

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Key Words: Decision 2001/471/EC, sheep carcasses, sampling methods

Introduction

Regular microbiological examinations of carcasses allow reliable conclusions to be drawn with regard to the long-term hygienic conditions in abattoirs (Mc Evoy et al. 2004, Zweifel et al. 2003, 2005). The EU Commission Decision (2001/471/EC) obliges fresh red meat and poultry meat operators to conduct regular checks on the general hygiene conditions of the production process, including microbiological controls of carcasses, and also makes HACCP methodology principles mandatory. For microbiological monitoring of carcasses Directive 2001/471/EC relies exclusively on total viable counts (TVC) as indicators of hygiene and Enterobacteriaceae as indicators of faecal contamination, and defines microbiological performance criteria for samples obtained by destructive sampling (excision technique). Recent studies suggest that swabbing with abrasive materials and applying the wet-dry double swab technique may be a suitable alternative to excision (Dorsa et al. 1996, Gill et al. 2000). However some authors and Federal authorities have suggested that the microbiological criteria for samples taken by the wet-dry double swab technique and abrasive materials should be set at 20% of the values set for excision samples (Anonymous 2002, Mc Evoy et al. 2004, Zweifel et al. 2005).

Objectives

The aim of the present study was to acquire extensive scientific issues on the hygienic status of sheep slaughtering process. The microbiological contamination of sheep carcasses at two slaughterhouse typologies, EU-approved high capacity slaughterhouses (H.C.S.) and low capacity slaughterhouses (L.C.S, which are allowed to slaughter a maximum of 20 units/week and/or 1,000 units/year), was evaluated by the comparison of the sampling methods recommended by Directive 2001/471/EC (excision and wet-dry swab) with an alternative non-destructive methods (sponge swabbing). Moreover, the practicability and reliability in routine use of these methods was evaluated.

Methodology

267 sheep carcasses were sampled in six slaughterhouses, three EU-approved H.C.S. and three L.C.S. 138 and 129 sheep carcasses were examined respectively at H.C.S. and L.C.S. abattoirs. 187 subjects were Sarda sheep, while 80 were imported from Spain. At each abattoir, sampling was performed weekly within a working day, after slaughtering and before chilling of the carcasses. On each visit, samples were collected involving ten sheep carcasses, randomly chosen. The carcasses were examined at three different sites of the four suggested by the Directive 2001/471/EC: flank (F), brisket (B) and rump (R). On the right side of carcasses, sampling was performed by the destructive method for five sheep (excision, EX) and by the non-destructive method for the remaining five (wet/dry double swab, SW). On the left side of all carcasses, samples were collected by the sponge swabbing method (SP). The sample collection by EX and SW techniques was performed according to the EU Decision criteria. The SP method was performed as following described: at each sampling site (100 cm²), a moistened (7 ml of Buffered Peptone Water, Oxoid-England) sponge (enviro sponge, Tecna, U.S.A.) was rubbed vertically, horizontally and diagonally across the site delineated by a sterile template. Microbiological analyses a) Total viable count (TVC-A.P.H.A., 2001); b) Enterobacteriaceae count (TEC) on Chromocult Coliform Agar (Merck, Germany), incubated at 37 °C for 24-48 h. In the comparison of EX vs SP, a single sponge for all sampling sites was used, while in the comparison of SW vs SP, a sponge for each sampling site was used. Analysis of variance were performed using the GLM procedures. The mean differences were evaluated using the LSD test (Statgraphics Plus, 5.1).

Results & Discussion

The microbiological results, expressed as mean \pm s.d. of log₁₀ cfu/cm² values, were compared to the criteria recommended by Decision 2001/471/EC. a) EX vs SP: the total mean results from 129 sheep carcasses are shown in table 1, while the allocation into the ranges defined by EU Decision is reported in tables 5, 6. Independent from the slaughtering capacity, TVC and TEC mean values were higher ($p < .01$) in the samples collected by the EX than those obtained by the SP. Only TVC mean values were significant higher ($p < .01$) in the L.C.S. than in the H.C.S. (table 2). b) SW vs SP: table 1 shows total mean results from 132 sheep carcasses, while the allocation into the ranges defined by EU Decision is reported in tables 3, 4. Microbiological criteria for samples collected by the SW and SP techniques have been set at 20% of the values set for EX samples (Anonymous, 2002). In the samples obtained by SP, TVC and TEC mean values were higher than those obtained by the SW ($p < .01$). Comparing the results (table 2) in relation to the slaughtering plants capacity, the TVC and TEC mean values were significant higher ($p < .01$) in the L.C.S. than in the H.C.S.. In relation to the sampling site, brisket was the most contaminated: TVC mean values were 2.63 ± 0.64 in the samples obtained by the SP and 1.62 ± 0.75 in those obtained by the SW. Differences between the methods were significant ($p < .01$). In the same sampling site, TEC mean values were 1.23 ± 1.15 and 0.40 ± 1.08 in the samples collected by SP and SW, respectively. As reported by other authors (Vanderlinde et al. 1999), such results are strongly linked to the slaughtering operations. In the flank, the TVC mean values were 2.39 ± 0.76 and 1.60 ± 0.81 in the samples collected by SP and SW respectively, while the TEC mean values were 1.23 ± 1.15 (SP) and 0.40 ± 1.08 (SW). The rump was less contaminated: TVC mean

values were 2.23 ± 0.80 (SP) and 1.48 ± 0.76 (SW), while TEC were 1.10 ± 1.11 (SP) and 0.10 ± 0.92 (SW).

Conclusions

The comparison of a destructive (excision) with a non-destructive method (sponge swabbing) shows significant differences ($p < .01$) between the two techniques. The recovery capacity of the sponge swabbing was lower for all the microbiological considered parameters. Moreover, the use of a single swab for the three sampling sites was unsuitable (dilution effect), particularly for the recovering of Enterobacteriaceae. The excision method was the most reliable and effective in terms of microbial recovering efficacy, but its use is limited because of the destructive effect (Dorsa et al. 1996, Gill et al. 2001, Byrne et al. 2005). Although the lower recovery efficacy, the non-destructive methods are effective and reliable for the hygienic assessment of carcasses at slaughterhouses, and are also suitable for routine use (Reid et al., 2002). With respect to the TVC criteria proposed by Directive 2001/471/EC, most of the carcasses sampled by the three methods were allocated into the acceptable category. Instead over 60 % of samples obtained by the EX were allocated into the unacceptable category for TEC. This percentage decreased until to 17,2% and 39,3% in the samples collected by SP and SW, respectively. The higher prevalence of TVC and TEC were detected in carcasses sampled at L.C.S., independent of the sampling method. The results show that the process management and the slaughterhouse capacity are the main factors affecting the level of sheep carcass contamination (Giuffrida et al. 2002).

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Tables and Figures

Table 1 - Results of the comparison of destructive (*EX*) and non-destructive (*SW* and *SP*) methods for microbiological sampling of sheep carcasses (mean \pm s.d.).

Parameter	EX vs SP		SW vs SP	
	Excision	Sponge swabbing	Dry/wet Swab	Sponge swabbing
Total Viable Count	3.36 \pm 0.76x	2.47 \pm 0.82y	1.57 \pm 0.77y	2.42 \pm 0.75x
<i>Enterobacteriaceae</i>	3.42 \pm 1.40x	1.54 \pm 1.19y	0.24 \pm 1.03y	1.31 \pm 1.15x

(x, y): the mean values in the same line not identified with the same letter are significantly different: = $p < .01$

Table 2 - Results of the comparison of destructive (*EX*) and non-destructive (*SW* and *SP*) methods for microbiological sampling of sheep carcasses (mean \pm s.d.) in relation to the slaughtering plants capacity

Parameter	Sampling Method	Capacity		Sampling Method	Capacity	
		L.C.S.	H.C.S		L.C.S.	H.C.S
		Total Viable Count	EX		3.55 \pm 0.68x	319 \pm 0.79y
	SP	2.82 \pm 0.82	2.16 \pm 0.68	SW	1.60 \pm 0.79x	1.54 \pm 0.76x
<i>Enterobacteriaceae</i>	EX	3.34 \pm 1.40	3.49 \pm 1.41	SP	1.56 \pm 1.28x	1.07 \pm 0.97y
	SP	1.77 \pm 1.15	1.33 \pm 1.19	SW	0.40 \pm 0.96x	0.09 \pm 1.07y

L.C.S.= low capacity slaughterhouses; H.C.S.= high capacity slaughterhouses; x, y = the mean in the same line not identified with the same letter are significantly different ($p < .01$)

Table 3 – Allocation of the Total Viable Counts results in the categories for process control verification, according to the modified criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		< 2.8	2.81-4.3	>4.30
Sponge swabbing	L.C.S.	63.1	36.9	0
	H.C.S.	89.6	10.4	0
Dry/wet swab	L.C.S.	100	0.1	0
	H.C.S.	100	0.1	0

L.C.S.= low capacity slaughterhouses; H.C.S.= high capacity slaughterhouses;

Table 4 – Allocation of the *Enterobacteriaceae* Counts results in the categories for process control verification, according to the modified criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		<0.5	0.5-1.8	>1.8
Sponge swabbing	L.C.S.	9.2	53.8	33.8
	H.C.S.	13.4	68.7	14.9
Dry/wet swab	L.C.S.	27.7	40.0	32.3
	H.C.S.	29.9	23.9	46.3

Table 5 – Allocation of the Total Viable Counts results in the categories for process control verification, according to the criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		< 3.5	3.5 - 5	>5
Excision	L.C.S.	42.6	55.7	1.63
	H.C.S.	58.8	41.1	0

Table 6 – Allocation of the *Enterobacteriaceae* Counts results in the categories for process control verification, according to the criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		< 1.5	1.5 - 2.5	> 2.5
Excision	L.C.S.	4.9	29.5	65.6
	H.C.S.	10.3	11.8	77.9

PREVALENCE OF SALMONELLA SPP. IN RED MEAT ABATTOIR LAIRAGES IN THE SOUTH-WEST OF ENGLAND, PRIOR TO ANIMALS ENTERING AFTER ROUTINE CLEANING OPERATIONS

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Introduction

Foodborne pathogens such as *Salmonella* spp are carried asymptotically in the intestines of healthy animals, and are shed into the environment when faeces are voided[1]. Animals sent for slaughter may contaminate the lairage holding and stunning facility through the shedding of pathogens in the faeces, but also through mechanical transfer of organisms carried on the coats[2, 3]. *Salmonella* spp may persist for a number of days in the environment[4], and can be transferred onto the coats and skins of animals in subsequent batches handled within the same facility[2, 3]. Legislation and good manufacturing practice demand that abattoir operators take steps to limit contamination, and cleaning regimes have been put into place in both the food processing area and in the lairage. These cleaning regimes vary widely, and may or may not entail the use of chemical cleaning products[4]. “Normal” cleaning programmes in UK lairages have been shown to be ineffective in elimination of foodborne pathogens[5], and indeed, an improved cleaning regime implemented in a pig lairage in the Netherlands similarly did not remove *Salmonella* contamination[6]. This study was carried out to investigate the prevalence of *Salmonella* contamination in the pre-harvest areas in a variety of commercial abattoirs in the UK.

Methods

Origin of Samples

A total of 432 samples were taken from various sites in red meat abattoir lairages. The samples were taken from the holding pens and stunning areas early in the morning before animals were delivered and processing began. The lairages had undergone routine cleansing operations at the end of the previous day’s processing. Within the holding pens, samples were taken from the floors, walls, edges (two-dimensional corner between floor and wall) and corners (three-dimensional corner between the floor and two walls), whilst in the stunning areas, samples were taken from the stun box walls, floors and corners (three-dimensional corner between the floor and two walls), and from the roll-out ramp in the case of cattle stun boxes or sheep restrainer-conveyor systems.

Five abattoirs participated in the study, and each was visited on two or more occasions to collect samples. Plant A was a medium sized sheep and cattle plant, processing approximately 700 steers/heifers, 150 bobby calves and 1000 sheep each week. In this plant, routine cleaning of the stun boxes and roll-out area involved the

use of pressure washing and quaternary ammonium cleaning products, whilst cleaning of the holding pens entailed removal of soiled bedding using a pitchfork and scraper, followed by the addition of fresh straw bedding. At the end of each week, all bedding was removed and the pens steam-cleaned and allowed to dry before fresh bedding was laid. Plant B was a small multi-species plant, processing 6 cattle, 10 pigs and 10 sheep each week, and all areas were cleaned at the end of the processing day using pressure wash with quaternary ammonium cleaning products. Plant C was a medium sized multi-species plant processing 1000 pigs, 2000 sheep and 500 bobby calves each week. The stun box was cleaned on a daily basis with a pressure washer and a hypochlorite solution, whilst the cleaning regime for the holding pens involved removal of soiled bedding and brushing out on a daily basis, and the pens were pressure washed using a broad-spectrum virucidal disinfectant solution once weekly, on a rotational basis. Plant D was a large sheep and cattle plant, processing 1500 steers/heifers and 5000 sheep each week; and plant E was a medium sized cull cow/bull plant, processing 800 cows/bulls each week. Plants D and E were owned by the same company and the cleaning regimes were identical. Stun boxes were cleaned at the end of each working day using a pressure wash, followed by detergent foam clean. This was then rinsed and a terminal quaternary ammonium sanitizer was applied. The holding pens were pressure washed after each batch of animals, but no chemicals were used.

The stunning facilities used for cattle in all abattoirs A, B, D and E comprised a race leading to an individual stunning box, from which the animal, once stunned, would roll out to be shackled and hoisted. In abattoir D, sheep were processed using a restrainer-conveyor system, with the stunned sheep rolling onto a bleeding table, whilst in abattoirs A, B and C, all the small species (sheep, pigs and calves) were processed through a group stunning pen, where the stunned animal would fall to the floor of the group pen and be shackled and hoisted there.

Collection of Samples

Samples were collected using gauze swabs (Readiwipes Super, Robinson Healthcare 5345) pre-soaked in 100 ml Buffered Peptone Water (BPW, Oxoid CM0509). Excess BPW was squeezed from the swab into the transport container, and the swab was rubbed vigorously over a measured area 50 cm by 50 cm before being returned to the transport container. These were then stored on ice and returned to the laboratory within 2 hours of collection.

Sample Processing

On return to the laboratory, the transport containers containing the swab and BPW were vigorously shaken, and 10 ml decanted into a universal container (UNI1). The original samples were then refrigerated at 4°C. From UNI1, a decimal dilution series was made in BPW, and this was incubated for 24 hours at 37°C. After this enrichment phase, 0.1 ml was taken from UNI1 and from the refrigerated original sample, and was inoculated into DIASALM selective enrichment medium (Merck 1.09803), and this was incubated at 41.5°C for 24 hours. The original sample and the dilution series were stored under refrigeration at 4°C. On the third day, a 10 µl loopful was taken from each of the DIASALM plates and streaked onto Rambach Chromogenic Agar (Merck 1.07500) for the identification of *salmonella* spp. and incubated at 37°C for 24 hours.

Where cultures showed a presumptive identification of *Salmonella* spp on Rambach Agar (cerise coloured colonies), the associated dilution series was removed from refrigeration and 0.1 ml from each dilution enriched and plated using DIASALM and Rambach Agar as outlined above. An estimation of the numbers of *Salmonella* organisms in the original sample could then be made, based on the lowest dilution at which *Salmonella* spp were identified on Rambach Agar (table 1).

Presumptive *Salmonella* isolates were confirmed using Api20e strips (Biomérieux 20100).

Results

Overall, 33 of the 432 (7.6%) samples taken were positive for *Salmonella* spp, and the numbers present ranged from below 10 to below 10,000 (table 2). No *Salmonellae* were found on the stun box walls or the roll-out ramps. High estimated numbers of organisms were not associated with any one particular sampling site, but positive samples originated from sites where the swab collected visual contamination, or where the integrity of the surface sampled had been broken due to corrosion of metal or shattering of concrete. These areas are those where cleaning had been insufficient to remove contamination, either due to lack of cleansing in pens where physical removal of bedding was the cleaning method employed, or due to the damage in the corners and edges of the lairage and pre-slaughter areas allowing contamination to collect and be by-passed by the cleansing process.

In the holding areas, it would be expected that abattoirs A and C, where physical removal of bedding was the main cleaning method, would have a greater incidence of *Salmonella* contamination than in abattoirs B, D and E. Abattoirs B and E yielded no *Salmonella* positive samples in the holding pens, suggesting that the cleaning regime in these lairages was sufficient to remove contamination, but in abattoir D, where the cleaning regime was identical to that of abattoir E, the incidence of *Salmonella* contamination in the holding pens (10.2%) was similar to that in abattoirs A and C (9.3% and 10.1% respectively) (table 3). Overall incidence of *Salmonella* contamination in the holding areas was 10%, the walls being the least often contaminated. Within individual abattoirs, the contamination of a particular sample site may be as much as 30.8% (holding pen corners, abattoir D). The difficulty of cleaning a corner may contribute to this high result, but also, at this particular abattoir, the drainage for the pens was situated in the pen corners, and it is possible that contamination gathers around the drains.

14% of samples taken from cattle stun boxes were positive for *Salmonella* spp, and the corners were more likely (20%) to harbour contamination than the floors (10%) and walls (0). Contamination in the cattle stun box in abattoir A was high (40%), but this facility in particular showed heavy corrosion in the corners and wear to the floor, making it difficult to clean thoroughly. The incidence of *Salmonella* spp in the stunning pens for small species was lower (4.9%) than that for cattle (14%), which was not expected, as pigs and calves would be expected to carry greater risk of excreting *Salmonella* spp than cattle. However, the number of samples taken in these areas is relatively low, and this may be an artefact of sample size, rather than an indication that cleaning was more effective in small species stunning facilities than in those for cattle.

Conclusions

The cleaning regimes in use in lairages at UK red meat abattoirs are often insufficient to remove *Salmonella* contamination from the holding pens and stun boxes. As a result, there is the risk of *Salmonella* spp persisting in the environment and potentially contaminating animals and carcasses processed on subsequent days. Abattoir managers should take care to ensure that the state of repair of the facility is such that cleaning can be carried out effectively, and put into place a system of monitoring of the effectiveness of cleaning in removing pockets of contamination.

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Tables and Figures

Table 1: Estimation of numbers of *Salmonella* organisms in original sample

Lowest dilution giving positive result

	Interpretation
Neat	1-10 organisms in the sampled surface area
-1	10-100 organisms in the sampled surface area
-2	100-1,000 organisms in the sampled surface area
-3	1,000-10,000 organisms in the sampled surface area
-4	10,000-100,000 organisms in the sampled surface area

Sample Site	Abattoir A		Abattoir B		Abattoir C		Abattoir D		Abattoir E		Total	
	Samples taken	Samples Positive	Samples taken	Samples Positive	Samples taken	Samples Positive	Samples taken	Samples Positive	Samples taken	Samples Positive	Samples taken	Samples Positive
Holding pen wall	20	0	5	0	25	0	15	1 (1-10)			65	1 (1-10)
Holding pen floor	20	3 (10-100)	5	0	25	3 (10-100)	15	0			65	6 (10-100)
Holding pen corner	12	1 (10-100)	12	1 (10-100)	23	2 (1-10)	13	4 (1-10)	6	0	54	4 (1-10)
		1(10 ² -10 ³)										1 (10-100)
Holding pen edge	12	0	26	5 (1-10)	26	1(10 ³ -10 ⁴)	13	1 (1-10)	4	0	55	6 (1-10)
								1 (10-100)				1 (10-100)
								1(10 ² -10 ³)				1(10 ² -10 ³)
Stun box wall	6 beef 5 sheep	0	12 beef 11 smalls	0	6 smalls	0	6 beef 6 sheep	0	9	0	33 beef 17 smalls	0
Stun box floor	6 beef 6 sheep	2 (10-100)	12 beef 11 smalls	0	9 smalls	0	6 beef	0	6	1(10 ² -10 ³)	30 beef 20 smalls 6 sheep	1 (1-10) 2 (10-100) 1(10 ² -10 ³)
Stun box corner	4 beef 4 sheep	1 (1-10) 100	6 beef 6 smalls	0	5 smalls	1(10 ² -10 ³)	4 beef	1(10 ² -10 ³)	6	1 (10-100)	20 beef 11 smalls 4 sheep	1 (1-10) 2 (10-100) 1(10 ² -10 ³)
Roll-out ramp	12 beef	0	11 beef	0			8 beef 2 sheep	0	6	0	39 beef 2 sheep	0

Totals

432

33

Table 3. Percentage of samples containing *Salmonella* spp by sampling site

Sample Site	Abattoir A	Abattoir B	Abattoir C	Abattoir D	Abattoir E	Total	Overall
Holding pen wall	0	0	0	6.7%	0	1.5%	
Holding pen floor	15%	0	20%	0	0	12.3%	10.0%
Holding pen corner	16.7%	0	0	30.8%	0	11.1%	
Holding pen edge	0	0	23.1%	23.1%	0	16.4%	
Total Holding Pens	7.8%	0	11.1%	14.3%	0		
Cattle							
Stun box floor	33.3%	0	0	0	16.7%	10%	14%
Stun box corner	50%	0	0	25%	16.7%	20%	
Total Stun Box	40%	0	0	10%	16.7%		
Small Species (Pigs, Sheep, Calves)							
Stun box floor	16.7%	0	0	0	0	3.8%	4.9%
Stun box corner	0	0	20%	0	0	6.7%	
Total Stun Box	10%	0	7.1%	0	0		
Overall Lairage Incidence	9.3%	0	10.1%	10.2%	5.4%		7.6%

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MICROBIOLOGICAL CONDITIONS AT LOCAL SLAUGHTERHOUSES IN HIDALGO, MEXICO

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Key Words: slaughterhouse, hygiene, carcass, microbial contamination

Introduction

Microbial contamination of meat occurs since animal is slaughtered when the microorganisms deposited on the surface of livestock, slaughter facility equipment, food handlers or present in the intestinal tract or in the environment contact with the meat as a consequence of deficient hygienic conditions during the slaughtering process. Pathogens commonly isolated from raw beef and pork include *Salmonella* spp., *E. coli*, *Campylobacter jejuni* and *Listeria monocytogenes* (Eisel et al., 1997) and *Pseudomonas*, *Enterobacteriaceae*, *Brochothrix thermosphacta* and lactic acid bacteria are considered frequent spoilage microorganisms present in meat (Gustavsson and Borch, 1993).

In this way, safety programs have been adopted in the slaughtering plants in order improve meat safety. Good manufacturing practices (GMP) emphasize sanitary effectiveness and hygienic practices during the processing of foods. Hazard analysis critical control point (HACCP) is mainly directed to identify and control foodborne pathogens (Eisel et al., 1997). In USA every slaughter plant that operates under federal inspections is committed to establish and carry out a HACCP program as well as apply sanitation standard operating procedures (SSOP) (USDA, 1996). In the European Union the UE Commission Decision (2001/471/EC) requires validated HACCP systems in the slaughter plants and conduct regular checks on general hygiene.

In México almost 50% of the slaughtering is done at the local slaughterhouses while TIF (Federal Inspection Type) slaughterhouses where more hygienic conditions are observed, account only for the 25% of the slaughter (SAGARPA, 2002). However no SSOP or HACCP programs are compulsory in any slaughterhouse in this country. So microbial counts in these establishments are expected to be quite high but no studies are related to the subject, especially Hidalgo State where there is not located any TIF slaughterhouse.

Objectives

To assess the microbial conditions of the slaughtering process at four small slaughterhouses located in Hidalgo State, Mexico.

Methodology

Four local slaughterhouses located in Hidalgo State (Mexico) were involved in the study where swine and cattle is slaughtered everyday. Data found for one of the studied slaughterhouses were previously reported (Hernández et al., 2004). Every slaughtering establishment was sampled 3-4 times both pork and beef lines. Every sampling time, nine carcasses were randomly selected immediately after slaughter and dressing and sampled. Four sites of 100 cm² were strongly swabbed with cheese cloth premoistened with buffered peptone and placed in a same sterile Stomacher bag constituting a single composite sample. In case of swine swabbed areas were ham, back, belly and jowl and in beef carcasses the swabbed zones were ham, belly, breast and jowl. Also knives used for the bleeding, scrapping, skinning and evisceration as well as saws used to split the sternum and carcasses of beef and hands of different personnel working were sampled by swabbing technique. The water from the scalding process and used to wash the carcasses was also analysed.

The swab samples were cultured and enumerated for the presence of total viable count (TVC), coliforms, *E. coli* and *Salmonella*. Plate count agar (PCA) was used to enumerate total viable count, Petrifilm Coliforms/*E. coli* (3M) was used to enumerate coliforms and *E. coli*. For detection of *Salmonella spp.*, 100 ml of buffered peptone water was used for pre-enrichment at 37°C for 18 h. Selective enrichment was done in Rappaport-Vassiliadis (RV) broth and Tetrathionate broth. Isolation and identification were performed by plating on xylose lysine desoxycholate (XLD) agar and modified brilliant green agar (BGAM). One suspect colony on each plate was inoculated on triple sugar iron agar (TSI) and lysine iron agar (LIA) for identification. Also multivalent serum agglutination was performed to confirm the presence of *Salmonella*.

Results & Discussion

A total of 467 samples were analysed (227 samples corresponding to pork lines and 240 samples corresponding to beef lines). Results of TVC (total viable count), coliforms, *E. coli* and incidence of *Salmonella* are shown in tables 1 and 2 for pork and beef samples, respectively. Total viable counts, coliforms and *E. coli* were recovered from the majority of samples indicating faecal contamination except in water samples where coliforms and *E. coli* were only detected in samples from one of the slaughterhouses. In that plant the origin of faecal contamination of water could be explained because the cleaning water is deposited in an opened container prior to be used and could be contaminated during the slaughtering process.

In general average TVC from carcasses, utensils and workers were quite similar in both lines around 4.5 Log cfu/cm² and values ranged from 2.08 to 7.90 Log cfu/cm² in pork samples and from not detected to 7.88 Log cfu/cm² in beef samples. Similar results for both lines were also observed for coliforms and *E. coli* in samples from workers and utensils but average counts were below 2 Log cfu/cm². Presence of coliforms and *E. coli* in utensils and workers hands indicates absence of good manufacturing practices which facilitate the contamination of carcasses although this effect was more noticeable in pork carcasses than in beef. These microbiological results were higher to those observed by Gill et al. (2000) in a small abattoir where different animal species were slaughtered.

The overall prevalence of *Salmonella* (18%) in the study was a rather high value similar to those observed by Korsak et al. (1998) in four pork slaughterhouses

however these authors did not find *Salmonella* spp in the beef slaughterhouses sampled. In our study prevalences of *Salmonella* were also irregular since this microorganism was hardly recovered in one of the plants which had a low rate of pork slaughter whereas its presence was more important in the slaughterhouses with a higher rate of pork slaughtering. It is well documented that *Salmonella* enters into the slaughterhouse through the animals, especially swine and although scalding step could reduce the presence of this pathogen, posterior operations as dehairing manual scraping and polishing and evisceration contribute significantly to cross contamination and could explain the prevalence of *Salmonella* in the carcasses, knives and hands of the workers in both lines in the rest of the slaughterhouses studied.

Conclusions

Elevated microbial counts present in carcasses, utensils and personnel working as well as high presence of coliforms and *E. coli* indicate poor hygienic conditions in all slaughtering establishments and confirm that hygiene standards and good management practices are extremely important to contain the rate of carcass contamination. In addition the implementation of some kind of post-slaughter treatment like hot water pasteurizing or spraying with one or several organic acids would be recommended to reduce the levels of microorganisms (Jay, 1996; Gill et al., 1999).

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Tables and Figures

Table 1. Microbial results in Log cfu/cm² or Log cfu/mL of the samples obtained from carcasses, utensils, workers and water at pork slaughtering process in the slaughterhouses sampled.

Number of samples	Sampling site	Microbial count	Mean	Standard deviation	Minimum	Maximum	Incidence (%)
95	Carcasses	TVC	4.65	0.75	3.28	6.90	100
		Coliforms	1.60	0.99	-0.60	4.64	100
		<i>E. coli</i>	1.18	0.78	-0.60	3.92	100
		<i>Salmonella</i>	-	-	-	-	17
58	Workers	TVC	4.46	1.05	ND	7.90	98
		Coliforms	1.63	1.14	ND	4.49	97
		<i>E. coli</i>	1.13	0.96	ND	3.76	95
		<i>Salmonella</i>	-	-	-	-	36
48	Utensils	TVC	4.68	1.06	2.08	7.08	100
		Coliforms	1.67	1.26	ND	4.94	90
		<i>E. coli</i>	1.16	0.99	ND	3.78	81
		<i>Salmonella</i>	-	-	-	-	13
13	Scalding water	TVC	3.20	1.14	ND	5.30	100
		Coliforms	0.09	0.34	ND	1.23	8
		<i>E. coli</i>	0.04	0.13	ND	0.48	8
		<i>Salmonella</i>	-	-	-	-	0
13	Cleaning water	TVC	2.29	1.32	ND	4.46	85
		Coliforms	0.19	0.62	ND	2.23	15
		<i>E. coli</i>	0.12	0.42	ND	1.52	8
		<i>Salmonella</i>	-	-	-	-	0

ND = Not detected

Table 2. Microbial results in Log cfu/cm² or Log cfu/mL of the samples obtained from carcasses, utensils, saws, workers and water at beef slaughtering process.

Number of samples	Sampling site	Microbial count	Mean	Standard deviation	Minimum	Maximum	Incidence (%)
100	Carcasses	TVC	4.50	1.14	2.31	7.41	100
		Coliforms	0.79	1.22	ND	6.01	86
		<i>E. coli</i>	0.12	0.85	ND	3.44	76
		<i>Salmonella</i>	-	-	-	-	18
58	Workers	TVC	4.69	0.88	2.52	6.90	100
		Coliforms	1.63	1.21	ND	5.63	91
		<i>E. coli</i>	1.10	1.05	ND	3.37	84
		<i>Salmonella</i>	-	-	-	-	19
43	Utensils	TVC	4.65	1.27	ND	7.88	98
		Coliforms	1.46	1.30	ND	7.26	74
		<i>E. coli</i>	0.87	1.09	ND	2.63	56
		<i>Salmonella</i>	-	-	-	-	19
26	Saws	TVC	4.38	1.30	2.28	7.45	100
		Coliforms	1.30	1.44	ND	6.40	92
		<i>E. coli</i>	0.17	0.83	ND	3.30	58
		<i>Salmonella</i>	-	-	-	-	15
13	Cleaning water	TVC	3.07	1.49	ND	5.32	92
		Coliforms	1.52	1.84	ND	4.76	31
		<i>E. coli</i>	1.21	1.62	ND	3.64	31
		<i>Salmonella</i>	-	-	-	-	0

ND = Not detected.

STUDIES ABOUT THE OCCURRENCE OF SHIGATOXIN-PRODUCING ESCHERICHIA COLI (STEC) IN MEAT PROCESSING COMPANIES

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Key Words: STEC, short fermented raw sausages, asymptomatic STEC-shedders

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) belong to a group of gut-pathogenic microorganisms, which were made responsible for food-poisoning first in 1982 (CDC, 1982). Since that time it has been world wide a number of outbreaks and sporadic illnesses. First of all, meat-products are of great importance as a zoonotic spread of EHEC. In 1994 raw sausage proved to have redeemed a group disease with EHEC in the United States (CDC, 1994). In 1995 more than 20 cases of HUS (hemolytic uremic syndrome) were reported from Australia after the consumption of raw Bologna sausage (CDC, 1995). Furthermore the significance of the infectious potential via human to human contact, respectively the contamination of food increases.

Several virulence factors can contribute to the pathogenicity of EHEC/STEC. The major factors, which also define the STEC group, are the Shiga toxins (Stx) comprising Stx 1 and Stx 2 with subtypes (Pierard et al., 1998; Schmidt et al., 2000). Other important virulence factors are the intimin- (encoded by *eae*-gene) and the hemolysin-production (encoded by *hly*-gene) and were closely associated with HUS (Bockemühl and Karch, 1996; Fruth et al., 2002).

Objectives

The aim of the studies was to investigate the contamination of meat products such as short fermented spread able raw sausages with Shiga toxin-producing *Escherichia coli* (STEC) and to determine the entry sources of STEC into meat processing companies.

Methodology

Sampling: From 1997 to 2002 samples were taken from sausage manufacturing plants and local supermarkets during five studies. Origins, kinds and number of the samples are shown in table 1. Stool samples and hand swabs were collected from healthy staff members working in the production line of the plants.

Screening for STEC: The samples were enriched in modified Tryptic soy broth (mTSB) and tested for the encoding gene for shigatoxin production (*stx*) by PCR according to Karch and Meyer (1989). After subcultivation of *stx*-positive sample

enrichments on Sorbitol MacConkey agar (SMAC) *stx*-positive colonies were isolated and characterised by biochemical methods. STEC confirmed as *E. coli* were further differentiated for other virulence factors by PCR (see table 2) and serotyped.

Results & Discussion

In order to investigate the occurrence of STEC in meat processing companies stool samples (n=1566) and hand swab samples (n=2366) from healthy employees of processing areas, swab samples from sanitary (n=699) and working areas (n=2850), samples of raw material (n=717) and short fermented raw sausages (n=2748) were collected in regular intervals from meat processing companies during five studies (1997 – 2002). STEC were detected in all sample materials except swab samples of sanitary areas.

Most of the STEC were isolated from product samples (1.5%, see figure 1). Some large EHEC-outbreaks were associated with contaminated beef or beef products (Bell et al., 1994). Short fermented raw sausages were involved in one outbreak: In 1995 a total of 23 cases of HUS were reported from Australia after the consumption of raw Bologna sausage (CDC, 1995).

In this work about 7% asymptomatic STEC-shedders (6% to 15%) were detected among the staff (n=233). The serotyping resulted in serotypes, which have been connected with human EHEC-diseases (e.g. O26, O91, and O103). These results are shown in figure 2.

Human shedders are important sources for STEC-infections (Carter et al., 1987; Pavia et al., 1990; Beutin and Niemer, 1995). However, there is a small knowledge about the prevalence of STEC in healthy people. Stephan et al. (2001) studied the occurrence of STEC in employees of food processing companies in Switzerland and found positive results in 3.5 %.

A total of 140 STEC-strains were isolated during the studies. About 58% of them (n=84) possessed the gene for production of shigatoxin 1 (*stx1*), 30% (n=44) of the strains had the *stx 2*-gene and 9% possessed *stx 1* together with *stx 2* (n=13). Strains with the *eae*-gene (n=15) were isolated only from stool and product samples (see table 3).

Stx 1 is regarded as less toxic for humans as *stx 2* and more connected with enteritis than with HUS (Pierard et al., 1998; Fruth et al., 2002). The other important virulence factors closely associated with HUS like the *eae*-gene and the *hly*-gene (Bockemühl and Karch, 1996; Fruth et al., 2002) were found in 10 and 42%, respectively. However, EHEC strains lacking *eae*- and *hly*-gene can also cause diseases in humans (Fruth et al., 2002). Therefore, it is not possible to differentiate high pathogenic EHEC from STEC by analysis of virulence factors. In consequence several authors required to consider food borne STEC as potential EHEC (Azavedo et al., 1994; Bockemühl and Karch 1996).

Conclusions

The results of the studies document the importance of asymptomatic carriers as a possible source of entry for EHEC/STEC into meat producing companies. Therefore regular examinations of staff are necessary to guarantee product safety. The source of infection for staff members could not be ruled out in the studies.

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Tables and Figures

Table 1: Origins, kinds and number of samples

plant	sampling period	sample material						
		stool	swabs of sanitary areas	hand swabs	swabs of working areas	raw material	meat juice	products**
I	4-8/1997	574 (100)*	285	-	522	-	-	-
II	4/1997- 12/1998	467 (22)*	256	-	762	-	168	623
retail	3/1999- 3/2000	-	-	-	-	-	-	139
III	10/2001- 9-2002	193 (59)*	53	609	473	224	-	813
IV	10/2001- 9-2002	158 (20)*	53	669	624	234	198	501
V	10/2001- 9/2002	174 (32)*	52	1088	469	259	-	672
Total	1997- 2002	1566 (233)*	699	2366	2850	717	366	2748

* in brackets: analyzed staff members (people, who came in contact with raw material or finished products)

** products: short fermented raw sausages like „Teewurst“

Table 2: Targets, primers, PCR conditions and references

Target	Primer	Reference
<i>stx</i>	MK1/MK2	Karch and Meyer, 1989
<i>eae</i>	SK1/SK2	Schmidt et al., 1993; Yu and Kaper, 1992
<i>hlyA</i>	hlyA1/hlyA2	Schmidt et al., 1995
<i>stx 1</i>	KS7/KS8	Rüssmann et al., 1995
<i>stx 2</i>	LP43/LP44	Cebula et al., 1995
<i>stx 2, stx 2c*</i>	GK3/GK4	Schmidt et al., 1994
<i>*after Hae III-restriction</i>		Schmitt et al., 1998
<i>stx 2d</i>	VT2-cm/VT2-f	Pierard et al., 1998
<i>stx 2e</i>	slt2v start/slt2v stop	Weinstein et al., 1988
<i>ast A</i>	AstA1/AstA2	Savarino et al., 1993
<i>col D 157</i>	col D1/col D2	Hofinger et al., 1998
<i>esp P</i>	Esp A/Esp B	Brunder et al., 1997
<i>etp D</i>	D1/D13R	Schmidt et al., 1997a
<i>ile X</i>	356/595	Schmidt et al., 1997b
<i>kat P</i>	wkatB/wkatF	Brunder et al., 1996

Table 3: Numbers, origins and virulence factors of the isolated STEC strains
sample materials

Isolates	Stool	swabs of sanitary areas	THand	swabs of working areas	Raw materials	Meat juices	Products	Total
n	63	1	11	4	9	3	53	144
<i>stx1</i>	53	1	3	2	4	2	19	84
<i>stx2</i>	8	-	7	1	6	1	21	44
<i>stx1+2</i>	1	-	1	1	-	1	9	13
<i>eae</i>	10	-	-	-	-	-	5	15
<i>Ehly</i>	39	-	1	1	-	-	19	60
<i>astA</i>	15	1	3	1	1	2	12	35
<i>colD157</i>	6	-	-	-	-	1	5	12
<i>katP</i>	3	-	-	-	-	1	4	8
<i>espP</i>	7	-	5	1	3	-	11	27
<i>etpD</i>	12	-	-	-	-	-	5	17
<i>ileX</i>	1	-	2	-	-	-	4	7

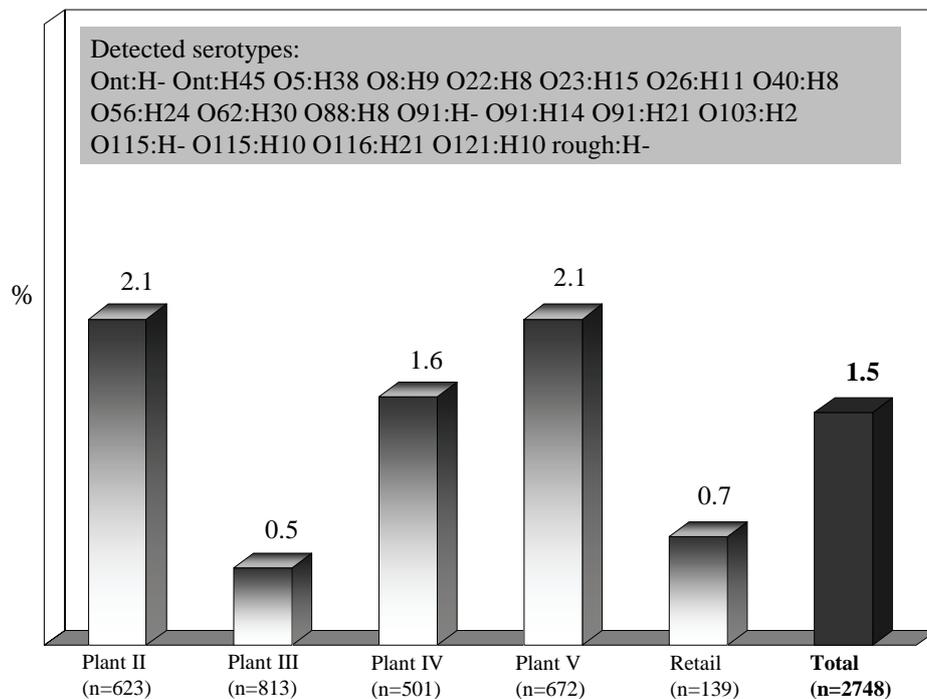


Figure 1: STEC in short fermented raw sausages (1997 – 2002)

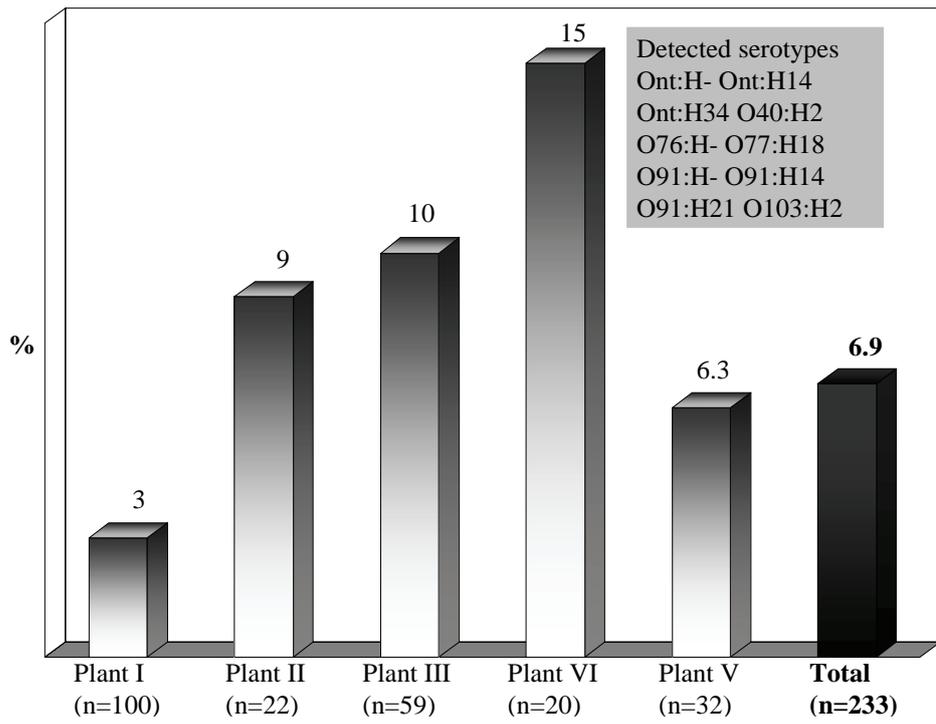


Figure 2: STEC shedders in meat processing plants (1997 – 2002)

GENERALIZED MODELING FOR THERMAL INACTIVATION OF SALMONELLA IN MEAT AND ENHANCEMENT OF THE AMI PROCESS LETHALITY SPREADSHEET

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Key Words: Modeling, Salmonella, Meat products, Thermal inactivation, AMI process lethality spreadsheet, Lethality performance standards

Introduction

In order to ensure the microbial safety of ready-to-eat (RTE) meat and poultry products, the U.S. Department of Agriculture (USDA) has required a 6.5-Log reduction in *Salmonella* spp. for cooked beef, RTE roast beef, and cooked corned beef products, and a 7-Log reduction in certain fully and partially cooked poultry products (USDA 1999). Additionally, USDA has proposed to extend the performance standards to all RTE meat products (USDA 2001). However, the ability of the industry to produce reliable and uniform thermal process validations on a broad scale has not been tested.

The existing tools for calculating process lethality do not account for all of the variables that might affect pathogen survival, and the acceptability of these tools for regulatory compliance has been recently questioned (USDA 2002). For example, the current version of the widely used American Meat Institute process lethality spreadsheet (AMI-PLS) calculates the F-value of a process, given time-temperature data, a reference temperature, and a z value. The user then must calculate log reductions, given a reference D value. Additionally, the AMI-PLS does not account for product characteristics, and therefore the user must know that the D and z values are valid for the product of interest. Unfortunately, a large percentage of the AMI-PLS users likely input the example D and z values provided in the spreadsheet, without knowledge of their validity for their specific products.

In general, temperature, fat content, and moisture content are important factors for thermal inactivation of *Salmonella*. Juneja (2001) conducted research on thermal inactivation of a *Salmonella* cocktail (Thompson, Typhimurium, Heidelberg, Enteritidis, Montevideo, Copenhagen, and Hadar) in different kinds of meat products, such as ground chicken, ground turkey, and ground beef. The general D-values at 58, 60, 62.5, and 65°C were around 7, 5, 1.5, and 0.6 min, respectively (depending on the product types). Murphy (2003) worked with a *Salmonella* cocktail in ground chicken breast and obtained D-values at 56, 60, 62, and 63°C of 3.2, 0.6, 0.31, and 0.18 min, respectively. Humphrey (1997) tested *Salmonella* Typhimurium DT 104 in ground beef and reported D-values at 58 and 60°C of 2.62 and 1.35 min, respectively. Table 1 (poultry) and Table 2 (beef) show the D-values for thermal inactivation of *Salmonella*, depending on the fat content, moisture content, and temperature.

Overall, the hypothesis of this project was that the D-value of *Salmonella* in meat products could be successfully modeled in a general way, as a function of temperature, fat content, and moisture content.

Objectives

The specific objectives were: 1) To develop and validate a complete secondary model for logD as a function of temperature, fat content, and moisture content, with a 95% confidence limit as reference, and 2) To improve the AMI-PLS by integrating the new logD model and adding a user-friendly front-end.

Methodology

1. Data

Data Source Previous work (before 2004) was evaluated in the general area related to the project objective - product factors affecting thermal resistance of *Salmonella* in different kinds of meat (poultry, beef and pork). Most of related studies were identified through Combase, journal indexes, and in-house research data.

Data Selection Data were selected only when they met the following requirements: 1) temperature of 50-70°C; 2) experimental methods stated clearly; 3) fat content and moisture content analyzed; 4) D-value reported or could be calculated from the data; 5) good correlation ($R^2 > 0.85$) for logN and time in the data. After this screening, data from 18 different studies were selected for further analysis.

2. Model

Based on the temperature, fat content, and moisture content, the complete secondary model was selected by using back elimination ($\alpha = 0.05$). The variables were Fat, Moisture, and Temperature; the dependent variable was logD. The model was

$$\text{LogD} = \alpha + \beta_{11}T + \beta_{12}F + \beta_{13}M + \beta_{21}TF + \beta_{22}TM + \beta_{23}FM + \beta_{31}T^2 + \beta_{32}F^2 + \beta_{33}M^2 + \varepsilon$$

where D is the D value (min); T is temperature (°C); F is the fat content (%); and M is the moisture content (%).

3. AMI Spreadsheet Improvement

Currently, a user of the AMI-PLS must provide time-temperature data, along with D and z values at a reference temperature; however, the spreadsheet does not directly calculate log reduction for a given process. Our modification of the AMI-PLS includes: 1) development of a user-friendly front-end, via Visual Basic, for input of product attributes; 2) integration of the new generalized model for the D value; and 3) direct calculation of log reduction, and the confidence interval for those calculations. The front-end asks the user for the product species, product type, and product composition (fat, moisture, and pH). After selecting the product species and product type and inputting product compositions, clicking the submit button calculates the parameters for the logD model, which are imported directly into the spreadsheet. According to the data input, the spreadsheet reports the reference D-value at 62.8°C ($\pm 95\%$ confidence interval), and the accumulated log reduction at each processing time.

Results & Discussion

Data Analysis *Salmonella* Senftenberg is the most thermally resistant species among all the species in *Salmonella*, but it is seldom the source of outbreaks related to meat and poultry. Therefore, data sets that included *Salmonella* Senftenberg were ignored in this study. Also, because the research methods were different from one laboratory to the other, and the range of D-value and z-value were large, some outlier data (where predicted minus actual $\log D > 0.3 \log[\text{min}]$) were excluded, due to the need for more data to verify those values. The data used for the model are listed on Table 1 (poultry, 5 different studies from 4 researchers, 58 data sets) and Table 2 (beef, 3 different studies from 3 researchers, 28 data sets). The ranges of temperature, fat content, and moisture content for poultry were 55 - 65°C, 1 - 14.2%, and 67 - 75% respectively; the ranges of temperature, fat content, and moisture for beef were 55 - 65°C, 4.8 - 24%, and 57 - 72.4% respectively.

Model Selection The final model for poultry was:

$$\log D = -92.589 + 0.750F + 1.633M + 1.240T - 0.0035F^2 - 0.0101M^2 - 0.01T^2 - 0.0099FM + 0.0024MT$$

The R^2 was 0.97, and observed vs. predicted Log D is shown in Figure 1. The root mean squared error (RMSE) was 0.08518 log(min). The final model for beef was

$$\log D = -237.59 + 2.307F + 3.309M + 4.086T - 0.0048F^2 - 0.0375FT - 0.0569MT$$

The R^2 was 0.97, and observed vs. predicted Log D is shown in Figure 2. The RMSE was 0.1365 log(min).

AMI Spreadsheet Improvement The user-friendly front-end (Figure 3) includes the product species selection, product type selection, and product parameters input. After selecting the product species and product type and inputting product parameters, clicking the submit button leads the user to the spreadsheet (Figure 4). The data currently included in the model encompass only beef and poultry, no variation in product structure (e.g., whole vs. ground samples), and no meaningful variation in pH. Therefore, other species and product structure are currently non-active choices in the input box, and the pH input is merely returned in the output worksheet.

Conclusions

According to the data analysis and modeling, the D-value could be successfully modeled in a general way, as a function of temperature, fat content, and moisture content. However, further research is needed to validate the model with independent results for more accurate prediction. Currently, the confidence intervals (CI) for the D-value are still quite wide. Therefore, more data sets are needed to narrow the CI and improve model accuracy, and the enhanced version of the AMI-PLS described here is not yet ready for distribution. Additionally, we are working on incorporating data for pork and different product structure (ground or whole muscle) into an expanded model set, which will enhance the AMI-PLS functionality.

Acknowledgement

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Tables and Figures

Table 1. List of product parameters and D-values for thermal inactivation of *Salmonella* in poultry.

Log D	Fat	Moisture	Temp	D value	Log D	Fat	Moisture	Temp	D value
0.875061	1	72	58	7.5	0.897627	1.1	72.3	55	7.90
0.658965	1	72	60	4.56	1.026125	1.1	72.3	55	10.62
0.184691	1	72	62.5	1.53	1.118595	1.1	76.3	55	13.14
-0.22915	1	72	65	0.59	0.895975	13.02	64.5	55	7.87
0.887054	7	72	58	7.71	0.913814	13.02	66.5	55	8.20
0.693727	7	72	60	4.94	1.039811	13.02	66.5	55	10.96
0.267172	7	72	62.5	1.85	0.994757	13.02	68.5	55	9.88
-0.25964	7	72	65	0.55	0.975891	1.1	72.3	55	9.46
0.839478	10	67	58	6.91	1.168203	1.1	71.5	55	14.73
0.710117	10	67	60	5.13	1.151982	1.1	71.9	55	14.19
0.161368	10	67	62.5	1.45	1.067443	1.1	71.4	55	11.68
-0.24413	10	67	65	0.57	0.993877	1.1	72	55	9.86
0.869818	12	68	58	7.41	1.079543	1.1	72.5	55	12.01
0.7348	12	68	60	5.43	1.016616	1.1	72.4	55	10.39
0.25042	12	68	62.5	1.78	1.089552	1.1	71.9	55	12.29
-0.22915	12	68	65	0.59	1.034227	13.02	68.5	55	10.82
0.870404	8.85	70.2	58	7.42	-0.29243	6.3	72	65	0.51
0.683047	8.85	70.2	60	4.82	0.931458	9	68	58	8.54
0.178977	8.85	70.2	62.5	1.51	0.732394	9	68	60	5.4
-0.09691	8.85	70.2	65	0.8	0.064458	9	68	62.5	1.16
0.665581	14.2	73	57	4.63	-0.27572	9	68	65	0.53
0.130334	14.2	73	60	1.35	0.956168	12	69	58	9.04
0.868056	2	75	58	7.38	0.740363	12	69	60	5.5
0.683947	2	75	60	4.83	0.113943	12	69	62.5	1.3
0.056905	2	75	62.5	1.14	-0.30103	12	69	65	0.5
-0.38722	2	75	65	0.41	0.850033	8.45	71.75	58	7.08
0.865104	6.3	72	58	7.33	0.716003	8.45	71.75	60	5.2
0.670246	6.3	72	60	4.68	0.133539	8.45	71.75	62.5	1.36
0.064458	6.3	72	62.5	1.16	-0.22915	8.45	71.75	65	0.59

Table 2. List of product parameters and D-values for thermal inactivation of *Salmonella* in beef.

Log D	Fat	Moisture	Temp	D value
0.507856	7	71	58	3.22
0.390935	12	65	58	2.46
0.396199	18	62	58	2.49
0.206826	24	57	58	1.61
0.956649	4.8	72.4	55	9.05
0.354108	4.8	72.4	58	2.26
-0.24413	4.8	72.4	61	0.57
-0.82391	4.8	72.4	64	0.15
1.023252	4.8	72.4	55	10.55
0.332438	4.8	72.4	58	2.15
-0.38722	4.8	72.4	61	0.41
-1.1549	4.8	72.4	64	0.07
1.01157	4.8	72.4	55	10.27
0.313867	4.8	72.4	58	2.06
-0.36653	4.8	72.4	61	0.43
-0.85387	4.8	72.4	64	0.14
1.342028	19.1	63.4	55	21.98
0.419956	19.1	63.4	58	2.63
-0.18709	19.1	63.4	61	0.65
-0.79588	19.1	63.4	64	0.16
1.270912	19.1	63.4	55	18.66
0.5302	19.1	63.4	58	3.39
-0.24413	19.1	63.4	61	0.57
-0.69897	19.1	63.4	63	0.2
0.937016	12.45	65.5	58	8.65
0.738781	12.45	65.5	60	5.48
0.176091	12.45	65.5	62.5	1.5
-0.17393	12.45	65.5	65	0.67

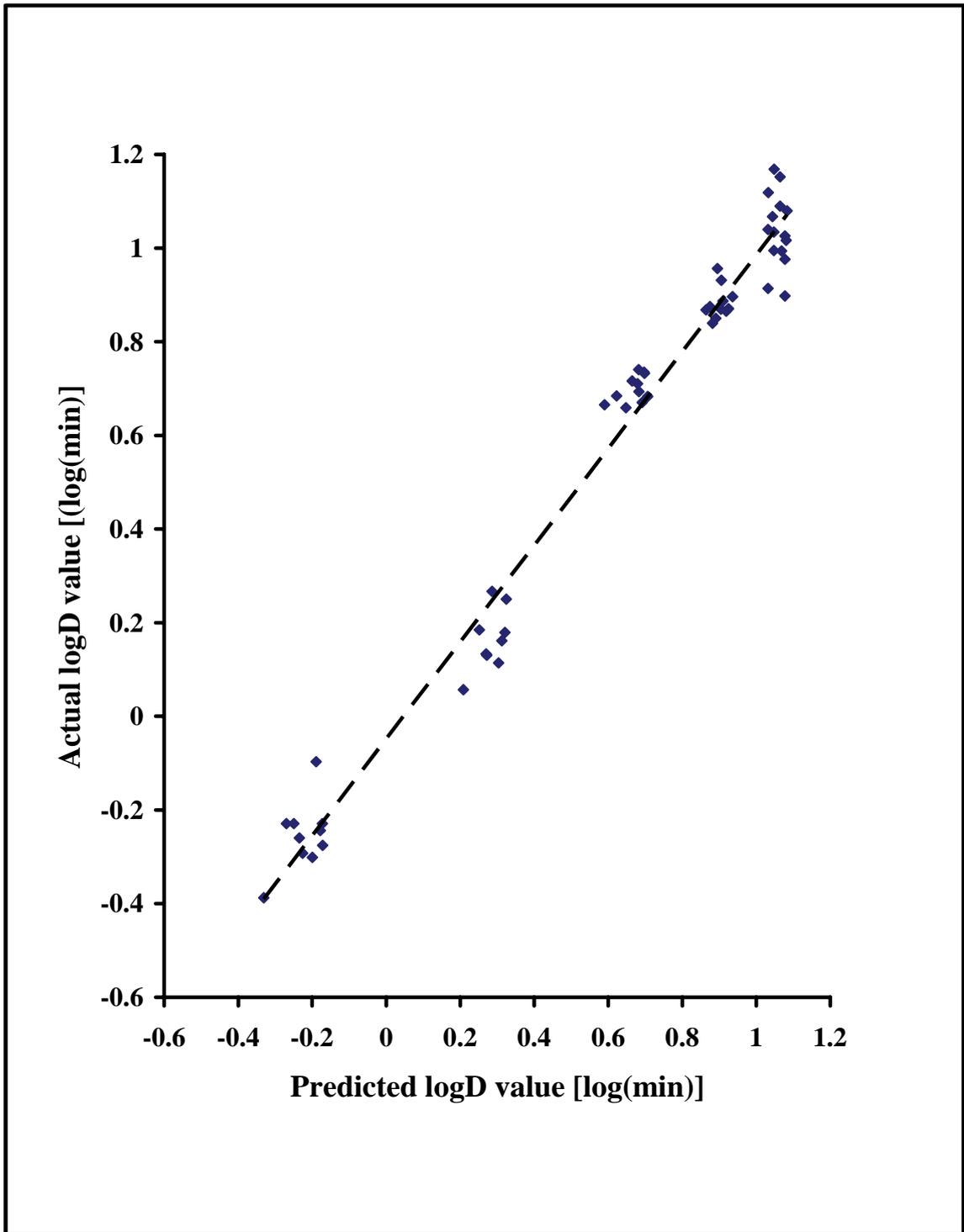


Figure 1. Observed vs. predicted log D from generalized model for thermal inactivation of *Salmonella* in poultry.

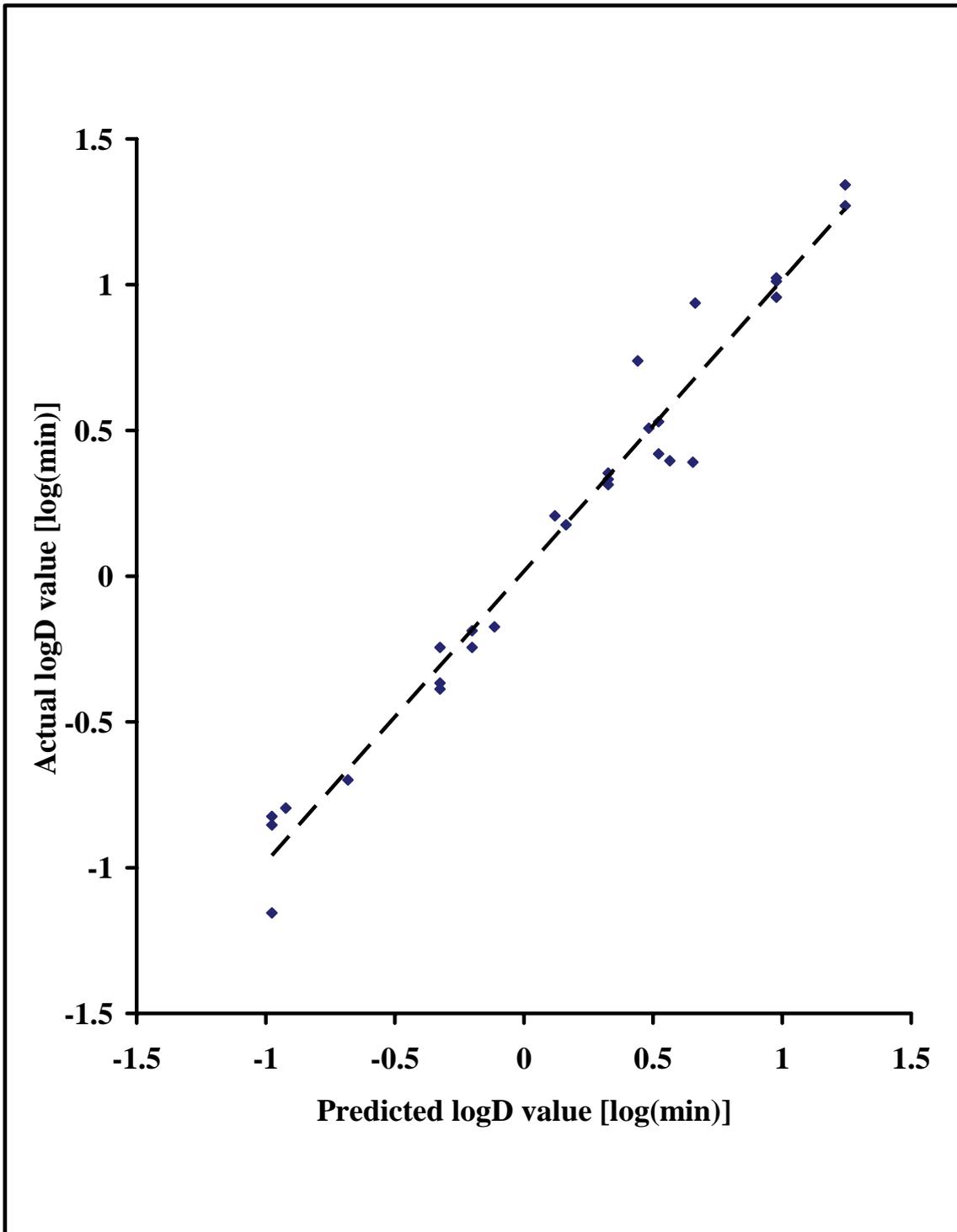


Figure 2. Observed vs. predicted log D from generalized model for thermal inactivation of Salmonella in beef.

AMI--Process Lethality For Salmonella

Product Attributes --Input

SELECT: Product

Beef
 Pork
 Poultry
 Other

SELECT: Product Type

WholeMuscle
 Restructured
 Ground
 Other

ENTER:

Fat Content %:	<input type="text"/>	4.8--24
Moisture Content%:	<input type="text"/>	57--72.4
pH:	<input type="text"/>	5.5--7.5

Figure 3. The front-end for input to the enhanced process lethality spreadsheet.

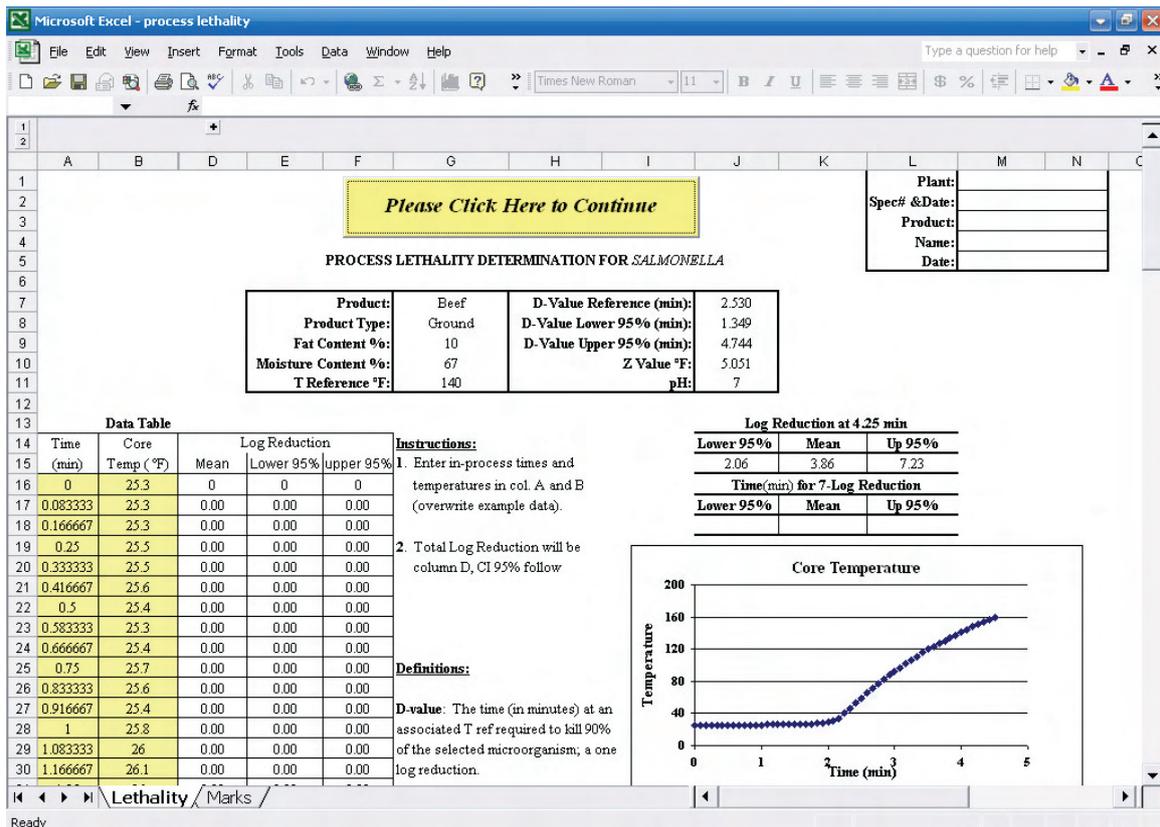


Figure 4. The spreadsheet showing product attributes, reference D-value with 95% confidence limit, and resulting log reductions with confidence limits.

EVALUATING THE USE OF PREDICTIVE MODELS FOR CRITICAL LIMIT VALIDATION IN RAW PORK PRODUCTS

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Key Words: Salmonella, Escherichia coli O157:H7, Staphylococcus aureus, raw pork, predictive model

Introduction

Under the Hazard Analysis Critical Control Point (HACCP) system, Critical Control Points (CCP's) identified by the processor are steps in a food process at which control can be applied in order to prevent, eliminate, or reduce the likelihood of a food safety hazard. (USDA 1996). The major CCP in HACCP plans for raw meat and poultry products is often the production step at which the product is the warmest or a later cooling step. Critical limits at a CCP must be met to ensure control of hazards previously identified by the processor. For raw meat and poultry products, these hazards are the growth of the infectious pathogens *Escherichia coli* O157:H7 and *Salmonella* spp., and excessive growth of *Staphylococcus aureus* which may result in the production of heat-stable enterotoxin. Ideally, critical limits associated with these pathogens would be validated using experimental challenge studies, but this is not feasible for most very small processors. An alternative validation method would be to analyze processing parameters using computer-generated predictive models of pathogen behavior such as the USDA Pathogen Modeling Program (PMP 7.0; Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA). A shortcoming of this method is that it is based on isothermal growth conditions using laboratory media and not an actual meat product. The goal of our project was to develop a model based on isothermal growth conditions in raw pork and to evaluate the resulting model with a series of challenge studies.

Objectives

Develop a predictive model for pathogen growth during the short-term temperature abuse of pork products using lag-times experimentally determined in isothermal studies using ground pork

Conduct pathogen challenge studies and evaluate safety predictions derived from our resulting model and PMP 7.0.

Methodology

Preparation of Inoculum. Five-strain cocktails of *Escherichia coli* O157:H7, *Salmonella* spp., and *Staphylococcus aureus* were used to inoculate meat products. Inoculation cultures (20-24 h, 35°C) were prepared in Brain Heart Infusion broth (BHIB; Difco, Becton-Dickinson, Sparks, MD) from working culture plates. To prepare multi-strain cocktails, the five strains for each pathogen were combined into a

50 mL centrifuge tube (Falcon Brand, Fisher Scientific, Itasca, IL) and centrifuged for 12 minutes at 5,000 x g. The supernatant in each tube was decanted and the pellet was re-suspended in 25 ml of Butterfield's phosphate diluent (BPD, Nelson Jameson, Marshfield, WI). From each of the 5-strain pathogen cocktails, 10 mL were combined into another 50 mL centrifuge tube, creating a 15-strain, 3-pathogen cocktail which was then diluted 1:100 in BPD to make the final inoculum. Separate inocula were prepared for each trial as described above.

Isothermal Studies. Ground pork was used in developing a conservative isothermal-based model (IBM) for predicting growth in a wide range of pork-containing products, such as bacon, frankfurters, and sausage, that may contain inhibitory ingredients such as spice, salt, and cure agents. Boneless pork loin roasts were obtained from a local retailer and ground (4 mm grinding plate). Ground product was then stored at -20 °C until 24 h before use when the product was thawed at 5°C. Thawed ground pork (25 g) was packed into 50 mL centrifuge tubes and a hole ~ 3-4 mm in diameter was placed in the center of the pork using a sterile bent plastic spreader (Daigger, Vernon Hills, IL) to a depth of ~ 2 cm for inoculation. Isothermal studies were conducted at 2.8 °C (5 °F) intervals ranging from 21 - 49°C (70 - 120 °F). Meat-packed centrifuge tubes were held at a specific temperature until a calibrated temperature probe (K-type, Dickson, Inc.; Chicago, IL) indicated that the center of the meat reached the test temperature. Each sample was then inoculated in the hole created in the center of the meat mass with 100 µL of the pathogen cocktail. Samplings were at time-zero and hourly thereafter for three concurrent trials with one tube per trial sampled at each time.

Challenge Study. To evaluate the IBM, pathogen behavior in ground hot-boned pork and spiced pork sausage containing ground hot-boned pork (obtained from a processor) was evaluated during cooling and abusive storage. The ground hot-boned pork had pH of 6.1 and contained 4.3% water-phase salt, and spiced pork sausage had pH of 6.2 and contained 4.6% water-phase salt. Preparation and inoculation of product was done identically to the isothermal studies except one trial was done with two tubes prepared for each sampling time. Two cooling curves, differing in cooling rate, were developed for each product (Table 1) to mimic commercial practice. Each product was warmed to the starting temperature, inoculated and then cooled to 3°C (34.7°F) in 7 h by gradually decreasing the temperature of an incubator. Another study with spiced pork sausage, involved abusing the product by holding it at 21°C for 17 hrs. At several time intervals throughout the cooling and holding processes, duplicate samples were analyzed for inoculum cell numbers.

Enumeration of Inoculum Organisms. For microbial analysis, the contents of a centrifuge tube were transferred to a filter sampling bag, suspended with 99 mL of BPD and stomached at medium speed for 30s. Subsequent dilutions were made in BPD and spread-plates were prepared (one plate per dilution) on Sorbitol MacConkey agar (SMAC; Oxoid, Inc., Ogdensburg, NY), XLD agar (Oxoid), and Baird-Parker agar base (B-P; Difco) with tellurite egg yolk supplement (Difco) for enumeration of *E. coli* O157:H7, *Salmonella* spp, and *Staphylococcus aureus*, respectively. Plates were incubated at 35°C for 24 h (SMAC and XLD) and 48 h (B-P). Typical colonies were counted and log CFU/sample was calculated. For challenge studies 3M™ Petrifilm™ Staph Express plates (PF-SE; 3M Microbiology, St. Paul, MN) were used to enumerate *S. aureus*. The PF-SE plates were incubated at 35°C for 24 h. Only red-to-purple colonies were observed on PF-SE plate after 24 h for all samples; these were counted as presumptive *S. aureus* and the thermonuclease disk analysis was not necessary. It has been shown that there is no significant difference between counts on

PF-SE and B-P for meat products (Ingham et al, 2003). Throughout the study, the identity of all presumptive isolates was confirmed.

Data Analysis and Model Development. From the isothermal study data, a Critical Time (CT) was defined for each pathogen at each temperature. For *Salmonella* spp. and *E. coli* O157:H7, CT was defined as lag time (time until one doubling or 0.3 log CFU increase). Because *S. aureus* is only a significant hazard under conditions allowing growth to high levels and production of enough enterotoxin to cause illness (Jay, 1992), CT for this pathogen was defined as the time until a 1.3 log CFU increase in population had occurred, i.e. lag time plus 1.0 log CFU increase. The change in population at each sampling time (relative to time-zero) for each independent trial was calculated. The mean change in log CFU/sample and standard deviation were then calculated for each sampling time for the three independent trials. Using a one-sided t-test (Snedecor and Cochran, 1980), the CT values at each test temperature were determined, and an IBM was developed to predict growth for each challenge study (Table 2). In order to use the IBM to predict pathogen behavior in ground hot-boned pork and spiced pork sausage, the temperature profile for each trial was divided into 2.8°C (5°F) intervals and the time that the temperature was within each interval was determined. In chronological order, the time for which the product was in a given temperature interval was divided by the CT for the lowest temperature in the interval. The resulting value, multiplied by 100, equaled the % of CT elapsing in the time interval. For example, if a product was between 23.8 and 21°C for 25 minutes and the CT for *S. aureus* at 21°C was 400 minutes, then the % CT for that interval would be $6.7 = [(25 / 400) \times 100]$. With each successive temperature interval during product cooling, the % of CT in that interval was calculated, along with the cumulative % CT. When cumulative % CT exceeded 100 for a pathogen, the IBM predicted that the process was unsafe.

For comparison, the same product temperature intervals were used in two different applications with the PMP 7.0 model. In the first application (PMP 7.0-plain) compositional values of pH 6.2, % water-phase salt of 0.5, and 0 ppm ingoing nitrite were assigned. A second application (PMP 7.0- product) used the actual product pH and % water-phase salt values. The pathogen initial level was set to 3.0 log, and the “level of concern”, or LC, was 0.3 log CFU higher for *Salmonella* spp. and *E. coli* O157:H7, and 1.3 log CFU higher for *S. aureus*. When aerobic and anaerobic models existed for a pathogen, the more conservative, i.e. more likely to predict growth, aerobic model was chosen. In chronological order, the time in each temperature interval was divided by the predicted LC time, and then multiplied by 100 to result in the % of LC time. A cumulative % LC time was calculated as each temperature interval was analyzed. When this value exceeded 100 for a pathogen, the process was predicted to be unsafe.

The log CFU/sample data from the challenge studies was statistically analyzed as described above for the isothermal studies and the mean change in log CFU/sample at each sampling time was evaluated to determine whether the CT had been exceeded.

The predicted CT of IBM and the predicted LC time of the PMP 7.0-plain model for the challenge studies were evaluated by a paired t-test (Snedecor and Cochran, 1980) with a 5% significance level. When a model predicted no CT or LC time for a given trial, a value of the length of the trial plus 1 minute was used.

Results & Discussion

No significant pathogen growth occurred in either pork product during cooling (Table 3), suggesting that use of critical limits based on the cooling curves would ensure product safety. However, significant growth did occur in spiced pork sausage held at 21.1°C for 17 h. Our IBM and PMP 7.0-plain predicted growth of *Salmonella* spp. and *E. coli* O157:H7 for all cooling curves. The PMP 7.0-plain also predicted growth of *S. aureus* during slow cooling of ground hot-boned pork. Growth of all three pathogens was predicted by IBM and PMP 7.0-plain for 21°C storage of spiced pork sausage. It is clear that both IBM and PMP 7.0-plain are conservative in predicting pathogen growth. PMP 7.0-product, using the actual pH and % water-phase salt of both products, did not predict growth for the cooling curve challenge studies and agreed with experimental data. However, PMP 7.0-product failed to predict growth of *E. coli* O157:H7 and *S. aureus* which occurred in the 21°C storage experiments with spiced pork sausage. Thus, the use of this application of PMP 7.0 can be fail-dangerous and therefore inappropriate for processors to use, while our model and PMP 7.0-plain were fail-safe. IBM and PMP 7.0-plain were not significantly different in predicting CT and LC time. Thus, IBM effectively validates use of the PMP 7.0-plain model. However, processors must perform and interpret several calculations in using PMP 7.0-plain to evaluate their process safety. Further development of IBM will result in the processor only having to input time and temperature data to obtain an easily understandable output of “safe” or “not safe”.

Conclusions

In conclusion, our isothermal-based model (IBM) and the PMP 7.0-plain application of PMP 7.0 are not significantly different when applied to the cooling and holding of raw ground pork products. Both models are conservative in their predictions, which will help processors ensure a safe product. Further development of IBM will make it simple for processors to use in predicting process safety.

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Tables and Figures

Table 1. Incubator settings for challenge study cooling of ground hot-boned pork and spiced pork sausage.

Ground hot-boned/Rapid		Ground hot-boned/Slow		Spiced pork sausage/Rapid		Spiced pork sausage/Slow	
temp (°C)	time (min)	temp (°C)	time (min)	temp (°C)	time (min)	temp (°C)	time (min)
37.7	Start	37.7	Start	21.0	Start	21.0	Start
32.2	30	32.2	45	15.6	45	18.3	45
26.7	30	29.4	30	10.0	45	15.6	45
21.0	30	26.7	30	8.9	30	12.8	45
15.6	45	23.9	30	7.8	30	10.0	45
10.0	45	21.0	30	6.7	30	8.9	30
8.9	30	18.3	45	5.6	30	7.8	30
7.8	30	15.6	45	4.4	30	6.7	30
6.7	30	12.8	45	3.0	180	5.6	30
5.6	30	10.0	45			4.4	30
4.4	30	7.2	30			3.0	90
3.0	90	4.4	30				
		3.0	15				

Table 2. Isothermal-based model (IBM) used to predict process safety

Temp (°C)	Time (min) at given temperature to reach Critical Limit		
	<i>E. coli</i> O157:H7	<i>Salmonella</i> spp	<i>S. aureus</i>
37.8	120	240	480
35.0	120	240	480
32.2	180	240	480
29.4	180	300	480
26.6	300	300	420
23.8	300	300	720
21.0	360	420	720

Table 3. Experimental and predicted growth of *Escherichia coli* O157:H7 (EC), *Salmonella* spp. (SALM), and *Staphylococcus aureus* (SA) in cooling/warm holding of two pork products. Predicted values were obtained from our isothermal-based model (IBM), the USDA ARS PMP 7.0 model with (PMP 7.0-product) and without (PMP 7.0-plain) adjustment for actual product pH and % salt.

Pathogen Growth: - (safe; no growth) or time (min) at which growth observed/predicted

Product/Cooling	Pathogen	Experimental	IBM	PMP 7.0 plain	PMP 7.0 product
Ground hot-boned pork/Rapid	<i>EC</i>	-	274	241	-
	<i>SALM</i>	-	367	351	-
	<i>SA</i>	-	-	-	-
Ground hot-boned pork/Slow	<i>EC</i>	-	242	166	-
	<i>SALM</i>	-	337	312	-
	<i>SA</i>	-	-	362	-
Spiced pork sausage /Rapid	<i>EC</i>	-	360	396	-
	<i>SALM</i>	-	420	408	-
	<i>SA</i>	-	-	-	-
Spiced pork sausage mix/Slow	<i>EC</i>	-	360	396	-
	<i>SALM</i>	-	420	408	-
	<i>SA</i>	-	-	-	-
Spiced pork sausage /21°C hold	<i>EC</i>	660	360	396	-
	<i>SALM</i>	900	420	408	936
	<i>SA</i>	1020	720	606	-

PREDICTIVE MODEL FOR OUTGROWTH AND GERMINATION OF *CLOSTRIDIUM PERFRINGENS* SPORES IN CURED AND UNCURED PORK HAM DURING COOLING

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Key Words: *Clostridium perfringens*, pork ham, cooling, predictive model, performance standard

Introduction

Clostridium perfringens is a ubiquitous organism normally present in a variety of meat and poultry products and frequently associated with foodborne disease outbreaks (Doyle, 2002; ICMSF, 1996; Labbe, 2000). The U.S. Centers for Disease Control and Prevention (CDC) estimates more than 248,000 cases of foodborne illness due to Cp annually in the United States (Mead et al, 1999). The major contributing factors leading to food poisoning by Cp include its ability to form heat resistant spores that survive commercial cooking operations and its very rapid growth rate at relatively high temperatures (Labbe, 2000). Germination and outgrowth of Cp spores during cooling of thermally processed meat products has been reported extensively in the literature (Doyle, 2002; Labbe, 2000, ICMSF, 1996). Predictive models for its growth in some meat systems are currently available in the literature and as electronic databases such as the USDA's Pathogen Modeling Program and ComBase (Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001). These predictive models are used as decision-making tools to determine potential growth of the target organism and assert product disposition. USDA-FSIS published performance standards for lethality and stabilization of meat and poultry products (USDA, 1999, 2001). Further, the agency provided guidelines as safe harbors for cooling of these products. The stabilization performance standards focus on the potential survival of sporeformers during traditional thermal processing schedules and their germination and outgrowth during cooling. The stabilization guidelines state that processed meat and poultry products be cooled from 54.5°C (130°F) to 26.6°C (80°F) within 1.5 h and from 26.6°C (80°F) to 4.4°C (40°F) within 5 h. While cured products are required to be cooled from 54.5°C to 26.6°C within 5 h and from 26.6°C to 4.4°C in 10 h. Because of the risk of Cp growth under improper cooling conditions; meat processors incorporate cooling regimes as critical control points in their HACCP plans. However, equipment malfunctions or power failures may occur in commercial processing operations and cause cooling deviations, resulting in growth of Cp. Because of this, more information is necessary on the growth characteristics of this organism subjected to different rates of cooling in commercially formulated products. expected to be more accurate in describing the behaviors of Cp in commercially processed products

Objectives

Hypothesis: Predictive models developed under commercial conditions of thermal processing and cooling will accurately describe outgrowth of Cp spores in artificially inoculated pork ham subjected to different scenarios of cooling.

Objectives: The objectives of this study were (i) to develop predictive models to describe germination and outgrowth of Cp spores in commercially formulated pork ham; and (ii) to validate these models under commercial (exponential) cooking and cooling conditions

Methodology

Bacterial cultures. Procedures outlined by Juneja et al. (1993) were followed to prepare a 3-strain-cocktail (NCTC 8238, 8239 and ATCC 10388) of enterotoxin-producing Cp strains that have been implicated in foodborne illness. Strains were selected based on the growth rate and resistance to thermal processing.

Sample preparation. Five gram portions of commercially prepared pork ham per treatment were inoculated with 0.1ml of the spore-cocktail (ca. 3.0 log CFU/g of meat), vacuum sealed, massaged and stored under refrigeration as previously described (Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001).

Heat activation. Samples were submerged in refrigerated water baths programmed to cook product exponentially from 4.4°C to 71.1°C in ca. 10 h. Temperature changes were simultaneously registered by the water bath thermometer and external data loggers.

Microbial Enumeration. Samples were diluted in buffered peptone water and pour-plated on tryptose-sulphite-cycloserine (TSC) agar plates (Oxoid). Solidified plates were overlaid with 5ml of TSC and were incubated for 24h at 37°C in an anaerobic chamber (Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001).

Predictive modeling. A 3-step modeling approach was performed according to previous publications ((Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001):

(i) Development of primary models to describe kinetics of Cp growth under **isothermal** conditions at 10, 15, 17, 25, 30, 35, 40, 43 and 47°C. A total of 810 samples were analyzed (2 ham formulations x 9 isotherms x 15 sampling times x 3 replications). Populations of Cp were converted to *ln* CFU/g of pork ham and fitted to the Baranyi's non-autonomous differential equation (Baranyi and Roberts, 1994; Baranyi et al, 1995; Huang, 2003) by non-linear regression (PROC NLIN procedure of SAS®).

(ii) Development of secondary models to describe the effect of **temperature changes** on growth parameters using the square-root function by non-linear regression (Huang, 2003; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001).

(iii) Solution of the first order differential equations describing the combined dynamic model with the fourth-order Runge-Kutta method (numerical technique of Matlab®).

Dynamic cooling validations. Sample bags were subjected to commercial thermal processing and cooling cycles to chill the product from 71.1 to 7.2°C for 9, 12, 15, 18, 21 and 24 h. A total of ca. 540 samples were examined (2 ham formulations x 6 cooling cycles x 15 sampling times x 3 replications).

Results & Discussion

Very few studies on germination and outgrowth of Cp in pork products are available. This may be due to the fact that very few reported outbreaks have been associated with pork products. Low numbers of cases associated with pork products may be related to high concentrations of salt (> 3%) in pork hams and other derivatives (Zaika, 2003). Cp has been detected in the liver and fluid from the body cavity of pork carcasses as well as 100% of samples from scalding vat water (Doyle, 2002). Therefore, presence of the organism in pork products such as ham is possible and potential outgrowth during cooling must be elucidated.

Isotherms. Characteristic (sigmoid) growth curves of Cp in commercially processed pork ham were obtained in this study. No Cp growth was observed in uncured or cured ham incubated at 10 or 15°C for up to 30 days. As previously reported (Gough and Alford, 1965), cured samples showed longer germination times when compared to non-cured counterparts thus supporting the reasoning behind the addition of curing salts to these products. It also supports the fact that there is no history of Cp diarrhea associated with cured meat products since the organism is relatively sensitive to sodium chloride and nitrite (Gibson and Roberts, 1986; ICMSF, 1996; Labbe, 2000).

Primary modeling. Three replications per temperature were used for model fitting with the Baranyi function to a sigmoidal growth curve for each isothermal evaluated. The parameters of this function allow the calculation of lag phase duration, generation times and exponential growth rate (Baranyi and Roberts, 1994; Baranyi et al, 1995; Huang, 2003).

Secondary modeling. The expanded square-root Ratkowsky model was used to fit all the values for maximum specific growth rate derived from each replication that were estimated by the Baranyi function. Maximum specific growth rate for Cp in **pork ham** subjected to thermal processing as a function of temperature can be described by Equation 1, while Equation 2 describes the model for **cured pork ham**.

$$\text{Eq. 1} \quad \sqrt{\mu_{\max}} = 0.0625(T - 9.7806)$$

$$\text{Eq. 2} \quad \sqrt{\mu_{\max}} = 0.0563(T - 8.8253)$$

Where μ_{\max} is the exponential growth rate and T is the temperature (°C) as a function of time. Regression coefficients and estimated parameters for maximum and minimum temperatures for growth are also provided.

Validations. Dynamic cooking and cooling profiles obtained from a commercial processor were used to validate predictive models. Cp spores were able to germinate and grow in uncured pork ham samples from an initial population of ca. 2.91 log CFU/g by 2.09, 2.19, 3.31, 4.80 and 4.83 log CFU/g subsequent to 9, 12, 15, 18 and 21 h exponential chill rates from 54.5 to 7.2°C, respectively. Growth observed in cured pork ham samples from an initial population of ca. 2.87 log CFU/g was 0.68, 1.46, 2.94, 3.05 and 3.41 log CFU/g subsequent to 12, 15, 18, 21 and 24h exponential cooling from 54.5 to 7.2°C. Predicted versus observed data for the 21 h cooling cycle of uncured pork ham is displayed in Figure 1.

There are some studies that evaluated outgrowth of Cp in processed uncured pork products. Formulated pork injected with salt, phosphates and starch at commercial

levels and inoculated with Cp spores was evaluated during the cooling from 54.5 to 7.2°C after a heat shock treatment (Thippareddi et al, 2003). Growth of ~3.5 and 4 log₁₀ CFU/g when cooling took 18 and 21 h respectively in control samples was inhibited by the addition of organic acid salts (buffered sodium citrate and sodium diacetate) at levels higher than 1%. Slightly higher growth levels were observed in this study 4.80 and 4.83 log CFU/g for 18 and 21 h cooling; differences that may be associated with the activation method. In the Thippareddi study a heat shock protocol (constant heat of 75 °C for 20 minutes) was used while an exponential heating method was used in this study.

There are very few studies in cured pork-products that evaluated Cp outgrowth. *C. perfringens* cook-activated spores were capable of outgrowing rapidly in cured frankfurters (50% pork, 50% beef) when incubated at 37 and 23°C. Growth levels diminished at 15 and 12 and no growth was observed at ≤ 10°C (Solberg and Elkin, 1970). Cp inoculated into frankfurter emulsion containing salt, curing agents and spices; survived processing at 69°C for 30 to 48 min. Outgrowth was observed under anaerobic conditions at 23 and 37°C but minimal or no growth was observed at < 15°C. Similar results were observed in this study where low temperature of incubation during the isothermal analysis also inhibited growth significantly in cured samples.

Slow cooling of ham has been recognized as potentially hazardous (Doyle, 2002). *C. perfringens* inoculated in ham with curing brine survived both curing and smoking. Even a secondary heat treatment of 121°C for 10 min only delayed but did not prevent outgrowth of spores (Gough and Alford, 1965). Zaika (2003) evaluated the influence of salt content and cooling rate on the outgrowth of Cp spores in cooked ham. Concentrations of ≥ 3.1% NaCl inhibited the growth of *C. perfringens* when hams were cooled exponentially (54.5 to 7.2°C) for 15, 18 and 21 h. The salt levels used in this study were lower and permitted growth of the organism in both cured and uncured samples.

Conclusions

Two predictive models to estimate germination and outgrowth of *C. perfringens* spores in thermally processed pork ham (cured and uncured) during the entire temperature range of commercial cooling of processed meats is provided. In general, the Cp predictive model for uncured pork ham had a measure of performance ±0.56 log CFU/g, however it underpredicted (fail dangerous) when the cooling took 9, 12 and 18 h. On the other hand the model for cured pork ham had a measure of performance of ±0.86 log CFU/g and underpredicted in the cooling validations of 12 and 18h. Summarizing, both models developed perform relatively well to describe outgrowth when compared to validation experiments simulating dynamic cooling scenarios. The use of these equations in commercial settings may enable processors and regulators to evaluate the safety of commercially produced pork ham.

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Tables and Figures

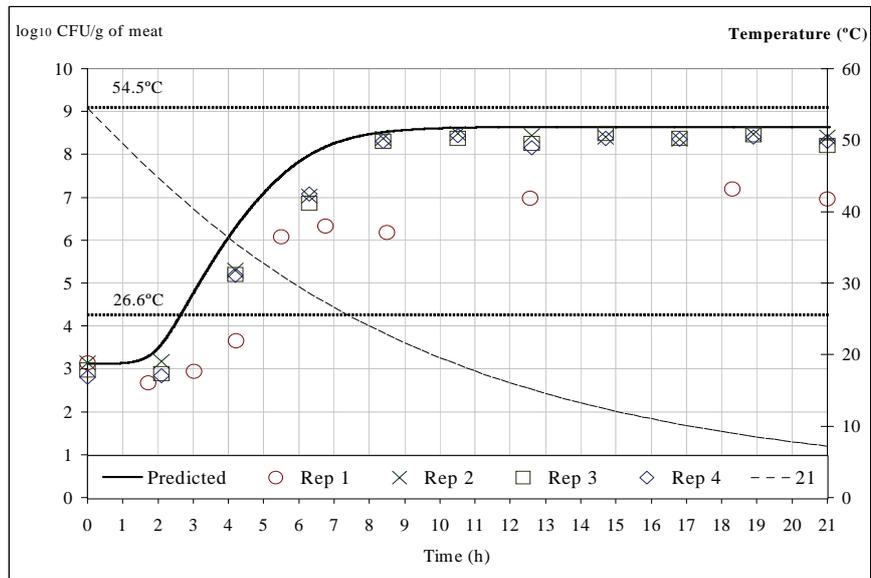


Figure 1. Experimental growth data (symbols) for heat-activated Cp spores during dynamic exponential cooling of pork ham from 54.5 to 7.2°C in 21 h. Solid line represents growth of Cp as predicted by the pork ham model. Dotted line represents the temperature profile.

MANUFACTURING A MODEL MEAT MATRIX TO INVESTIGATE THE SENSITIVITY OF RAPID TESTING METHODS ON CAMPYLOBACTER SPP.

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Key Words: reference material, *Campylobacter* spp., poultry meat, rapid testing method

Introduction

For the examination of meat referring to the occurrence of *Campylobacter* spp. many different testing methods are developed. Rapid testing methods (e.g. PCR, ELISA) give faster results and are easy to handle compared to cultural methods. The food industry and the administration of food surveillance make use of these advantages. Therefore the validity of the results, got by rapid testing methods, has to be proved by comparing with the results of cultural methods. Validating new methods includes the examination of the sensitivity and specificity. The investigations have to be done with naturally contaminated material and additionally with artificial contaminated materials for receiving the detection limit.

Objectives

There are no standardized instructions for the production of a reference material, which could be used for the validation of rapid testing methods on *Campylobacter* spp.. Seasonally poultry meat has a high contamination rate with *Campylobacter* spp.. To produce reference material poultry meat would be suitable as a model matrix.

Methodology

For the testing procedure a mixture of poultry meat (turkey, chicken) was minced (3 mm). The absence of *Campylobacter* spp. was examined using cultural and rapid testing method.

Until the tests started the meat was stored at -18° C and was defrosted at 2° to 4° C 24 hours before use. Preliminary tests (without adding the test germ) were made in order to find out which machine is more suitable to get the best homogenisation. The meat was mixed/chopped in the universal mixing machine UM12 (Company Stephan, Germany) or in the 10L circuit-cutter (chopper) (Company Mueller, Germany). The selection of the machine which should be used for further procedure was made by comparing the cutting result and the rising of the temperature.

In further tests 1.8 kg meat was artificially contaminated with *Campylobacter jejuni* ssp. *jejuni* DSM 4688 (ATCC 33560) and chopped by using the 10L circuit-

cutter (chopper). Additionally 0.2 kg ice was added. A germ-suspension of 20 ml was inoculated, so that the matrix contained about 3.2×10^4 to 5.0×10^6 cfu/g *Campylobacter jejuni*. The matrix was chopped at knife-speed 1500 rpm. After 50, 100, 200 seconds 10 g samples were taken, diluted into 90 ml 0,1% buffered Peptone and mixed by stomacher (lab blender Seward Ltd., UK). The dilution was investigated quantitatively on Karmali-Agarplates by using the spiral-plater (Meintrup DWS, Germany). The Agarplates were incubated microaerophilic at 42° C for 48h. The evaluation of the characteristic colonies was done by analysis equipment ProtoCOL (Meintrup DWS, Germany).

In the main tests the meat matrix was handled the same way (1.8 kg meat + 0.2 kg ice). The inoculation level of *Campylobacter jejuni* was about 4.2×10^2 to 4.1×10^{-1} cfu/g meat matrix. The chopping time at 1500 rpm took 150 seconds.

Afterwards samples of 25 g were diluted in 225 ml Preston-broth for qualitative determination. Additionally quantitative investigation of the enrichment broth was made by spiral-plating on Karmali Agarplates. The broth and the agarplates were incubated microaerophilic at 42° C for 48h.

Results & Discussion

In the preliminary tests the temperature gradient in the meat matrix was determined during processing with the UM12 and the circuit cutter. The temperature of the meat was higher by using the UM12 machine (fig. 1). The circuit-cutter had a lower temperature increase and showed a better homogeneity of the meat. The preliminary tests showed that circuit-cutter is assessed as the better system to produce the model meat matrix.

Further tests showed a good distribution of the microorganism after short chopping time. The germ concentration varied within tolerable ranges in the meat matrix. The determined standard-deviation of log cfu/g were from $s = 0.05$ to 0.24 . A longer chopping time showed, that the isolated colonie forming units (cfu) decreased (tab.1). A reason could be that the matrix was warming up and the intake of oxygen, which might transference *Campylobacter* cells into VNBC-form.

In the main tests the qualitative determination in all samples was performed by a minimum inoculated concentration of *Campylobacter jejuni* of 4.0×10^0 cfu/g. At the given conditions meat with a contamination rate lower than 4.0×10^0 cfu/g showed culturally positive and negative results (tab.2).

The incubation of the enrichment broth for 24 hours determines presumptive positive results. If the enrichment broth was incubated for 48 hours, presumptive results would be significant positive with a contamination rate of *Campylobacter jejuni* over 1×10^4 to 1×10^7 cfu/ml. Therefore a 48 hour enrichment step is recommended according to the comparison of the sensitivity of detection methods.

If technological parameters are not adjusted, e. g. temperature of the meat below 0° C, stress might have an effect on the organisms and this could lead to negative results for meat with an even higher contamination rate. By optimisation of technological parameters the qualitative determination in artificial contaminated meat might possible be at a lower rate than 4.0×10^0 cfu/g.

Conclusions

The suitability of the circuit-cutter (chopper) for producing a meat matrix as a reference material for the determination of *Campylobacter jejuni* has been shown. The

samples could be used to investigate the sensitivity of rapid screening methods. The results of the cultural method are crucial for the determination of the sensitivity of rapid screening methods.

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Tables and Figures

Table 1. quantitative determination after chopping

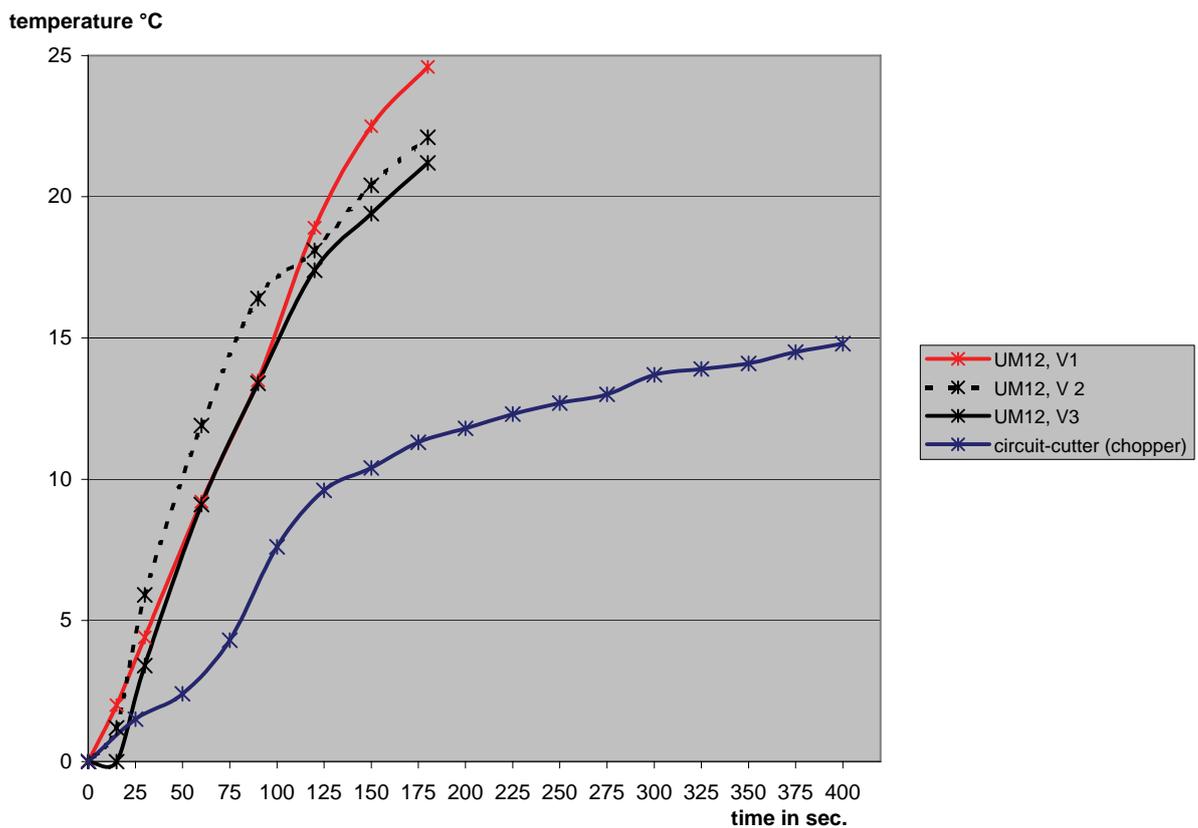
Test No.	chopping time (1R =5 Sek.)	samples (n)	average cfu/g	standard-deviation log10 –transformed (s)
B	10	6	$3,6 \times 10^6$	0,23
	20	6	$4,8 \times 10^6$	0,05
	40	6	$3,4 \times 10^6$	0,06
C	10	6	$2,8 \times 10^6$	0,06
	20	6	$1,8 \times 10^6$	0,05
	40	6	$1,4 \times 10^5$	0,24
D	10	6	$1,0 \times 10^5$	0,05
	20	6	$7,9 \times 10^4$	0,04
	40	6	$5,0 \times 10^4$	0,08
E	10	6	$1,6 \times 10^5$	0,04
	20	6	$6,5 \times 10^4$	0,04
	40	6	$2,6 \times 10^4$	0,20
F	10	10	$1,2 \times 10^4$	0,09
	20	10	$8,9 \times 10^3$	0,07
	40	10	$5,7 \times 10^3$	0,07
G	10	10	$1,0 \times 10^4$	0,14
	20	10	$5,6 \times 10^3$	0,07
	40	10	$3,1 \times 10^3$	0,10

Table 2. qualitative determination of *Campylobacter jejuni* in the main tests

Test No.	conc. inoculation (cfu/ml)	calculated average conc. in meat matrix (cfu/g)	samples (n)	24 h enrichment positive samples	48 h enrichment positive samples
H	$4,2 \times 10^4$	$4,2 \times 10^2$	6	6	6
I	$3,1 \times 10^4$	$3,1 \times 10^2$	6	6	6
J	$5,6 \times 10^3$	$5,6 \times 10^1$	6	5	6
K	$3,0 \times 10^3$	$3,0 \times 10^1$	6	5	5
L	$4,5 \times 10^2$	$4,5 \times 10^0$	6	1*	2
M	$4,0 \times 10^2$	$4,0 \times 10^0$	6	6	6
N	$8,5 \times 10^2$	$8,5 \times 10^0$	12	12	12
O	$5,2 \times 10^1$	$5,2 \times 10^{-1}$	12	3 +3*	6
P	$4,0 \times 10^1$	$4,0 \times 10^{-1}$	12	0 +12*	5 +7*
Q	$5,7 \times 10^1$	$5,7 \times 10^{-1}$	20	1 +3*	8 +10*

* presumptive

Figure 1. Temperature gradient by mixing/chopping the meat matrix in preliminary tests



INHIBITORY EFFECT OF SODIUM BENZOATE, POTASSIUM SORBATE AND METHYL P-HYDROXYBENZOATE ON FUNGI RECOVERED FROM TWO TYPES OF PORTUGUESE SMOKED DRY SAUSAGES

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Key Words: Portuguese smoked dry sausages; Spoilage fungi; Preservatives; Inhibitory effect; Modified atmosphere package; Shelf-life

Introduction

Mould spoilage in Portuguese smoked dry sausage (chouriço) packaged in modified atmosphere package (MAP) may occur, reducing the shelf life and causing substantial financial losses to manufacturing companies (Matos et al., 2003a). Presence of moulds also could constitute a potential hazard to human health considering that some mould strains might be toxigenic (Sweeney & Dobson, 1998). In Portugal, as in the E.U., benzoates, sorbates and the esters of p-hydroxy-benzoic acid may be used in surface treatments of dry sausages casings. However, resistance of fungi to these preservatives depends on factors such as pH, type and strain, preservative concentration, inoculum level, product composition, water activity, other additives, physical treatment of processing and smoke application, storage temperature, length of storage, storage atmosphere, and type of packaging. (Jay, 2000; Asehraou et al., 1997; Sofos, 1989; Hotchkiss, 1989; Lueck, 1980). It is therefore, interesting to investigate the effect of these additives at pH 6.5 on the spoilage fungi of these meat products.

Objectives

Mainly goals of this work were to identify some important spoilage fungi isolated from two types of Portuguese chouriço after producer-defined shelf life period (120 days at 20±5°C) in MAP (55% N₂ / 45% CO₂), which might decrease storage period and compromise product safety and, to study the effect of potassium sorbate, sodium benzoate and methyl p-hydroxybenzoate on the growth rate of representative mould isolates in vitro conditions.

Methodology

The experiments were conducted at a commercial meat plant and the study was based on 12 samples (each sample was composed by a mixture of three sausages, a total of 36 sausages) randomly drawn from two batches. Representative sausages of each experiment were produced as outlined by Matos *et al.* (2003b) and were packed separately in modified atmosphere (55% N₂/45% CO₂) and stored at 20±5°C for 120 days (shelf-life period). Identification of fungi was performed according conventional

mycological methods based on morphological and physiological characterization using taxonomic tables.

Each mould strain was tested in triplicate in peptone water (PW):g l⁻¹): 30 g of bacto proteose peptone (Difco, 0120-17-6) per l of distillate water, pH of 6.5 and incubation at 25°C for 5 days. Salt solutions were prepared in distillate water with addition of 0.1 g, 0.5 g, 1.0 g, 1.5 g and 3.0 g 100 ml⁻¹ salt in order to reach the final concentration in the microwell of 0.01, 0.05, 0.1, 0.15 and 0.3% (a ten fold dilution (v/v) corresponding to 25 ìl /250 ìl), respectively. From each spore suspension, 25 ìl was dispensed into wells of a 96-microwell plate containing 200 ìl of peptone water (PW) and 25 ìl of each salt solution to give a final volume of 250 ìl in each well. In the positive control salt solutions were replaced by sterile water and, in the negative control the corresponding volume of spore suspension was replaced by PW-medium. Microplates were covered with special lids, to avoid evaporation of water during incubation period.

Growth curves of the mould batches in each well of the microplates were obtained by reading optical density at 650 nm (OD₆₅₀) twice a day for 5 days on an EL 808 – Ultra Microplate Reader (Bio-Tek Instruments, Inc. Winooski, VE). The specific growth rates, μ , were calculated as the slope of the linear part of the ln-plots, representing the exponential growth phase. Results are presented as mean \pm standard deviation (n=3) of the growth rate (\hat{i} h⁻¹). Negative control was subtracted from each result.

Results & Discussion

Identification of moulds yielded 11 groups: *Penicillium terrestres* (43.4%), *Penicillium* sp. (13.3%), *Fusarium* sp. (10%), *Aspergillus glaucus* (10%), *Aspergillus versicolor* (6,8%), *Monilia fruticola* (3.3%), *Absidia* sp. (3.3%), *Cephalosporium* sp. (3.3%), *Rhizopus stolonifer* (3.3%) and *Fusarium tricinctum* (3.3%). Differences founded between identified species in our study and from those reported by several authors (Andersen, 1995; Kivanc *et al.*, 1992; Mutti *et al.*, 1992; Dragoni *et al.*, 1991) could be related with the applied processing and packaging technology and also with the house ambient, which is the main source of moulds (López Díaz *et al.*, 2001). In our case, mould fermentation is not promoted and, the species reported here, were isolated after shelf life period in MAP.

The methodology used to quantify *in vitro* fungal growth was quite different from the one traditionally applied for studies of fungal/mould growth. In the present study, we observed mycelium growth on top of liquid media to be an exponential function of time when registered as OD₆₅₀ values. We were thus able to quantify growth of fungal mycelia using classical microbiological growth equations, whereby the evaluation of inhibitory effects of the tested salts was straightforward. This approach has to our knowledge not been reported before.

MHB showed greater inhibitory effect than SB and PS in all fungi isolates with exception in *Aspergillus glaucus* [Tm30(A)], in which the inhibitory effect of MHB was similar to PS (Figure 1). At 0.05% (w/v) all fungi were inhibited with MHB with exception for *Rhizopus stolonifer* [Tm25(A)] which, only with a concentration higher than 0.1% started to decrease the growth rate. In what concerns PS and SB, PS was more effective to inhibit mould growth than SB, with exception in *Absidia* sp [Tm16(R)], in which both presented similar inhibitory effect.

Conclusions

Although contributions from other fungi than the ones isolated can not be excluded, *Penicillium terrestres* (43.4%), *Penicillium* sp. (13.3%), *Fusarium* sp. (10%), *Aspergillus glaucus* (10%), *Aspergillus versicolor* (6,8%), *Monilia fruticola* (3.3%), *Absidia* sp. (3.3%), *Cephalosporium* sp. (3.3%), *Rhizopus stolonifer* (3.3%) and *Fusarium tricinctum* (3.3%) were found in varieties Alentejano and Ribatejano of Portuguese chouriço after 120 days at 20±5°C in MAP.

Addition of MHB at 0.1% (w/v) could represent an alternative to be considered in order to assure the safety of the final product and to reduce the substantial financial losses felt by manufacturing companies. At pH 6.5, MHB showed greater inhibitory effect than SB and PS in all fungi isolates with exception for *Aspergillus glaucus* [Tm30(A)], in which the inhibitory effect of MHB was similar to PS. In what concerns PS and SB, PS was more effective to inhibit mould growth than SB, with exception in *Absidia* sp [Tm16(R)], in which both presented similar inhibitory effect. However, at pH 6.5, PS and SB did not exhibit their entire antifungal properties, which are effective antifungal agents only at pH=6. In this way, before more specific conclusions could be drawn, mycotoxin formation and its inhibition should also be investigated and, further studies performed in the product, should be carried out.

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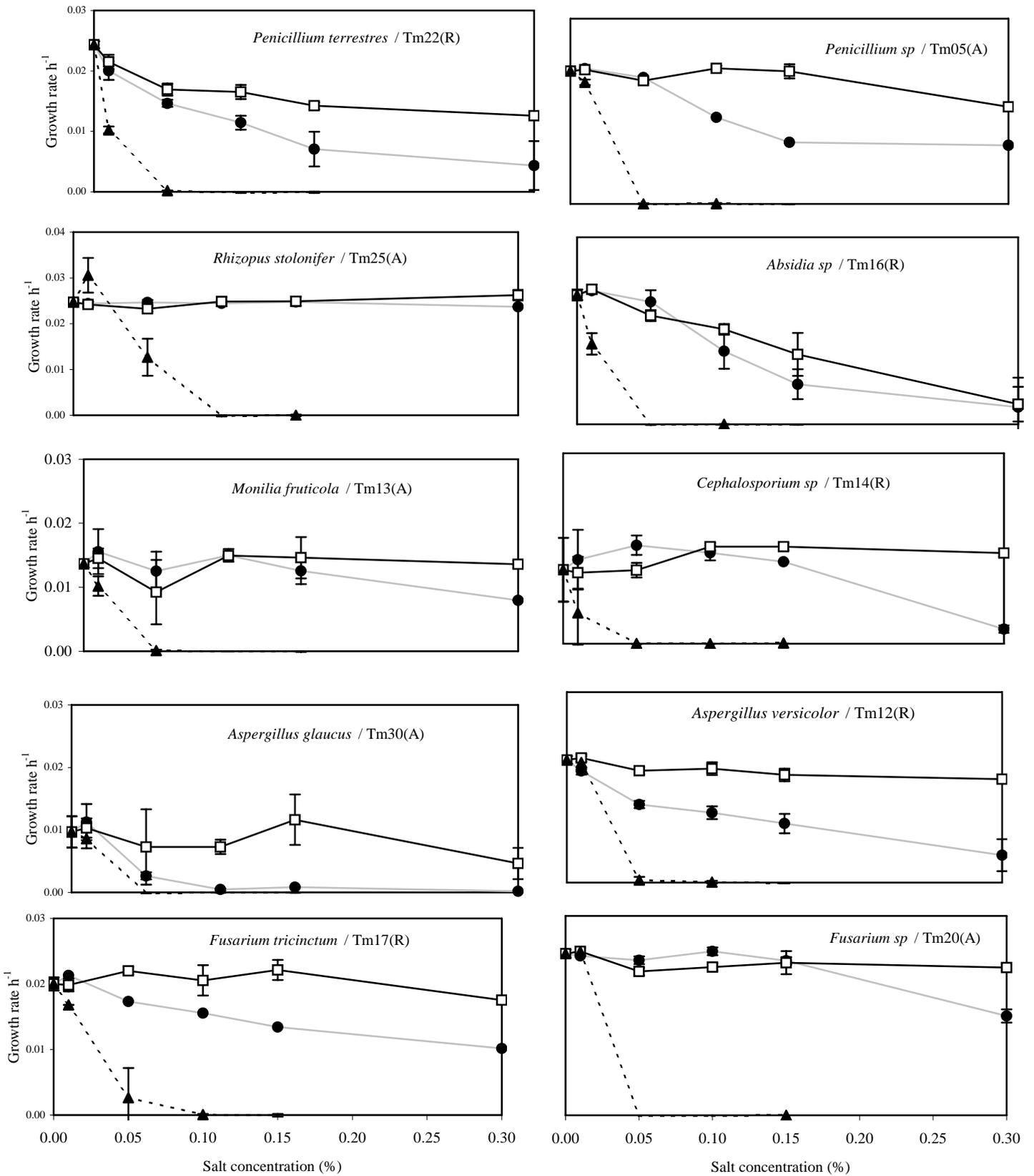
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Tables and Figures

Figure 1. Growth rate (mean values \pm SD, n=3) of representative mould isolates recovered from chouriço type Alentejano (A) and from type Ribatejano (R) in PW medium (pH of 6.5 and incubation at 25°C) with addition of potassium sorbate (?) and sodium benzoate (?) at five different concentrations and of methyl *p*-hydroxybenzoate (?) at four different concentrations. Growth rate h^{-1} was determined twice a day for 5 days (growth period). For each salt concentration, values of growth rate with the same letter do not differ significantly ($p>0.05$).



INFLUENCE OF FRESH GARLIC AND SPICE EXTRACTS IN MARINADES ON THE FORMATION OF HETEROCYCLIC AROMATIC AMINES IN FRIED BEEF PATTIES

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Key Words: Heterocyclic Aromatic Amines, HAA, PhIP, MeIQx, Norharman, Harman, garlic, garlic extract, wood garlic, meat products, patties

Introduction

Epidemiological studies showed that the daily diet can be responsible for the initiation of different kinds of cancer. In the search for a possible relationship between diet and cancer, the highly mutagenic Heterocyclic Aromatic Amines (HAA), present in cooked food, were found by Japanese scientists nearly 30 years ago (Sugimura et al., 1977). These substances are found especially in the crust of fried, broiled and cooked meat and fish. The compounds were shown to be carcinogenic in long-term animal studies on rodents and monkeys (Adamson et al., 1990). The International Agency for Research on Cancer classified several HAA as possible (class 2B) or probable carcinogens (class 2A) and recommends a reduced dietary intake of these compounds (IARC, 1993). The major known HAA in fried meat products are MeIQx, 4,8-DiMeIQx and PhIP. These substances are responsible for most of the observed mutagenic activity in these foods and are usually formed as products of the Maillard reaction. The precursors are creatine or creatinine and Maillard products from free amino acids and hexoses. The important parameters of the HAA formation are the temperature and the heating time (Arvidsson et al., 1997), but the heat transfer to the surface of the product as well as the mass transport of the precursors outwards to the crust of meat also affect the formation of HAA. Some studies showed that the concentrations of HAA can be reduced by addition of compounds, which possess antioxidant potential (Murkovic et al., 1998). A number of spices are known for their antioxidant activity. Especially, the influence of fresh minced or crushed garlic and onion, as inhibitors on the formation of HAA, was reported (Murkovic et al., 1998, Gibis et al., 1999, Shin et al. 2002).

Objectives

The aim of this study was to examine the possibility of reducing the formation of HAA in beef patties by using several oil marinades with different concentrations of fresh garlic (*Allium sativum*), garlic extract or wood garlic extract (*Allium ursinum*). In addition, the beef patties should have an appetising colour and a non over spiced flavour.

Methodology

Material: Prepared deep frozen beef patties 70 g (Salomon Hitburger, Großostheim, Germany) (8 mm thick x 113 mm x 105 mm). The composition of the patties is shown in Table 1.

Specifications of extracts: (Raps, Kulmbach, Germany) 1. Wood garlic extract: wood garlic (*Allium ursinum*) 33 %, water, salt, stabilizer gum tragacanth (E 413), potassium sorbate (E 202), citric acid (E 330), modified corn starch.

2. Garlic extract: Garlic and salt, relationship extract to spice 1:3.

Heating devices: The two grill plates of a double contact grill (Nevada, Neumärker, Hemer, Germany) have a temperature of 230 °C. The deep frozen patties were coated with sunflower oil (refined) or different marinades (5g per side). The following concentrations of fresh minced and or crushed garlic, garlic or wood garlic extract were used (Table 2). The substances are added to 67.75 % sunflower oil (refined), 32 % water and 0.25 % emulsifier (mono- and diglycerides of fatty acids E 471, Gruenau, Illertissen, Germany). The patties were immediately fried on both sides simultaneously to a core temperature of 72 °C and a surface temperature < 190 °C at the end of the frying process. The beef patties were laid between tin foil and fried for 2:40 min.

Determination of HAA: The method included the 15 polar and apolar HAA. The method of HPLC analysis with some modifications was based on the method described by Gross and Grüter (1992). The peaks of HAA, as well as Norharman and Harman, in samples were identified by comparing the retention times and UV-spectra with standards.

Results & Discussion

Only MeIQx, PhIP and the β -carbolines Norharman and Harman were detected in all fried beef patties. The effect of the varying additions on the formation of HAA is demonstrated in the Figures 1, 2 and 3. Especially the content of the compound PhIP was significantly reduced by about 90 %, near the detection limit by using marinades with the addition of spice or extracts in the highest concentration. The first control batch fried with sunflower oil had the highest concentration of PhIP in comparison to the batches with spice addition. The second control batch fried with the emulsified marinade had similar concentrations of PhIP. Comparable to PhIP, the formation of the compound MeIQx was inhibited. The concentration of MeIQx in the fried beef patties was reduced by about 40 % through using fresh garlic and about 50 % by using garlic extract. Also the frying with the wood garlic marinades effected in a significant reduction of 50 % of the MeIQx content. The co-mutagenic Norharman and Harman were found in all beef patties, but the concentrations partly increased with higher addition of these extracts. These compounds do not possess any mutagenic activity as such, but become mutagenic together with non-mutagenic aromatic amines and can enhance the mutagenic potential of other HAA (Sugimura et al., 1982).

Fresh garlic has a number of compounds with sulphhydryl groups, which were mainly formed by an enzymatic reaction after mincing. These substances showed a reducing effect on the formation of MeIQx and PhIP in other studies (Murkovic et al., 1998, Gibis et al., 1999, Shin et al. 2002). Likewise, garlic extract and wood garlic extract had the same reducing effect on the formation of HAA. The advantages of garlic extract consist in the better application and dosage in comparison to the fresh garlic. The inhibiting effect on the HAA formation is approximately the same between fresh garlic and extract, if the ratio (1:3) is considered in the specification of the producer.

An inhibiting effect of different organosulphur compounds such as cysteine, acetylcysteine and glutathione on MeIQx formation in meat matrix based model system was reported (Schoch et al., 1998).

The application of marinades has the advantage, that the beef patties have a pleasant flavour. The products are not over spiced. This marinating of meat is a traditional preparation before grilling in Mediterranean countries.

Conclusions

The application of garlic or garlic extract and wood garlic extract can inhibit the formation of HAA during the frying process. Especially, the concentration of PhIP in fried beef patties is nearly reduced up to the detection limit with the highest added concentration of spices or extracts.

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Abbreviations:

- HAA: Heterocyclic Aromatic Amines,
 MeIQx: 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline,
 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline,
 PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
 Norharman: 9H-pyrido[3,4-*b*]indole
 Harman: 1-methyl-9H-pyrido[3,4-*b*]indole

Tables and Figures

Table 1. Composition of the frozen raw beef patties

Moisture	Mineral matter	Protein	Fat	Hydroxyproline	Creatine	Creatinine
[g/100g]	[g/100g]	[g/100g]	[g/100g]	[g/100g]	[g/100g dm]	[g/100g dm]
60.7	0.071	17.6	21.5	0.331	0.343	0.01

dm: dry matter

Table 2. Used concentrations of wood garlic extract, garlic extract and fresh minced garlic in oil marinades

Wood garlic extract [g/100 g marinade]	Garlic extract [g/100 g marinade]	Fresh garlic [g/100 g marinade]
1.5	0.15	0.5
3	0.3	1
4.5	0.6	3
6	1.2	6
	1.8	9
		12

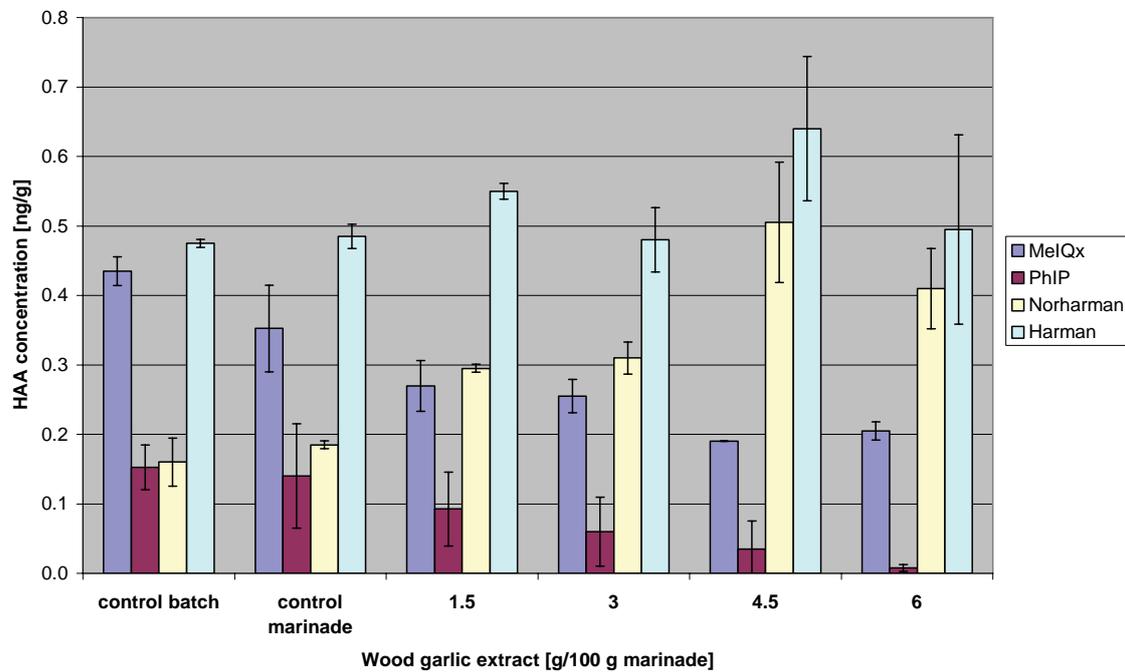


Figure 1: HAA concentrations of fried beef patties after application of marinades with wood garlic extract

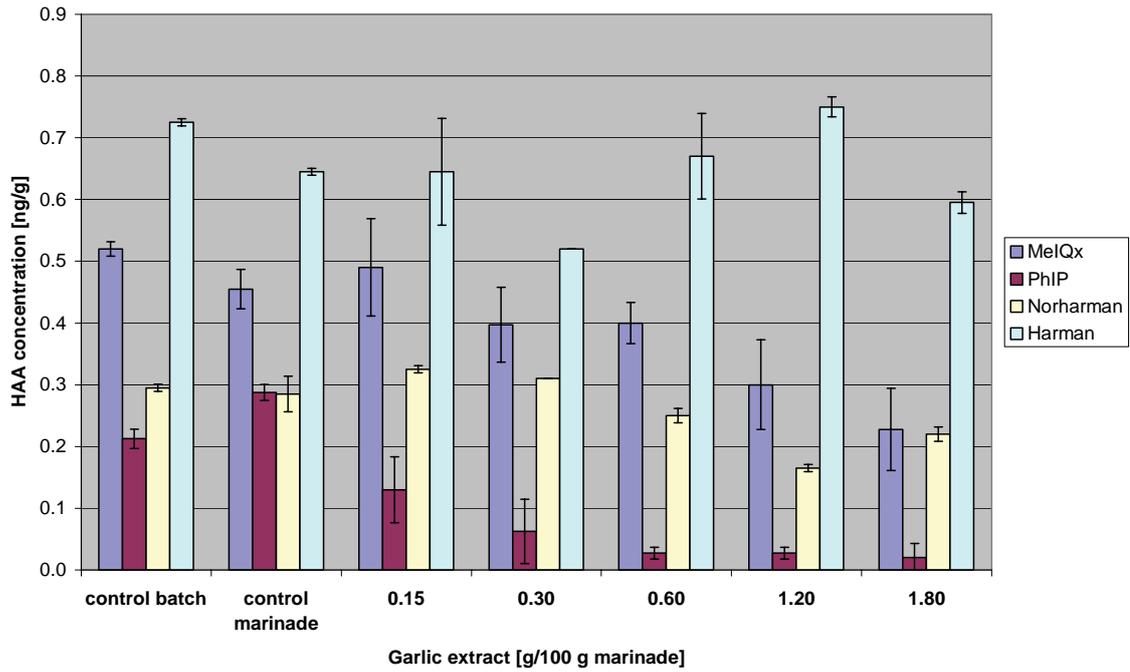


Figure 2: HAA concentrations of fried beef patties after application of marinades with garlic extract

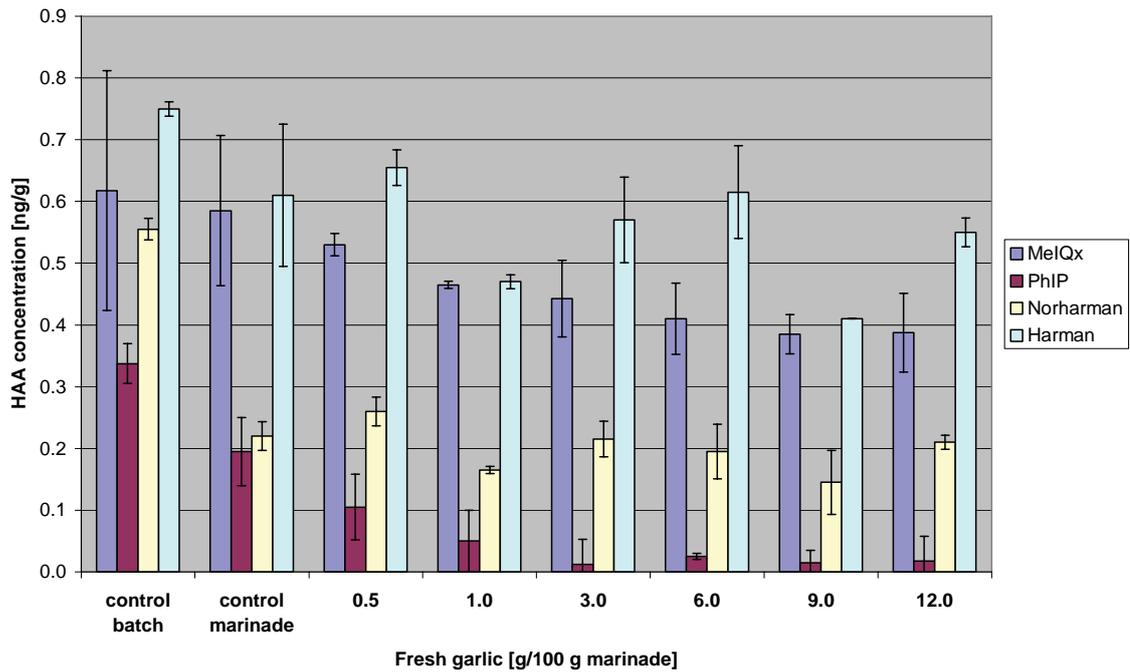


Figure 3: HAA concentrations of fried beef patties after application of marinades with fresh garlic

SENSITIVITY OF THREE PATHOGENIC BACTERIA TO TURKISH CEMEN PASTE AND ITS INGREDIENTS

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Introduction

Meat spoils readily unless kept at temperatures in the proximity of 0 °C or is processed immediately. Therefore traditional methods of meat preservation, such as salting and drying, still continue to play an important role in many of the world's less developed countries (Norman and Corte, 1985). Salted and dried meat products with specific textural and eating quality characteristics could be produced to have a useable shelf-life of 3-5 months without noticeable deterioration with unique taste and flavor which makes them a delicacy (Leistner 1987).

Pastirma is one of the salted and partially fermented dry cured meat product which is pasted (outside covered with paste which makes it different from its counterparts) and stored for several months without refrigeration, and it is highly regarded and very popular in most of the Middle East countries (El-Khatib et al. 1987; Tekinsen and Dogruer 2000; Aksu and Kaya 2001). The end product is highly tasty and nutritious, and it contains an average of 45% moisture, 30% protein, 15% fat, 5% carbohydrate (from paste) and 5% ash under normal conditions and may be consumed either raw or cooked. The paste, called cemen used to cover pastirma is prepared from following herbs; 50% fenugreek (ground), 35% garlic (fresh and ground), and 25% red pepper (ground) are mixed with water to make a slurry like paste. It is then placed on the surface of the meat block, approximately 0.3-0.5 cm thick (Gokalp et al. 1999).

There have been some studies for the microbiological stability of pastirma because microbiological contamination may occur during the pastirma production, thereby the risk of spoilage or public health would increase. Cemen paste is the first hurdle to protect the product, but there was very limited research on the cemen paste and its ingredients for their antibacterial activity. For example, El-Khatib et al. (1987) stated that cemen paste had about 1.0×10^7 total bacteria and $<10^2$ Enterobacteriaceae per gram sample. Nevertheless there is no information on the survival of some well known pathogens in the cemen paste.

One of the main ingredient of the cemen is fenugreek (*Trigonella foenum-graecum*) which is a leguminous plant grown in northern Africa, the Mediterranean, European, western Asia and northern India. Fenugreek seed have been used medicinally and as a spice (food) for many years (Billaud and Adrian 2001). Many research reports have indicated that fenugreek seed reduced the total serum cholesterol levels and lowered blood glucose and peroxidation lipids in plasma (Anuradha and Ravikumar 1998). There have also been many reports about antibacterial effects of garlic (Harris et al. 2001; Sallam et al. 2004; Fernandez-Lopez et al. 2005). The objective of the present study was to evaluate the effects of cemen paste and its basic ingredients for their potential to inhibit some common pathogenic

bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*) encountered in foods.

Material and Methods

Plant materials: Basic cemen ingredients; fenugreek (*Trigonella foenum-graecum*), garlic (*Allium sativum*) and red hot pepper powder (*Capsicum annum*) were purchased from retail markets in Kayseri, Turkey.

Preparation of test bacteria: Three bacterial species including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 2392 and *Yersinia enterocolitica* ATCC 1501 were used to determine the antibacterial activity of each sample. Bacterial species were obtained from stock cultures and were grown in nutrient broth. *E. coli* and *S. aureus* were incubated at 37°C for 18 h, *Y. enterocolitica* was grown in nutrient broth and incubated at 22°C for 18 h. Broth cultures were prepared overnight in nutrient broth with final cell concentrations of approximately 10⁶-10⁷ cfu/mL.

Preparation of cemen paste: Experimental cemen was made of fenugreek, garlic, red hot pepper powder (RHPP) and water. The 29.1% of fenugreek, 20.4% of garlic, 8.7% of red hot pepper powder and 41.8 of fresh drink water were homogenously mixed and the mixture was aseptically kneaded. Then they were packaged and stored at 4 oC during the analysis.

Chemical analyses of cemen paste: Ten g of cemen was mixed with 10 mL of distilled water, and the pH was measured using a pH meter (Hanna Ins., Italy). Total solid (%) contents were determined by heating at 105 oC to a constant weight. Colour of cemen paste were measured by colorimeter (Lovibond, UK) as L, a and b values.

Determination of inhibitory effects: The inhibitory effects of cemen paste and basic ingredients against bacterial species were measured using serial dilution method. The fenugreek, garlic and red hot paper powder (RHPP) were prepared at the 29.1%, 20.4%, 8.7% concentrations in nutrient broth, respectively. Then, these samples were sterilized in an autoclave. Additionally, the 58.2% (CP1) and 29.1% (CP2) concentrations of cemen paste were mixed in nutrient broth to provide basic nutrients of the bacteria and then they were autoclaved. All the samples were separately inoculated of %1 of fresh bacterial culture (*E. coli*, *S. aureus* and *Y. enterocolitica*). Flasks inoculated with *E. coli* and *S. aureus* were incubated at 37 °C for 4 days, *Y. enterocolitica* was incubated at 22 °C for 4 days. Every day, the number of colonies in the tubes was counted using serial dilution method in nutrient agar. The growth inhibition level (%) caused by each the ingredient of the cemen and the cemen paste on test bacteria was determined according to the following equation:

$$\text{Growth inhibition level (\%)} = \frac{\text{control population} - \text{treated population}}{\text{control population}} \times 100$$

The control flask was prepared as described above except that the flask contained no added ingredients (Sagdic 2003). Bacterial counts were reported as log cfu/g or cfu/mL.

Results and Discussion

In the physicochemical analysis, the color values of cemen paste were L= 36.49, a= 22.26 and b=40.65, and total solids of cemen samples were average 56.51% with 4.83 pH values.

In this study, mainly the effects of cemen paste and its basic ingredients for their potential to inhibit common pathogens *E. coli*, *S. aureus* and *Y. enterocolitica* were evaluated. The growth inhibition levels of cemen ingredients and cemen pastes inoculated with the three pathogenic bacteria are shown in Table 1. In the study, the CP1 (full cemen paste), CP2 (50% diluted cemen paste) and garlic had strongly antibacterial activities against all the bacteria tested. The fenugreek and especially red hot pepper powder (RHPP) had a small inhibitory effect on the test bacteria (Table 1). The effectiveness of the inhibitor materials followed the sequence: CP1>CP2>garlic>fenugreek>RHPP.

Table 1. Growth inhibition levels (%) of cemen paste and its ingredients against some pathogenic bacteria

Cemen ingredients and cemen paste	<i>E. coli</i> time (days)				<i>S. aureus</i> time (days)				<i>Y. enterocolitica</i> time (days)			
	1	2	3	4	1	2	3	4	1	2	3	4
Fenugreek	18	33	54	66	26	54	72	100	34	51	69	78
Garlic	50	74	100	100	82	100	100	100	40	62	87	100
RHPP	10	10	11	12	11	14	13	22	10	10	12	7
CP1	63	100	100	100	61	100	100	100	52	73	100	100
CP2	60	86	100	100	42	85	100	100	47	70	100	100

RHPP: Red hot pepper powder, CP1: full cemen paste, CP2: 50% diluted cemen paste

All the samples had a low or high inhibitory effect against all test bacteria during the 4 days. In general, fenugreek and RHPP had a bacteriostatic effect when compared to the control treatment while the CP1, CP2 and garlic showed bactericidal effect. Again, the CP1, CP2 and garlic samples completely inhibited the tested bacteria in one of the storage time (Table 1). The CP1 was the most active on all bacteria in serial dilution method. Additionally, *S. aureus* was the most sensitive bacterium to the cemen paste or its ingredients while *Y. enterocolitica* was the most resistant bacterium against those samples.

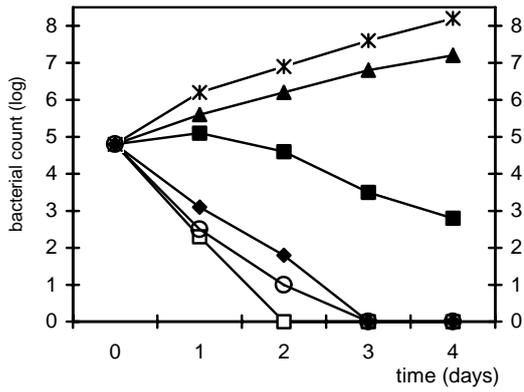


Figure 1. The inhibitory effect of fenugreek, garlic, RHPP, cemen paste against *E. coli*

■ fenugreek ▲ RHPP □ CM1
 ✕ control ◆ garlic ○ CM2

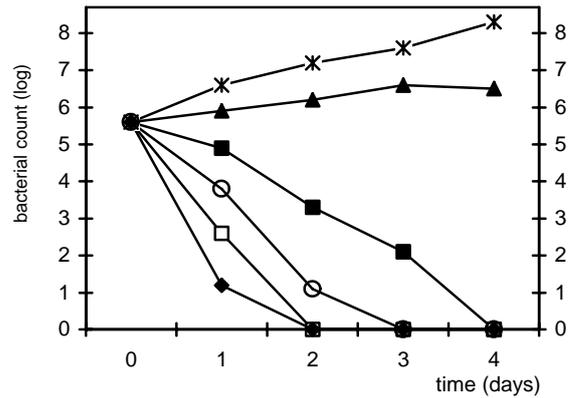


Figure 2. The inhibitory effect of fenugreek, garlic, RHPP, cemen paste against *S. aureus*

■ fenugreek ▲ RHPP □ CM1
 ✕ control ◆ garlic ○ CM2

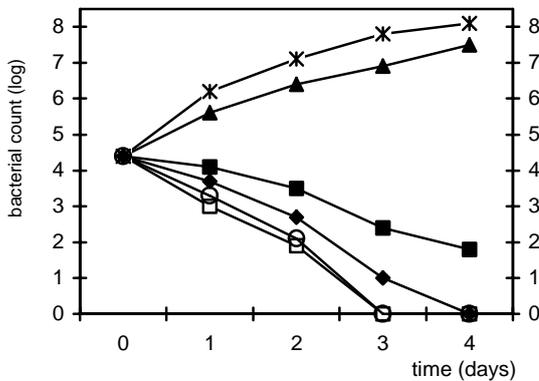


Figure 3. The inhibitory effect of fenugreek, garlic, RHPP, cemen paste against *Y. enterocolitica*

■ fenugreek ▲ RHPP □ CM1
 ✕ control ◆ garlic ○ CM2

The antibacterial activities of the cemen ingredients and cemen paste against *E. coli* are shown in Fig. 1. Among the tested materials, cemen paste had the highest inhibitory effect against *E. coli*. While CP1 had a bactericidal effect against *E. coli* at the second day, garlic and CP2 had bactericidal effect at the third day for this bacteria (Fig. 1). The inhibitory effect of cemen paste against *E. coli* was higher than that of garlic, this result might be due to the synergistic effect of cemen ingredients. The fenugreek and RHPP showed a slightly bacteriostatic effect to *E. coli* (Fig. 1).

Figure 2 shows the effects of the cemen ingredients and cemen paste against *S. aureus*. The antibacterial activities of cemen paste and garlic were about the same against *S. aureus*. While CP1 and garlic showed bactericidal effect to *S. aureus* at the second day, CP2 and fenugreek had bactericidal effect at the third and/or fourth day (Fig. 2). *S. aureus* was the most sensitive bacterium against cemen paste and its ingredients. Fenugreek had bactericidal effect against *S. aureus* but it had bacteriostatic effect to *E. coli* and *Y. enterocolitica*.

The inhibitory effects of the cemen paste and its ingredients against *Y. enterocolitica* are shown in Fig. 3. *Y. enterocolitica* was the most resistant bacterium against the cemen paste and its ingredients. Cemen paste had the highest inhibitory effect against *Y. enterocolitica*. A significant observation was that the garlic had bactericidal effect to *Y. enterocolitica* at the fourth day while CP1 and CP2 showed some effect at the third day (Fig. 3). The inhibitory effect of cemen paste against *Y. enterocolitica* was higher than that of garlic, again this might be resulted from the synergistic effect of cemen ingredients.

As the fenugreek had strong bacteriostatic effect against *Y. enterocolitica*, RHPP showed a quite poor bacteriostatic effect to *Y. enterocolitica*.

Some researchers indicated that garlic had antibacterial activity (Harris et al. 2001; Sallam et al. 2004; Fernandez-Lopez et al. 2005). According to Sagdic et al. (2003) the extract of red pepper had no activity against some pathogenic bacteria. However, there has been a very limited research on the antibacterial effects of the cemen paste and fenugreek. For example, Mansouri (1999) reported that fenugreek had no inhibitory effect against *S. aureus* using ethanolic extract. Current results were different from previous reports for the fenugreek and red pepper (Mansouri 1999, Sagdic et al. 2003). It has been well known that the antimicrobial effects of the cemen ingredients and cemen paste are depended on the species and concentrations of the ingredients, regional conditions and test bacterium strains. Additionally the method used to test antibacterial activity could have an effect on the results, and the differences between the previous reports might be due to these variations.

Based on all of these results, cemen paste on the three pathogens showed the strongest inhibitory effect compared to the cemen ingredients alone. Dogruer et al (1998) reported that cemen paste had a positive effect on the microbiological quality of pastrami, and this resulted from the antimicrobial effects of garlic. El-Khateib et al. (1987) indicated that cemen paste inhibited the growth of moulds on Turkish pastirma. However, antimicrobial activities of cemen paste on pathogenic bacteria were missing.

4. Conclusion

Based on the results of this study, cemen paste and garlic appeared to inhibit the growth of the three pathogens tested. In general, the inhibitory effect of cemen paste was higher than that of garlic. This might be resulted from the synergistic effect of

cemen ingredients. Cemen paste and garlic had completely inhibitive effect on the bacterial growth in broth culture. The results of this study confirmed the protective effect of cemen paste and garlic in food preservation, especially Turkish pastirma a traditional dry cured meat product and sauce. Antibacterial effects of the cemen paste against *E. coli*, *S. aureus* and *Y. enterocolitica* suggest microbiological safety for public health; also low pH (4.83) value would be another hurdle for organism growth. It can be suggested that similar studies should be conducted on actual meat systems to confirm above findings.

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**THE EVALUATION OF AVGARD™ TO DETERMINE ITS
EFFECTIVENESS AT REDUCING MICROBIAL INDICATOR COUNTS
WHEN APPLIED
AT DIFFERENT COMMERCIAL PARAMETERS**

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Key Words: Avgard™, carcass decontamination, microbial reduction

Introduction

Since 1993, *Escherichia coli* O157:H7 has been a costly challenge to the beef industry. In an effort to improve the safety of beef, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) published a Final Rule that required meat and poultry plants to implement a Hazard Analysis Critical Control Point (HACCP) plan (USDA-FSIS 1996). In 2002, the FSIS required a reassessment of an establishment's HACCP system to determine whether *E. coli* O157:H7 contamination is a hazard "reasonably likely to occur" in their production process. If reassessment results determine that *E. coli* O157:H7 contamination of the carcass is a food safety hazard "reasonably likely to occur" in the establishment's production process, it then must be addressed in a HACCP plan (USDA-FSIS, 2002). To comply with these regulations, packers and researchers have worked diligently to develop interventions effective at improving the microbiological quality of carcasses. Through this effort, many effective interventions have been identified such as trimming, spraying with hot water, and the application of organic acids (Castillo, 1998; Graves Delmore, 1997). Although these treatments have been shown to be effective individually, use in a "multiple hurdles" decontamination system have produced greater improvements in microbiological quality of carcasses (Graves Delmore, 1998; Bacon, 1999). Improvement in carcass decontamination systems has resulted in a safer and higher quality product for consumers. In 2005, CDC reported that human cases of *E. coli* O157:H7 currently are below a target level set for 2010 which can, in part, be attributed to improved carcass decontamination interventions.

Objectives

To evaluate Avgard™ and determine its effectiveness at reducing microbial indicator counts when incorporated as a sanitizer utilized in a carcass decontamination intervention.

Methodology

Pieces (area greater than 10 cm x 10 cm) of fresh beef adipose tissue (N = 270) were obtained from a commercial beef processing facility before application of the

“final in-plant intervention” and immediately transported, in a warm insulated container, to the Pathogen Reduction Laboratory at Colorado State University. Warm adipose pieces were then cut into identical samples (10 cm x 10 cm) and inoculated with a fecal slurry (fecal slurry was created using equal portions of sterile distilled water and warm fresh feces collected through rectal palpation of a steer receiving a finishing ration), allowed to dwell for 10 min to insure bacterial attachment and to simulate commercial exposure times, and finally subjected to one of the following treatments:

Control (No Treatment)

Water Control; <5 psi

Water Control; 20 psi

Water Control; 40 psi

0.1% Avgard™; <5 psi

0.1% Avgard™; 20 psi

0.1% Avgard™; 40 psi

0.3% Avgard™; <5 psi

0.3% Avgard™; 20 psi

0.3% Avgard™; 40 psi

0.6% Avgard™; <5 psi

0.6% Avgard™; 20 psi

0.6% Avgard™; 40 psi

1.0% Avgard™; <5 psi

1.0% Avgard™; 20 psi

1.0% Avgard™; 40 psi

1.5% Avgard™; <5 psi

1.5% Avgard™; 20 psi

1.5% Avgard™; 40 psi

2.0% Avgard™; <5 psi

2.0% Avgard™; 20 psi

2.0% Avgard™; 40 psi

4.0% Avgard™; <5 psi

4.0% Avgard™; 20 psi

4.0% Avgard™; 40 psi

All treatments were applied for 5 seconds at an ambient room temperature ($23\pm 2^{\circ}\text{C}$) utilizing a small replicate spray wash cabinet (Chad, Co., Olathe, KS) that incorporated four stationary spray nozzles located at a constant distance of 20 to 25 cm from the inoculated sample surface. Following application of the Avgard™ spray treatments, samples were then sponge-sampled for microbiological analysis.

Microbiological Analysis

Inoculated pieces of adipose tissue were sponge-sampled following the application of Avgard™ so that bacterial enumeration could be achieved. Immediately prior to sponge sampling, sterile sponges (BioPro Enviro-Sponge Bags, International BioProducts) were aseptically hydrated with 10 ml of sterile, Butterfield's Phosphate Buffer (International BioProducts). Consequently, the hydrated sponge was positioned in a manner that a corner of the sponge was orientated towards the opening of the bag so that an individual, wearing sterile latex gloves, could easily grip the sponge. Sponge-sampling of each sample was achieved with 10 vertical passes (up-and-down being considered as one pass) and 10 horizontal passes (side-to-side being considered as one pass) while applying pressure equivalent to that which would be required to remove dried blood from a surface. Following sponging, the used sponge was returned to its bag along with an additional 15 ml of sterile, Butterfield's Phosphate Buffer (International BioProducts) to bring the total volume of buffer to 25 ml. Contaminated latex gloves were then discarded and replaced with new sterile gloves before touching another sponge intended for a subsequent sample.

Following tissue sampling, sponge samples were pummeled in a stomacher (Masticator, IUL Instruments, Barcelona, Spain) for 2 min before microbiological determinations were conducted. Samples were then serially diluted in 0.1% buffered peptone water (Difco, Becton Dickinson & Co., Sparks, MD; BPW). Appropriate decimal dilutions were plated onto Tryptic Soy Agar (Difco; TSA) in duplicate, for determination of Total Plate Count (TPC). TSA plates were incubated at 35°C for 24 h and, following incubation, colonies were enumerated. Additionally, appropriate decimal dilutions were plated onto Petrifilm™ *E. coli*/Coliform count plates (3M Microbiology & Products, St. Paul, MN) in duplicate for determination of *E. coli* Biotype I Count (ECC) and Total Coliform Count (TCC). Petrifilm™ was incubated at 35°C for 24 h and colonies were enumerated. *E. coli* Biotype I (ECC) colonies were determined by counting the blue colonies that were associated with a gas bubble, where as TCC were determined by counting both the red and blue colonies closely associated with gas bubbles. For all analyses, average counts of colonies on duplicated plates were transformed to $\log \text{CFU}/\text{cm}^2$.

Statistical Analysis

Data were analyzed and Least squares means (LSM) were generated using the General Linear Models (GLM) procedures of SAS (1999) to determine which concentration and pressure combinations resulted in the greatest reductions in TPC, TCC, and ECC compared to inoculated, untreated controls and inoculated water treated controls. Least squares means were separated and differences were determined using an alpha-error level of 0.05 ($P < 0.05$).

Results & Discussion

Least squares means for TPC and differences in TPC compared to untreated controls and water treated controls are presented in Table 1. Generally speaking, all treatments incorporating Avgard™ reduced, numerically, total plate counts when compared to the negative and water treated controls, with the exception of the 0.3% concentration applied at <5 psi. When all concentrations of Avgard™ were applied at 20 or 40 psi, the treatments resulted in significant reductions when compared to the negative controls. However, only concentrations greater than 0.6% of Avgard™ regardless of application pressure were found to differ ($P < 0.05$) from the water controls. When compared to the water treated controls, all Avgard™ concentrations higher than 0.6% applied at <5 psi and the 2.0% level applied at 20 psi resulted in the greatest reductions of TPC.

Least squares means and reductions for TCC and ECC when compared to negative and water treated controls are reported in Tables 2 and 3, respectively. Concentrations of 2.0 and 4.0% Avgard™ were the most effective as they resulted in the greatest reduction of TCC when compared to negative and water treated controls, regardless of application pressure. Similarly, 2.0 and 4.0% concentrations of Avgard™ applied at all pressures also resulted in the largest reduction of ECC when compared to the negative and water treated controls. When evaluated against the reductions of all the Avgard™ concentrations and application pressures, the 4.0% level of Avgard™ reduced ($P < 0.05$) TCC and ECC levels by at least 5.6 and 5.4 log CFU/cm², respectively.

Conclusions

Sodium metasilicate, a basic chemical, is the active ingredient in Avgard™. Sodium metasilicate has been approved for contact with beef carcass surfaces at levels not exceeding 4.0% (FSIS Directive 7120.1, Amendment 1). Although Avgard™ was not evaluated for its efficacy at reducing known pathogens, it has been shown effective at lowering microbial populations that are regarded as indicator organisms. It stands to reason that if the level of an indicator organism is reduced, the threat of pathogenic bacteria also being prevalent is reduced. These laboratory results demonstrated that use of a 4.0% concentration of Avgard™ applied at 20 or 40 psi was an effective means to lower TCC and ECC while reducing moderately TPC, and of Avgard™ use should be considered as an effective carcass decontamination intervention.

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Tables and Figures

Table 1. Least squares means \pm standard error for Total Plate Counts (TPC) (log CFU/cm²), difference in TPC (log CFU/cm²) from negative controls (NC) and difference (log CFU/cm²) in TPC from water-treated controls (WC) from inoculated samples treated with 7 concentration of AVGARD™ at 3 pressures (PSI).

Treatment	Pressure	Log APC	Diff from NC	Diff From WC
NC		9.5 \pm 0.03 ^{ab}	0.0 \pm 0.03 ^{ab}	0.5 \pm 0.03 ^{bc}
WC		9.0 \pm 0.11 ^b	-0.5 \pm 0.11 ^b	0.0 \pm 0.11 ^{cd}
0.1%		9.0 \pm 0.05 ^b	-0.5 \pm 0.05 ^b	0.0 \pm 0.05 ^{cd}
0.3%		9.6 \pm 0.13 ^a	0.1 \pm 0.13 ^a	0.6 \pm 0.13 ^b
0.6%	<5	6.2 \pm 0.02 ^{efghij}	-3.3 \pm 0.02 ^{defgh}	-2.8 \pm 0.02 ^{ij}
1.0%		6.2 \pm 0.03 ^{efghij}	-3.2 \pm 0.03 ^{defg}	-2.7 \pm 0.03 ⁱ
1.5%		6.1 \pm 0.03 ^{efghij}	-3.4 \pm 0.03 ^{efgh}	-2.9 \pm 0.03 ^{ij}
2.0%		6.5 \pm 0.32 ^e	-3.0 \pm 0.32 ^{de}	-2.4 \pm 0.32 ^{hi}
4.0%		6.4 \pm 0.28 ^{efg}	-3.1 \pm 0.28 ^{def}	-2.6 \pm 0.28 ⁱ
NC		9.3 \pm 0.06 ^{ab}	0.0 \pm 0.06 ^{ab}	1.6 \pm 0.06 ^a
WC		7.6 \pm 0.06 ^{cd}	-1.6 \pm 0.06 ^c	0.0 \pm 0.06 ^{cd}
0.1%		7.5 \pm 0.03 ^{cd}	-1.8 \pm 0.03 ^c	-0.2 \pm 0.03 ^d
0.3%		7.2 \pm 0.22 ^d	-2.1 \pm 0.22 ^c	-0.4 \pm 0.22 ^d
0.6%	20	6.5 \pm 0.34 ^{ef}	-2.8 \pm 0.34 ^d	-1.2 \pm 0.34 ^e
1.0%		5.7 \pm 0.08 ^j	-3.6 \pm 0.08 ^{fgh}	-1.9 \pm 0.08 ^{fgh}
1.5%		6.2 \pm 0.23 ^{efghij}	-3.1 \pm 0.23 ^{def}	-1.4 \pm 0.23 ^{ef}
2.0%		4.3 \pm 0.50 ^k	-4.9 \pm 0.50 ⁱ	-3.3 \pm 0.50 ^j
4.0%		5.7 \pm 0.07 ^{ij}	-3.5 \pm 0.07 ^{fgh}	-1.9 \pm 0.07 ^{fg}
NC		9.6 \pm 0.03 ^a	0.0 \pm 0.03 ^{ab}	1.8 \pm 0.03 ^a
WC		7.8 \pm 0.19 ^c	-1.8 \pm 0.19 ^c	0.0 \pm 0.19 ^{cd}
0.1%		7.6 \pm 0.07 ^{cd}	-2.1 \pm 0.07 ^c	-0.3 \pm 0.07 ^d
0.3%		6.3 \pm 0.22 ^{efgh}	-3.3 \pm 0.22 ^{efgh}	-1.6 \pm 0.22 ^{efg}
0.6%	40	5.9 \pm 0.22 ^{ghij}	-3.7 \pm 0.22 ^{gh}	-1.9 \pm 0.22 ^{fgh}
1.0%		6.4 \pm 0.27 ^{efg}	-3.2 \pm 0.27 ^{defg}	-1.4 \pm 0.27 ^{ef}
1.5%		5.8 \pm 0.06 ^{hij}	-3.8 \pm 0.06 ^h	-2.0 \pm 0.06 ^{gh}
2.0%		5.9 \pm 0.17 ^{fghij}	-3.7 \pm 0.17 ^{gh}	-1.9 \pm 0.17 ^{fg}
4.0%		5.9 \pm 0.17 ^{fghij}	-3.7 \pm 0.07 ^{gh}	-1.9 \pm 0.17 ^{fg}

^{a,b,c,d,e,f,g,h,i,j,k} Means, within a column, lacking common superscript letters, differ ($P < 0.05$).

Table 2. Least squares means \pm standard error for Total Coliform Counts (TCC) (log CFU/cm²), difference in TCC (log CFU/cm²) from negative controls (NC) and difference (log CFU/cm²) in TCC from water-treated controls (WC) from inoculated samples treated with 7 concentration of AVGARD™ at 3 pressures (PSI).

Treatment	Pressure	Log TCC	Diff from NC	Diff From WC
NC		8.1 \pm 0.02 ^{bc}	0.0 \pm 0.02 ^a	0.5 \pm 0.02 ^{bc}
WC		7.5 \pm 0.06 ^{cd}	-0.5 \pm 0.06 ^{ab}	0.0 \pm 0.06 ^{cde}
0.1%		7.4 \pm 0.06 ^{cd}	-0.6 \pm 0.06 ^{ab}	-0.1 \pm 0.06 ^{cde}
0.3%		7.5 \pm 0.17 ^{cd}	-0.5 \pm 0.17 ^{ab}	0.0 \pm 0.17 ^{cde}
0.6%	<5	5.4 \pm 0.09 ^h	-2.7 \pm 0.09 ^{ef}	-2.2 \pm 0.09 ^{hi}
1.0%		4.6 \pm 0.39 ⁱ	-3.5 \pm 0.39 ^{ghi}	-2.9 \pm 0.39 ^j
1.5%		3.3 \pm 0.17 ^j	-4.8 \pm 0.17 ^{kl}	-4.3 \pm 0.17 ^k
2.0%		3.0 \pm 0.31 ^j	-5.1 \pm 0.31 ^{klm}	-4.5 \pm 0.31 ^k
4.0%		2.0 \pm 0.00 ^k	-6.1 \pm 0.00 ^{no}	-5.6 \pm 0.00 ^l
NC		8.5 \pm 0.25 ^b	0.0 \pm 0.25 ^a	1.2 \pm 0.25 ^b
WC		7.3 \pm 0.05 ^{de}	-1.2 \pm 0.05 ^{bc}	0.0 \pm 0.05 ^{cde}
0.1%		7.1 \pm 0.03 ^{de}	-1.4 \pm 0.03 ^c	-0.2 \pm 0.03 ^{cde}
0.3%		6.9 \pm 0.17 ^{de}	-1.6 \pm 0.17 ^{cd}	-0.3 \pm 0.17 ^{def}
0.6%	20	5.7 \pm 0.30 ^{gh}	-2.8 \pm 0.30 ^{efg}	-1.6 \pm 0.30 ^{gh}
1.0%		4.5 \pm 0.34 ⁱ	-4.1 \pm 0.34 ^{ij}	-2.8 \pm 0.34 ^{ij}
1.5%		4.0 \pm 0.28 ⁱ	-4.5 \pm 0.28 ^{jk}	-3.3 \pm 0.28 ^j
2.0%		2.9 \pm 0.37 ^j	-5.6 \pm 0.37 ^{mn}	-4.3 \pm 0.37 ^k
4.0%		1.2 \pm 0.21 ^l	-7.3 \pm 0.21 ^p	-6.1 \pm 0.21 ^l
NC		9.4 \pm 0.04 ^a	0.0 \pm 0.04 ^a	2.3 \pm 0.04 ^a
WC		7.2 \pm 0.19 ^{de}	-2.3 \pm 0.19 ^{de}	0.0 \pm 0.19 ^{cd}
0.1%		6.6 \pm 0.31 ^{ef}	-2.8 \pm 0.31 ^{efg}	-0.5 \pm 0.31 ^{ef}
0.3%		6.2 \pm 0.32 ^{fg}	-3.3 \pm 0.32 ^{fgh}	-1.0 \pm 0.32 ^{fg}
0.6%	40	5.5 \pm 0.21 ^{gh}	-3.9 \pm 0.21 ^{hij}	-1.7 \pm 0.21 ^{gh}
1.0%		5.6 \pm 0.34 ^{gh}	-3.8 \pm 0.34 ^{hij}	-1.6 \pm 0.34 ^{gh}
1.5%		4.1 \pm 0.32 ⁱ	-5.3 \pm 0.32 ^{lm}	-3.1 \pm 0.32 ^j
2.0%		2.9 \pm 0.40 ^j	-6.6 \pm 0.40 ^o	-4.3 \pm 0.40 ^k
4.0%		0.9 \pm 0.41 ^l	-8.5 \pm 0.41 ^q	-6.2 \pm 0.41 ^l

a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q

Means, within a column, lacking common superscript letters, differ ($P < 0.05$).

Table 3. Least squares means \pm standard error for *E. coli* Biotype I Counts (ECC) (log CFU/cm²), difference in ECC (log CFU/cm²) from negative controls (NC) and difference (log CFU/cm²) in ECC from water-treated controls (WC) from inoculated samples treated with 7 concentration of AVGARD™ at 3 pressures (PSI).

Treatment	Pressure	Log ECC	Diff from NC	Diff From WC
NC		7.8 \pm 0.02 ^{bc}	0.0 \pm 0.02 ^a	0.5 \pm 0.40 ^{bc}
WC		7.3 \pm 0.04 ^{cd}	-0.5 \pm 0.06 ^{ab}	0.0 \pm 0.06 ^{cd}
0.1%	<5	7.3 \pm 0.05 ^{cd}	-0.6 \pm 0.05 ^{ab}	0.0 \pm 0.05 ^{cde}
0.3%		7.3 \pm 0.15 ^{cd}	-0.5 \pm 0.15 ^{ab}	0.0 \pm 0.15 ^{cd}
0.6%		5.1 \pm 0.12 ^{ij}	-2.7 \pm 0.12 ^{fg}	-2.2 \pm 0.12 ^{hi}
1.0%		4.0 \pm 0.37 ^k	-3.8 \pm 0.37 ^{hi}	-3.3 \pm 0.37 ^j
1.5%		2.9 \pm 0.22 ^l	-4.9 \pm 0.22 ^{jk}	-4.4 \pm 0.22 ^k
2.0%		2.9 \pm 0.28 ^l	-5.0 \pm 0.28 ^k	-4.4 \pm 0.28 ^k
4.0%		2.0 \pm 0.00 ^m	-5.9 \pm 0.00 ^l	-5.4 \pm 0.00 ^l
NC			8.4 \pm 0.25 ^b	0.0 \pm 0.25 ^a
WC		7.2 \pm 0.04 ^{cde}	-1.2 \pm 0.04 ^{bc}	0.0 \pm 0.04 ^{cd}
0.1%	20	7.1 \pm 0.03 ^{def}	-1.4 \pm 0.03 ^{cd}	-0.1 \pm 0.03 ^{cde}
0.3%		6.5 \pm 0.26 ^{fg}	-1.9 \pm 0.26 ^{de}	-0.7 \pm 0.26 ^{ef}
0.6%		5.6 \pm 0.30 ^{hi}	-2.8 \pm 0.30 ^{fg}	-1.6 \pm 0.30 ^{gh}
1.0%		4.4 \pm 0.33 ^{jk}	-4.0 \pm 0.33 ⁱ	-2.8 \pm 0.33 ^{ij}
1.5%		2.9 \pm 0.22 ^l	-4.5 \pm 0.29 ^{ij}	-3.3 \pm 0.29 ^j
2.0%		2.9 \pm 0.28 ^l	-5.5 \pm 0.37 ^{kl}	-4.3 \pm 0.37 ^k
4.0%		2.0 \pm 0.00 ^m	-7.3 \pm 0.21 ⁿ	-6.1 \pm 0.21 ^m
NC			9.4 \pm 0.04 ^a	0.0 \pm 0.04 ^a
WC		7.1 \pm 0.19 ^{def}	-2.3 \pm 0.19 ^{ef}	0.0 \pm 0.19 ^{cd}
0.1%	40	6.6 \pm 0.32 ^{efg}	-2.8 \pm 0.32 ^{fg}	-0.5 \pm 0.32 ^{def}
0.3%		6.1 \pm 0.32 ^{gh}	-3.3 \pm 0.32 ^{gh}	-1.0 \pm 0.32 ^{fg}
0.6%		5.4 \pm 0.21 ^{hi}	-3.9 \pm 0.21 ^{hi}	-1.7 \pm 0.21 ^{gh}
1.0%		5.5 \pm 0.35 ^{hi}	-3.9 \pm 0.35 ^{hi}	-1.6 \pm 0.35 ^{gh}
1.5%		4.0 \pm 0.32 ^k	-5.3 \pm 0.32 ^{kl}	-3.1 \pm 0.32 ^j
2.0%		2.8 \pm 0.42 ^l	-6.6 \pm 0.42 ^m	-4.3 \pm 0.42 ^k
4.0%		0.9 \pm 0.40 ⁿ	-8.5 \pm 0.40 ^o	-6.2 \pm 0.21 ^m

a,b,c,d,e,f,g,h,i,j,k,l,m,n,o Means, within a column, lacking common superscript letters, differ ($P < 0.05$).

INFLUENCE OF WINE, NITRITE AND LACTIC ACID BACTERIA ON THE BEHAVIOUR OF FOOD POISONING BACTERIA IN MODEL SAUSAGES

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Key Words: dry fermented sausages; wine, nitrite, Lactic acid bacteria, food pathogens

Introduction

In fermented sausages the control of food poisoning bacteria is usually achieved by the low pH, the reduced water activity and the protective effect of nitrite and/or nitrate (Leistner, 1992). There are strong evidences that the presence of lactic acid bacteria plays a role in the safety of these products wider than the reduction of pH (Hugas, 1998; Lewus et al, 1991). Even with centuries of experience manufacturing these products, it is still observed punctually, foodborne outbreaks related to fermented sausages and other non-heated ready-to-eat meat products (CDC 2002, Moore, 2004). Even if the faults associated to those outbreaks are not always easy to establish, the strategies to prevent the potential risk that fermented dry sausages represents to health are well known through HACCP methodology. Nevertheless, its successful application lays in a deep knowledge of the product and the parameters that contributes to its conservation.

In Portugal there is a large variety of traditional dry fermented meat products. Among these products made from pork meat and fat, there is several using wine for seasoning, besides salt and other spices eventually present. There are two main ways to season the meat with wine, depending on the type of product. In those made with single pieces of meat – as “salpicao” - the meat can be submerged in wine for a few days before stuffing. In chorizo like products, the small pieces of meat are mixed with an amount of wine that is absorbed by the meat, and it stuffed with the batter after a resting period of, at least 24 hours.

The information in scientific literature related to sausages made from wine marinated pork is very scarce. But this procedure might have interesting consequences on the microbiological profile of the products, once, due to its composition, wine is a potential inhibitor for some pathogens. It is particularly important if we consider that some of these meat products are made at very different scales, from the large industries to small scale units associated to mountain rural environments. In those small units, the production is characterized by the use of “natural” methods, associated to absence of chemical additives or starter cultures. However, and even with no addition of sugar, those sausages present high numbers of lactic acid bacteria (LAB) and pH values moderately low, characteristics usually observed in fermented products. Those products manufactured in rural environments have an important potential to growth, but there are some aspects of its safety that are unknown, namely the effect of wine and added LAB in the food poisoning bacteria, as the consequences that the lack of nitrite have in the microbiological safety of these products.

Objectives

The aim of this work was to evaluate the effect of wine, nitrite and nitrate, and the addition of a starter culture of *L. sakei* on the behaviour of three pathogens frequently considered hazards in the preparation of not heated ready-to-eat sausages: *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes*.

Methodology

Bacterial strains and preparation of inoculum. Three strains of each pathogen tested were used (Table 1). One reference strain and two strains isolated from meat products or its production environment. Strains maintained at -18°C were subcultured twice in BHI before use and incubated 18 to 24 h at 37°C. Cultures to the inoculation were grown individually overnight in 30 ml of BHI, harvested by centrifugation, washed twice and resuspended in NaCl 0.85%. A mix of the 9 strains was prepared in order to achieve a level of inoculation of the model sausage about 2 log ufc /g of each pathogen.

Lactobacillus sakei BAL1343 previously identified from a fermented dry sausage (Patarata, 2002) were prepared basically as the pathogens, but using MRS. The level of inoculation was adjusted at 5 to 6 log ufc/g.

Model sausage: To screen more efficiently the effects in study, a model sausage was applied using meat with very low initial contamination obtained by ethanol immersion of a piece of pork, surface burning and aseptic excision of the inner part. Only not heated meat was used. Sterile pork fat was added (10%) and 2% of salt. To test the effect of wine, local red wine was used (pH 3.8, 11.5% ethanol) at a level of 7.5% of the batter, and a solution to imitate wine – thereafter called artificial solution (AS) - was prepared with the same amount of ethanol and 2.2 g/L of tartaric acid, 2.0 g/L lactic acid, 1.2 g/L malic acid, 1.2g/L succinic acid and 0.5 g/L acetic acid, as the mean values pointed by Curvelo-Garcia (1988) for a similar wine of the region. Both wine and the artificial solution were sterilized by filtration. The control of the effect “wine” was performed substituting it by the same amount of water. Nitrite and nitrate was prepared in solution in water to achieve a level of 150 mg/kg and 300 mg/kg of model sausage using 1 ml of solution.

After mincing the meat, the common ingredients were added and mixed in an aseptic environment. The last ingredients were added according to the experimental design and mixed with the model sausage. Units of 100 g were placed in polyethylene bags, and contaminated with the inoculum of pathogens. Homogenization was performed with a stomacher (3 min) and manually. After removing the air the bag was closed with a clip for stomacher bags. Sausage models were incubated at 15°C, as it is the mean temperature used by several small producers of meat products. Experimental design: A factorial design was performed for the three effects in study: Wine (local wine, artificial solution, water); Nitrite and nitrate (present or absent); LAB (present or absent). For each one of the 12 possible experimental units, 4 model sausages were prepared.

Bacterial enumeration: Samples were taken at 2 h, 1, 3, 7 and 14 days of incubation. Ten g were aseptically weighed and homogenised with 90 ml of buffered peptone water (Biokar 128) during 1.5 min in a stomacher. Appropriate serial dilutions in peptone water were spread on Compass *Listeria* (Biokar 06508), Compass *Salmonella* (Biokar 06608) and Baird Parker with RPF (Biokar 074) to count the three pathogens. After 24 to 48 h of incubation at 37°C, typical colonies were counted.

Lactic acid bacteria was enumerated in MRS (Cultimed 413785), Enterobacteriaceae in VRBG (Biokar 011) and Pseudomonas in CFC (Biokar 118), as described by Chevalier et al. (2005).

Results & Discussion

Two hours after the inoculation the microflora was enumerated in order to observe if the inoculation level was achieved. The count of *Salmonella* spp. and *S. aureus* were slightly higher than the previewed (2.44 ± 0.14 log ufc/g and 2.48 ± 0.16 log ufc/g, respectively). The contrary was observed with *L. monocytogenes*, presenting a mean counting of 1.5 ± 0.1 log ufc/g. No differences were observed among experimental unites in this sampling period, indicating that the level of inoculation was successful equilibrated among sausage models.

The results of analysis of variance for the three effects in study: wine, nitrite and nitrate and LAB, on the behaviour of *Salmonella* spp., *S. aureus* and *L. monocytogenes* after 1, 3, 7 and 14 days of incubation at 15°C are presented in table 2. The absolute values of the countings are not presented. To synthesize the main aspects of the results the percent of survivors between the sampling after inoculation and the last period (14 days) is presented in table 3.

As it can be observed in table 2, among the three effects tested, the presence of an inoculated LAB had important consequences in the counting of the three pathogens in almost all of the sampling periods (except for *Salmonella* at day 3). Samples with *L. sakei* presented less pathogens. The presence of nitrite and nitrate was also responsibly for the control of the growing of the pathogens. At the end of the incubation period, *Salmonella* and *L. monocytogenes* presented mean counting lower (significantly different $P < 0.001$) in samples treated with those chemicals additives. A similar effect was also observed with *S. aureus* after 3 and 7 days of incubation, but in the last period this effect disappeared. The effect of wine was observed directly only in *Salmonella* at day 7 and in *L. monocytogenes* at day 14. For *Salmonella* at day 7, the significant difference ($P < 0.01$) was established between samples treated with wine (2.96 ± 1.45 log ufc/g) and the control treated with water (3.64 ± 0.06 log ufc/g). Samples treated with AS were similar to both. The results observed with *L. monocytogenes* indicate that, at the end of 14 days of incubation, the counting was lower, significantly different ($P < 0.01$) in those samples treated with wine or AS (0.71 ± 0.86 log ufc/g; 0.41 ± 0.076 log ufc/g, respectively) than the control with water (1.37 ± 1.32 log ufc/g). Several interactions were observed to be significant, mainly related to the differences observed when more than one effect was in the control level. The reflex that the former analysis had on the behaviour of the microflora is synthesized in table 3. The mains aspects that must be stressed is that in almost all of the combinations of the effects under study, the number of *S. aureus* and *L. monocytogenes* was maintained under control, being the worst percent of survivors near 75%. The only situation that was problematic was the control with all the effects missing (no starter culture, no nitrite and nitrate and no wine). The results observed for *L. monocytogenes* are very interesting, considering that the simultaneous presence of *L. sakei*, nitrite and nitrate and wine - or the artificial solution - leads to the elimination of viable microflora. The situation observed for *Salmonella* is not as interesting as that observed for the Gram positive pathogens, once only the simultaneous presence of inoculated LAB nitrite and nitrate have effect in the suspension of its growth.

The LAB had higher growth rates in samples without the addition of *L. sakei*, and in those where it was added, it remained at the same level during all the period, as regarded for the percent of survivors near 100.

The spoilage bacteria enumerated revealed that both LAB and nitrite and nitrate have an interesting effect controlling the growth of Enterobacteriaceae and *Pseudomonas*. The simultaneous use of LAB, nitrite and wine is useful for the reduction of the counting of *Pseudomonas*.

Conclusions

The addition of a freshly grown *Lactobacillus sakei* to the model sausage revealed to be a procedure with important consequences on the control of the three pathogens studied during all the period of incubation. Nitrite and nitrate was confirmed as an important chemical additive controlling *Salmonella*, *S. aureus* and *L. monocytogenes*. The use of wine in the production of fermented dry sausages has a potential effect on the control of the development of *Salmonella* and *L. monocytogenes*, as observed from the results at day 7 or 14.

From the results of this work it should be stressed that, for the Gram positive pathogens studied, even with the lack of the effect of the drying, and consequent water activity reduction, the presence of, at least, one of the effects studied is enough to reduce its number during the period under study. From these results it is possible to infer that the apparent risk associated to the lack of nitrite might be solved by the presence of competitive LAB and/or wine. Considering these two pathogens, the most risky combination observed was the simultaneous lack of LAB, nitrite and wine.

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Tables and Figures

Table 1. Strains of Salmonella, *S. aureus* and *L. monocytogenes* implicated in the study

Strain	Origin
Salmonella Enteritidis	CECT 4155
Salmonella spp. T4Ah3c	Meat products
Salmonella spp. B4Rc4c	Raw meat to sausages
Staphylococcus aureus	ATCC25953
<i>S. aureus</i> SAt35	Dry fermented sausage
<i>S. aureus</i> SAt7	Environments of sausage production
<i>Listeria monocytogenes</i>	ATCC 7973
<i>L. monocytogenes</i> LMt1	Batter for dry fermented sausage
<i>L. monocytogenes</i> LMt4	Dry fermented sausage

Table 2. Results of the analysis of variance of the counting of the three pathogens in study in kmodel sausages with different composition regarding wine, nitrite and nitrate (NOx) and LAB.

Effect	Salmonella spp				S. aureus				L. monocytogenes			
	1	3	7	14	1	3	7	14	1	3	7	14
Wine	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	**
NOx	ns	**	***	***	ns	***	***	ns	ns	*	***	***
LAB	***	ns	***	***	***	*	***	**	***	***	*	***
Wine * NOx	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Wine * LAB	*	ns	ns	*	ns	*	**	ns	ns	ns	ns	*
NOx * LAB	ns	ns	**	***	ns	ns	***	ns	ns	ns	***	ns
Wine*NOx*LAB	ns	ns	**	*	ns	ns	ns	ns	ns	ns	ns	***

ns – not significant; * P<0.05; ** P<0.01; ***P<0.001.

Table 3. Percent of survivors determined between pos-inoculation (2 hours after) and 15 days of incubation

Effects			Salmon.	S. aureus	L. mono.	LAB	Ent.	Psed.
LAB	NOx	Wine						
-	-	-	337	162	291	282	396	388
-	-	AS	337	77	31	243	395	325
-	-	wine	290	70	60	245	358	319
-	present	-	125	70	29	236	155	281
-	present	AS	90	55	56	263	143	257
-	present	wine	115	64	73	262	126	270
LAB	-	-	208	54	43	112	299	201
LAB	-	AS	218	41	29	109	254	190
LAB	-	wine	181	39	43	108	214	182
LAB	present	control	0	42	20	107	124	91
LAB	present	AS	93	41	0	106	102	0
LAB	present	wine	22	44	0	104	103	0

**ANTIMICROBIAL EFFECT OF ROSELLE (*HIBICUS SABDARIFFA* LINN.)
EXTRACT AGAINST BACTERIAL PATHOGENS ASSOCIATED IN THAI
FERMENTED MEAT (NHAM)**

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Keywords: Roselle (*Hibicus sabdariffa* Linn.) extract, Nham, Thai fermented meat, *Staphylococcus aureus*, *Salmonella anatum*

Roselle, a Thai medicinal plant, has been known to exhibit antioxidant and antimicrobial activities. The study of antimicrobial activities of roselle extract (RE) at different concentrations (25, 50, 100, 150 and 200 mg/ml) were tested against 6 strains of bacteria associated in retailed meat and Thai fermented meat (Nham) [Four strains of gram-positive : *Staphylococcus aureus*, *Listeria innocua*, *Lactobacillus plantarum* and *Pediococcus pentosaceus*, and two strains of gram-negative : *Escherichia coli* and *Salmonella anatum*]. The results revealed that RE at 25 mg/ml showed an inhibitory effect only on *Lis. innocua*, while at 50 mg/ml inhibited *Lis. innocua* and gram-positive pathogen of *Staph. aureus*. The RE extract at 100 mg/ml exhibited wide range of inhibitory effect on both of gram-positive and gram-negative tested strains except two strains of lactic acid bacteria associated in the fermentation of Nham, while RE at 150 and 200 mg/ml could inhibited all the tested indicator strains. In order to confirm the inhibitory effects were not due to the lower pH of RE, three different concentrations of citric acid solution (CAS) with pH corresponding to the pH of RE at 100, 150 and 200 mg/ml were used to evaluate the inhibition of aforementioned 6 indicators. Wider inhibition zones were observed for RE compared to CAS at all concentrations evaluated. In addition, no inhibition zone for 2 LAB indicator strains was found with CAS. The concentration of RE at 25 – 50 mg/ml (w/v) in trypticase soy broth + 0.6 % yeast extract (TSBYE) exhibited a bactericidal effect on the cells of *Staph. aureus*, *Salm. anatum*, *Lis, innocua* and *E. coli*. RE concentration at 12.5 mg/ml showed a bactericidal effect only on *Staph. aureus* and *Salm. anatum*, while this same concentration implied a bacteriostatic effect on *Lis, innocua* and *E. coli*. This study implied that RE contain other components, in addition to organic acids, with antimicrobial activity. Moreover, the concentration of RE at 12.5 - 50 mg/ml in collaboration with important LAB as starter for Nham production may potentially be used to produce a safety of this Thai fermented meat product.

Introduction

Roselle (*Hibiscus sabdariffa* Linn.), one of Thai medicinal plants, is known to contain abundant phenolic compounds such as anthocyanin. The efficacy of this substance in Roselle extract against some gram-positive and gram-negative bacteria is well established when using in foods [1-3].

Nham, Thai traditional fermented meat, is produced when *Pediococcus cerevisiae*, *Pediococcus* spp. and heterofermentative lactobacilli reproduce rapidly during the first 3 days of the natural fermentation process, particularly when the incubation temperature is about 25-30°C [4]. The product is normally consumed after 3 days of fermentation without cooking. The incidence of salmonellae in the product [5 – 8] is therefore a serious public health concern. Since all ingredients to produce Nham cannot be pasteurized and, therefore, the fermentation flora has to compete with the fortuitous microorganisms. The application of starter cultures in this fermented meat product has become a means of controlling the fermentation process [9-10]. The fermented products have a safe reputation but, nevertheless, the process technology has to be directed to the prevention of the growth or even the elimination of food pathogens such as *Staph. aureus*, *Lis. monocytogenes* and *Salmonella*. Thus, the use of RE is one of the process technology to prevent the growth of associated food pathogens and lead to produce a safety of this Thai fermented meat product.

Objective

The study was to evaluate the antimicrobial activity of Roselle extract (RE) against some gram-positive and gram-negative bacteria associated in raw meat and Nham (Thai fermented meat). In order to study the potential use of RE for the safety prospect of Nham production, the effect of appropriate amount of RE on opportunistic food pathogens was also determined in an *In-vitro* trypticase soy broth + 0.6 % yeast extract (TSBYE).

Materials and Methods

Indicator strains and media

Two strains of gram-positive bacteria (*Staphylococcus aureus* and *Listeria innocua*) and 2 strains of gram-negative bacteria (*Escherichia coli* and *Salmonella anatum*) occasionally found in meat and Nham were cultured overnight at 35-37° C in trypticase soy broth + 0.6 % yeast extract (TSBYE). Two gram-positive of lactic acid bacteria (LAB) associated during Nham fermentation were cultured overnight at 35-37° C in MRS broth. Trypticase soy agar + 0.6 % yeast extract (TSAYE) and MRS agar were used for susceptibility test of RE against all indicators.

Roselle extract (RE) preparation

To prepare Roselle extract (RE), the dried flower of *Hibiscus sabdariffa* Linn. (purchased from local markets in Bangkok, Thailand) was ground to powder. A 25 g portion of powder was extracted by stirring with 250 ml of 80 % ethanol for 4 h and then filtered through filter Whatman No. 1 paper. The residue was re-extract

overnight with 250 ml of 80 % ethanol. The filtrates were pooled and subjected to the evaporation in a rotary evaporator under reduced pressure at 40° C. Concentrated RE was then freeze-dried. The dried extract was redissolved in water to obtain sample solution at various studies concentration (mg/ml). Citric acid solution was prepared at different concentrations with pH corresponding to the pH of RE solution at 100, 150 and 200 mg/ml.

Determination of antimicrobial activity of Roselle extract

The antimicrobial activity of RE was evaluated by the agar well diffusion method using the overnight culture suspension approximately $10^5 - 10^6$ Colony forming unit (CFU) [11 -12]. Melted agar media (TSAYE and MRS agar) were poured into a plate containing with overnight cultured suspension ($10^5 - 10^6$ CFU/ml). The wells then were made in each media using a sterile metallic borer. An aliquot of 150 μ l from each test concentration of RE, solvent control (negative control) and 30 mcg Chloramphenicol (positive control) were added in the wells. All plates were incubated at 35 - 37° C for 24 h. Results were expressed in terms of the inhibition zone surrounding the well. (Inhibition zone = diameter in mm. of the zone from the center of well – diameter in mm. of well). The experiment was performed in triplicate for each sample concentration.

In order to investigate the inhibition zone appeared to all tested bacteria by each concentration of RE were not due to the low pH, three different concentrations of citric acid solution (CAS) with pH corresponding to the pH of RE at 100, 150 and 200 mg/ml were used to evaluate the inhibitory effect on all tested strains by the agar well diffusion method as described above.

Determination of inhibitory effect of Roselle extract on tested opportunistic pathogens in trypticase soy broth plus 0.6 % yeast extract (TYBYE)

RE were filter-sterilized through a sterile 0.45 μ m membrane filter. The sterile RE were then dissolved in TSBYE at the final concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/ml (w/v). Each concentration RE was tested in triplicate for the inhibitory effect on *Lis. innocua*, *Staph. aureus*, *E. coli* and *Salm. anatum* (An initial load of each tested strain was $10^6 - 10^7$ CFU/ml) at 35 – 37° C for 24 h compared to the control TSBYE broth without RE.. After each tested strain inoculation in the broths, the cells counts were analyzed every 3 h interval during the incubation using spread technic on TSAYE and incubated overnight at 35 – 37° C.

Results and Discussion

Determination of antimicrobial activity of Roselle extract

The results in Table 1 revealed that RE at 25 mg/ml showed the inhibitory effects against only *Lis. innocua*, while at 50 mg/ml inhibited all gram-positive pathogens studied. Moreover, the RE at 100 mg/ml could inhibit both gram-positive and gram-negative pathogenic bacteria tested. However, the concentration of RE at 150 and 200 mg/ml inhibited not only the growth of pathogens but also that of lactic acid bacteria (LAB). The results indicated that the tested gram-positive (C and D) except LAB (E and F) were more sensitive to RE than gram-negative (A and B) bacteria.

Table 1. Antimicrobial property of Roselle extracts (RE) against some pathogenic bacteria

Test sample	A	B	C	D	E	F
RE 25 mg/ml pH 2.35	-	-	1.47	-	-	-
RE 50 mg/ml pH 2.25	-	-	2.56	2.83	-	-
RE 100 mg/ml pH 2.05	1.74	4.57	8.73	9.22	-	-
RE 150 mg/ml pH 2.02	5.87	6.22	11.53	12.56	1.43	1.49
RE 200 mg/ml pH 1.97	9.41	7.64	11.90	14.28	3.07	3.06
chloramphenicol	6.69	8.41	11.48	11.84	15.94	15.42
solvent control	-	-	-	-	-	-

Notes : - no inhibition, A = *E. coli*, B = *Salm. anatum*, C = *Staph. aureus*, D = *Lis. innocua*, E = , *Lb. plantarum*, F = *P. pentosaceus*

The results to investigate whether the inhibitory effects were due to the low pH of RE, three different concentrations of citric acid solution (CAS) with pH corresponding to the pH of RE at 100, 150 and 200 mg/ml were used to evaluate the inhibition of aforementioned 6 indicators. It was confirmed that, wider inhibition zones were observed for RE compared to CAS at all concentrations evaluated (Table 2). In addition, no inhibition zone for LAB was found with CAS. By these results, it is confident to inform that RE contain other components, in addition to organic acids, with antimicrobial activity.

Determination of minimum inhibitory concentration (MIC) of Roselle extract on opportunistic pathogens in trypticase soy broth plus 0.6 % yeast extract (TSBYE)

The results for MIC of RE on opportunistic pathogens in TSBYE (Figure 1) revealed that the best concentration of RE to diminish the cells of *Salm. anatum* (b) and *Staph. aureus* (c) in TSBYE within 24 h is upto and more than 12.5 mg/ml. The higher concentration of RE upto and more than 25 mg/ml is the most appropriate to be used for *E. coli* (a) and *Lis. innocua* (d) diminishment in TSBYE within 24 h. It is implied that the concentration of RE at 12.5 - 50 mg/ml collaborated with important LAB as starter for Nham production may potentially be used to produce a safety of this Thai fermented meat product.

Table 2. Antimicrobial property of Roselle extract (RE) compared to citric acid solution (CA) at pH corresponding RE to against some pathogenic bacteria.

Test sample	Zone of inhibition (mm)					
	A	B	C	D	E	F
RE 100 mg/ml pH 2.05	1.74	4.57	8.73	9.22	-	-
CA 2 % (v/v) pH 2.05	3.42	2.84	5.48	4.08	-	-

RE 150 mg/ml	pH 2.02	5.87	6.22	11.53	12.56	1.43	1.49
CA 2.25 % (v/v)	pH 2.02	4.06	3.36		6.64	4.61	-
RE 200 mg/ml	pH 1.97	9.41	7.64	11.90	14.28	3.07	3.06
CA 3.3 % (v/v)	pH 1.97	7.08	4.13	8.67	6.05	-	-
chloramphenicol		6.69	8.41	11.48	11.84	15.94	15.42
solvent control		-	-	-	-	-	-

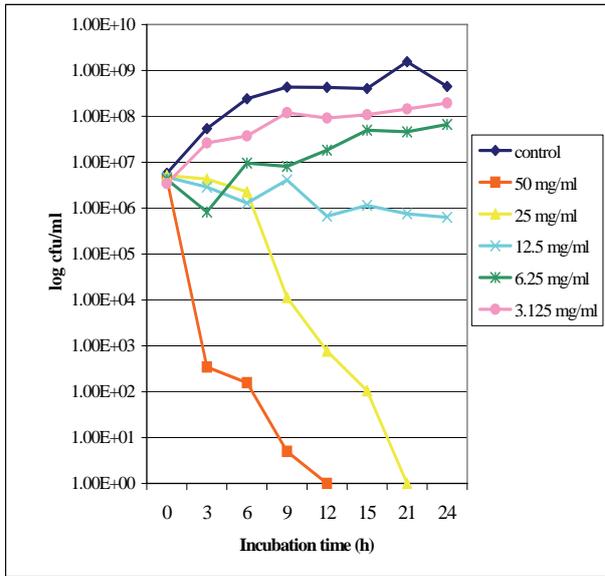
Notes : - no inhibition, A = *E. coli*, B = *Salm. anatum*, C = *Staph. aureus*, D = *Lis. innocua*, E = , *Lb. plantarum*, F = *P. pentosaceus*

Conclusions

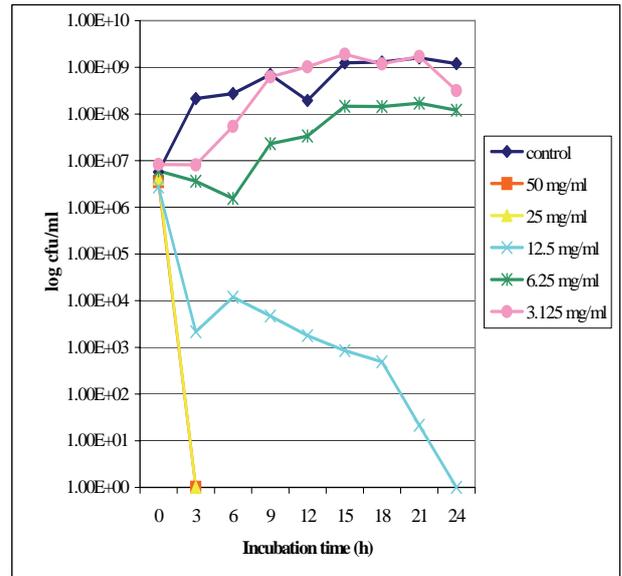
The study concludes that :

1. Roslle extract contains other components, in addition to organic acids, with antimicrobial activity.
2. *Salm. anatum* and *Staph. aureus* 2 opportunistic pathogens associated in Nham product were more sensitive to RE extract than *E. coli* and *Lis. innocua*
3. The concentration of RE at 12.5 upto 100 mg/ml implied to be used in collaborated with important LAB as starter to reduce some associated pathogens during Nham fermentation.

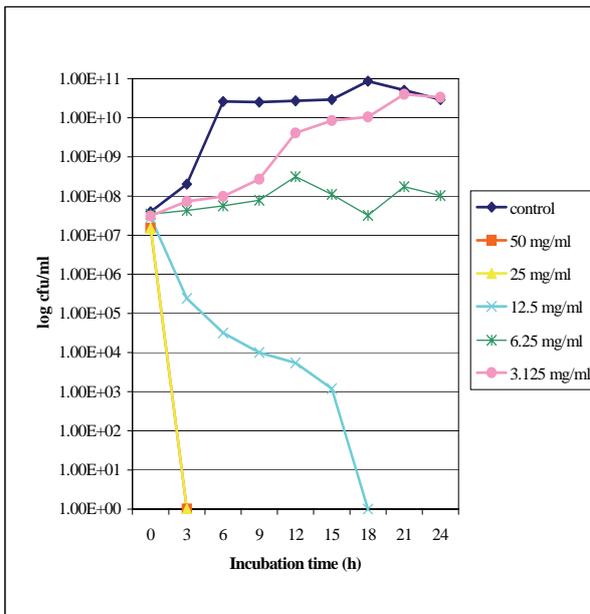
Figure 1 : Effect of various RE concentrations on *E. coli* (a), *Salm. anatum* (b), *Staph. aureus* (c) and *Lis. Innocua* (d) in TSBYE at 35-37° C



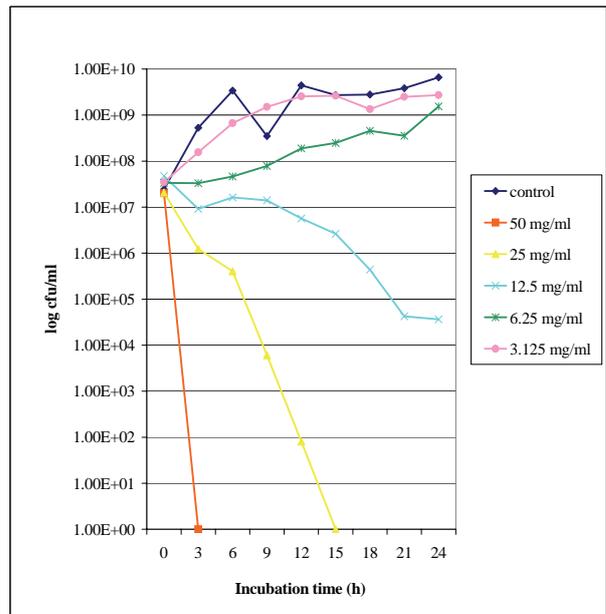
(a)



(b)



(c)



(d)

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STABILIZATION OF DRY-FERMENTED “LUKANKA” TYPE SAUSAGES WITH NATURAL ANTIOXIDANTS BLEND

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Background

One of the main reasons for the quality deterioration of the dry-fermented sausages is the lipid peroxidation. This process causes discoloration, appearance of unusual odour and taste and formation of potential toxic compounds (Morrissey et al., 1994; Gray et al., 1996). At less extent the disadvantage called "Worm over flavour" (WOF) exists as a problem in some types of meat foods (Spainer et al., 1992).

The dry-fermented "Lukanka" type sausages, traditional for Bulgaria, appear to be an attractive model of investigation, as the meat raw materials are minced and mixed with grinded salt, additives and spices, and after that filled in casings, fermented (matured), and dried. During their processing the sausages are exhibited to significant impact of prooxidant factors. As a result three types of lipid derivatives are formed: primary products – lipid hydroperoxides, secondary products - expressed as free malondialdehyde (free MDA) and oxidized cholesterols.

The stabilization of the "Lukanka" type sausages against lipid peroxidation depends on the technological treatment and meat storage. During the "Lukanka" production the abovementioned methods for lipid peroxidation limitation are either inapplicable or insufficiently effective. This is a reason different blend of antioxidants and synergists to be added to the minced meat. These types of blends have not universal application, because their effect depends on the composition of oxidized substrate (Raharjo et al., 1993). The effective stabilization of the lipids in dry-fermented "Lukanka" type sausages can be realized only after determination of the impact of endogen pro- and antioxidant factors.

Objectives

The objective of this study is stabilization of the lipid peroxidation occurring in dry-fermented "Lukanka" type sausages, produced from 3 months stored at -18°C frozen meat raw materials, by the addition of optimized composition of natural antioxidants (Dragoev et al., 2004, Dragoev and Balev, 2004) in two forms – liquid and dry.

Materials and Methods

The model system of "Lukanka" was prepared from frozen meat raw materials: beef type CL 95; pork sort 50/50 and bacon, stored 3 months *post mortem* at -18°C.

The experiments were carried out with "Monastery's lukanka" which is largely covered representative of dry-fermented sausages in Bulgaria, and is distinguished

with comparatively high fat content. The last one is a precondition for the most expressive changes of lipid fraction in comparison with other assortments lukanka.

The recipe of the “Monastery’s lukanka” control sample is presented in Table 1.

For the aim of the experiments the following antioxidants were supplied:

LRSE 1 – liquid rosemary extract, supplied by “Aromena” Ltd. (Sofia, Bulgaria). This extract is in accordance with the indicators of the TS (Technological Specification) No 3267-2000 – 55-60 % undistilled alcohol extract, which is a liquid with hard phase, yellow-brown colour and a characteristic rosemary odour. The dry content is 3.00 – 3.10 %. Flavonoides content is 1.15 – 1.30 %. Refraction coefficient n_D^{20} is 1.3605 and its relative density d_{20}^{20} is 0.7651.

DRSK – dry powder rosemary concentrate, supplied by “Aromena” Ltd. (Sofia, Bulgaria). This concentrate is in accordance with the indicators of the TS (Technological Specification) No 3267-2000 and it is a powder with yellow-brown colour and slightly expressed rosemary characteristic odour. Flavonoides content is about 42 %, and the acid value – 67.87.

RT – chemically pure rutine, purchased from E. Merck (Darmstadt, Germany).

DKFK – dry extract of Japanese acacia (*Sophora japonica*) flower bud, produced in the Department of Biotechnology in the University of Food Technologies – Plovdiv, Bulgaria. The dry finely grinded mass is hydrolysed 8 hours at 60°C and pH 4.5. The obtained hydrolyse product is triple extracted with 80 % ethanol and dried (Valkova and Bahcevanska, 2003). The extract contains 53.33 % quercetine – aglicon of the natural glycoside rutine.

SE – sodium erythroate, supplied by F.I.A. Food Ingredients Anthes GmbH (Teising, Germany).

For the analysis the following reagents were purchased: 2-thiobarbituric acid, distilled pure chloroform and methanol - from Sigma Chemical Company Ltd. (St. Louis, USA, Deisenhofen, Germany); potassium iodide, sodium thiosulfate and silver iodide were supplied by Fluka Chemie AG (Buchs, Swaziland). All the rest of chemicals and solvents were AR and GPL grade and were supplied by Aldrich Chemical Co (Steinheim, Germany).

Parallel samples were produced as following:

Control sample – without antioxidants.

First experimental sample – with addition of 0.124 % (1.24 g/kg) natural antioxidants composition No 1 (liquid form), containing 48.39 % LRSE 2 – liquid rosemary extract, which is 28 - 30 % undistilled alcohol extract with flavanoides content of 2.12 – 2.64 %, combined with 35.48 % rutine and 16.13 % sodium erythroate.

Second experimental sample – with addition of 0.112 % (1.12 g/kg) natural antioxidants composition No 2 (dry form), containing 3.58 % DRSK – dry powder rosemary concentrate, containing about 42 % flavonoides, combined with 78.57 % DKFK – powder extract of Japanese acacia (*Sophora japonica*) flower bud, containing 53.33 % quercetine – aglicon of rutine and 17.85 % sodium erythroate.

The samples of “Monastery’s lukanka” were processed by use of traditional Bulgarian technology.

The lipids of the samples were extracted by the Bligh and Dyer method (1959), according the recommendations of Smith et al. (1990).

The oxidative stability of the extracted lipids was determined by Rancimat method (Ranfft et al., 1988), based on conductometric measuring of the lipid peroxidation volatile secondary products. The analyses were carried out on the Metrohm 679 Rancimat apparatus (Metrohm AG, CH – 9100, Switzerland).

The hydroperoxide concentration was determined by measuring the peroxide value (POV), expressed as $\text{meqvO}_2 \cdot \text{kg}^{-1}$ lipids (AOAC, 1990).

Lipid peroxidation secondary products content, expressed as free malondialdehyde (MDA) was estimated by TBARS indicator according the water-acid extraction method (Schmedies and Holmer, 1989). TBARS were presented as MDA $\text{mg} \cdot \text{kg}^{-1}$ product.

Microbiological analyses. The total number of micro organisms and the share of oxidase reducing bacteria were estimated according standard methods (Boshkova, 2000).

Statistical analyses. The data was processed using the Microsoft Excel, Version 5 software. The ANOVA was made. The statistical significant differences were determined by Fischer's test.

Results and Discussion

The addition of natural antioxidants blend (in both examined forms) partially stabilizes the lipids when frozen meat materials are used. Higher and statistically significant differences ($p < 0.05$) are estimated only in the first 15 – 20 d of the maturing and drying of the product. In the final product – at 30 d, all the examined samples show induction period close to 0 h (Fig. 1). The two forms of the antioxidants composition stabilize almost identically the sausage lipids.

The addition of antioxidant composition at the abovementioned concentrations significantly suppresses the formation of the primary products of lipid peroxidation (Fig. 2), where in the maturing and drying process (15 d) the levels of the formed hydroperoxides reduce with 22 – 25 %. In the final product (30 d) the sample with an addition of liquid form No 1 of the composition shows approximately three times lower levels of hydroperoxides with comparison to the control sample, while the sample with an addition of dry form No 2 of the composition shows about 65 % reduction of the hydroperoxides level with comparison to the control sample.

Irrespectively of the significant detention of the lipid peroxidation in the experimental samples of “Monastery's lukanka”, produced from frozen raw materials, the levels of the hydroperoxides are higher than 3 meqvO_2/g lipids. These results show that once the lipid peroxidation process during the meat raw materials storage progresses, it is difficult to get control over it during the further technological treatment of the sausages.

It is estimated that both blend forms do not show statistically different results ($p > 0.05$) concerning the hydroperoxides levels, but they are statistically significantly lower ($p < 0.05$) with comparison to the control sample.

With the examination of the effect of antioxidant blend on limitation of the lipid peroxidation secondary products formation, similar results are obtained (Fig. 3). The addition of the antioxidant blend reduces to great extent the levels of the free malondialdehyde. On the 15 d the levels of the lipid peroxidation secondary products formed are reduced with 20 – 22 %, and in the final product (30 d) – with about 25 %.

The sample with the addition of liquid form No 1 of the blend shows statistically significant ($p < 0.05$) lower levels of malondialdehyde on the 15 d of the drying in relation to the sample with addition of dry form No 2 of the blend, but in the final product in both experimental samples the levels of TBARS are statistically indifferent ($p > 0.05$).

It is estimated that both examined forms of the blend do not show statistically different results ($p > 0.05$) in relation to the TBARS levels, but they are statistically significantly lower ($p < 0.05$) than these of the control sample.

The results show that the total number of aerobic mesophylic microorganisms decrease during the sausage drying. This trend of decrease is kept both in the control samples (produced without antioxidants addition) and experimental samples (with dry and liquid form of antioxidant blend addition) (Fig. 4). The addition of natural antioxidant blend contributes to the slight, but statistically significant decrease ($p < 0.05$) of the total number of microorganisms in “Monastery’s lukanka”, as both in the processes of maturing and drying, as well as in the final product. Little higher are the results of the samples with addition of the liquid form No 1 of the antioxidant blend, in relation to the dry form No 2, but these differences are statistically insignificant ($p > 0.05$) (Fig. 4). The total number of microorganisms in the samples of filling mass of “Monastery’s lukanka”, produced from frozen raw materials (0 d) is 3.8×10^4 . The addition of antioxidant blend does not effects statistically significantly the change of the total number of mesophylic aerobic microorganisms in the samples, produced from frozen raw materials. This shoes, that the antioxidant blend used has not clear bactericide effect, irrespectively of the form (dry or liquid).

During the process of maturing and drying of the sausages, produced from frozen meat raw materials, the number of the oxidase positive strains increases (Fig. 5) and in the control sample of the final product (30 d) the increased percentage of these microorganisms is statistically significantly greater ($p < 0.05$) in relation to the both experimental samples. The results obtained show, that the addition of antioxidant blend contributes to the significant decrease of the number of oxidase positive strains and detains the oxidative processes in the sausages.

Conclusions

The two experimental samples of the natural antioxidant blend stabilize the sausage’s lipids and reduce the levels of hydroperoxides and TBARS. The examined blend of natural antioxidants, irrespectively of its forms (liquid or powder), has not clear bactericide action and slightly effects the changes of total number of aerobic microorganisms. It mainly influences the share of oxidase reducing strains and thus suppresses the initialization of free radicals which cause lipid peroxidation processes.

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Table 1. Raw materials of 100 kg filling mass for “Monastery’s lukanka” processing

Main and supplementary raw materials	Mass, kg
1. Beef leg and shoulder	55.000
2. Semi fat pork	25.000
3. Firm dorsal bacon	20.000
4. Salt	2.200
5. Nitrate potassium	0.040
6. Black pepper	0.300
7. Cumin	0.200
8. Red pepper	0.200
9. Artificial casings “Fibrous” type 55 Ø mm	70 m

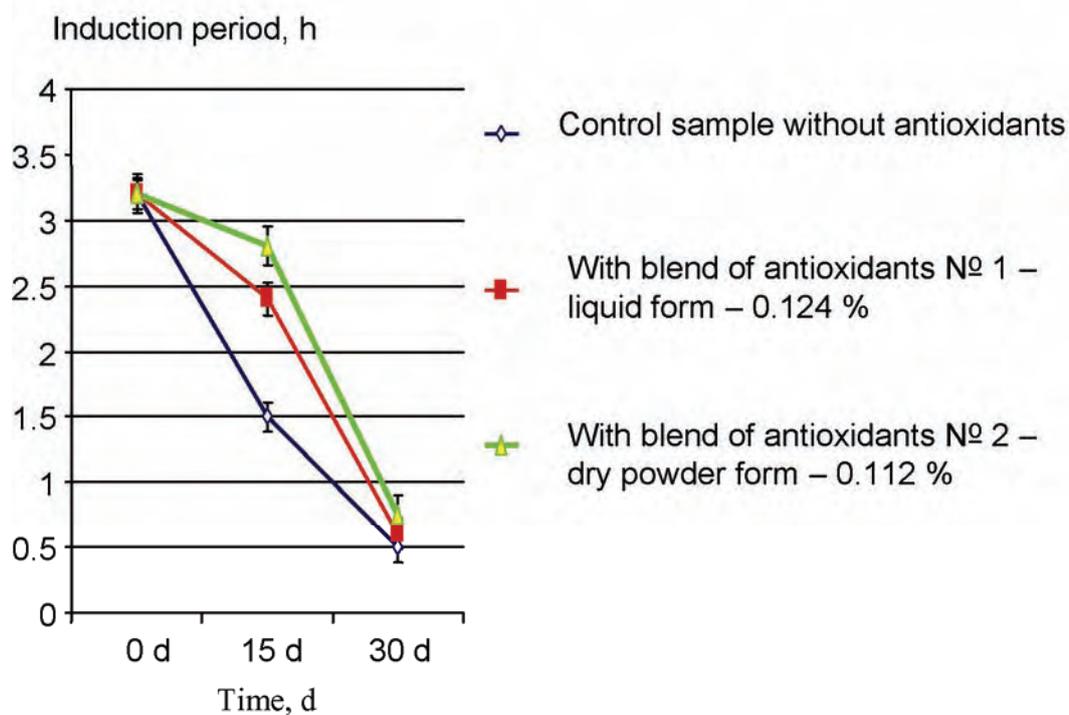


Figure1. Oxidative stability of lipids, extracted from “Monastery’s lukanka” processed from frozen to minus18°C 90 d stored raw materials with addition of antioxidant blend

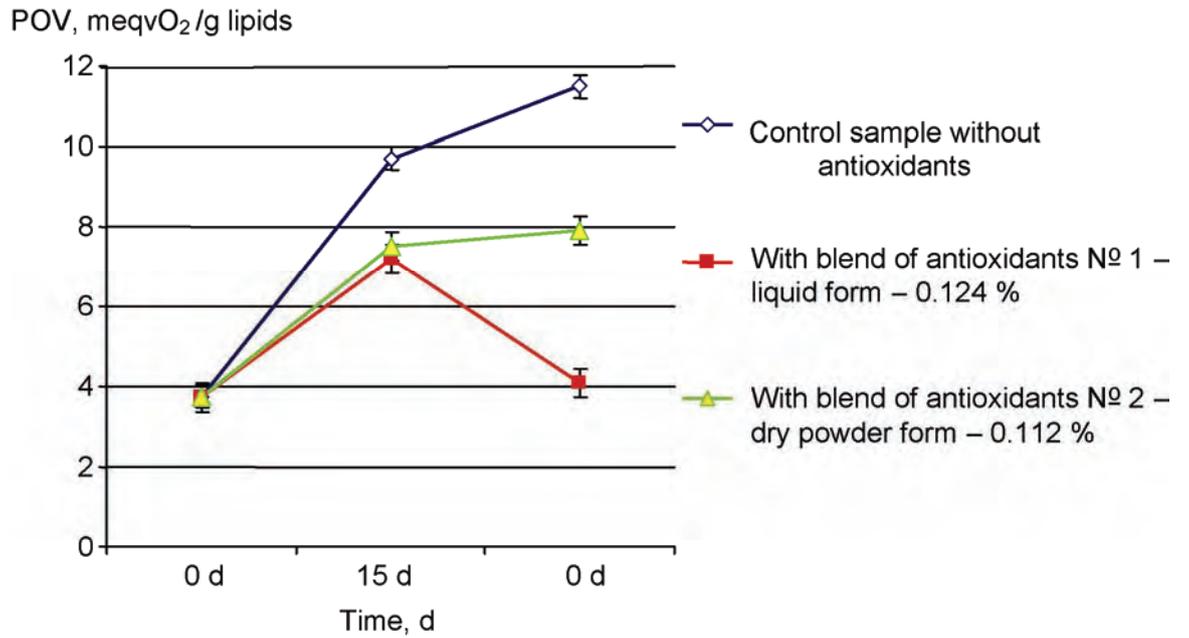


Figure 2. Peroxide value (POV) of lipids, extracted from “Monastery’s lukanka” processed from frozen to minus 18°C 90 d stored raw materials with addition of antioxidant blend

TBARS, free MDA mg/kg

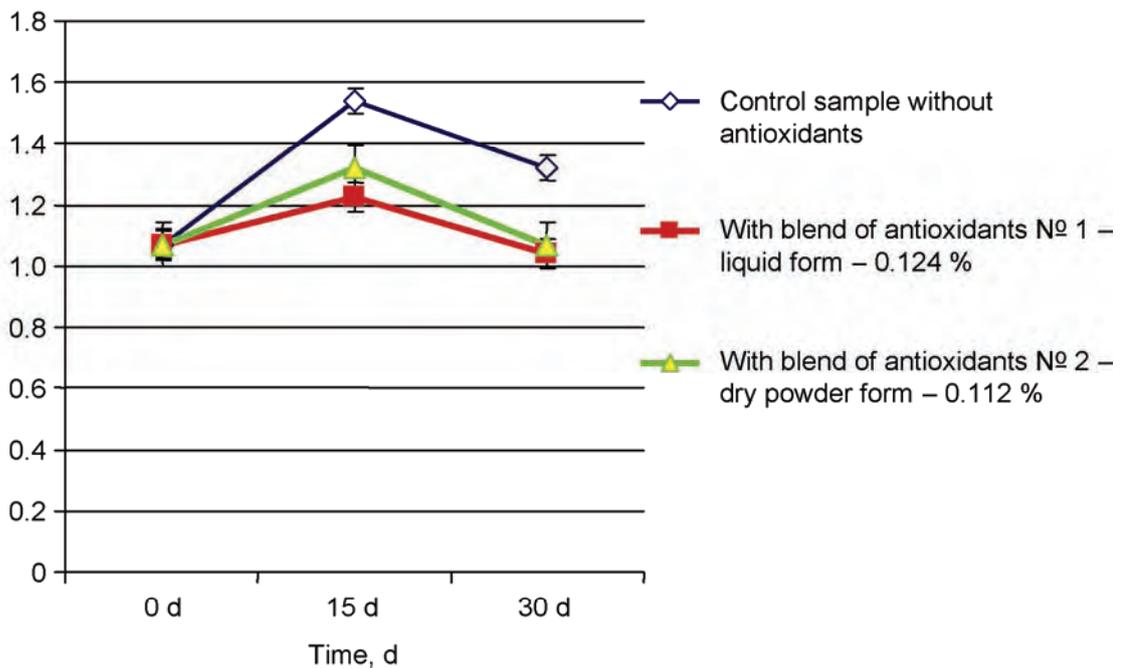


Figure 3. TBARS of lipids, extracted from “Monastery’s lukanka” processed from frozen to minus 18°C 90 d stored raw materials with addition of antioxidant composition

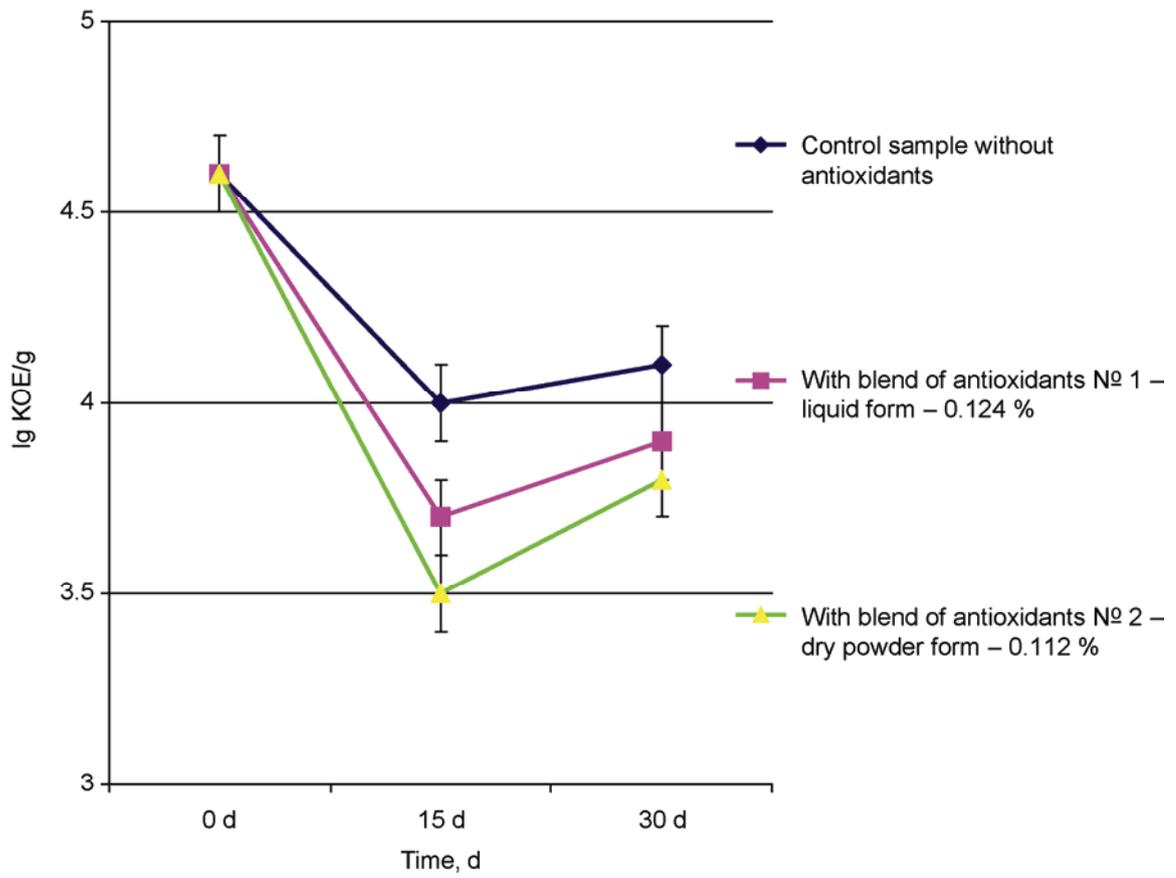


Figure 4. Changes of total number of micro organisms (logKOE/g) in "Monastery's lukanka" processed from frozen 90 d stored raw materials with addition of antioxidant blend

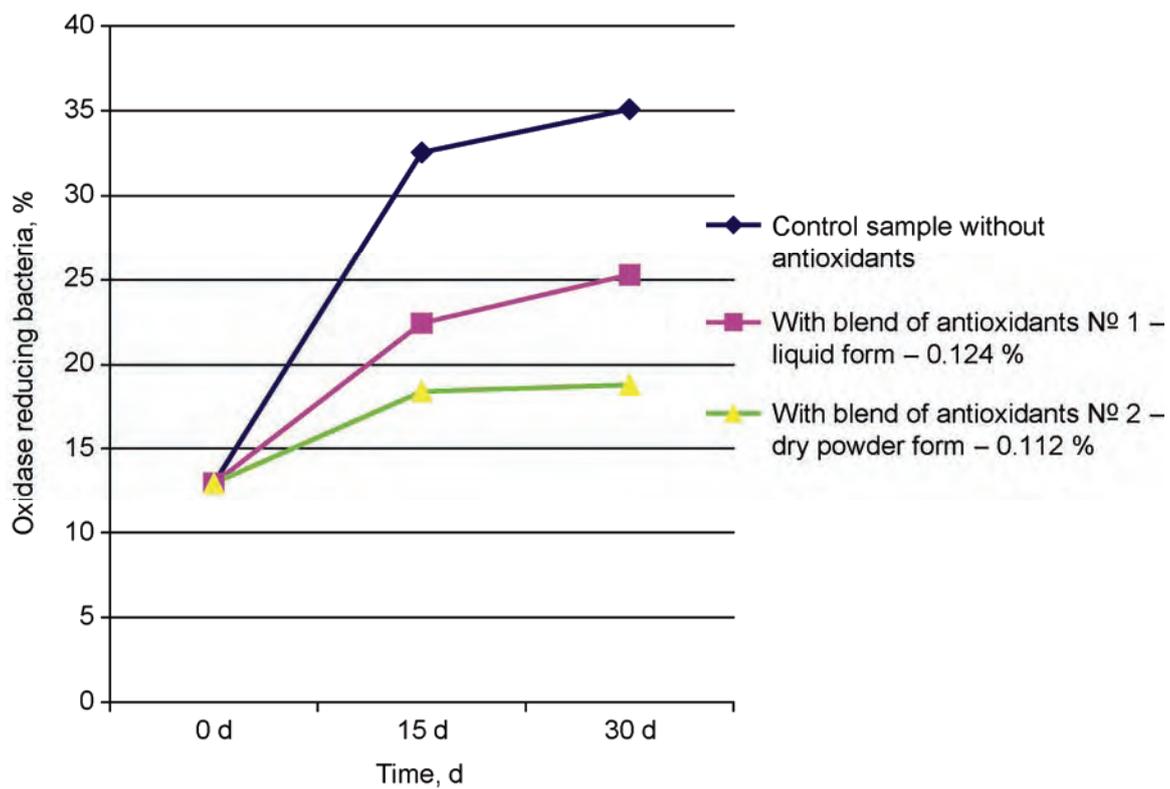


Figure 5. Percentage of ORB in relation to total number of microorganisms in "Monastery's lukanka" processed from frozen to minus 18°C 90 d stored raw materials with addition of antioxidant blend

APPLICATION OF BACTERIOCIN PRODUCING LACTOBACILLI FOR THE CONTROL OF *LISTERIA* IN ITALIAN SALAMI

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Key Words: Fermented sausage, starter culture, acidification, staphylococci, LAB, bacteriocin, *Listeria*, PCR

Introduction

All over the world *Listeria* contamination is a potential hazard in fermented, dry sausages produced without heat treatment. As heat treatment alters the meat structure such a product is not perceived as a traditional fermented, dry sausage by consumers. Normally, if present, the level of *Listeria* in fermented, dry sausages is relatively low and should not cause health problems when fermented sausages are consumed. Nevertheless, regulation in food requirements, as safety criteria, calls for absence of *Listeria monocytogenes* in 25 g food, and consequently, efforts are accomplished to prevent *Listeria* being present in traditionally produced fermented sausages. Commercial bacteriocin producing lactic acid bacteria (LAB) have successfully been tested on applied *L. monocytogenes* in fermented sausages (Andersen, 1999) but few data on effect on indigenous *Listeria* with such LAB strains are available (Hugas et al, 2003).

Some of the characteristics of Italian salami are high final pH, moulded surface, and pronounced meaty flavour. It is well-known that staphylococci enhance the development of meaty flavour but also that they are inhibited by lowering in pH (Tjener, 2003). Therefore, an adequate anti-*Listeria* LAB starter culture should not lower pH so much that it influences the development of required flavour compounds and the sensory assessment.

Objectives

The aim of this work was, in commercially manufactured sausages, to test the inhibition of indigenous *Listeria* by a class II bacteriocin producing *Lactobacillus plantarum* BG-112 (called Code 2) in parallel with a non-bacteriocin producing culture blend consisting of *Lactobacillus sakei* Ls and *Pediococcus pentosaceus* Pp MIX (called Code 3). Both cultures were applied with *Staphylococcus xylosus* (called SX) as well. Additionally, a control product without bacteria cultures applied was followed (called Code 1). The growth of staphylococci, LAB, pH, weight loss, and the presence of indigenous *Listeria* were monitored.

Methodology

As the experiment was conducted at a commercial manufacture all details in composition of the ingredients used were not revealed. Neither were the details in the production procedure. The sausages were produced with the normal recipe and processing procedure used for production of big calibre Milano type salami.

Recipe

Meat: Pork meat with approximately 30% back fat added. The meat and fat were grinded. Ingredients: Commercial blend of sugar (approximately 0.5%), spices, curing salt and salt was applied just after grinding. During mixing the culture solution was applied to the meat.

Casing: Approximately 110 mm collagen casing.

Processing

Sausage mince was produced and cold stored at 4°C for two days.

1. Mince was stuffed (just about 2 kg/sausage) and cold stored at 4°C for five days.

Day 0 in the registered data.

2. On day 4 the sausages were sprayed with a mould solution (*Penicillium nalgiovense*). The sausages were hereafter stored in climate chamber at 24°C and 55-65% r.h.

3. The following six days the temperature was lowered with 2°C per day.

4. On day 13 the temperature was 10°C and min. 85% r.h. The drying process was continued until a weight loss of roughly 26% was obtained.

5. On day 69 the test was terminated.

Sausage codes

Code 1: Control without culture applied

Code 2: Added 2.5x10⁶ CFU BG-112/g meat and 5x10⁶ CFU SX/g meat

Code 3: Added 2.5x10⁶ CFU Ls/g meat, 2.5x10⁶ CFU Pp/g meat, and 5x10⁶ CFU SX/g meat.

Bacteriological investigation

Staphylococci were detected by surface plating on S-110 (Oxoid), aerobically incubated for two days at 30°C. LAB were detected on MRS -5.4 (Oxoid adjusted to pH 5.4), anaerobically incubated for three days at 30°C. All analyses were performed on a sample size of approximately 35 g finely chopped sausage.

Identification of Listeria in sausage samples

The presence of *Listeria* was determined as follows: 25 g of sausage were added 225 g Listeria Enrichment Medium, homogenized and incubated over night at 37°C. DNA was extracted from the enrichment broth as described by Matarante (Matarante et al. 2004). PCR for the identification of *Listeria* species was carried out as described by Bubert (Bubert et al. 2003). The expected size of amplicons were 457 bp for *L. monocytogenes*, 472 bp for *Listeria innocua*, and 600-610 bp for *Listeria seeligeri*,

Listeria welshimeri, and *Listeria ivanovii*. The analyses were performed in duplicate per code at the samplings days mentioned below.

Analytical programme

On the day of manufacturing a meat mince sample from each code were bacteriological analysed detecting LAB and staphylococci. pH was registered continuously (Cinac apparatus) for 5 days in about 400 g meat mince in a container with lid placed in water bath at 25°C. During industrial production of the sausages sampling was performed as follows: Two randomly picked sausages of each code (A and B) were collected at the manufacture for external bacteriological survey on day 0, 4, 5, 6, 7, 8, 11, 12, 20, 27, 41, 55 and 69. They were analysed monitoring LAB & staphylococci, and pH (Knick instrument) was measured. Furthermore, the same two sausages were weighed at the manufacture on analytical days to follow the weight loss. 1/3 of each sausage was stored frozen at -18°C for later determination by PCR of indigenous *Listeria* contamination.

Results & Discussion

Continuous pH measurement is a good tool to investigate lag phase and acidification profile and to compare the performance relatively between starter cultures. Nevertheless, it is a model system with e.g. optimal heat transfer at a favourable temperature for the cultures and no water loss. Consequently, it will not reflect what will happen in the sausages during maturation. In Figure 1 the continuous pH measurements are illustrated.

The lag phase of the two starter cultures was similar. After 40 hours BG-112 acidified slightly faster than MIX and after approximately 90 hours the acidification was finalised for both cultures. The difference in acidification profiles was not important for technological purposes. Indigenous LAB from the raw meat, did probably not contribute considerably to the acidification in Code 2 and 3, given that Code 1 showed a longer lag phase and slower acidification profile (Figure 1 and 2).

The pH kinetics (average of sample A and B) during sausage fermentation is illustrated in Figure 2.

The slight initial pH increase was probably due to the storage at low temperature, and consequently, neither indigenous nor culture LAB initiated acidification. On day 6 to 7 the decrease in pH was initiated. The acidification in Codes 2 and 3, with starter cultures applied, was more pronounced than in the control (Code 1). BG-112 and MIX showed rather similar acidification kinetics and approximately at day 11 pH 5.3 were reached. As a guideline pH 5.3 can be considered as safe for initiating the drying but also approximately the pH at which staphylococci are inhibited. After day 27 pronounced raise in pH was observed and continued to more or less the same extend until the trial was terminated. This change in acidification kinetics could be connected to the processing temperature that was lowered and kept at 10°C from day 13. On day 30 for Code 3 and day 37 for Code 2 pH was again above 5.3. The control followed the same pattern only more slowly and to lesser extend.

The growth dynamics of LAB (average of sample A and B) during sausage processing is illustrated in Figure 3.

LAB initiated growth after five days, when temperature was raised, and the numeral increase was closely related to the acidification process. In Code 1, control, the initial LAB level was close to 10⁴ CFU/g. Approximately at day 11 the level of

LAB was 10⁸ CFU/g in all codes with the count of LAB remaining at a high level throughout the processing in all three codes.

Growth dynamics of staphylococci (average of sample A and B) during processing is illustrated in Figure 4.

The growth of staphylococci was enhanced by the initial raise in temperature and then retarded by the acidification. They probably adapted to the pH being approximately 5.1 and re-started further growth supported by the raise in pH later. Consequently, the staphylococci were able to continue the production of functional enzymes anticipated to benefit colour stability (catalase) and flavour formation.

Weight loss (average of weighing of two sausages) during the processing is illustrated in Figure 5.

The weight loss followed the pH decline in the sausages with BG-112 having a slightly faster weight loss than MIX which again was faster than the control code. Due to the big calibre and recipe the sausages were still rather soft with only approximately 26% of weight loss when the test was terminated.

The presence of indigenous *Listeria* in the sausages was analysed using PCR primers and condition which were able to distinguish among different *Listeria* species. The discoveries are reported in Figure 6, 7 and 8.

In all three codes *Listeria* was detected in 25 g sausage during the first weeks of fermentation. In Code 1, control, *Listeria* appeared on day 6 and was present all through the processing. *L. monocytogenes* was also detected at the end of the sausage production.

In Code 2 *Listeria* also appeared on day 6 but disappeared on day 12 and did not reappear. *L. monocytogenes* was detected only in one sample after 7 days of fermentation.

In Code 3 *Listeria* also appeared on day 6 and was detected throughout the ripening period. A faint band of *L. innocua* was as well detected at the end of the processing.

Conclusions

The development of pH had influence on the growth of staphylococci, and consequently, the initial decrease in pH, requested for safety purposes as well as initiation of the drying process, had negatively impact of the development. Thereafter, an increase in pH, probably related to the processing, re-activated the staphylococci which should augment the production of essential enzymes. Applying bacteriocin producing LAB was demonstrated to be an additional safety hurdle as the presence of indigenous *Listeria* was diminished. *Listeria* appeared early in the processing in all three codes but was later suppressed and not detected by PCR after day 12 in the product applied *L. plantarum* BG-112 producing a class II anti-listerial bacteriocin.

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Figures and Tables

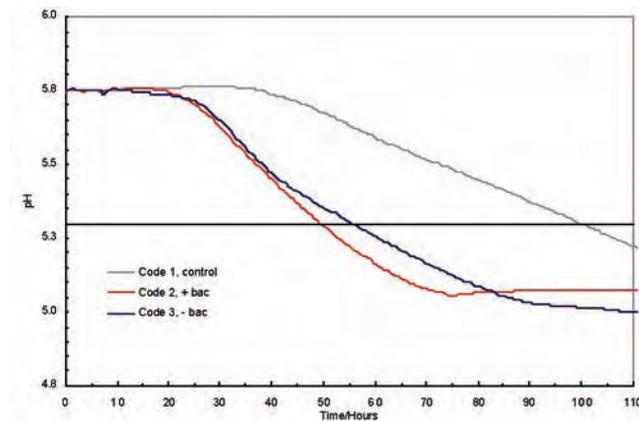


Figure 1. Continuous pH measurement at 25°C in meat mince

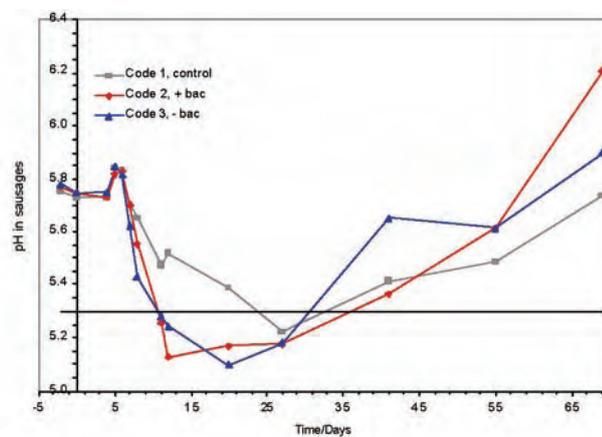


Figure 2. Development of pH in sausages during production

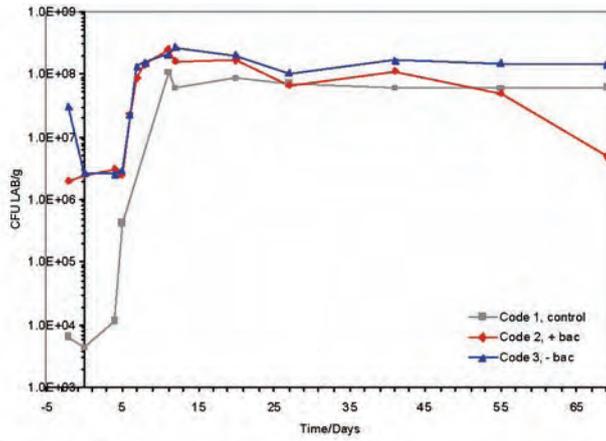


Figure 3. Enumeration of LAB in sausages during production

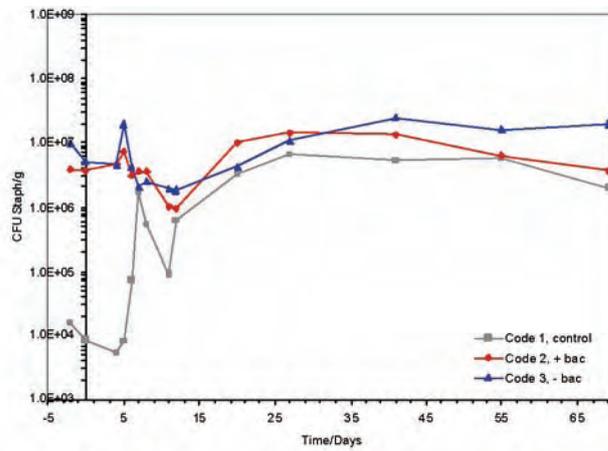


Figure 4. Development of staphylococci in sausages during production

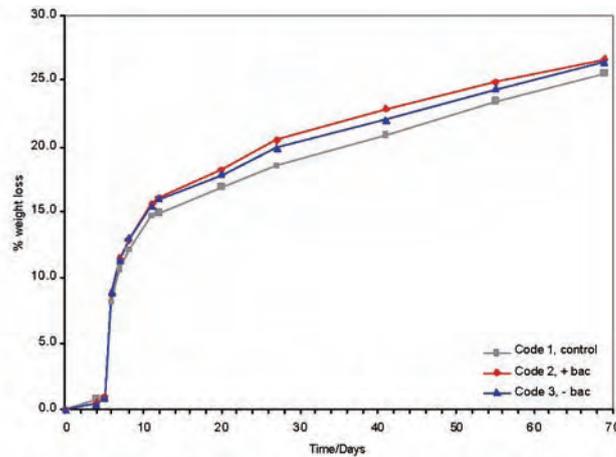


Figure 5. Development of weight loss of sausages during production

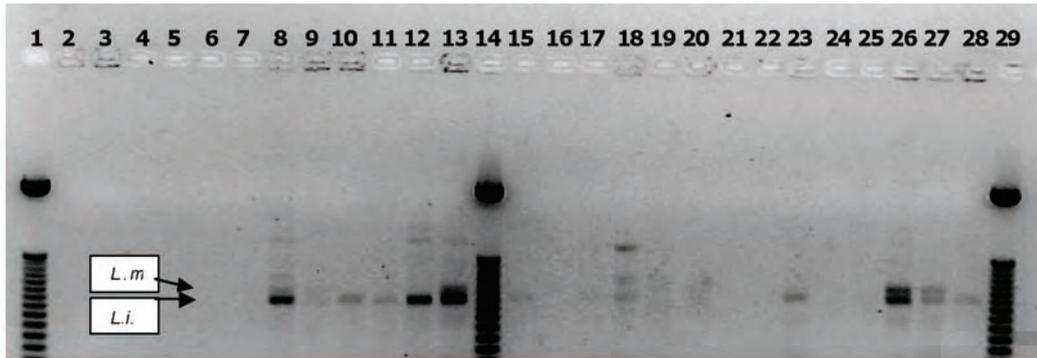


Figure 6. Detection of *Listeria* in Code 1, control. The band corresponding to *L. monocytogenes* and *L. innocua* are indicated with L.m and L.i., respectively. Sample lanes were as follows:

1	DNA MARKER	8	Day 6	A	16	Day 11	B	24	Day 41	B
2	Day 0	9	Day 6	B	17	Day 12	A	25	Day 55	A
3	Day 0	10	Day 7	A	18	Day 12	B	26	Day 55	B
4	Day 4	11	Day 7	B	19	Day 20	A	27	Day 69	A
5	Day 4	12	Day 8	A	20	Day 20	B	28	Day 69	B
6	Day 5	13	Day 8	B	21	Day 27	A	29	DNA MARKER	
7	Day 5	14	DNA MARKER		22	Day 27	B			
		15	Day 11	A	23	Day 41	A			

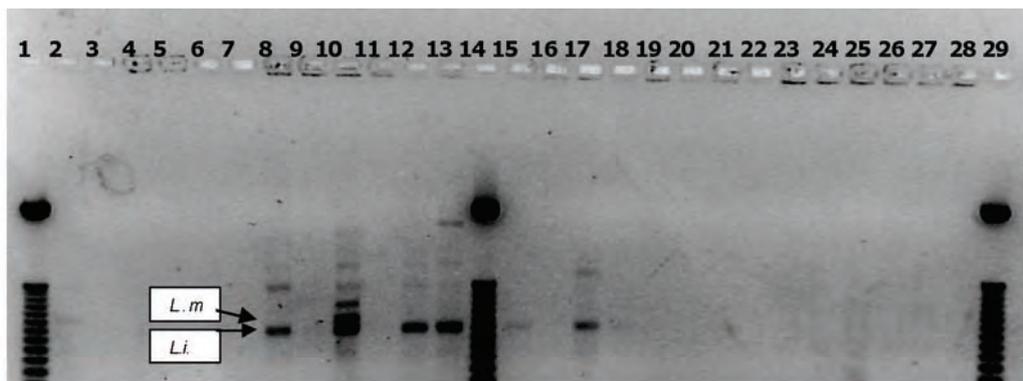


Figure 7. Detection of *Listeria* in Code 2, + bac. The band corresponding to *L. monocytogenes* and *L. innocua* are indicated with L.m and L.i., respectively. Sample lanes were as follows:

1	DNA MARKER	8	Day 6	A	16	Day 11	B	24	Day 41	B
2	Day 0	9	Day 6	B	17	Day 12	A	25	Day 55	A
3	Day 0	10	Day 7	A	18	Day 12	B	26	Day 55	B
4	Day 4	11	Day 7	B	19	Day 20	A	27	Day 69	A
5	Day 4	12	Day 8	A	20	Day 20	B	28	Day 69	B
6	Day 5	13	Day 8	B	21	Day 27	A	29	DNA MARKER	
7	Day 5	14	DNA MARKER		22	Day 27	B			
		15	Day 11	A	23	Day 41	A			

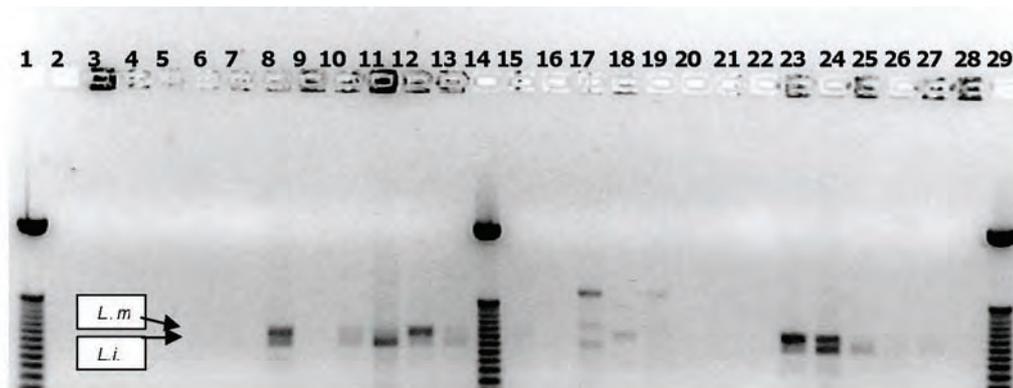


Figure 8. Detection of *Listeria* in Code 3, - bac. The band corresponding to *L. monocytogenes* and *L. innocua* are indicated with L.m and L.i, respectively. Sample lanes were as follows:

1	DNA MARKER	8	Day 6	A	16	Day 11	B	24	Day 41	B
2	Day 0	9	Day 6	B	17	Day 12	A	25	Day 55	A
3	Day 0	10	Day 7	A	18	Day 12	B	26	Day 55	B
4	Day 4	11	Day 7	B	19	Day 20	A	27	Day 69	A
5	Day 4	12	Day 8	A	20	Day 20	B	28	Day 69	B
6	Day 5	13	Day 8	B	21	Day 27	A	29	DNA MARKER	
7	Day 5	14	DNA MARKER		22	Day 27	B			
		15	Day 11	A	23	Day 41	A			

EFFECT OF MONOCAPRYLIN ON KILLING *E. COLI* O157:H7 IN UNDERCOOKED GROUND BEEF PATTIES

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Key Words: *E. coli* O157:H7, monocaprylin, ground beef, heat sensitivity

Introduction

Escherichia coli O157:H7, a pathogenic strain of *E. coli* that cause foodborne illness remains a challenge for the beef industry. Cattle have been implicated as the principal reservoir of this pathogen (Zhao et al., 1999) and about 50% of the reported outbreaks in the United States have been associated with consumption of undercooked beef burgers (Meng and Doyle, 1998).

In an attempt to minimize the impact of this pathogen on consumer health, the United States Department of Agriculture, and Food Drug Administration (FDA) have recommended that patties be cooked to a minimum internal temperature of 160°F (71.1°C) or 68°C for 15 seconds, respectively. However, the degree to which this cooking temperature is achieved is complicated by the lack of homogeneity in patty composition and the complexity of temperature monitoring (D'sa et al., 2000). Further, the use of thermometers for cooking beef patties by consumers is limited (NCBA, 1999) due to the inconvenience of the procedure, consumer ambivalence, and a lack of consumer confidence in a thermometer's ability to ensure food safety (USDA, 1998). Most consumers determine doneness by observing the color and texture of cooked beef patties (Rhee et al., 2003). However, premature browning in ground beef can lead to inadequate cooking by consumers who are misled by the cooked color (Warren et al., 1996), allowing potential survival of *E. coli* O157:H7. Effective methods that kill *E. coli* O157:H7 in undercooked ground beef patties would reduce the likelihood of food-borne outbreaks of this pathogen, and decrease economic losses to the beef industry.

Free fatty acids and their monoglycerides have been found to be inhibitory towards an array of pathogenic microorganisms (Kabara et al., 1972). Caprylic acid is an eight-carbon (C:8) fatty acid present in breast milk, bovine milk (Jensen et al., 2000), and coconut oil (Sprong et al., 2001); it is a food-grade chemical approved by the FDA as Generally Recognized As Safe (GRAS) (CFR 184.1025). Monocaprylin is the monoglyceride ester of caprylic acid. Nair et al. (2004) observed that caprylic acid and monocaprylin were effective in killing *E. coli* O157:H7 and *Listeria monocytogenes* in milk.

Objectives

This study was conducted to determine the effect of monocaprylin on increasing the heat sensitivity of *E. coli* O157:H7 in ground beef cooked to an internal temperature of 60°C, 65°C or 68°C, and on the color acceptability of monocaprylin containing ground beef patties.

Methodology

Bacterial Culture. A five-strain mixture of *E. coli* O157:H7 was used; each strain was induced for resistance to 50 µg/ml nalidixic acid and 50 µg/ml rifampicin. The cell suspension was prepared by transferring 100 µl of each strain from stock culture to 10 ml Tryptic Soy Broth (TSB; Difco, Detr, Mich.) and incubating at 37°C for 24 hrs. TSB was supplemented with 50 µg/ml of nalidixic acid (Sigma Aldrich Chemical Co., St. Louis, Mo.) and rifampicin (Fischer Scientific, Fair Lawn, N.J.). After two consecutive transfers, equal volumes from each strain were combined and harvested by centrifugation (3600 x g, 12 min) at 4°C, washed twice in sterile phosphate-buffered saline (PBS, pH 7.4) and the cell pellet resuspended in PBS. The bacterial count of the 5-strain mixture was confirmed by plating 100 µl of appropriate dilutions on Tryptic Soy Agar (TSA; Difco, Detr, Mich.) plates containing the antibiotics, and by incubation at 37°C for 24 hrs.

Preparation of beef patties. Fifteen kg of coarse ground beef (90% lean and 10% fat) was purchased locally. The beef was fine ground and a total of 60 patties (50 g ea) was randomly assigned into one of the three treatments, Control (0 mM monocaprylin), 20 mM monocaprylin and 25 mM monocaprylin (Nu-Check Prep, Inc., Elysian, MN).

Inoculation and treatment. Each patty was inoculated with 500 µl of the five-strain mixture of *E. coli* O157:H7 to obtain a final concentration of 6 log₁₀ CFU/g. Monocaprylin (0, 20 mM and 25 mM) was added to the inoculated patties, mixed thoroughly and formed into patties using a micro-petri dish (1.5 cm thick, 5.5 cm dia.). The patties were placed on foam trays, overwrapped with fresh meat film and stored at 4°C for 5 days.

Cooking. On each sampling day (0, 1, 3, 5), the inoculated beef patties were cooked singly in a double-sided George Foreman Grill (Salton Inc., China) until desired internal temperature (60°C, 65°C or 68°C) was reached. Internal temperature was monitored by using an Acutuff Model 34 Atkins 2 mm probe meat thermometer (Koch Supplies, N. Kansas, MO) placed in the geometric center of each patty. Patties were turned three times during cooking with the first turn at 30°C, the second turn at 40°C and the third turn at 50°C.

Enumeration of surviving *E. coli* O157:H7. Upon reaching the desired internal temperature, patties were removed from the grill, submerged individually in 100 ml sterile ice-cold PBS, and homogenized in a stomacher for 1 min. Two hundred µl of appropriately diluted meat homogenate was surface-plated on duplicate TSA plates supplemented with 50 µg/ml nalidixic acid and 50 µg/ml rifampicin. Plate counts were determined after incubation at 37°C for 24 to 48 hours.

Determination of pH: Ten grams ground beef from each treatment was homogenized with 30 ml deionized water in a Waring™ blender, and pH was measured using a pH meter (model 720, Orion Research, MA) standardized with pH 4 and 7 buffers.

Sensory analysis: Triangle test (Larmond, 1977) and the 9-point Hedonic color acceptability test (Lawless and Heymann, 1999) were used to evaluate the color of patties. Both evaluations were conducted with twenty-five untrained panelists. Pre-coded control and treated patties were cut in half longitudinally, randomly arranged on a sensory plate, and served to each panelist.

Statistical Analysis. Four patties per treatment per cooking temperature were used in this study. The entire study was replicated two times. The collected data (microbial, pH, and sensory) from independent replicate trials were pooled and analyzed using the general linear model of Statistical Analysis Software (SAS Institute, Inc., N.C). Significant differences ($P < 0.05$) between the treatments and controls were determined by the least significant difference (LSD) test.

Results and Discussion

Bacterial counts. At 60°C, *E.coli* O157:H7 counts in control patties were decreased by 0.6 log CFU/g, whereas significant reductions ($P < 0.05$) of 1.4 log CFU and 1.9 log CFU/g were achieved in patties containing 20 mM and 25 mM monocaprylin, respectively.

On day 0 of storage, cooking the patties to 65°C reduced the pathogen load by approximately 2.0, 4.0, and 4.1 log CFU/g in patties containing 0, 20 mM, and 25 mM monocaprylin, respectively. Further, at 65°C *E.coli* O157:H7 was completely inactivated in the samples containing 25 mM monocaprylin (on days 3 and 5).

Cooking the patties to 68°C reduced *E.coli* O157:H7 populations by 4.8 log CFU/g in control samples, whereas complete inactivation of the pathogen was observed at this temperature in patties treated with 25mM monocaprylin.

pH. There were no significant differences in the pH of control and treated patties ($P > 0.05$).

Sensory evaluation. The triangle test showed that 66% of panelists detected differences in color between treated and control patties. Mean acceptability scores for control and 25 mM treated patties were 6.68 and 6.80, respectively. These scores were equivalent to “like slightly” to “like moderately” on the Hedonic rating scale used.

Conclusions

The *E.coli* O157:H7 population in ground beef patties cooked to the three suboptimum temperatures (60°C, 65°C and 68°C) was significantly ($P < 0.05$) reduced by the addition of 20 mM and 25 mM monocaprylin without compromising color acceptability. This is especially important since the infectious dose of *E. coli* O157:H7 in humans is very low.

Acknowledgements

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LOCATION OF SALMONELLA SPP. IN WHOLE-TURKEY MUSCLE AFTER MARINATION

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Key Words: marination, turkey muscle, *Salmonella*, transmission electron microscopy

Introduction

Poultry marketing in the U.S. has changed considerably in the past 20 years, and poultry products are now the second most consumed animal-based protein behind pork (Roenigk 1999). Consumer trends show an increasing demand for ready-to-eat (RTE) products, which include marinated whole-muscle products (Russell, 2002). Value-added processing, such as marination, is frequently used to improve the quality of whole-muscle meat and poultry products.

The processes of marination and mechanical tenderization are used throughout the meat industry to increase palatability of lesser quality cuts of meat (Johnston 1978). Methods implicated for meat marination/tenderization include blade tenderization, vacuum tumbling, and needle injection. One of the concerns that arise with these procedures is the introduction of bacterial pathogens from the meat surface into the interior of value-added meat products (Phebus et al., 1999). In addition, when internalized, these bacteria may exhibit enhanced thermal resistance, which depends upon various factors, including meat species, muscle type, pH, fat content, and additives (Orta-Ramirez et al., 2003). Warsaw (2003) concluded that bacteria could penetrate into intact muscle with or without the aid of vacuum during marination.

Salt and phosphates, which induce changes in muscle structure, are commonly incorporated in marinades to increase product yield and palatability. As the marinade penetrates into the interior of the muscles, it may act as a vehicle for microbial contamination. Water in the marinade may contribute to bacterial penetration by increasing the water content between muscle fibers and therefore increasing penetration (Thomas et al., 1987). Phosphates in the marinade could also contribute to an increase water absorption. Pyrophosphates act as a fluidizing agent in muscle, dissociating actin and myosin, which leads to increased water uptake (Xiong and Kupski 1999).

Salmonella is responsible for an estimated 1.4 million cases of foodborne illness each year in the U.S. *Salmonella* is the second most common cause of foodborne illness behind *Campylobacter*, both of which are commonly found in poultry products (CDC, 2001). According to the Food Safety Inspection Service, raw turkey is a common source of *Salmonella* in the U.S. food supply (FSIS, 2002a). About 30% of ground turkey sampled from July 1999 to June 2000 was positive for *Salmonella* (FSIS 2002b). An infective dose can be as little as 10 to 100 cells in susceptible persons leading to symptoms of salmonellosis (nausea, abdominal cramps, diarrhea and vomiting).

Therefore, the overall goal of this research was to improve the safety of marinated poultry products. In order to observe bacterial penetration during marination process,

the effects of marinade composition on muscle microstructure need to be studied. Transmission electron microscopy (TEM) was used to observe changes in muscle structure and also the attachment of *Salmonella* spp. after marination.

Hypothesis

Marination of turkey muscle with a salt, phosphate marinade containing an 8-strain *Salmonella* cocktail results in penetration of *Salmonella* into the intact muscle.

Objectives

Determine the location of *Salmonella* in whole-muscle turkey, with respect to tissue structure, after marination with a *Salmonella*-inoculated marinade.

Specific aims: 1) To document changes in the turkey muscle microstructure after marination and 2) To view the position of *Salmonella* in marinated turkey muscle using TEM

Methodology

Marinade preparation

The marinade solution contained 95.8% water (filtered and deionized), 3.2% NaCl, and 1% mixed phosphate solution (w/w). Salt was incorporated into the water before adding the phosphate solution, in order to ensure total dispersal. Aliquots (525 mL) of marinade were poured into glass bottles with plastic screw caps and autoclaved for 15 min at 121 °C to ensure sterility.

Inoculum preparation

The *Salmonella* cultures were propagated by transferring frozen culture to 9 mL of tryptic soy broth (TSB). The strains were maintained separately by transfer to fresh TSB followed by 24 hr of incubation at 37°C. On the day of experiment, the cultures were combined and centrifuged at 6000 x g for 20 min at 4°C. The supernatant was removed and the bacterial pellet was resuspended in marinade to give a final concentration of $\sim 10^8$ CFU/mL.

Turkey preparation

Frozen turkey breasts (whole, intact, irradiated) were thawed 24 hr at 4°C. A hand-coring device was used to aseptically remove cores from the turkey breast (2-3 cores/treatment) aseptically. Cores were submerged in treatments including water, marinade, and an 8-strain *Salmonella*-inoculated marinade for 20 min at 4 °C. Treated cores were then removed from the treatments placed in Petri dishes and sectioned into smaller pieces measuring approximately 5 x 5 x 2 mm (W x L x H). Samples were then processed for electron microscopy.

Sample preparation for bright-field and transmission electron microscopy

Samples for bright field and electron microscopy were fixed in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) overnight at 4°C. After fixation, samples were washed with cacodylate buffer and postfixed with 2% osmium tetroxide and dehydrated using a graded series of acetone and embedded in Poly/Bed 812 resin. Thin sections were obtained with a diamond knife (90-110 nm thickness) on a Power Tome XL ultramicrotome (RMC). Uranyl acetate (2% in 50% ethanol) and lead citrate (Reynolds formula) were used as a positive stain for the thin sections. After staining, turkey muscle samples were observed under a transmission electron microscope, JEOL 100CX, at an accelerating voltage of 100 kV.

For bright-field microscopy, in order to determine the location of the bacteria in the muscle, thick sections (~ 500 nm thickness) were cut using a glass knife and stained with 1% toluidine blue. Bright-field transmitted images were taken with a Zeiss LSM5 Pascal microscope using a 633 nm laser.

Results & Discussion

The ultrastructure of whole turkey breast muscle subjected to marinade provides an initial view of the marination effects on turkey muscle structure. The changes of muscle structure after each treatment were observed using TEM. Figures 1 and 2 illustrate representative micrographs of turkey samples that were subjected to water and marinade treatments, respectively. The TEM images of the longitudinal section of muscle fibers indicate greater fiber size (width) in the marinated sample, compared to the water-treated sample. The changes in muscle fiber size of marinated turkey samples may be due to the contribution of salt and phosphate incorporated in the marinade solution. Offer and Trinick (1983) explained that increased moisture retention ability by phosphates is achieved through muscle fiber expansion (swelling) caused by electrostatic repulsions, which allows more water to be immobilized in the myofibril lattices. The action of phosphates in improving water holding capacity (WHC) appears to be twofold: (1) raising the pH; and (2) causing an unfolding of muscle proteins, consequently making more sites available for water binding (Pearson and Gillett 1999). In addition, the combination of salt and phosphate in marinade recipes has the primary function of WHC.

TEM is also a useful method to locate *Salmonella* attached inside turkey muscle. Figure 3 is a transmission electron micrograph of a longitudinal section of whole muscle turkey breast subjected to *Salmonella*-inoculated marinade for 20 min at 4°C. We observe the attachment of *Salmonella* to be parallel to the orientation of the muscle fibers. This result is also supported by Gill and Penney (1977), who indicated that *Salmonella* likely penetrated the tissue between muscle fibers. Thomas and others (1987) described changes in poultry muscle post-slaughter where gaps between muscle fibers are created by radial shrinkage of the muscle fibers due to increases in muscle osmolality from lactic acid formation post-slaughter. Water in the marinade may contribute to bacterial penetration as the water content between muscle fibers increases and therefore increases penetration of the *Salmonella*.

In order to obtain an overall view of the bulk tissue samples for bacterial penetration/attachment, bright-field transmitted images of longitudinal and cross sections of turkey muscle bundles were also taken from thick sections (Figures 4 and 5). Turkey samples were subjected to the *Salmonella*-inoculated marinade treatment

for 20 min at 4°C. The images indicate where *Salmonella* are likely to attach between turkey muscle bundles.

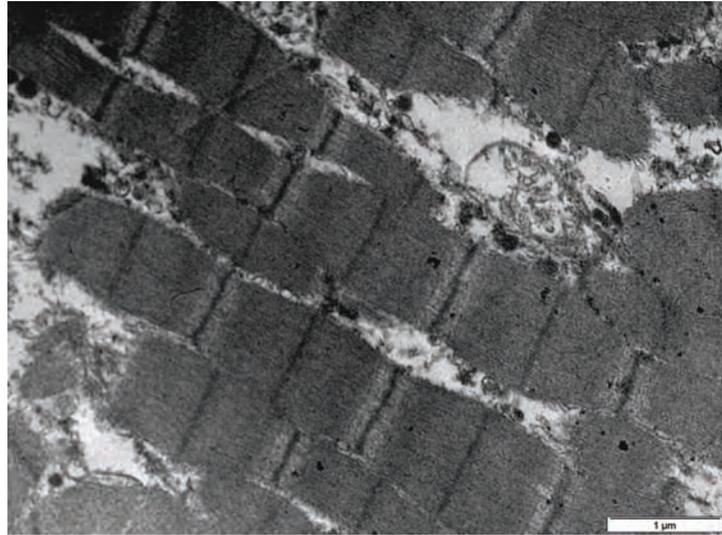


Figure 1. Transmission electron micrograph of a longitudinal section of whole muscle turkey breast subjected to water-only marination for 20 min at 4°C. Magnification 20,000x; bar indicates 1 μm.

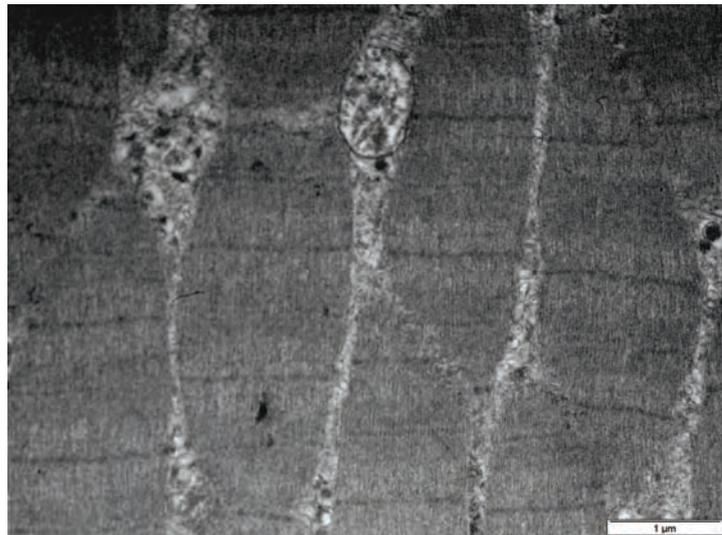


Figure 2. Transmission electron micrograph of a longitudinal section of whole muscle turkey breast subjected to marination treatment for 20 min at 4°C. Magnification 20,000x; bar indicates 1 μm.

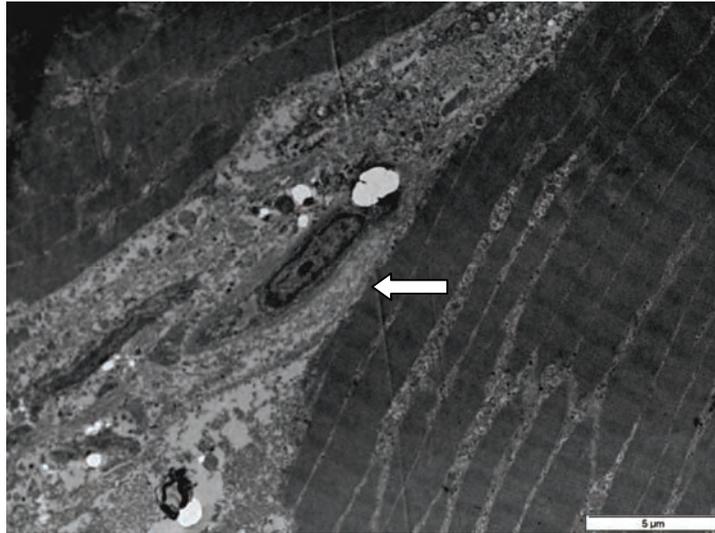


Figure 3. Transmission electron micrograph of longitudinal section of whole muscle turkey breast subjected to *Salmonella*-inoculated marinade (with salt and phosphate) treatment for 20 min at 4°C. Magnification 5,000x; bar indicates 5 μm. The arrow points to *Salmonella*, which has penetrated the muscle.

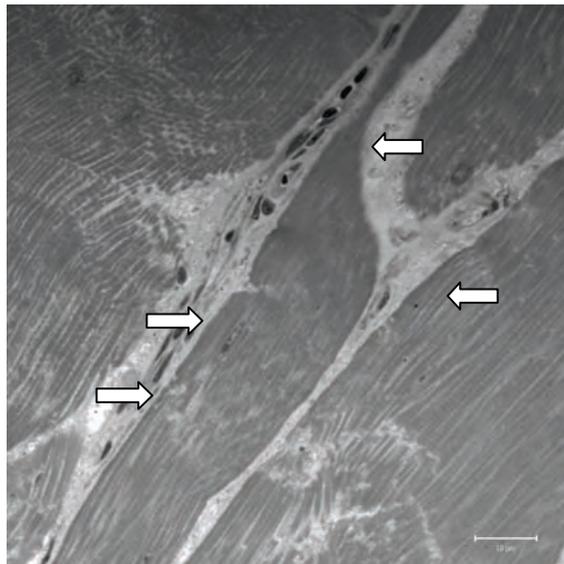


Figure 4. Bright-field transmitted image of longitudinal section of turkey muscle bundles subjected to *Salmonella*-inoculated marinade treatment for 20 min at 4°C. Bar indicates 10 μm. The arrows point to *Salmonella*, which has penetrated the muscle.

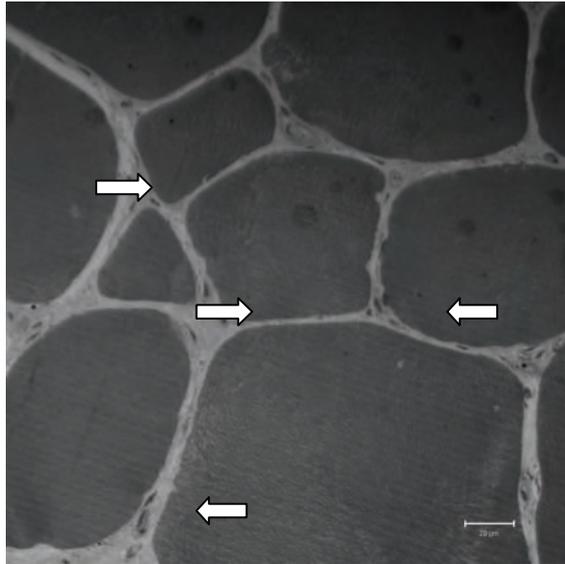


Figure 5. Bright-field transmitted image of cross section of turkey muscle bundles subjected to *Salmonella*-inoculated marinade treatment for 20 min at 4°C. Bar indicates 20 µm. The arrows point to *Salmonella*, which has penetrated the muscle.

Conclusions

The TEM technique conducted in this study provides a way to observe marinade absorption and *Salmonella* attachment in turkey at the microscopic level. *Salmonella* present in inoculated marinade migrated into the interior of intact, whole-muscle turkey breast during marination. Marinade composition, especially salt and phosphate, was a significant factor contributing to marinade penetration and absorption. Pathogen migration into whole muscle meat products may be characterized by the increase in water uptake and may be a function of the numbers of *Salmonella* present. As a result, the cooking times and temperatures currently recommended for pathogen inactivation, especially for marinated value-added meat products, may need to be reevaluated.

Acknowledgements

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SURVIVAL OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA* SPP. AND *LISTERIA MONOCYTOGENES* ON BEEF JERKY

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Key Words: *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, Jerky

Introduction

Jerky is a common ready to eat product that can be purchased at just about any gas station, grocery store and small processing facility in the United States. Many small and very small plants manufacture jerky. It is important that these small and very small plants be able to validate that the product they manufacture is safe and free from pathogens. Many of the small processors use smokehouses with minimal controls, especially for humidity which are similar to home dehydrators. Faith et al. (1998) evaluated ground and formed jerky that had been dried using processes that were similar to home style dehydrators. They reported that viability of bacteria was reduced as the drying temperature increased and drying time increased. In the past, control of pathogens for jerky was due to reduced water activity. Control of most bacteria occurs at water activities below 0.90 (Jay, 1986). Calicioglu et al. (2003a) reported a 5 log reduction in *Escherichia coli* O157:H7 inoculated post-drying after 7 days of storage. The water activity of the jerky post-drying was between 0.564 and 0.696. Water activity below 0.85 has been used in other countries to establish safety of jerky (Agriculture and Agri-Food Canada, NZFSA). New guidelines from the USDA suggest lowering water activity to 0.80 to control *Salmonella* and *E. coli* O157:H7 along with processing with high humidity at the first of the manufacturing process.

Listeria monocytogenes has been a new problem that most small processors are now dealing with on ready to eat products. *Listeria* is normally a problem because of post processing contamination. Calicioglu et al. (2002) reported that *Listeria* numbers were reduced during the drying process and that the use of acid marinades helped increase this reduction. Työppönen et al (2003) observed that traditional processing procedures for dried, and fermented and dried sausages were not sufficient to prevent the survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7. Therefore it is important to determine the effect of different water activities on the survivability of select pathogens. With the uncertainty of survival of different pathogens in dried products, it is important for processors to validate the process they are using to manufacture jerky that is being labeled shelf stable.

Objectives

The objective of this study was to determine the survivability of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* on whole muscle jerky dried without humidity and stored in a vacuum package.

Methodology

Beef (*semimembranosus* muscle) was purchased from a small commercial facility for the manufacture of jerky. Meat was tempered at 4°C for 24 h and sliced. A formulation containing beef (89.58% w/w), soy sauce (5% w/w), dextrose (2% w/w), salt (1.8% w/w), garlic powder (0.5% w/w), black pepper (0.5% w/w), onion powder (0.4% w/w), Cure #1 (0.18% w/w) and sodium erythorbate (0.04% w/w) was used to marinate the jerky. Each slice was inoculated by dipping in a cocktail of pathogens (Salmonella (4 strains *S. typhimurium* 04121V and 0363V, *S. abatebua* 0817V and *S. choleraesuis* 0902V from Fisher Scientific, Denver, CO) , 3×10^6 ; *Listeria monocytogenes* (4 strains FSL J1-110, FSL C1-115, FSL J2-064, and FSL J2-054 from Martin Weidman, Cornell University) 6.25×10^7 and *E. coli* O157:H7 (one strain 0617V, Fisher Scientific, Denver, CO) 3.3×10^7). Three raw slices were tested to determine inoculation level. Jerky was placed on racks and dried in an ALKAR smokehouse (Alkar Smokehouses, Lodi WI) at 60°C with dampers open and fans running at maximum for the whole drying cycle. Samples were taken at 3, 6, 9, and 12 hours of drying to give three slices of jerky for each of five storage periods (0, 3, 6, 9, or 12 weeks). Each sample was tested for microorganism survival utilizing selective media. Internal temperature and water activity was also determined. Internal temperature was determined using hypodermic thermocouples (Omega Scientific, Tarzana, CA) inserted into 6 slices in each of three different full smokehouse loads. Water activity was determined with a Series 3 Decagon Aqualab water activity meter (Pullman, WA) on two different samples from each jerky slice at each drying and storage period. Three jerky slices from different places in the smokehouse were analyzed at each storage time for bacterial survival and water activity.

Ten grams of jerky slice per drying time were aseptically transferred into sterile plastic bags (Fisher Scientific, Denver). A 90-mL aliquot of 0.1% sterile peptone buffer (Difco) was added to each sample bag prior to pummeling with a stomacher for 2 min at room temperature. Serial decimal dilutions were made and pour-plated onto each of duplicate plates of each agar medium. Three different jerky slices per drying time were analyzed at each storage period. Bacteria were enumerated using tryptic soy agar (Difco) plus 0.1% sodium pyruvate (TSAP, total plate count), PALCAM agar (Difco) (*Listeria monocytogenes*), xylose-lysine-tergitol 4 agar (Difco) (XLT4, *Salmonella*), MacConkey sorbitol agar (Difco) (SMAC, *E. coli*) and modified eosin methylene blue agar (Difco) (MEMB, *E. coli*). PALCAM and half of the TSAP plates were incubated at 30°C while the other plates were incubated at 35°C. When numbers of the pathogen decreased to <10 cfu/g by direct plating, the presence/absence of the pathogen was determined by enrichment as described by Calicioglu et al. (2003a, b) for *Listeria* and *Salmonella*.

Data were analyzed using GLM of SAS. The absence of pathogens on selective agar was scored as 9 cfu for statistical analysis if there were no colonies on a plate. LSMEANS was used to separate means.

Results & Discussion

The internal temperature of jerky slices was similar to the smokehouse temperature after three hours of drying (Fig. 1). Calicioglu and co-workers (2003b) reported that the internal temperature of jerky slices were similar to the dehydrator

temperature after 6 hours of drying. The difference between these two reports is probably due to increased air flow and better temperature control in the smokehouse used in this study when compared to the home dehydrator used by Calicioglu et al. (2003b). The total number of colony forming units declined as drying time increased (Fig. 2). The total number of *E. coli*, *Salmonella* and *Listeria* also significantly declined as drying time increased. Drying of jerky resulted in a 2 log reduction of *Listeria* after 6 hours of drying which increased to a 3 log reduction after 12 hours. Drying of jerky slices inoculated with *E. coli* O157:H7 resulted in a 3 log reduction after 3 hours of drying and a 4 log reduction after 12 hours. After vacuum storage for three weeks there was no *Listeria* or *E. coli* recovered from the jerky strips. Furthermore after enrichment there was no *Listeria* or *E. coli* resuscitated. *Salmonella* however did survive storage up to 3 and 6 weeks of storage but was reduced to below detectable limit after 9 and 12 weeks of storage. *Salmonella* was recovered after enrichment, but in very low numbers (5 cfu). Water activity reduced as the drying time increased (Table 1) however at all drying times it was low for jerky products.

Table 1 Effect of drying and storage on the water activity of jerky

	Water activity	SEM
Drying Time (hrs)		
0	0.886	0.012
1	0.823	0.012
3	0.548	0.005
6	0.406	0.005
9	0.320	0.005
12	0.278	0.005
Storage (weeks)		
0	0.624	0.004
3	0.580	0.006
6	0.426	0.006
9	0.585	0.006
12	0.503	0.006

Conclusions

Drying procedures currently used by most small processors will reduce the number of pathogens on the jerky, however drying does not reduce the number enough to meet the 7 log reduction USDA uses as a kill step. Drying and storing in a vacuum package does result in reduction of pathogens to below the detectable limit. The cause of the reduction of pathogens in storage is not clear. Some will be due to the lowered water activity but some may be due to pathogens being injured during drying and not being able to adapt to the vacuum packaged atmosphere.

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Figure 1 Internal temperature of jerky slices during drying at 60°C.

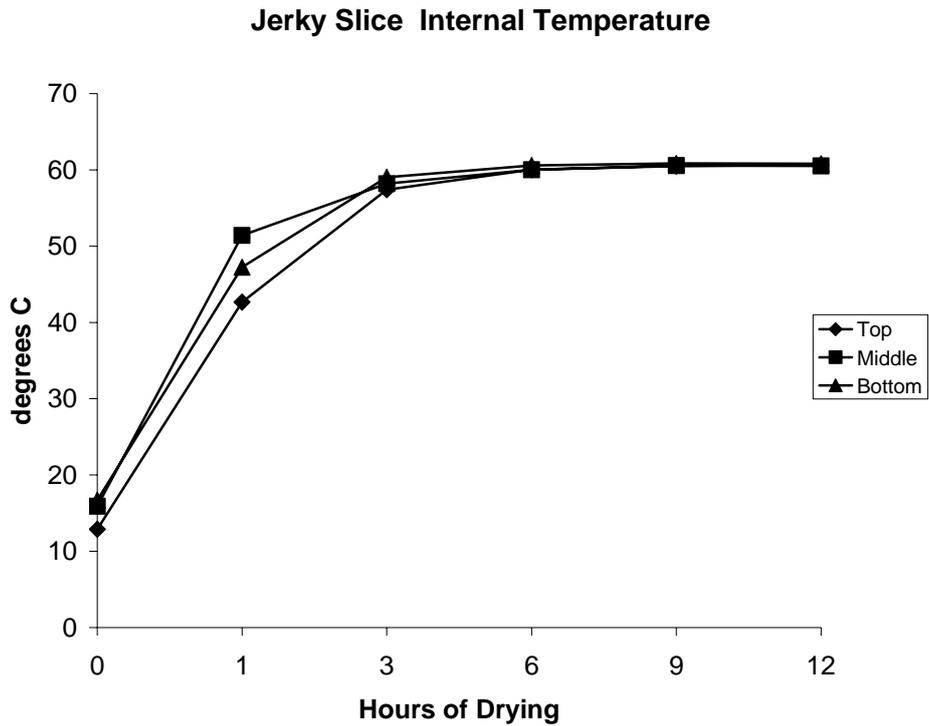


Figure 2. Effect of drying at 60°C on the survival of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*.

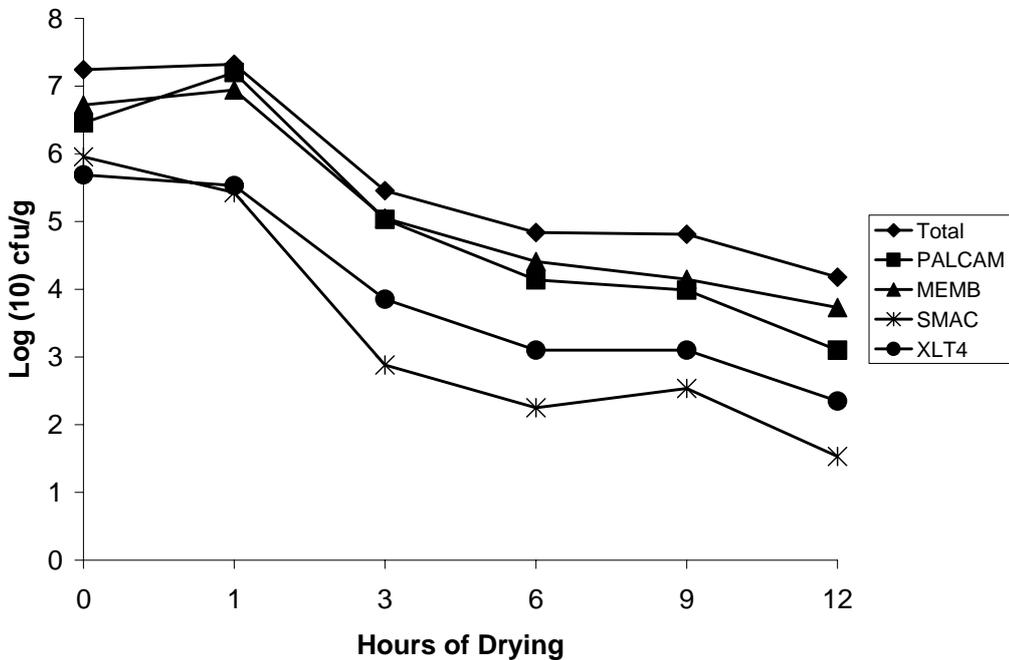
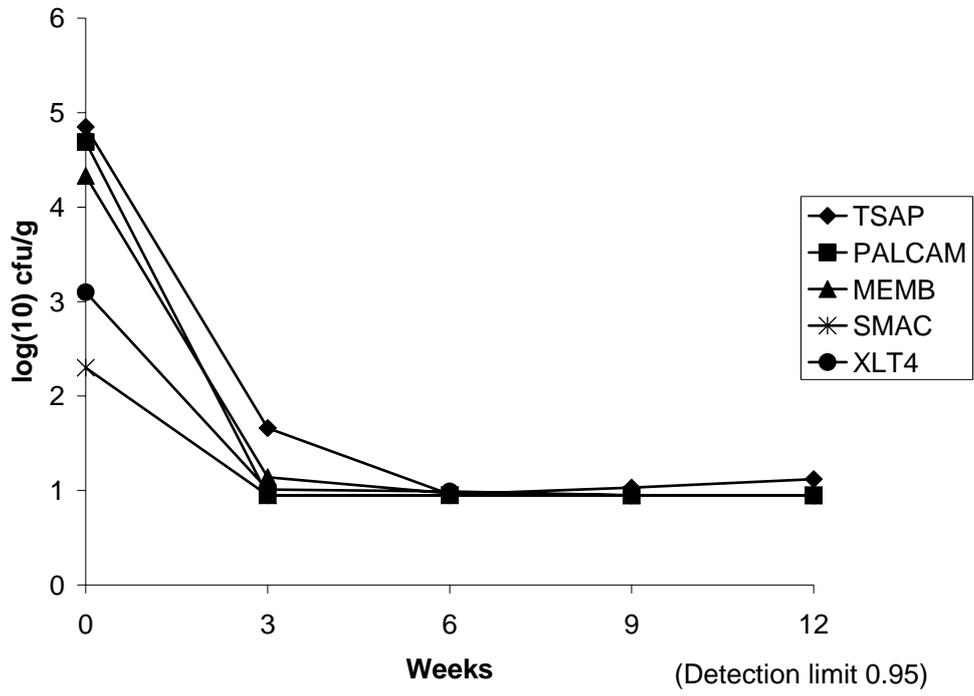


Figure 3. Effect of storage on the survival of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*.

Storage



**DEATH OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP.
DURING PROCESSING OF WHOLE-MUSCLE BEEF JERKY**

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Key Words: *Salmonella* spp., *Escherichia coli* O157:H7, jerky, lethality

Introduction

Beef jerky processing, using whole muscle or restructured ground meat, is unique compared to the processing of other ready-to-eat meat products because during heating considerable drying is done to attain the desired texture and shelf-stability. This drying may reduce the process lethality against pathogenic bacteria in beef, and outbreaks of salmonellosis have indeed been linked to the consumption of beef jerky. Previous research has suggested that sub-lethal drying conditions may lead to increased heat-resistance in pathogens such as *Salmonella* spp. Furthermore, evaporative cooling during drying may lessen the effective temperature to which pathogens are exposed. All of these factors have resulted in heightened scrutiny of the lethality of the heating and drying steps typically used during jerky processing. United States Department of Agriculture (USDA) officials have issued several compliance guidelines for jerky processors that stress the importance of maintaining high humidity during thermal processing in order to ensure sufficient destruction of *Salmonella* spp. and *Escherichia coli* O157:H7. Processors have had difficulty either complying with USDA guidance or developing and validating adequate alternative processes. Development of validated heating/drying guidelines for processors of whole-muscle jerky has been complicated by variables such as thickness of jerky strips, whether the strips have been marinated and, if so, the composition and conditions of marination, and the type of smokehouse or oven used for heating/drying.

Objectives

The objective of this study was to develop and validate sufficiently lethal processes for heating and drying whole-muscle beef jerky.

Methodology

Five-strain cocktails of *Salmonella* spp. and *E. coli* O157:H7 were used to inoculate beef strips prior to jerky processing. Inoculation cultures were prepared for each strain by transferring a loopful of growth from working culture plates (maintained at 4°C on Brain Heart Infusion agar (BHIA; Difco, Becton-Dickinson, Sparks, MD) and prepared monthly from frozen stock cultures) to 9 ml of Brain Heart Infusion Broth (BHIB; Difco) and incubating at 35°C for 20-24 h. To prepare a 5-strain inoculum cocktail of each organism, the BHIB cultures of each organism were combined into one 50-ml sterile plastic centrifuge tube, and centrifuged for 12 minutes at 5,000 x g. The supernatant in each tube was decanted and the pellets were

resuspended with approximately 20 ml of Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). Beef strips (5 – 7 mm thick) were placed in a biosafety hood on aluminum foil that had been previously sanitized with 70% (v/v) ethanol. To inoculate each strip, 0.4 ml of the undiluted cocktail was pipetted onto the product surface and distributed as evenly as possible using a sterile plastic spreader. Aluminum foil was placed over the strips in a tented manner to minimize the amount of drying during microbial attachment (30 min). After the initial attachment phase, strips were turned over and the inoculation/attachment process was repeated on the other side. For each trial, one group of beef strips was inoculated with *Salmonella* spp., another group was inoculated with *E. coli* O157:H7, and a third group of uninoculated strips was used to monitor yield and water activity throughout thermal processing. Initial pathogen levels on inoculated beef strips were approximately 10^8 CFU per beef strip. Each group of beef strips was then tumbled manually for 5 minutes in a non-acidic (pH 5.3) spice-containing marinade applied at a level of 15% (w/w) intended to result in a pre-processing level of 2% (w/w) sodium chloride, 2% sucrose, 156 ppm sodium nitrite (w/w) in the meat. Following marination, strips were held for 22-24 h at 5°C. The next day, strips were arranged on racks placed in the center of a commercial one-truck smokehouse for processing. Pans of water were placed on the lower level of the rack and a low fan speed was used to simulate as much as possible a drying rate consistent with a smokehouse full of beef strips. The smokehouse dry-bulb and wet-bulb temperatures were monitored using thermocouples. Smoke was not introduced to the beef strips during processing and the dry-bulb temperature controller was set at 62.8°C (145°F) in the first 15 minutes and then at 76.7°C (170°F) during the next 15 minutes. Next, humidity (steam or water) was introduced into the smokehouse via the wet-bulb temperature controller to obtain targeted increases in wet-bulb temperatures, referred to as “wet-bulb spikes”. The process lethality was determined for a series of trials conducted using early-process wet-bulb spikes of 51.7°C (125°F) for 60 min, 54.4°C (130°F) for 60 min., 57.2°C (135°F) for 30 min., and 60°C (140°F) for 10 min. Following completion of wet-bulb spikes, no further humidity was introduced into the smokehouse chamber as the product was further dried at 76.7°C (170°F).

The numbers of *Salmonella* spp. and *E. coli* O157:H7 on beef strips were determined prior to marination, after the early-process wet-bulb spike in the smokehouse, and following drying when the beef strips had reached an average water activity of ≤ 0.90 . One beef strip comprised a sample and three samples were analyzed at each sampling time. Each sample was placed in a whirl pak filter bag, BPD (99 ml) was added, and the bag contents were stomached for 2 minutes at medium speed (Stomacher 400 Circulator lab blender; Seward) or samples were manually massaged for 1 minute and shaken for 1 minute. This initial dilution was arbitrarily defined as 10^{-1} . Serial decimal dilutions were made in BPD as needed. For the initial dilution, 1.0 ml was distributed for spread-plating among three plates of BHIA. From the original dilution and each subsequent dilution 0.1 ml was spread on one BHIA plate per dilution. Plates were incubated at 35°C for 1 h to allow for repair of injured cells, and then overlaid with MacConkey Sorbitol agar (SMAC; Difco) or XLD agar (Difco) for selective/differential enumeration of *E. coli* O157:H7 and *Salmonella* spp., respectively. After 20-24 h incubation at 35°C, plates were examined for typical *E. coli* O157:H7 or *Salmonella* spp. colonies. Selected presumptive *Salmonella* spp. and *E. coli* O157:H7 colonies were transferred to BHIA, incubated at 35°C for 20-24 h, and then tested to confirm colony identity. The log CFU for a given pathogen was calculated for each sample with a mean log CFU calculated for each

sampling time. A value of 9 CFU (0.95 log CFU) was assigned when no colonies were present for the least dilute plating.

Results & Discussion

Earlier research has established the fact that sub-lethal drying can make pathogens such as *Salmonella* spp. more resistant to heat (Goepfert et al., 1970). This phenomenon was likewise observed in several early jerky-making trials (data not shown). Therefore, a variety of early-process wet-bulb temperature spikes were applied to determine the extent of elevated-humidity heating conditions necessary to achieve desired lethality while maintaining product quality. During the 54.4, 57.2, and 60°C (130, 135, and 140°F) wet-bulb spikes, the interior temperature of a beef strip (measured by inserting a probe into the meat or by folding the strip around the probe) was generally quite similar to the wet-bulb temperature. However, in the 51.7°C (125°F) wet-bulb spike, the product temperature rose to a temperature several degrees higher than the wet-bulb temperature. Of the two pathogens studied, *E. coli* O157:H7 was better able to survive early-process wet-bulb spike treatments employed in making whole-muscle beef jerky. However, foodborne illness outbreaks linked to beef jerky have primarily involved *Salmonella* spp.; it is therefore prudent for any validation of a jerky-making process to involve both pathogens. Presently, the USDA has indicated that a jerky-making process has sufficient lethality if it results in a 5-log reduction of *Salmonella* spp. However, USDA guidance for certain other beef products specifies a 6.5 log reduction in *Salmonella* spp. All early-process wet-bulb spike treatments tested resulted in a ≥ 6.4 log reduction in both pathogens by the end of the complete process (including final drying). Three wet-bulb spike treatments achieved a ≥ 5.2 log reduction in *Salmonella* spp. but caused smaller decreases in *E. coli* O157:H7 numbers at the end of the wet-bulb spike (Table 1). These treatments were 51.7°C (125°F) for 60 min, 57.2°C (135°F) for 30 min, and 60°C (140°F) for 10 min. However, a ≥ 6.4 log reduction of both *Salmonella* spp. and *E. coli* O157:H7 was achieved by the end of drying after each of these wet-bulb spike treatments. One wet-bulb spike treatment did not cause a > 5.0 log reduction in either pathogen by the end of the web-bulb spike but subsequent drying resulted in sufficient overall lethality. This treatment, 54.4°C (130°F) for 60 minutes resulted in decreases of 3.2 – 3.9 and 2.0 – 2.1 log CFU for *Salmonella* spp. and *E. coli* O157:H7, respectively. By the end of drying after these treatments, reductions of ≥ 6.9 log had occurred for both pathogen species. Although USDA guidance (2004) recommended 90% relative humidity (RH) during the early heating period in jerky processing, it also stated that such a high humidity may not be necessary if alternative procedures are validated. In the studies conducted here using a wet-bulb temperature of 76.7°C (170°F), early-process wet-bulb spikes resulted in 28 - 48% RH. When followed by drying at 76.7°C (170°F), these processes were sufficient to provide ≥ 6.4 log reduction in numbers of *Salmonella* spp. and *E. coli* O157:H7. Processors could therefore monitor wet-bulb spike treatments directly with a wet-bulb thermometer or indirectly with a hygrometer.

Conclusions. Taking into account current USDA expectations for jerky processing lethality, processors using the conditions employed in this study [non-acidic marinade, initial dry-bulb smokehouse target temperatures of 62.8°C (145°F) for the first 15 min and 76.7°C (170°F) for the next 15 min] could employ any of the early-process wet-bulb spike treatments listed in Table 1 followed by drying at 76.7°C (170°F) as scientifically validated processes for making safe whole-muscle beef jerky.

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Tables and Figures

Table 1. Process lethality against *Salmonella* spp. (S) and *Escherichia coli* O157:H7 (EC) during the heating and drying of whole-muscle beef jerky. Inoculated beef strips were marinated in a non-acidic marinade for 22-24 h at 5°C, and then exposed to pre-heating [smokehouse heated to 76.7°C (170°F) dry-bulb temperature in 30 minutes], followed by an early-process wet-bulb spike (addition of humidity to attain desired wet-bulb temperature), and then final drying [continued exposure to 76.7°C (170°F) with no added humidity] processes. Reduction in log CFU per sample is relative to initial pathogen levels prior to beef strip marination.

Early-Process				Post-spike Drying	Lethality (reduction in log CFU) and Water Activity (a_w)					
Wet-Bulb Spike				Time at 76.7°C	For Sample After					
Temperature		Time	RH ^a	(170°F)	Wet-Bulb Spike			Drying		
(°C)	(°F)	(min)	(%)	(min)	S	EC	Mean a_w	S	EC	Mean a_w
51.7	125	60	28	60	5.6	5.6	0.87	6.5	6.7	0.81
51.7	125	60	28	60	5.2	3.8	0.89	6.4	7.1	0.78
54.4	130	60	38	120	3.2	2.0	0.93	6.9	7.1	0.88
54.4	130	60	41	120	3.9	2.1	0.92	6.9	7.0	0.87
57.2	135	30	40	120	6.4	2.7	---	7.0	7.1	0.86
57.2	135	30	40	90	5.3	3.1	0.93	7.0	7.1	0.90
60	140	10	48	120	6.2	3.8	0.96	7.0	7.2	0.90
60	140	10	48	120	6.8	2.2	0.96	6.9	7.0	0.84

^aMean percent relative humidity during wet-bulb spike.

**DEATH OF *SALMONELLA* SPP., *ESCHERICHIA COLI* O157:H7, AND
LISTERIA MONOCYTOGENES DURING THE MANUFACTURE OF
BASTURMA, A DRIED DRY-CURED PRODUCT**

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Key Words: *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, Basturma

Introduction

Basturma refers to various meat products originating in Armenia and neighboring countries. However, in this study basturma refers to a commercial product made from intact beef eye of round subprimals (hereafter referred to as rounds) that are dry-cured, rinsed, pressed, and dried with a spice paste coating added. The finished product is sold under refrigeration as a raw product. Basturma made by the process typically has a final water activity of 0.80 to 0.85, below the water activity commonly regarded as the lower limit for pathogenic bacterial growth (3). Provided this product also has a Moisture:Protein Ratio (MPR) of ≤ 1.9 , it could legally be considered shelf-stable (9) and, if the processor desired, the product could be sold as a ready-to-eat (RTE) item. Currently, this commercial basturma would be in the “raw product, not ground” category in the Hazard Analysis Critical Control Point (HACCP) food safety system mandated by the United States Department of Agriculture (USDA; 7). If the product were to be marketed as shelf-stable and ready-to-eat, it would fall under the “not heat-treated, shelf-stable” HACCP plan category. Regardless of the HACCP category, critical limits must be met at designated Critical Control Points to ensure control of significant hazards, namely *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Although USDA critical limit guidance exists for destroying *Salmonella* spp. during cooking (8), no USDA guidance exists for critical limits associated with prevention of *Salmonella* spp. and *E. coli* O157:H7 growth or destruction of these pathogens using non-thermal processes.

USDA guidance states that decreasing a product’s water activity to < 0.92 will prevent *L. monocytogenes* growth (10). However, this guidance is generally applied to previously cooked products. The basturma evaluated in this study received no thermal process and little is known about *L. monocytogenes* survival during non-thermal processing steps leading to reduced water activity.

Objectives

The objective of this study was to determine the fate of *Salmonella* spp., *E. coli* O157:H7, and *Listeria monocytogenes* throughout a basturma-making process used by a collaborating industry partner.

Methodology

Inoculum Preparation. A cocktail was prepared using five strains each of *E. coli* O517:H7, *Salmonella* spp., and *Listeria monocytogenes*.

Meat Product and Inoculation. In each of three trials, eighteen commercial beef eye of the round subprimals were used. Rounds ranged from 1.81 kg to 2.72 kg with typical dimensions of 25.4 cm x 12.7 cm x 10.2 cm. Rounds were not trimmed of external fat in trials 1 and 2, but were trimmed prior to inoculation procedures in trial 3. To inoculate each round, a 0.25 ml volume of the undiluted cocktail was pipetted onto one side of the product surface and distributed as evenly as possible over the entire side using a sterile plastic spreader. The inoculated rounds were allowed to dry for 30 minutes to aid microbial attachment.

Basturma-Making Process. Following inoculation, 45.4 kg of rounds were manually rubbed by hand with approximately 6 kg of curing mixture containing proprietary amounts of salt, sucrose, glucose and sodium nitrite, and packed into plastic 52.1 cm x 29.2 cm x 20.3 cm lugs. Lugs were stored at 6.7°C, 50% relative humidity (RH) for 7 days. After one week, accumulated fluid was drained from the lugs; and the rounds were manually dry-rubbed again with a second batch of the curing mixture and allowed to cure for 14 more days.

At the end of the dry-curing phase, rounds were rinsed for 1 h with tap water (approximately 18°C): lugs were filled with water to a level that covered the rounds, allowed to soak for 15 min, and then the water was drained from the lugs. This process was repeated four times. After rinsing, rounds were hung to dry for 2 days at 24°C, 21°C, or 27°C for trials 1, 2, and 3, respectively. The % RH levels for trials 1 and 2 cycled every 12 h between 65 and 80% RH. The % RH for trial 3 was held constant at 70. After drying for 2 d, rounds were pressed for 12 h at 2.2°C to remove additional moisture. Pressures of 517 kg per m² were achieved in Trials 1 and 2, while a pressure of 167 kg per m² was obtained in Trial 3. When pressing was complete, rounds were re-hung and allowed to continue drying for 4 d. Following the 4 d of drying, rounds were coated with a spice paste consisting of proprietary amounts of fenugreek, cayenne or red pepper, garlic powder, paprika, and water; and were hung to dry for 4 d in trial 1, 5 d in trial 2, and 6 d in trial 3. Finished product samples were sent to a commercial testing laboratory for water activity, MPR, pH, and % water-phase salt determinations (Table 1).

Enumeration of Surviving Cells. Samples were obtained at different stages in the basturma-making process. At each sampling time, three rounds were sampled with three individual samples (3.8 x 3.8 x 0.5 cm thick, by excision) being tested per round. Each sample was placed in a whirl pak filter bag, along with 99 ml BPD, and stomached for 2 minutes at medium speed (Stomacher 400 Circulator lab blender; Fisher). This initial dilution was arbitrarily denoted as 10⁻¹. Initial and subsequent dilutions were spread-plated on MacConkey Sorbitol agar (SMAC; Difco), XLD agar (Difco), and Listeria Selective Agar base (LSA; Oxoid, Ogdensburg, NY) with added Listeria Selective Supplements (Oxford formulation; Oxoid). Plates were incubated at 35°C and then examined for typical colonies of the relevant pathogen.

Data analysis. No difference in numbers of surviving pathogens was noted between sampling sites on individual rounds, so the mean log CFU was calculated for each round. A mean and standard deviation were then calculated for the three rounds at each sampling time. A value of 0.95 log CFU/g (1 CFU less than the 10 CFU detection limit) was assigned when no colonies were present for the least dilute plating.

Results & Discussion

Techniques used in the three trials were very consistent through the dry-curing and rinsing steps. However, the range of pressing and drying conditions purposely used over the three trials resulted in different finished product MPR, % water-phase salt, and water activity values (Table 1). In trial 2, case-hardening may have limited moisture loss.

As seen in Table 2, there was a general trend of pathogen death throughout the process. Through the first 21 days of processing, the reductions in log CFU per sample of *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* were 2.1 – 3.6, 1.8 – 3.4, and 1.8 – 3.2, respectively. The pathogens may have died during the dry-curing or, may have been physically removed from the beef rounds during rinsing. The temperature employed during dry-curing, 6.7°C, is above the minimum growth temperature for *L. monocytogenes* (2) and slightly below the accepted minimum growth temperatures for *Salmonella* spp. and *E. coli* O157:H7 (2) but the presence of salt and sodium nitrite may have resulted in growth inhibition.

Further reduction in levels of *E. coli* O157:H7 and *Salmonella* spp. occurred in the two days of drying and pressing that following rinsing (Table 2). *Salmonella* spp. levels decreased by an additional 0.7 and 2.3 log CFU in trials 1 and 3. However, in trial 2, levels of *Salmonella* spp. were 0.9 log CFU higher after pressing compared to post-rinsing values. Reductions in *E. coli* O157:H7 levels ranged from 0.1 log CFU in trial 2 to 2.4 log CFU in trial 3. Levels of *L. monocytogenes* fell by 0.8 and 0.9 log CFU in trials 1 and 3, respectively.

The initial six days of drying after pressing, with the surface-application of the spice paste after four days, resulted in further pathogen reductions, with levels of *E. coli* O157:H7 and *Salmonella* spp. in trial 3 falling below the detection limit. Levels of *L. monocytogenes* also continued to fall during the first six days of drying. The continued lethal effect of drying was evident during the final 4 – 6 days of drying. No detectable *E. coli* O157:H7 or *Salmonella* spp. were detected on finished products in trials 1 and 3 and very low levels were found in trial 2. Levels of *L. monocytogenes* also continued to fall during the last 4 – 6 days of processing, with overall reductions of 4.0 – 4.9 log CFU by the end of the basturma-making process.

Of the three pathogens studied, *L. monocytogenes* is clearly the most likely to survive the basturma-making process. However, when the finished product water activity is considered along with the lethality achieved during the basturma-making process, it seems that basturma made as in this study presents little risk as a vehicle for food-borne listeriosis.

Previous microbiological surveys have found that *Salmonella* spp., when detected, is only present at very low levels in ground beef (6). Quantitative data on *E. coli* O157:H7 in ground beef is not available. Given this finding and the low prevalence of *E. coli* O157:H7 and *Salmonella* spp. on post-intervention beef carcass samples (4), it is highly unlikely that *Salmonella* spp. or *E. coli* O157:H7 would be present at high levels on beef rounds. Thus, our results strongly suggest that basturma prepared by dry-curing, rinsing, pressing, and drying, presents little risk of foodborne infection due to these pathogens, regardless of whether the product is cooked prior to eating.

Other researchers have noted the lethality of dry-curing and drying on Enterobacteriaceae (1, 5, 11), but pathogen studies have not been done. Clearly, dry-curing and drying of whole muscle products as applied in the present study causes substantial destruction of pathogenic bacteria and is worthy of closer study.

Conclusions

Basturma prepared by dry-curing, rinsing, pressing, and drying steps as done in this study, presents little risk of foodborne infection due to *Salmonella* spp., *Escherichia coli* O157:H7, or *Listeria monocytogenes*, regardless of whether the product is cooked prior to eating.

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Tables and Figures

Table 1. Water activity (a_w), Moisture:Protein ratio (MPR), pH, and % water-phase salt (% WPS) of basturma.

Trial	a_w	MPR	pH	% WPS
#1	0.87	1.92	5.6	13.1
#2	0.95	2.00	6.0	8.3
#3	0.84	1.52	5.6	18.0

Table 2. Lethality of the basturma-making process against *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Each value is the mean log CFU/piece for three tested beef rounds. Each beef round was sampled in three locations, with the mean log CFU/piece determined. The standard deviation is shown in parentheses. Reduction in log CFU per piece was calculated by subtracting the mean log CFU per piece at a given step from that determined at day zero.

		<i>E. coli</i> O157:H7		<i>Salmonella</i> spp.		<i>L. monocytogenes</i>	
Sampling Time		Mean Value	Reduction	Mean Value	Reduction	Mean Value	Reduction
Day 0							
	Trial 1	5.8 (0.3)	---	5.6 (0.4)	---	6.5 (0.3)	---
	Trial 2	6.5 (0.1)	---	6.4 (0.2)	---	6.4 (0.1)	---
	Trial 3	6.3 (0.2)	---	6.0 (0.2)	---	6.4 (0.2)	---
Day 21 (after rinse)							
	Trial 1	3.6 (0.7)	2.2	3.5 (0.6)	2.1	4.3 (0.9)	2.2
	Trial 2	3.1 (0.8)	3.4	2.8 (0.5)	3.6	3.2 (0.7)	3.2
	Trial 3	4.5 (0.1)	1.8	3.7 (0.2)	2.3	4.6 (0.2)	1.8
Day 23 (after pressing)							
	Trial 1	2.5 (0.8)	3.3	2.8 (0.8)	2.8	3.5 (0.1)	3.0
	Trial 2	3.0 (0.6)	3.5	3.7 (0.2)	2.7	Not tested	
	Trial 3	2.1 (0.4)	4.2	1.4 (0.3)	4.6	3.7 (0.3)	2.7
Day 29 (2 d after spice paste applied)							
	Trial 1	Not tested		Not tested		Not tested	
	Trial 2	1.9 (0.2)	4.6	1.7 (0.4)	4.7	2.7 (0.9)	3.7
	Trial 3	0.95* (0)	5.4	0.95* (0)	5.1	2.5 (0.1)	3.9
Finished product							
	Trial 1	0.95* (0)	4.9	0.95* (0)	4.7	2.5 (0.6)	4.0
	Trial 2	1.5 (0.4)	5.0	1.9 (0)	4.5	1.8 (0.2)	4.6
	Trial 3	0.95* (0)	5.4	0.95* (0)	5.1	1.5 (0.1)	4.9

* Value for log CFU per piece assigned when no colonies were observed on least dilute plate.

**IMPACT OF TRANSPORTATION OF FEEDLOT CATTLE TO THE
HARVEST FACILITY ON THE PREVALENCE OF *E. COLI* O157:H7,
SALMONELLA SPP AND TOTAL AEROBIC MICROORGANISMS**

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Key Words: cattle, transportation, *Salmonella*, *Escherichia coli* O157:H7

Introduction

The main pathogens of concern found in fresh meat products include *E. coli* O157:H7 and *Salmonella* spp. The prevalence of *E. coli* is used as an indicator of fecal contamination in meat products and its incidence can be highly variable. Because outbreaks of *E. coli* infection have been linked to beef more than any other food product, it is inherent to determine the prevalence levels in the live animal population (Jay 2000). Once determined, measures can be taken to reduce the risk of pathogen contamination in beef products and the need for multiple intervention steps later in processing.

Salmonella spp vary greatly by strain as to how they will affect and survive in an animal and human environment. According to Mulder (1995), the reduction and spread of *Salmonella* in the preslaughter environment can affect the contamination prevalence on swine and poultry carcasses. In addition, Bacon and others (2002) showed that the prevalence of *Salmonella* on the hides of beef animal entering the slaughter facility serves as an indication of contamination that could be transferred to sterile equipment and the environment during the dehiding process, which may not be an indication of the number of animals carrying or shedding *Salmonella*.

Total aerobic microorganisms can be representative of overall cleanliness in the beef processing environment and may be an indicator of spoilage organisms present in the final beef product.

Many factors that affect the microbial prevalence levels in fresh meat and poultry include but are not limited to feeding, transportation, the slaughter process and the use of antimicrobials (Hardin et. al 1995).

The handling and transportation of livestock can cause animals to become stressed, which can increase the shedding of fecal material and pathogens (Fischer 1996, Hails 1978). Research conducted by Williams and Newell (1970) investigated the affect of transport, overcrowding in holding pens, and rough handling before slaughter in swine species. They showed that animals differ in the *Salmonella* excretion patterns, preceding and following the stress of transport. This can result in hidden or masked *Salmonella* infections to become more prevalent. Increased stress and changes in fecal patterns can increase the many points of contamination as animals are transported to slaughter facilities.

Previous research (Barham 2002) indicates an increase in pathogen prevalence on hides of cattle during transportation from the feedyard to the packing plant. The transportation trailer was reported as a possible source of the increased prevalence, but it is not known how cleaning and sanitizing trailers prior to animal transportation will affect contamination on beef hides entering the slaughter plant. A study

conducted by Childers et al. (1977) showed the prevalence levels of bacteria obtained from animal midlines was reduced in those taken from pigs transported and held in sanitized trailers and holding pens, but there was no information collected regarding prevalence levels inside the trailers. In 1998, Rajkowski and others concluded that washing and sanitizing after animal unloading significantly reduced the incidence of *Salmonella* and *E. coli* found in trailers, but corresponding animal data was not collected. Interventions could be expected to decrease the microbial prevalence levels in feedyards, trailers and holding pens; therefore decreasing cross-contamination between farms, animal to animal contact, reduced contamination of the slaughter plant, and decreased microbial loads in fresh meat products. It is also unknown and hypothesized that animals transported on the lower level of the trailer will have increased microbial prevalence levels compared to those transported on the upper level.

Objectives

1. Determine the impact of transportation of beef animals to the harvest facilities on the prevalence of *E. coli* O157:H7, *Salmonella* spp. and total aerobic organisms.
2. Determine the impact of the animal location (upper vs. lower level) during transportation on the prevalence of *E. coli* O157:H7, *Salmonella* spp. and total aerobic organisms.
3. Determine the effectiveness of trailer washing as a means of minimizing hide contamination during transportation of beef animals on the prevalence of *E. coli* O157:H7, *Salmonella* spp. and total aerobic organisms.

Methodology

Sample Collection. A random sample of 40 animals from the same pen (Caprock Feeders, # 6, Lockney, TX) were evaluated and tagged prior to loading on each of 8 days. Spongesicles (SSL 100, International BioProducts, Muncie, IN) hydrated with 10 mL of Butterfields Phosphate Buffer (BPB, Difco Laboratories, Detroit, MI) were used to swab the midline and withers of all animals. Clean trucks were cleaned and sanitized at Excel, Plainview, TX using 4QUAT (K-Klean Chemical Co., INC, Dallas, TX) prior to animal loading. Samples of clean and dirty trailers were collected from the front, right side, left side and floor of each level prior to loading. Ten tagged animals were loaded on the upper and lower compartment of the clean and dirty trailers with the remaining trailer space filled with cattle pen mates. After transportation to the harvest facility, cattle were unloaded and kept in their treatment groups. Trailers swabs were repeated after unloading and animals swabs repeated after exanguination. All swabs were done in duplicate; one used for the *E.coli/Salmonella* analysis and one for the total aerobic plate count (TPC) analysis.

Microbiological Analysis. Within 6 hours of collection, samples were returned to Texas Tech University and enriched for further analysis. The spongesicle swab used for *E.coli/Salmonella* spp was cut in half and each half was placed in a separate bag for enrichment procedures specific for *E. coli* O157:H7 and *Salmonella* spp. An additional 15 mL of BPB was added to the total aerobic plate count (TPC) samples for dilution.

E. coli O157:H7 swabs were enriched with 45 mLs of GN Broth (Difco Laboratories, Detroit, MI). GN broth specific to organism was made by adding 50 um

Cefixime, 1 mL Vanomycin and 1 mL Cefsulodin to 1 L prepared GN broth. Enriched samples were incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 6 hours and stored in a refrigerated cooler at $1-4^{\circ}\text{C}$. Samples were further enriched in Brain Heart Infusion Broth (Difco Laboratories, Detroit, MI) for 3 hours prior to automated *E. coli* O157:H7 detection. The automated detection of *E. coli* O157:H7 was performed by Polymerase Chain Reaction assay using the BAX System (Model #1200, Qualicon, Inc., Wilmington, DE).

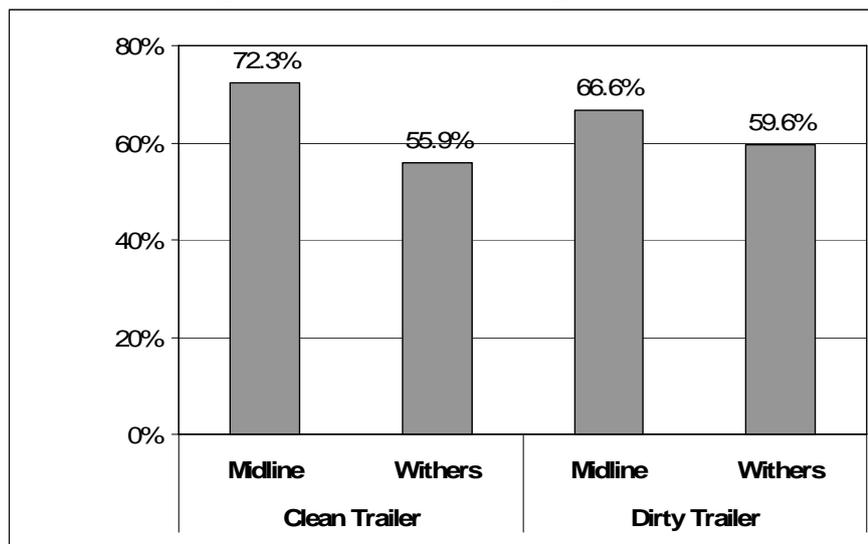
Salmonella spp swabs were enriched using 45 mLs of Tryptic Soy Broth (Difco Laboratories, Detroit, MI) prepared according to manufacturers instructions. Enriched samples were incubated at $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 2 hours, then $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for an additional 6 hours and stored in a refrigerated cooler at $1-4^{\circ}\text{C}$ until automated detection using the BAX System.

Serial dilutions of the TPC samples were made using 1 mL dilutions of BPB and plated on 3M Petrifilm Aerobic Count Plate (3M Corporation, St. Paul, MN) following AOAC Official Methods 998.08. Petrifilm plates were incubated for 48 hours \pm 3 hours at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and red colonies were enumerated using a 3M Petrifilm Plate Reader (Model # 6499, 3M Corporation, St. Paul, MN).

Experimental Design and Statistical Analysis. The experiment was conducted in 8 replications (days) with 10 experimental units per treatment. Data analysis was performed using the PROC MIXED application of SAS Version 8 (SAS Institute, Cary, NC). The design was completely random with model including the main effects of truck, animal location (level), hide swab location and day. Means were separated using the appropriate error terms with a significance level set at $P < 0.05$.

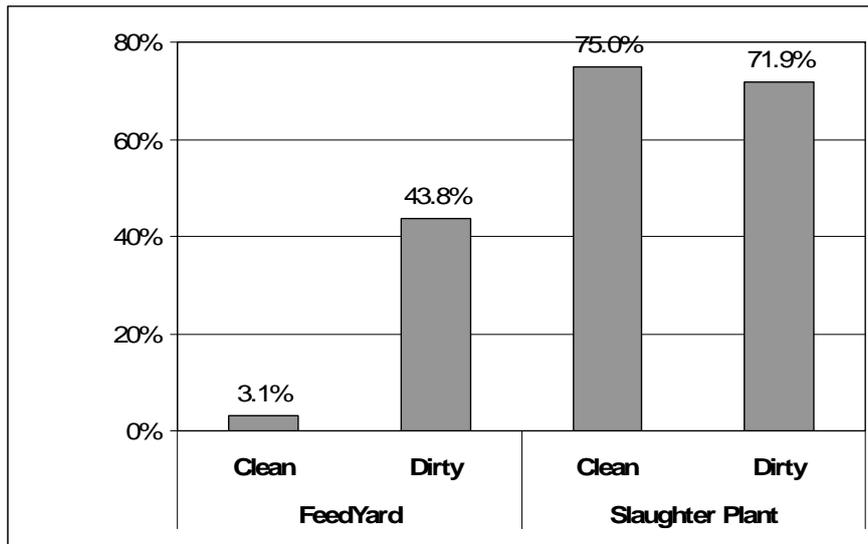
Results & Discussion

Salmonella spp. Animal swabs collected showed a significant interaction for trailer and hide swab. Swab samples collected from the midline had a higher ($P = 0.038$) percentage of *Salmonella* positive samples than those collected from the withers for animals transported on both trailers (Figure 1).



Animal swabs taken at the plant had significantly more positive samples than those taken at the feed yard at both hide swab locations. Trailer swabs collected for *Salmonella* spp analysis showed a significant interaction for location x trailer. At the feedyard, swabs taken from the clean truck (3.1%) had a lower ($P < 0.001$)

percentage of positive samples than those collected from the dirty truck (43.8%). However, at the plant swabs taken from the clean truck (75.0%) had a higher ($P = 0.001$) percentage of positive samples than those collected from the dirty truck (71.9%, Figure 2).



In addition, an interaction for location x level in trailer was observed for trailer swabs collected. At the feedyard, a significant difference was found as the top level reported only 15.6% positive samples while the bottom level had 31.3% positive samples.

Another interaction for the prevalence of *Salmonella* spp in trailer swabs taken was the trailer x level. The clean trailer had a lower percentage of positive samples than those found in the dirty trailer for the bottom level, which could be expected. However, the trailer swabs collected from the top level were similar ($P = 0.524$) for the clean and dirty truck. The main effect of swab location within the trailer also showed significant differences in the prevalence of positive samples. The floor samples collected were significantly higher ($P = 0.001$) than those collected from the right, left and front sides of the clean and dirty trailers.

***E. coli* 0157:H7.** The swab location on the animal hides had a higher ($P = .051$) percentage of positive samples at the plant (1.3%) when compared to the feedyard (0.3%). Trailer had no effect on prevalence as seen with 256 swabs taken from the trailers, less than 2% of samples were positive for *E. coli* 0157:H7.

Total Aerobic Organisms. The location swabs taken at the plant had a higher ($P = 0.003$) CFU log count than those taken at the feedyard. Also, hide swabs showed higher ($P = 0.008$) counts at the midline location compared to the withers (Table 7).

A significant 3-way interaction for location x trailer x trailer swab was determined for TPC from truck samples (Table 8). The dirty trailers had higher counts of aerobic organisms at all trailer locations (floor, front, right and left) at both the feedyard and the slaughter plant. In addition, trailer swabs collected from the top deck (7.50 log CFU) had less ($P = 0.008$) aerobes compared to the bottom deck (7.86 log CFU).

Other research has shown that the number of *Salmonella* spp positive animals increased from entry into feedyard when compared after 30 days in the feedyard (Corrier et. al 1990, Cray et. al 1998). This suggests that animal to animal contact may be a contributing factor to microbial transmission. Current data agrees with this suggestion as the *Salmonella* prevalence increased at the slaughter facility compared to the feedyard. However, there are not any current time and space parameters known for the transmission of *Salmonella* spp from animal to animal.

Even though the prevalence of *Salmonella* spp and total aerobic organisms in the trailer showed some significant interactions, there does not appear to be a direct relationship between the cleanliness of trailer, the level in which the cattle were transported and the actual microbial prevalence found on the hide of the cattle. Barham and others (2002) showed that the prevalence of *Salmonella* spp increased due to transportation and the current data agrees with these previous findings. However, current data shows that the trailer itself is not the source of the increased contamination. In addition to animal contact, other possible sources of contamination include the dirt/dust present in the loading area at the feedyard, holding areas at the feedyard just prior to loading, holding areas at the plant prior to slaughter and equipment/personnel found inside the plant during stunning and exanguination.

Conclusions

Even with increased levels of *Salmonella* spp and aerobic organisms at the harvest facility, positive samples for microorganisms found in trailers did not relate to the contamination found on animal hides. Neither the cleanliness of trailers nor level in which the animals were transported affected the contamination present on the animal at the harvest facility. Increased levels found on animal hides agrees with previous research, but is not caused by the trailer itself. Other sources of contamination include animal to animal contact, dirt/dust present in the loading area at the feedyard, holding areas at the feedyard just prior to loading, holding areas at the plant prior to slaughter and equipment/personnel found inside the plant during stunning and exsanguinations.

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**APPLICATION OF ANTIMICROBIAL TREATMENTS IN A COMMERCIAL
SIMULATION TO REDUCE *ESCHERICHIA COLI* O157:H7 AND
SALMONELLA SPP. IN BEEF TRIM AND GROUND BEEF**

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Key Words: Acidified sodium chlorite, acetic acid, lactic acid, sterile water, pathogens, beef.

Introduction

Animals are natural reservoirs of food-borne pathogens including *Salmonella* spp. and *Escherichia coli* O157:H7. The muscle of a healthy animal is essentially sterile, but even under the most stringent conditions, it becomes contaminated during the slaughter process from the environment, hide or from direct contact with the intestinal tract contents. This contamination ultimately can cause consumer illness if the product is not appropriately handled by the processor or the consumer. Pathogens are of great concern for processors for both food safety issues and for economic reasons. While beef trimmings and ground beef are to be cooked by the consumer, the processor must recall the raw product if testing indicates the presence of *Escherichia coli* O157:H7. Processors have very few interventions for beef trimmings and ground beef.

A limited amount of research has been completed to determine the effectiveness of interventions under commercially simulated conditions on beef trim to reduce pathogens. Additionally, some results are conflicting and do not address “practical” issues that are encountered in a processing environment. It is essential that the long-term effects on the product quality be evaluated to determine if the product has a similar shelf-life and sensory qualities as non-treated products.

In previous studies, lactic acid and acidified sodium chlorite (ASC) have been effective in reducing pathogen loads on beef carcasses and to a limited extent, on beef trim. Castillo et al. (1999) reported that acidified sodium chlorite reduced populations of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 up to 3.9 log cycles when applied to beef carcass cuts after water wash. The Food and Drug Administration (FDA) has recently approved the use of ASC as a food additive to reduce pathogens loads on both pre-chill and post-chill meat and poultry products. However, the effectiveness of antimicrobials in a commercially simulated application on reduction of pathogens on beef trim has not been thoroughly reported in peer-reviewed journals. A limited amount of industry generated and non-peer reviewed data exist, but the effectiveness of the product needs to be studied under controlled conditions that mimic production conditions so processors can make informed, science-based decisions about the use of this product.

Studies related to the use of acetic and lactic acids as decontaminants for beef trim have been conflicting. Conner et al. (1997) reported that 2 and 4% organic acid sprays did not significantly reduce populations of *Escherichia coli* O157:H7 or *Listeria monocytogenes* in beef trim or in the ground beef produced from that trim. On the other hand, Castillo et al (2001) reported that applying a 4% lactic acid spray to cold carcasses and then subsequently producing ground beef from the carcasses reduced

Escherichia coli O157:H7 and *Salmonella* spp. populations by 3.3-3.4 log cycles and additional reductions in the ground beef made from these carcasses. Kang et al. (2001) reported that multiple antimicrobial treatments were effective in reducing microbial populations on beef trim. They utilized hot water, hot air, and lactic acid to produce the antimicrobial effect on the trim and subsequently in the ground beef. They evaluated the effects of the treatments on naturally occurring microorganisms and did not report the effect on microbial pathogens. The numbers of aerobic organisms, psychotrophs, coliforms, and lactic acid bacteria were significantly lower in the treated samples.

Many currently published studies are conducted in a very controlled laboratory conditions and do not mimic the actual processing environment. For example, some studies report that trim was treated, held for 24 h, and then ground (Connor et al.). It is highly unlikely that this practice would be followed in industry. Additionally other studies report that the pieces of beef trim are treated evenly on all sides with the intervention. Again, it is unlikely that an on-line intervention process for beef trim will allow complete coverage of the pieces with the antimicrobial treatments. If the pieces of trim are dipped then they will likely contain too much of the antimicrobials and will adversely affect the product quality. If they are sprayed, then only a portion of the surface will be covered. Processors need validation in a setting that is similar to actual processing environments. At Texas Tech University we have a Pathogen Processing Area with processing equipment and facilities to produce both fresh and processed meat products that are pathogen inoculated. We are in a unique position to validate current interventions using “real world” conditions and generate data that are directly applicable to the meat processing industry. We recently completed a real world simulation at Texas Tech that showed the effectiveness of organic acids and acidified sodium chlorite in reducing the levels of both *Salmonella* spp. and *Escherichia coli* O157:H7. The results given in the final report to National Cattlemen’s Beef Association (NCBA) show promising results; however, an automated spray application system that flips the trim needs to be validated for their effectiveness in application of the pathogen reducing interventions.

Objective

The objective of this study was to validate the effectiveness and application of acidified sodium chlorite (1000 ppm), acetic and lactic acids (2% and 5%) and sterile water in reducing *Escherichia coli* O157:H7 and *Salmonella* spp. levels on beef trim prior to and after grinding in a simulated processing environment utilizing a belt turning and spray application.

Methodology

The antimicrobial effects of organic acids and acidified sodium chlorite (ASC) were evaluated by inoculating beef trim *Escherichia coli* O157:H7 or *Salmonella* spp. allowing for pathogen attachment, treating the trim with one of the interventions and collecting samples of trim before treatment (control) and at the following points during production: (1) immediately after treatment (20 min); (2) immediately after grinding (6 h); (3) 24 h after grinding. The experiment was conducted in the pathogen processing facility in the Food Technology Building at Texas Tech University under simulated industry conditions.

From a commercial beef-packing facility, 163.29 kg. of beef trim were obtained with an 80% lean and 20% fat blend. For each replication of *Escherichia coli* O157:H7 inoculated samples (n=3) and *Salmonella* spp. inoculated samples (n=3), 81.65 kg. of trim was processed. In the pathogen processing area, 81.65 kg. of the trim was inoculated with a cocktail mixture of a streptomycin-resistant strains of either *Salmonella* spp. (strains 1 and 2) (University of Nebraska, Lincoln, NE) or *Escherichia coli* O157:H7 by dipping each piece of trim into a sanitized container containing the pathogen mixture along with a buffer solution. The target population on the beef trim was 1×10^5 cfu/g, on the surface of the trim.

Prior to inoculation a sample of trim was taken to ensure no pathogens were present on the trim prior to inoculation. After the inoculation dip, the trim was held for 20 min on sanitized stainless steel mesh racks to allow for pathogen attachment. Control samples of trim were taken before intervention application. After attachment, trim for each study was divided into equal portions (4.54 kg.). Trim was fed to the grinder in simulated processing environment utilizing a belt turning and spray application. The trim was treated by spraying one of the antimicrobial treatments onto its surface as it moved down a conveyor system towards the grinder. Trim was fed to the grinder using a conveyor belt similar to those used in the industry. Trim was treated by spraying with an automatic premixed spray system, onto the surface of one side of the trim as it moves down a conveyor. The trim was flipped on to the same conveyor and the other side was sprayed prior to grinding. The individual portions were sent down the conveyor and treated with one of the six treatments: (1) 2% acetic acid; (2) 5% acetic acid; (3) 2% lactic acid; (4) 5% lactic acid (Fisher Scientific International Inc., Hampton, NH); (5) acidified sodium chlorite (1000 ppm) (Sanova®, Alcide Corporation, Redmond, WA); and (6) sterile water. The conveyor system and grinder were cleaned and sanitized with a 0.05% chlorine solution in between treatments within a replication. All processing equipment was cleaned with the application of a 0.05% chlorine solution and sanitized with Bi-Quat (Birko Corp., Henderson, CO) between replications.

Samples of trim were taken after the intervention application and immediately prior to grinding. The remaining trim was ground and ground samples were collected immediately after grinding for microbiological analysis. The remaining ground beef was divided into two equal portions, vacuum packaged and the two portions were stored at 4°C in the processing lab for either 6 or 24 h. Ground beef samples were processed at 6 and 24 h after processing. Both *Escherichia coli* O157:H7 and *Salmonella* spp. inhibition were evaluated separately.

Microbiological Analysis

During the sampling process 10 g of the sample were collected and placed in a sampling bag (Model 400 Bags 6041, Stomacher Lab System Seward Limited, London, UK). Buffered Peptone Water (99 ml) was added with the sample in the sampling bag. The bag and contents were placed in a laboratory blend stomacher (Model 400, Seward Medical, London UK) and processed at a normal speed for 60 s. Afterwards, 3 ml of the sample was collected and placed into a spiral-platter sampling cup. Samples were automatically plated using the Spiral Biotech Autoplate® (Spiral Biotech, Norwood, MA). The 10^5 samples were exponentially plated with 50 ul of the sample on two Trypticase Soy Agar (TSA) plates and two TSA (TSAST) plates with the addition of 50 ug of streptomycin antibiotic.

The streptomycin antibiotic inhibited growth of the background flora while allowing for pathogen growth. Total aerobic plate counts and coliform counts were determined using Trypticase Soy Agar with the absence of the streptomycin antibiotic. Plates were incubated for 48 h at 37°C. 10^5 plates were counted using the Spiral Biotech Q Count™ (Version 2.0, Spiral Biotech, Norwood, MA).

Results

Trim Samples

The antimicrobial treatments on the surface of beef trim showed no effects ($P < 0.05$) for *Escherichia coli* O157:H7.

The antimicrobial treatments of 5% acetic acid and acidified sodium chlorite (1000 ppm) on the surface of beef trim showed significant reductions ($P < 0.05$) for *Salmonella* spp. (Figure 1).

Ground Beef Samples

The antimicrobial treatments of: sterile water, 5% lactic acid, 2% acetic acid, 5% acetic acid, and acidified sodium chlorite significantly reduced *Escherichia coli* O157:H7 ($P < 0.05$) in ground beef when compared to the control at 6 h. 5% lactic acid showed the most effectiveness 6 h after processing. However, 2% acetic acid and acidified sodium chlorite (1000 ppm) showed the most effectiveness in reducing *Escherichia coli* O157:H7 24 h after processing (Figure 2).

The antimicrobial treatments of: sterile water, 2% lactic acid, 5% lactic acid, 2% acetic acid, 5% acetic acid, and acidified sodium chlorite significantly reduced ($P < 0.0001$) pathogen loads of *Salmonella* spp. on the ground beef after both 6 and 24 h after processing (Figure 3).

Reduction of *Escherichia coli* O157:H7 in ground beef

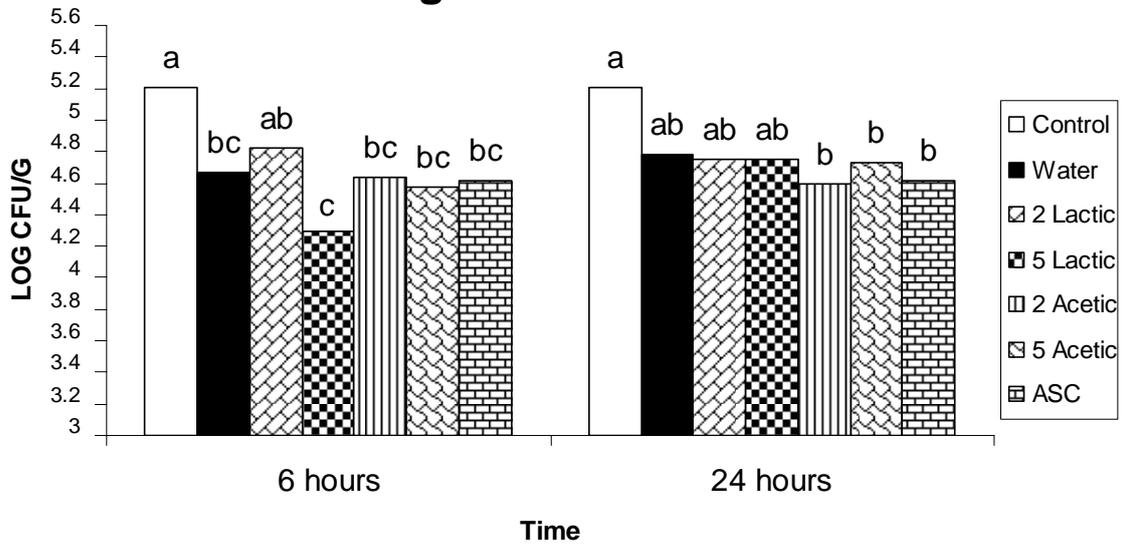


Figure 1. Effects of sterile water, acetic acid, lactic acid, and acidified sodium chlorite in reducing *Escherichia coli* O157:H7 in ground beef stored at refrigerated temperatures.

Reduction of *Salmonella* spp. in beef trim

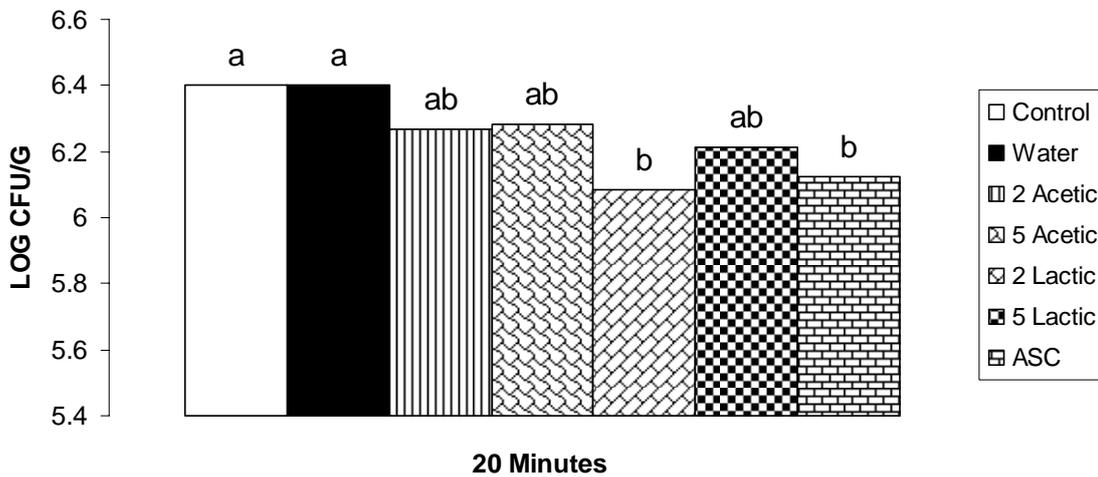


Figure 2. Reduction of *Salmonella* spp. in beef trim after treatment with sterile water, acetic acid, lactic acid and acidified sodium chlorite.

Reduction of *Salmonella* spp. in ground beef

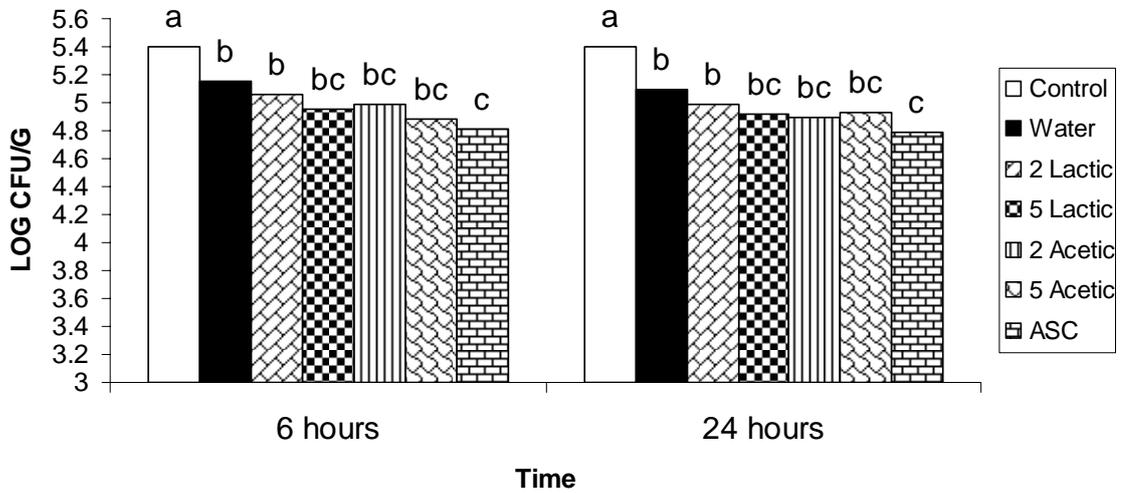


Figure 3. Effects of sterile water, acetic acid, lactic acid, and acidified sodium chlorite in reducing *Salmonella* spp. in ground beef stored at refrigerated temperatures.

Conclusion

In conclusion, research from this present study indicates that industries can utilize antimicrobial interventions under commercially simulated conditions on beef trimmings to reduce pathogens in ground beef.

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ANTIBACTERIAL EFFECT OF SODIUM CAPRYLATE ON *ESCHERICHIA COLI* O157:H7 IN CATTLE DRINKING WATER

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Key Words: *E.coli* O157:H7, sodium caprylate, cattle drinking water

Introduction

E.coli O157:H7 is a major food-borne pathogen in the United States. Cattle serve as the principal reservoir of *E.coli* O157:H7, excreting the pathogen in feces, thereby contaminating food, water, and the environment (Chapman *et al* 1993, Laegreid *et al.*, 1999, Shere *et al.*, 1998; Zhao *et al.*, 1998). Environmental persistence of *E.coli* O157:H7 is critical in its epidemiology on farms (LeJeune *et al.*, 2001). Several researchers have isolated *E.coli* O157:H7 from cattle water troughs, indicating that water troughs on farms could serve as a potential long-term reservoir of the pathogen. Persistence of *E.coli* O157:H7 in cattle water troughs can potentially act as a source of re-infection of cattle, birds, flies, and rodents, which, in turn can act as vectors of the pathogen (McGee *et al.*, 2002). Thus, there is a need for an effective and practical method for killing *E.coli* O157:H7 in cattle water troughs. Inactivation of *E.coli* O157:H7 in water at farm will potentially shut down one source of infection to cattle, thereby leading to a reduced carriage of *E.coli* O157:H7 in cattle. This in turn will translate into improved farm and animal hygiene, and a reduced contamination of beef products with *E.coli* O157:H7. Finally, a safe supply of beef products is critical for the economic viability of the beef industry.

Caprylic acid is a natural, eight-carbon fatty acid present in breast milk, bovine milk (Jensen *et al.*, 2002), and coconut oil (Jensen *et al.*, 1990, Sprong *et al.*, 2001). Caprylic acid is a food-grade chemical approved by the FDA as GRAS (CFR 184.1025). Previous research conducted in our laboratory indicated that caprylic acid was highly effective in killing *E.coli* O157:H7 in bovine rumen fluid (Annamalai *et al.*, 2004).

Objectives

To determine the antibacterial effect of sodium caprylate to kill *E.coli* O157:H7 in cattle drinking water.

Materials and Methods

Bacterial strains and media

Four strains of green fluorescent protein (GFP)-labeled *E.coli* O157:H7 were used in the study. The four strains of GFP-labeled *E.coli* O157:H7 were individually

cultured in 10 ml of Tryptic soy broth (TSB, Difco) containing 100 µg/ml of ampicillin (Sigma-Aldrich Chemical) at 37°C for 24 h with agitation (150 rpm). Following incubation, the cultures were sedimented by centrifugation (3600 X g for 15 min), washed twice, and resuspended in 10 ml of sterile deionized water. Equal portions from each of the four cultures were combined, and 100 µl (approximately 10⁸ CFU) of the four-strain mixture was used as the inoculum.

Sample inoculation and treatments

The efficacy of sodium caprylate for killing *E.coli* O157:H7 was determined in water with and without bovine feces or feed. Water was obtained from a local dairy farm, and aliquots of 100 ml each of water were dispensed into 250 ml sterile containers. Appropriate quantities of sodium caprylate (Sigma-Aldrich Chemical) were added to each water sample to obtain a final concentration of 75, 100 or 120 mM. Samples without sodium caprylate (0 mM) were used as controls for the study. In addition, a set of water samples containing bovine feces (1% w/v) (McGee et al., 2002) or feed (1% total mixed ration, TMR) were also included to determine the effect of feces/feed on the antibacterial property of caprylate. Each treatment and control water sample was inoculated with the four-strain mixture of *E.coli* O157:H7 to obtain an inoculation level 10⁶CFU/ml of water. The containers were loosely covered with plastic lids to enable free passage of air. The samples were incubated at 21°C, 10°C or 4°C. Triplicate samples of each treatment and control were included at each of the specified temperatures, and the entire study was duplicated.

Enumeration of E.coli O157:H7

The population of surviving *E.coli* O157:H7 in each water sample was determined by plating 0.1-ml portions of the samples directly or after serial dilutions (1:10 in phosphate buffered saline, PBS, pH 7.4) on duplicate Tryptic soy agar (TSA) plates containing 100 µg/ml of ampicillin. The plates were incubated at 37°C for 24 h and viewed under ultra violet light to enumerate *E.coli* O157:H7 (Vialette et al., 2004). At each sampling time, 1 ml of water from each container was also transferred to separate 250-ml flasks containing 100 ml of sterile TSB for enrichment at 37°C for 24 h. When growth was observed in TSB, the culture was streaked on TSA containing 100 µg/ml of ampicillin. The pH of each treatment and control sample was determined using an Accumet pH meter (Fisher Scientific, Pittsburgh, PA).

Statistical analysis

For each treatment and control, the data from independent replicate trials were pooled, and analyzed using a split-plot design with repeated sampling over time. The model included the treatment, concentrations, storage temperature and days. Significant differences ($P < 0.0001$) in bacterial counts due to treatment, concentrations, storage temperature and days were determined.

Results & Discussion

The magnitude of *E.coli* O157:H7 inactivation in water significantly ($P < 0.0001$) increased with increase in caprylate concentration and storage temperature. At 120 mM, sodium caprylate completely inactivated *E.coli* O157:H7 in all samples,

excepting those containing feces at 4°C. Feces or feed also had a significant effect ($P < 0.0001$) on the antibacterial property of caprylate. At all the storage temperatures, bovine feces substantially reduced the killing of *E.coli* O157:H7 by caprylate, whereas inactivation of the pathogen was rapid in presence of TMR.

Conclusions

This study indicated that sodium caprylate is effective in killing *E.coli* O157:H7 in cattle drinking water especially at higher environmental temperatures. This is important since fecal excretion of *E.coli* O157:H7 by cattle has been reported to be higher in summer months than in winter (Heuvelink et al., 1998; Jackson et al., 1998). Our future studies will focus on the palatability of water containing sodium caprylate to cattle.

Acknowledgement

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**INNOVATIVE APPLICATION OF ANTIMICROBIAL COMPOUNDS
DURING HIDE REMOVAL AS A MEANS TO REDUCE CARCASS
CONTAMINATION BY PATHOGENIC MICROORGANISMS**

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Key Words: Antimicrobials; Beef hides; Carcass contamination

Introduction

Hides are considered an important source of pathogenic organisms during slaughter because of fecal contamination experienced during holding (Castillo, Dickson, Clayton, Lucia, & Acuff, 1998). Prevalence levels of *Salmonella* on the hides of cattle have been determined to be as high as 15.4% pre-slaughter (Bacon, Sofos, Belk, Hyatt, & Smith, 2002). Bacteria present on hides can eventually be transferred to underlying “sterile” carcass tissue during the hide removal process. Contamination can occur when manure on the hide surface that has not been washed away before slaughter is carried onto the underlying carcass tissue (Delazari, Iaria, Riemann, Cliver, & Jothikumar, 1998). Because errors in slaughter and dressing have been implicated as the primary vehicles for contamination of beef carcasses (Bacon, Belk, Sofos, Clayton, Reagan, & Smith, 2000), many processors have incorporated hide washes into their systems to reduce levels of microbial contamination in the final product; however, these systems often increase the solubilization and migration of pathogenic bacteria on hide surfaces. Selective application of antimicrobial compounds to hide opening areas targets the specific site on the hide that is of most concern to potentially minimize pathogen transfer from the hide to carcass tissues during harvest without increasing solubilization.

Objectives

To determine the effectiveness of selected antimicrobial agents in reducing pathogenic microorganisms on hides and to verify the efficacy of selected antimicrobial agents in a commercial facility.

Methodology

Trial I

Fresh beef hides (n = 12; 4 per rep) were cut into 900-cm² sections with a minimum of 12 sections removed from each. Half of these sections were blown dry and shaved using Oster ClipMaster[®] clippers (Sunbeam Products, Inc., Boca Raton, FL), and the other half remained unshaved. The following day, hide sections were stretched over plastic clipboards and inoculated over a 400-cm² area with a bovine

fecal slurry (10g bovine feces and 10 mL 0.1% sterile peptone water, Difco Laboratories, Detroit, MI) containing approximately 10^6 CFU/g. Inoculum was allowed a 20 min attachment period before gross fecal material was washed off using a handheld, compressed-air sprayer standardized to deliver approximately 1 L of distilled water over 90 sec.

Microbiological samples were collected from each untreated hide following water wash using a sterile sponge (BioPro Sampling System; BioTrace International, Bothell, WA) to determine pre-treatment counts on hide surfaces. Prior to sampling, a sponge was moistened with 25 mL of sterile 0.1% peptone water, and one sample collection then was achieved by firmly rubbing the damp sponge over a 100-cm² area of the hide section. The sponge then was transferred to a plastic bag until analysis. Following pre-treatment sampling, sections were subjected to one of six antimicrobial treatments: distilled water; isopropyl alcohol; 3% hydrogen peroxide (Aaron Industries, Inc., Clinton, SC); 2% L-Lactic acid (Purac[®], Rotra International, Wood Dale, IL); 10% Povidone-iodine (Vetadine, Vedco, Inc., St. Joseph, MO); and 1% cetylpyridinium chloride (Zeeland Chemicals, Zeeland, MI) using saturated (50 mL), sterile sponges.

Following treatment, hides sections were again sampled as described previously. Each sponge sample then was hand-massaged inside its plastic bag for 1 min before examination for aerobic plate counts (APCs) and coliform and *E. coli* counts. Coliform and *E. coli* counts were generated by plating appropriate dilutions of the sponge sample onto Petrifilm *E. coli* count plates (3M Microbiology & Products, St. Paul, MN). Samples were incubated for 24 ± 2 h at 35°C before colonies were counted. *E. coli* colonies appeared blue with a gas bubble, while total coliform count was achieved by counting both blue and red colonies with a gas bubble. Aerobic plate counts were determined by plating appropriate dilutions of the sponge sample onto Petrifilm aerobic count plates (3M), incubating at room temperature (25°C) for 48 h, and then counting all colonies.

Trial II

Beef carcasses (n = 18) with hides attached were selected from a small commercial processor for use in Trial II. Following exsanguination, approximately 100-cm² of the hide in the brisket area was sampled with a pre-moistened, sterile sponge as described in Trial I to determine pre-treatment counts on hide surfaces. Following sampling, hides were shaved in approximately a 400-cm² area in the brisket region of the carcass. Cattle then were assigned to receive one of three antimicrobial treatments (2% L-lactic acid, 3% hydrogen peroxide, and 1% CPC), and treatments were applied using saturated (50 mL), sterile sponges.

After application of the designated treatment, hides were sampled again as described above to determine post-treatment counts. Following hide removal, 100-cm² of the carcass surface in the brisket area was sampled using a pre-moistened, sterile sponge as described previously to determine carcass counts. Sponge samples were placed in an insulated shipping container with refrigerant to keep them cool for transport to Texas A&M University's Food Microbiology Laboratory (College Station, TX). The following day, sponge samples were hand-massaged inside their plastic bags for 1 min before examination for APCs and coliform and *E. coli* counts. Data were analyzed using PROC GLM of SAS (SAS Institute, Cary, NC). Least-squares means were generated for each main effect and separated using the PDIF option when appropriate.

Results & Discussion

Trial I

Least-squares means for the interaction of shaving × antimicrobial agent on APC reduction for hide sections inoculated with 10⁶ CFU/g fresh bovine feces are reported in Table 1. Within non-shaved samples, 1% CPC and hydrogen peroxide produced among the greatest reductions, and within shaved samples, 1% CPC, 2% L-lactic acid, and hydrogen peroxide produced among the greatest reductions. Least-squares means for the interaction of shaving × antimicrobial agent on coliform reduction for hide sections inoculated with 10⁶ CFU/g fresh bovine feces are reported in Table 2. Within non-shaved samples, 1% CPC produced the greatest reduction at 5.3 log₁₀ CFU/100 cm², followed by 2% L-lactic acid, iodine, hydrogen peroxide. Within shaved samples, 1% CPC, 2% L-lactic acid, and hydrogen peroxide produced among the greatest reductions with 4.5, 4.1, and 3.9 log₁₀ CFU/100 cm² reported, respectively.

Table 1. Least-squares means for the interaction of shaving × antimicrobial agent on APC reduction

<i>Antimicrobial</i>	log ₁₀ CFU/100 cm ² reduction ^a	
	Non-shaved	Shaved
Water	0.9c	0.6c
Alcohol	0.5c	1.8bc
1% CPC	4.1a	4.6a
Iodine	1.3c	1.8bc
2% L-lactic acid	2.7b	4.1a
Hydrogen peroxide	4.5bc	4.4a
SEM	0.48	0.48

LS means lacking common letters (a-c) differ ($P < 0.05$).

^aLog reduction = (log₁₀CFU/100 cm² reduction on untreated hide area) – (log₁₀CFU/100 cm² reduction on treated hide area).

Least-squares means for the treatment effects of shaving and antimicrobial agents on *E. coli* reduction on hide sections inoculated with 10⁶ CFU/g fresh bovine feces are reported in Table 3. Non-shaved samples had a mean reduction of 2.0 log₁₀ CFU/100 cm², and shaved samples had a mean reduction of 2.8 log₁₀ CFU/100 cm². Within the antimicrobials tested, 1% CPC produced the greatest reduction, followed by 2% L-lactic acid and hydrogen peroxide.

Across all treatments, shaving appeared more advantageous than not shaving when applying antimicrobial agents to reduce bacterial counts on the hide surface. After completion of Trial I, shaving together with 1% CPC, 2% L-lactic acid, and hydrogen peroxide were determined to be the three most effective shaving/antimicrobial combinations, and were selected for further evaluation in Trial II.

Table 2. Least-squares means for the interaction of shaving × antimicrobial agent on coliform reduction

<i>Antimicrobial</i>	$\log_{10}\text{CFU}/100 \text{ cm}^2$ reduction ^a	
	Non-shaved	Shaved
Water	-0.9d	0.5d
Alcohol	0.2d	1.8c
1% CPC	5.3a	4.5ab
Iodine	2.4c	2.5c
2% L-lactic acid	2.8c	4.1b
Hydrogen peroxide	2.2c	3.9bc
SEM	0.43	0.43

LS means lacking common letters (a-d) differ ($P < 0.05$).

^aLog reduction = ($\log_{10}\text{CFU}/100 \text{ cm}^2$ reduction on untreated hide area) – ($\log_{10}\text{CFU}/100 \text{ cm}^2$ reduction on treated hide area).

Table 3. Least-squares means for treatment effects of shaving and antimicrobial agents on *E. coli* reduction

Treatment effects	$\log_{10}\text{CFU}/100 \text{ cm}^2$ reduction ^a
Shaving	
Non-shaved	2.0b
Shaved	2.8a
SEM	0.17
<i>Antimicrobial</i>	
Water	0.2d
Alcohol	0.9d
1% CPC	4.5a
Iodine	2.4c
2% L-lactic acid	3.3b
Hydrogen peroxide	2.9bc
SEM	0.30

LS means within treatments lacking common letters (a-d) differ ($P < 0.05$).

^aLog reduction = ($\log_{10}\text{CFU}/100 \text{ cm}^2$ reduction on untreated hide area) – ($\log_{10}\text{CFU}/100 \text{ cm}^2$ reduction on treated hide area).

Trial II

The average initial hide counts before treatment application were 8.1 \log_{10} CFU/100 cm^2 for APC, 4.2 \log_{10} CFU/100 cm^2 for coliforms, and 4.5 \log_{10} CFU/100 cm^2 for *E. coli*. Least-squares means for APCs, coliform, and *E. coli* counts and log reductions on brisket areas of hides before and after treatment are reported in Table 4. For APCs, 1% CPC produced the greatest reduction on the hide with 3.9 \log_{10} CFU/100 cm^2 reported. For coliforms and *E. coli*, there were no ($P > 0.05$) differences among treatments for hide reductions. Though few differences existed between antimicrobial treatments, all three resulted in approximately a 3 \log_{10} CFU/100 cm^2 reduction when applied to shaved hide surfaces in the brisket region of the carcass.

Table 4. Least-squares means for APCs, coliform, and *E. coli* counts and log reductions on brisket area of shaved hides before and after treatment with 1% CPC, 2% L-lactic acid, or hydrogen peroxide

Indicator organism	Treatment	log ₁₀ CFU/100 cm ²		
		Before	After	Reduction ^a
<i>APC</i>	1% CPC	8.2a	4.4c	3.8a
	2% L-lactic acid	7.5b	5.2b	2.3b
	Hydrogen peroxide	8.7a	6.5a	2.2b
	SEM	0.22	0.21	0.28
<i>Coliform</i>	1% CPC	4.6b	1.3b	3.3a
	2% L-lactic acid	3.7c	1.1c	2.6a
	Hydrogen peroxide	5.2a	2.6a	2.6a
	SEM	0.20	0.27	0.29
<i>E. coli</i>	1% CPC	4.3b	1.3a	3.0a
	2% L-lactic acid	3.2c	1.1b	2.1a
	Hydrogen peroxide	5.1a	2.1a	3.0a
	SEM	0.24	0.29	0.33

LS means lacking common letters (a-c) differ ($P < 0.05$).

^aLog reduction = (log₁₀CFU/100 cm² reduction on untreated hide area) – (log₁₀CFU/100 cm² reduction on treated hide area).

Conclusions

By shaving and applying an antimicrobial agent directly to the hide opening area in the brisket region, we were able to reduce bacterial counts on hide surfaces. This method targets a specific area on the hide that is very susceptible to fecal contamination, yet very critical when opening up the hide for removal. Selective application of these antimicrobials to shaved hide opening sites can reduce bacterial counts on hide surfaces, and therefore potentially reduce final carcass counts in these areas by decreasing the bacterial load before opening. Further research should be conducted to determine effectiveness along additional areas of the hide surface, and to evaluate the practicality of this process outside of a research setting.

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EFFICIENCY OF SEVERAL DECONTAMINATION HURDLES ON HYGIENIC QUALITY OF BEEF CARCASSES

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Key Words: beef, decontamination, hurdles, steam vacuuming, lactic acid, dedagger, meat quality

Introduction

Cattle arriving for slaughter can be very dirty, depending on the season and stabling conditions both on the farm and at the meat plant. Traditionally cattle are not cleaned before dehidng. This may cause a risk of pathogenic microorganisms spreading to the carcass during slaughter unless careful slaughter processes are applied. Another source of contamination of the carcass with pathogenic bacteria is the faecal contamination that can occur in connection with evisceration.

In recent years, a number of methods designed to improve hygiene on the slaughter line have been tested at Danish beef plants. Tests were performed to determine whether reduced scoring of the hide on the forelegs, neck and brisket and a new hide puller that pulls off the hide of the forepart of the carcass without scoring were capable of reducing the spread of *E. coli* to the brisket (Nersting & Jensen, 2002). It was concluded that reduced scoring and the new hide puller in combination significantly reduced the spread of *E. coli* to the brisket.

In the USA, steam vacuum is frequently used at several points along the beef slaughter lines, but the method needs national approval in EU member states if it is to be used to remove contamination before the veterinary inspection. In a Danish study on beef, steam vacuum was found more efficient than knife trimming for removal of dirt and bacteria from the surface (Dalsgaard et al. 2003). Used at strategic points along the slaughter line, steam vacuum is an efficient tool for removal of contaminations on carcasses on beef slaughter lines.

Dirty hides imply that the dehidng process must be carefully conducted by the operators. Removal of dust, dirt and dags after sticking and prior to dehidng can improve the hygienic quality of the carcass. In a previous study a 'De-dagger' was tested for its effect on the microbiological count (Rasmussen et al., 2004). The Australian 'De-dagger' removes visible dirt by mechanical work, vacuum and with or without water, without damaging the hide. It was concluded that cleaning dirty cattle with the 'De-dagger' significantly reduced the *E. coli* count on the carcasses.

DMRI has investigated the use of different hurdles such as lactic acid and hot water on the slaughter line for decontamination of beef carcasses (Tørngren, 2005a). Seven different combinations of acid, acid concentrations, water, temperature, method of application and time of treatments were tested. The most efficient method appeared to be spraying/flushing with 2% lactic acid at a temperature of 55°C for 10 seconds at a pressure of 1.5 bars through the nozzle, corresponding to a flow of 10 l/min.

Until now, the different hurdle technologies have only been studied one by one. In this Nordic project, the combined effect of several initiatives was studied.

Objectives

To assess the extent to which the microbiological quality of beef carcasses is improved by using several hygiene-improving interventions on the slaughter line:

- GMP (good working routines for slaughter)
- Optimised dehidng
- Cleaning dirty cattle on the slaughter line after sticking using a De-dagger.
- Steam vacuuming in selected areas with a risk of primary contamination
- Decontamination with lactic acid

Methodology

The experiments took place over a period of four slaughter days under normal production conditions in a representative Nordic meat plant with a slaughter line speed of 40-50 heads/hour. The design shown in fig. 1 included the following sampling of carcasses:

- 80 carcasses (20 carcasses/day), normal slaughter procedures (reference samples).
- 80 carcasses (20 carcasses/day), using GMP, De-dagger, Steam Vacuum at four locations (test samples)
- 80 carcasses (20 carcasses/day), using GMP, De-dagger, Steam Vacuum at four locations + lactic acid spray (brisket) (lactic acid samples)

All operators on the slaughter line were instructed to pay particular attention to GMP. This included specific focus on the procedures that could lead to contamination of the carcasses.

After shackling and sticking, dirt was removed from the belly with a De-dagger. The 'De-dagger' was developed by MLA, Australia and is now marketed in the EU by SFK Meat Systems, Denmark. The operator followed the slaughter line speed, but it is estimated that a trained operator can follow EU slaughter line speeds.

Before loosening the udder on lactating cows, a plastic apron was attached to the belly with bulldog clamps to allow the milk to flow on the apron instead of on the belly.

The Danish Meat Research Institute (DMRI) has developed a light (approx 300 g) Steam Vacuum handle (now marketed by SFK Meat Systems, Denmark) with nozzles that ensure an even temperature distribution all over the head of the handle. It is flexible for the operator to use in e.g. leg areas on the carcass. The admission of steam is controlled through the handle of the equipment to ensure that the operator does not burn himself. The plant was already applying steam vacuum on the outside round. Four additional steam vacuum handles were installed and used after scoring the hind legs, after scoring the belly, on the back after hide pulling and around the bung area.

A limited area (25 x 30 cm) of *M. cutanies trunci* (brisket) was sprayed with 0.8 l 55°C 2% lactic acid for a period of 10 seconds using four TEEJET TG SS 2.8 W nozzles from Spraying Systems CO with a pressure of 1.5 bars. The present use of lactic acid treatment requires dispensation in the EU, which is why the treated area was removed after microbiological testing.

For microbiological testing, samples were taken prior to chilling by swabbing each carcass in three locations measuring 600 cm² and in one location measuring 500 cm² with sterile gauze swabs. The locations were brisket (A), back (B), round (C) and pelvic region (D, 500 cm²) (see figure 2). For the lactic acid treatment, only the brisket was swabbed. The sterile gauze swabs were moistened with 0.85% NaCl

buffered peptone water before sampling. Each swab was suspended in 25 ml of 0.85% NaCl buffered peptone water, stomached for 1 min. and then analysed for *E.coli* and APC. *E. coli* was obtained on Petri-film™ EC, incubated at 37°C for 48 hours. APC was obtained in Plate Count Agar, incubated at 20°C for 5 days (NMKL nr. 86, 3rd edition, 1999).

All counts were transformed to log values. These calculations and calculations of the standard deviations were made in Microsoft Excel, 2000. The statistical analysis was made with Proc. GLM or, where some counts were below the detection limit, with Proc. Lifereg (SAS Institute).

In connection with this experiment, the eating quality of 20 of the lactic acid treated samples were compared to 20 non-treated adjacent samples after vacuum-aging the brisket for 10 days at 2°C (Tørngren, 2005b). After opening the vacuum bags, four quality specialists evaluated the visual appearance, colour and raw meat odour on a fourpoint scale. In addition, a sensory profile was made of samples, which were oven cooked in roasting bags at 160°C for 60-90 min to reach a core temperature of 75°C.

A trained panel evaluated the cooked meat flavour and odour.

Results & Discussion

There was a significant difference between the experiment and the reference for the aerobic count for the sample location Brisket (0,4 log units), while the difference between the experiment and the reference for the other sample locations was small and unsystematic (Figure 3).

The study also shows that decontamination with lactic acid is an efficient means of reducing the aerobic count as lactic acid resulted in a significant reduction of 3.5 log units compared to the reference before chilling (Figure 3).

Figure 4 shows the percentage of samples that were positive for *E. coli* before chilling. Only a few samples were positive - and those samples had very low counts - making it impossible to estimate average counts. However, there is a tendency towards a reduction in *E. coli* after lactic acid treatment.

The reference levels for aerobic count (1,0 – 2,6 log units before chilling) as well as for *E. coli* (14-38 % of samples positive for *E. coli* before chilling) were much lower in this project than in previous experiments including optimised dehiding (Nersting & Jensen, 2002), steam vacuuming (Dalsgaard et al., 2003) and use of De-dagger (Rasmussen et al., 2004), which indicates a considerable improvement in slaughter hygiene. This is considered to be the main reason why there were no significant differences between the reference and the test samples in this experiment. The slaughter of the reference group was performed under optimally hygienic conditions – and in order to further reduce the plate count level, decontamination is needed (e.g. lactic acid treatment).

Prior to this project, several hygiene-improving initiatives had already been implemented on the slaughter line at the host meat plant: limited scoring + optimal hygienic dehiding as well as steam vacuuming of all outside rounds just before the veterinary inspection. The results of the project indicate that the initiatives that have already been implemented are sufficient to obtain good slaughter hygiene.

To verify these findings, references were taken from three other Nordic beef plants, using identical sampling techniques. The results showed that the hygiene level

at two of these meat plants was just as good. On the other hand, the hygiene level at one of the meat plants, was slightly inferior.

Bacon *et al.* (2000) investigated microbiological quality of the hides and cattle carcasses at different stages in meat plants where 'multiple-hurdle-technology' was used for decontamination. Gill & Landers (2003) studied the microbiological effects of decontamination at four cattle meat plants. McEvoy *et al.* (2004) has written an article on microbial contamination of beef in relation to hygiene assessment based on the criteria stipulated in EU regulation 2001/471/EC. The methodologies, sample numbers and locations in these experiments are not fully comparable with the present study. Furthermore, the slaughter line speed was much higher in North America than in Ireland and the Nordic countries. However, the levels of APC and *E. coli* in the present study indicated good slaughter hygiene that could well match the results reported above, even without applying costly hurdle technologies.

Hence, good slaughter hygiene levels at European slaughter line speeds can be obtained through good operator training, careful instruction and motivated operators. The hurdle technologies can be justified under conditions in which operations get out of control, the animals are extremely contaminated and dirty, and acceptable microbiological results cannot be achieved by management alone. However, application of hurdle technologies must be customized individually according to the specific situation at the meat plant concerned.

At present, the use of the De-dagger is an expensive process that requires an extra operator. DMRI is looking into the possibilities of automating the De-dagger.

The use of steam vacuuming is a more effective method of removing faecal matter and impurities than trimming with a knife. Thus, it is recommended to install steam vacuum systems for removal of faecal matter and impurities.

In the EU, the use of lactic acid - or other organic acids - for decontamination is not legal presently. Regulatory changes must be made before these methods can be applied. Furthermore, when considering whether the introduction of treatment with organic acids is cost effective, it must be taken into account that the treatment involves investing in spray cabins.

Quality effects of lactic acid treatment

The results showed that the 2% lactic acid treatment did not affect the quality of the raw meat odour. The appearance of the meat was slightly inferior but still at an acceptable level. Lactic acid slightly reduced the cooked odour for the greasy and acidic odour parameters, and meat odour was somewhat reduced. The lactic acid treatment reduced the flavour intensity for the parameters meat, greasy and acidic flavour. However, the quality effects found in the study were considered to be of only marginal commercial importance.

Conclusions

There was only a limited reduction in the microbiological count for samples taken after the use of hygiene-improving interventions compared to the samples taken after the normal routine in the meat plant. The meat plant where the study was conducted already had a high level of slaughter hygiene. For that reason, the effect of the hygiene improving initiatives did not appear clearly.

Good slaughter hygiene can be obtained through care and consideration - also without the use of decontamination tools.

The lactic acid treatment with 55°C 2% lactic acid significantly reduced the count. The quality effects of the lactic acid treatment found in the study were considered to be of only marginal commercial importance. However, it must be questioned whether the introduction of the treatment with organic acids is cost effective when the microbiological load of the carcasses is as low as in this study.

Acknowledgements

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Tables and Figures

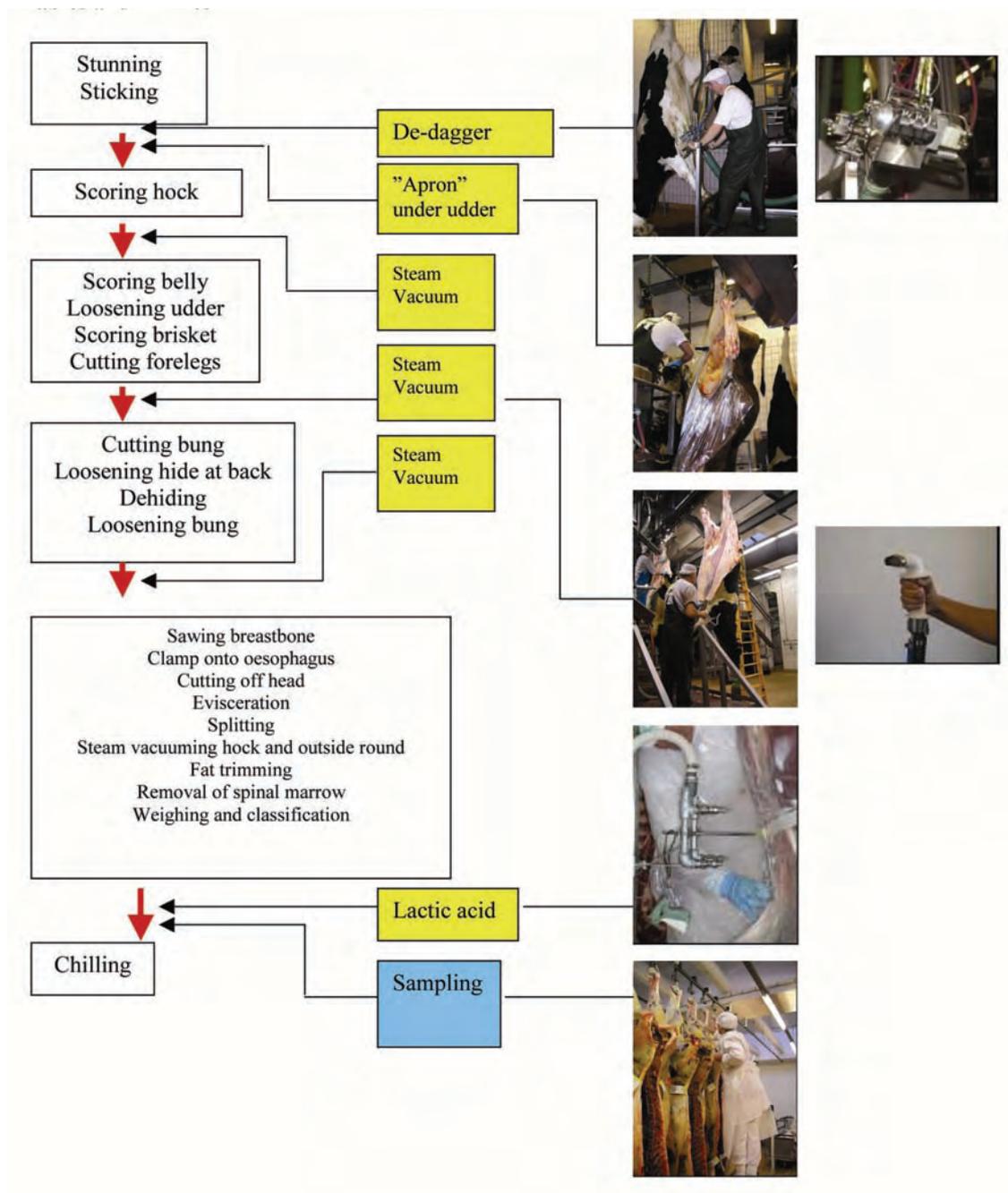


Figure 1: Schematic outline of the experimental design

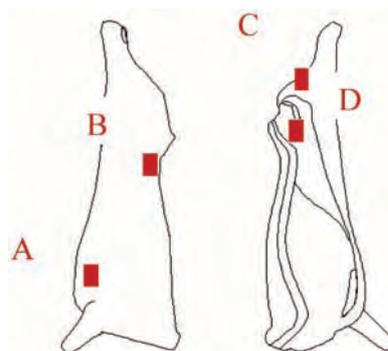


Figure 2: Microbiological sampling locations.

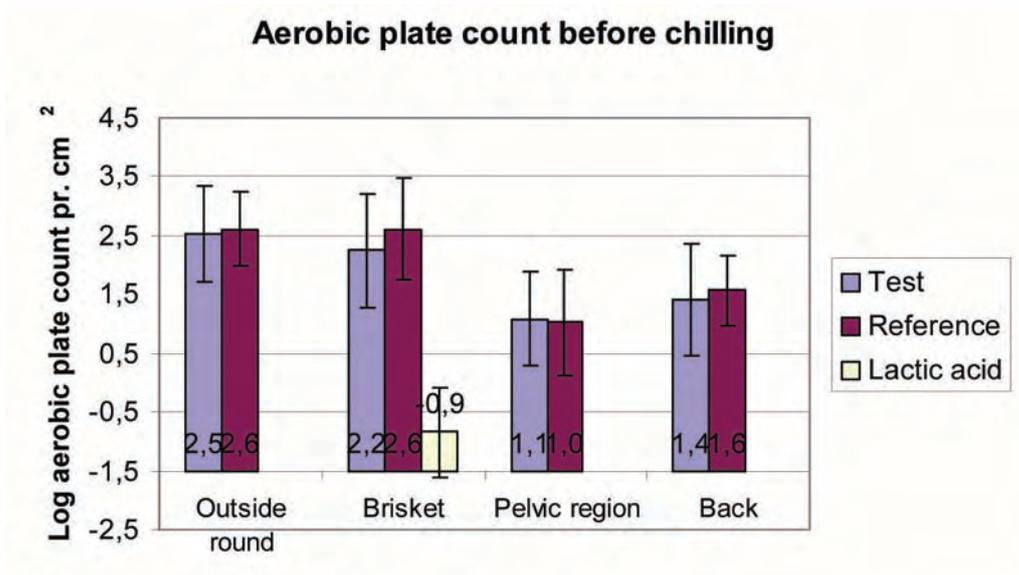


Figure 3: Aerobic plate count before chilling.
 The average level of aerobic count (per cm²). There is a significant difference between the experiment and the reference in the sample Brisket ($p=0.0062$) but not on the other three sample locations. There is a significant difference between the samples treated with lactic acid and the reference in the sample Brisket ($n=80$ per bar).

**% of E. coli positive samples
before chilling**

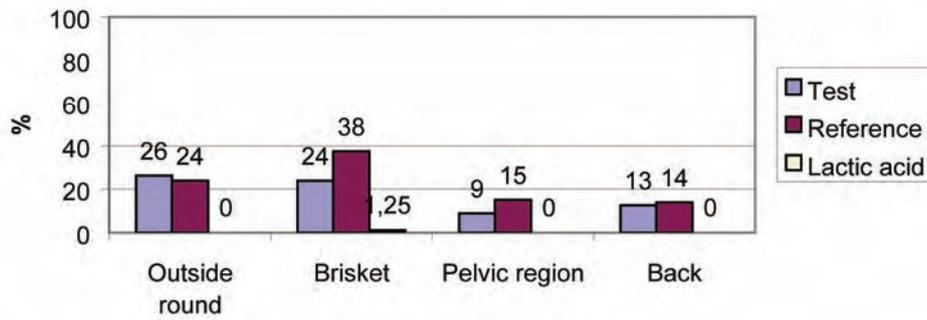


Figure 4: E. coli before chilling.

% samples positive for E. coli before chilling.

There is no significant difference between the experiment and the reference on any of the 4 sample locations. (n=80 per bar).

MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 HIDE CONTAMINATION ROUTES – FEEDLOT TO HARVEST FLOOR

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Introduction

Harvest decontamination interventions (usually applied in multiple-hurdle systems) are more efficacious if the proportion of incoming cattle with *E. coli* O157:H7 on their hides is reduced. Cattle can be a reservoir for *E. coli* O157:H7 (Chapman et al., 1993) and these organisms can be transferred from hide to meat during slaughtering/dressing (Sofos et al., 1999) such that hides of animals being harvested can be a significant source of contamination of resulting carcasses (Riddell and Kerkeala, 1993; Bell, 1997; Sofos et al., 1999; Byrne et al., 2000; McEvoy et al., 2000). According to Ransom et al. (2003), carriage of *E. coli* O157:H7 on pre-visceration carcasses was 7.1% if pre-harvest fecal prevalence was less than 20%, versus 12.5% if pre-harvest fecal prevalence was greater than 20%. Cattle hides may become contaminated with *E. coli* O157:H7 from feedlot pen floors (Dewell et al., 2003), contact between animals after departure from the farm (Tutenel et al., 2003), floors of lairage pens, or the stunning-box floor (Avery et al., 2002; Small et al., 2002, 2003).

Several studies have examined causality of carcass contamination at steps in the harvest process (Grau, 1987; Gill et al., 1996a, b; Bell, 1997; Gill and McGinnis, 1999), but such studies do not directly link sources with the introduction of specific organisms in terms of contamination events (Barkocy-Gallagher et al., 2001). The overall objective of this study was to identify genetic relationships of *E. coli* O157:H7 contamination found on cattle hides at the time of harvest, to *E. coli* O157:H7 found at associated pre-harvest sites, such as the feedlot, loading chutes, transport trailers, packing-plant holding pens, and feces obtained at various locations.

Materials and Methods

Samples were collected from Midwestern region commercial feedlots (N = 3), transporting cattle to packing facilities (N = 2), and Southwestern region feedlots (N = 3), transporting cattle to a Southwestern region packing facility. For each lot of cattle (defined as 1 feedlot shipment; N = 8), sets of ten sample types were collected: (1) feedlot pen floor composite, (2) feedlot pen water composite, (3) feed bunk and feed composites, (4) loading chute composite, (5) transport truck trailer wall and floor composite, (6) holding pen composite at beef plant, (7) plant pen water composite, (8) restrainer samples, (9) hide samples, and (10) corresponding colons. Sample sets were collected until at least 25 *E. coli* O157:H7 positive hides were confirmed as positive

(using biochemical means) for each of the Midwestern (N = 25) and Southwestern regions (N = 65).

Microbiological Analysis

Detection of *E. coli* O157:H7 in all samples was conducted according to the procedure in Barkocy-Gallagher et al. (2002).

Isolate confirmation was as follows; screening with the latex agglutination assay of the Oxoid *E. coli* O157:H7 Test Kit (Oxoid; Ogdensburg, NY), initial biochemical analysis of presumptive isolates (cellulose, triple sugar iron (TSI) slants, and motility), screening with O and H antigen agglutination test (RIM *E. coli* O157:H7 Latex test, Remel, Lenexa, KS), and the primary *E. coli* O157:H7 isolate from each sample also was subjected to VITEK analysis. Once confirmation testing was complete, isolates were stored in a 20% glycerol-TSB solution and frozen, pending molecular characterization testing.

Molecular Characterization of Recovered E. coli O157:H7 Isolates

Multiplex Polymerase Chain Reaction (PCR) of known *Escherichia coli* O157 gene targets (Gannon et al., 1997; Wang et al., 2002) and Pulsed-Field Gel Electrophoresis (PFGE) of restricted genomic DNA were used to identify recovered isolates.

Multiplex PCR

Each isolate was subjected to each of three multiplex PCR procedures, using different primers for amplification as follows: 1) for Shiga toxin genes 1 and 2 (*stx*₁ and *stx*₂) per Wang et al. (2002); 2) For enterohemorrhagic *E. coli* (EHEC) enterohemolysin (*hlyA*), *E. coli* somatic antigen O157 (*rfbE*_{O157}), and *E. coli* structural flagella antigen H7 (*fliC*_{H7}) per Wang et al. (2002); and 3) for intimin specific for *E. coli* O157:H7 (*eaeA*_{O157}) per Gannon et al. (1997). All reactions included a primer set for the 16S rRNA gene for *E. coli* to control to serve as an internal control per Wang et al. (2002).

Following cycler programs (iCycler, Bio-Rad, Hercules, CA), 2µl of sample and 1µl 10xbluejuice (Invitrogen, Carlsbad, CA) was added to a 100 ml 1xTBE buffer:1g agarose (Certified PCR Agarose, Bio-Rad, Hercules, CA) gel well, submerged in 80 ml 1xTBE buffer. A 20 well comb was used to cast each PCR gel (15 samples, 3 lambda ladder wells, one positive control, and one negative control). After electrophoresis (1 hr at 80v), gels were removed from buffer stained for 30 min with ethidium bromide, and de-stained for 20 min. A Gel Doc EQ (Bio-Rad, Hercules, CA) was used for ultra-violet photo imaging, exported gel images were analyzed using the QuantityOne (Bio-Rad, Hercules, CA) software program.

Pulsed-Field Gel Electrophoresis

Isolate cells at a 3.0 McFraland (Remel Colormeter Standards kit, Lenexa, KS) turbidity were suspended in Cell Suspension Buffer (CSB; 1.0 M NaCl, 10 ml 1.0 M Tris [Sigma, Desienhofen, Germany], pH 7.6) then added in a 1:1 ratio to 1.6% Low Melt Agarose (Bio-Rad, Hercules, CA) and CSB solution and set into individual plugs (10mm x 5mm x 1.5mm). Cells in plugs were then digested using 0.5 M EDTA (Bio-Rad, Hercules, CA), 1% Sodium Lauroyl Sarcosine (Sigma, Desienhofen, Germany),

and 0.5mg Proteinase K (Sigma, Desienhofen, Germany). After digestion, buffer was removed and plugs were washed/incubated (37°C for 30 min) 5 times with 5 ml TE buffer (10 mM Tris [Bio-Rad, Hercules, CA], 0.1 mM EDTA [Bio-Rad, Hercules, CA]).

Digested plugs, 400µl sterile H₂O, and 40µl 10X reaction buffer (Roche, Indianapolis, IN) were incubated, (4°C) overnight. Reaction buffer solution was removed/replaced, and incubation was allowed to continue, (24 ± 2 °C) for 4 h. After incubation, 6µl of *Xba*I (Roche Molecular Biochemicals, Indianapolis, IN) restriction enzyme was added to each centrifuge tube and incubated in a dry incubator, (37°C) overnight, then stopped by removing enzyme solution and adding enough TE buffer to cover the restricted plug followed by incubation, (37°C) for 1 hr.

Restricted plugs were inserted into solidified 1% Pulsed Field Certified Agarose (Bio-Rad, Hercules, CA) and 0.5X TBE buffer (Tris-Borate EDTA Buffer [Sigma, St. Louis, MO]) gel wells, then placed into 2L of 0.5X TBE buffer at 14°C in the electrophoresis chamber of the CHEF Mapper PFGE System (Bio-Rad, Hercules, CA) with the following electrophoresis parameters: 1) gradient 6.6 V/cm; 2) 120° Angle; 3) initial switch time of 2.16 sec; 4) final switch time of 54.17 sec; 5) linear ramp factor; and, 6) run time of 20 hours. Following ethidium bromide stain/de-staining, gels were placed in Gel Doc EQ (Bio-Rad, Hercules, CA) for ultra-violet photo imaging. Exported gel images were analyzed by the FingerPrinting II software program (Bio-Rad, Hercules, CA).

Results and Discussion

For all sample locations, with the exception of restrainer side walls and feed samples, *E. coli* O157:H7 was detected.

Multiplex-PCR

Using three Multiplex-PCR protocols (Gannon et al., 1996; Wang et al., 2002), all isolates were examined for known *E. coli* O157 gene targets identifying isolates that possess the capability to produce Shiga Toxins 1 and 2 (*Stx*1) and (*Stx*2), enterohemorrhagic *E. coli* hemolysin (EHEC *hlyA*), the gene which encodes for the *E. coli* O157 serotype (*rfbE*_{O157}) (Wang et al., 2002), the *E. coli* flagellum H7 serotype (*fliC*_{H7}), and the *eaeA*_{O157} gene which is responsible for the production of intimin and is involved in attaching and effacing adherence (Gannon et al., 1996; Wang et al., 2002).

At least one isolate recovered from the feedlot pen floors, feedbunks, loading chutes, transport trailers, processing plant holding pen railings and water tanks, possessed at least one of the *Stx*1, *Stx*2, *hlyA*, *rfbE*_{O157}, *fliC*_{H7}, and *eaeA*_{O157} genes (Table 1). Feedlot water and processing facility holding pen floor isolates possessed all genes screened for, with the exception of the gene for *Stx*2 production. Interestingly, over 50.0% of loading chute, transport trailer walls, holding pen railing and holding pen water derived isolates had all six genes present (Table 1).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was used to further evaluate the genetic relatedness of *E. coli* O157:H7 isolates recovered from hides, as well as from associated colon and companion samples. Isolate banding patterns were first separated

into factions of isolates related to the three processing facilities, and further separated into clusters of isolates that produced the same virulence gene profiles. Once environmentally linked isolates with identical gene marker profiles were grouped, individual band patterns were compared to the other patterns in that cluster. Since isolates were collected within a one year interval, and were restricted using a single restriction endonuclease, the method as described by Tenover et al. (1995) was used to analyze the isolate clusters. Tenover et al. (1995) suggests the following relationship classification when the band patterns of two isolates are compared: 0-1 band differences are considered genetically indistinguishable, 2-3 band differences are considered probably or closely related, 4-6 band differences are considered possibly related, and compared patterns with ≥ 7 band differences should be considered genetically different from one another. Indistinguishable relationships (0-1 differences) were expressed between two isolates derived from colons of animals from the same feedlot pen, and between two isolates of feedlot and plant holding pen origin (Table 2). Probable genetic relationships (2-3 differences) were observed between isolates derived from: hide and colon samples (cross-contamination); colons and feedlot locations; colons and transport trailers; and feedlot and plant receiving locations (Table 2). Possible genetic relationships (4-6 differences) were observed between: hide-derived isolates and feedlot, transport trailer and plant holding facility locations, as well as from other hides and colons (cross-contamination); colon-derived isolates and feedlot and transport trailer locations, as well as other hides and colons; different pens at the same feedlot; feedlot and plant holding pen locations; and plant holding and transport trailer locations (Table 2). As shown (Table 2), relationships were expressed between isolates cultured from samples that, upon initial analysis, did not have an epidemiological association (i.e. a relationship between isolates cultured from feedlot pen samples and transport trailer samples). However, matching bands do not always contain homologous genetic material (Davis et al., 2003), given that it is possible for genetically different restriction fragments to travel similar distances. There were also proposed relationships (2, 4, and 5 band differences, respectively) between 3 pairs of feedlot-derived isolates when compared to plant holding pen isolates. Although this improbable relationship could also be explained by non-homologous fragments at the same molecular weight distance, a number of these cattle feeding facilities used packer-owned transport trailers to haul cattle to the packing facility. These trucks haul cattle from similar feeders continually during the year, and there is potential for a dominant feedlot *E. coli* O157:H7 strain to establish itself in a plant holding pen environment if a sanitary atmosphere is not maintained.

Documented trailer condition ranged from new to excessively dirty; therefore, four categories of trailer condition were created. "Very Clean" (new, to adequately washed and dry, with no visible organic material present); "Moderately Clean" (poorly washed with little or no dry organic matter on floors and walls); "Moderately Unclean" (1-2 previous loads post-wash, excessive dry, or moderate wet organic matter present on floors and walls); and "Very Unclean" (3+ loads post-wash, with excessive amounts of wet organic matter present on trailer walls/floors). A total of 15 isolates were recovered from 7 (38.89%) of the 18 trailers. These 7 *E. coli* O157:H7 positive trailer samples were collected from 1 (14.29%) "Very Clean" trailer, 4 (57.14%) "Moderately Clean" trailers, 1 (14.29%) "Moderately Unclean" trailer, and 1 (14.29%) "Very Unclean" trailer, respectively. In that, 71.43% of the trailers from which *E. coli* O157:H7 isolates were cultured were considered "Very Clean" or "Moderately Clean"; this data should lead to the further investigation of current trailer cleaning and sanitation practices. A plausible explanation for the large number of

clean trailers that tested positive for pathogenic contamination could be the lack of competitive organisms found at the clean trailer sample site or possible sample contamination.

Conclusions

Escherichia coli O157:H7 isolates expressing the genes for virulence factors such as Shiga toxin production (*Stx1*, *Stx2*), known to cause the symptoms of Hemolytic Uremic Syndrome, the Intimin gene involved in adhering and effacement of the organism (*eaeA*_{O157}), and the virulent toxin enterohemolysin (*hlyA*_{O157}) were recovered from feedlot pen, loading chute, transport trailer, and packing plant holding facility sample types, as well as from cattle hides and colons at the time of slaughter. In this research, genetic relationships (Tenover et al., 1995) were expressed between hide derived *E. coli* O157:H7 isolates and isolates recovered from feedlot pen floors, feedlots pen water tanks, feedlot feedbunks, loading chutes, transport trailers, packing plant holding pens, holding pen water tanks, and from the hides and colons of other cattle.

In that, *E. coli* O157:H7 isolates with pathogenic capabilities were recovered from locations which also resulted in the transfer of similar organisms onto the hides of slaughter ready cattle, it is imperative that the feedlot and loading chute, transportation trailers and packing plant holding areas are all considered likely reservoirs of pathogenic *E. coli* O157:H7 organisms, which can result in hide contamination that may easily lead to contamination of beef carcasses subsequent and beef products.

Additionally, current methods used to wash cattle trailers after shipment may not be effective, as the largest numbers of isolates were recovered from trailers characterized as Very Clean and Moderately Clean. Existing cleaning protocols may need to be followed by a sanitation method to effectively remove pathogenic contamination from the walls and floors of trailers.

Table 1. Distribution of *E. coli* O157:H7 virulence genes (*eaeA*, *Stx1/2*, *hlyA*, *rfbE*, *fliC*) among each sample type.

Feedlot	# of Isolates	Virulence Genes						All
		<i>eaeA</i> _{O157}	<i>Stx1</i>	<i>Stx2</i>	<i>hlyA</i>	<i>rfbE</i>	<i>fliC</i>	
Water	3	66.7 (2/3)	66.7 (2/3)	0.0(0/3)	100.0(3/3)	100.0(3/3)	100.0 (3/3)	0
Pen floor	45	93.5(43/46)	65.2(30/46)	52.2(24/46)	95.7(44/46)	93.3(42/46)	93.5(43/46)	9
Feed	nd							0
Feedbunk	2	50.0(1/2)	50.0(1/2)	50.0(1/2)	50.0(1/2)	50.0(1/2)	50.0(1/2)	0
Loading chute	14	92.9(13/14)	50.0(7/14)	64.3(9/14)	92.9(13/14)	92.9(13/14)	92.9(13/14)	6
Transport								
Trailer walls	14	92.9(13/14)	57.1(8/14)	85.7(12/14)	92.9(13/14)	92.9(13/14)	92.9(13/14)	7
Trailer floor	5	60.0(3/5)	40.0(2/5)	60.0(3/5)	40.0(2/5)	60.0(3/5)	40.0(2/5)	1
Processing facility								
Pen floor	3	33.3(1/3)	33.3(1/3)	0.0(0/3)	33.3(1/3)	100.0(3/3)	33.3(1/3)	0
Pen side rails	8	87.5(7/8)	75.0(6/8)	75.0(6/8)	75.0(6/8)	100.0(8/8)	75.0(6/8)	6
Water	5	100.0(5/5)	80.0(4/5)	80.0(4/5)	100.0(5/5)	100.0(5/5)	100.0(5/5)	4
Restrainer	nd							0
Carcasses								
Hide	176	58.4(101/173)	34.1(59/173)	38.7(67/173)	50.9(88/173)	78.6(136/173)	53.2(92/173)	24
Colon	48	93.8(45/48)	47.9(23/48)	35.4(17/48)	83.3(40/48)	87.5(42/48)	83.3(40/48)	3
Totals	322							60

Table 2. Comparison of relationships between restriction band pattern differences of isolates recovered from different samples from the same collection lot, expressing similar genotypes (Multiplex-PCR). Relationships were categorized into four categories; Indistinguishable (0-1 differences), Probably Related (2-3 differences), Possibly Related (4-6 differences) and Different (Tenover et al., 1995).

Sample Type	Epidemiological Relationship (N of Relationships)		
	Indistinguishable	Probably Related	Possibly Related
Hide/Hide		X (3)	X (7)
Hide/Colon			X (1)
Hide/Feedlot pen floor		X (2)	X (3)
Hide/Feedlot pen water			X (1)
Hide/Trailer			X (2)
Hide/Plant pen water			X (1)
Colon/Colon	X (2)		X (2)
Colon/Feedlot pen floor		X (2)	X (5)
Colon/Trailer		X (2)	X (1)
Loading chute/Plant pen walls		X (1)	X (1)
Feedlot pen floor/Plant pen walls			X (1)
Feedlot pen floor/Plant pen floor	X (1)	X (2)	
Loading chute/Trailer			X (1)
Plant pen floor/Trailer			X (1)
Feedlot pen/Feedlot pen (same feedlot, different pens)			X (2)

ENUMERATION OF *ESCHERICHIA COLI* O157 IN CATTLE FECES USING IMMUNOMAGNETIC SEPARATION COMBINED WITH MOST PROBABLE NUMBER TECHNIQUES

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Key Words: *E. coli* O157, Enumeration, Feces, MPN and IMS.

Introduction

Escherichia coli O157 is a food borne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome in humans (1). In 1982 *E. coli* O157 was first identified as a human pathogen after the investigation of two outbreaks involving undercooked ground beef (8). Since these outbreaks, investigation of pre and post-harvest interventions has been conducted to ensure food safety of the beef supply. Direct or indirect contamination with ruminant feces is the leading predecessor to infections caused by *E. coli* O157 (6, 7). Most preventive measures involving the reduction of *E. coli* O157 in beef products have been at the post-harvest level. As new interventions have been investigated we have observed an increase in pre-harvest prevention of *E. coli* O157. Due to the direct correlation between positive animal samples (fecal and hide) and carcass contamination (4) pre-harvest intervention plays an important role in decreasing the pathogen load (1).

To date, the detection of *E. coli* O157 in feces of cattle has mainly been qualitative. Quantitative analysis of bovine feces naturally infected with *E. coli* O157 would be a valuable asset to determine the impact of interventions in the pre-harvest environment and to determine the risk of transferring the pathogen to beef products. We have taken into account previously reported methods (3, 5) and have developed a new methodology that is more specific and sensitive to *E. coli* O157 enumeration in bovine feces.

Objectives

The objective of this study was to develop an enumeration protocol that is sensitive and accurate in determining the amount of *E. coli* O157 present in bovine feces.

Methodology

Experimentation

Five separate studies were conducted as follows to determine the accuracy and specificity at both low-level and high-level inoculations in feces:

Study 1: Manure was sterilized and inoculated with a 10^2 *E. coli* O157:H7/g and populations were determined by direct plating of the cocktail (prior to inoculation of the feces) and the newly developed MPN/IMS methodology.

Study 2: Non-sterile, freshly collected manure was inoculated with a cocktail mixture of 10^3 *E. coli* O157:H7/g and populations were determined by direct plating of the cocktail (prior to inoculation of the feces) and the newly developed MPN/IMS methodology.

Study 3: Non-sterile, freshly collected manure was inoculated with a cocktail mixture of a 10^1 *E. coli* O157:H7/g and populations were determined by direct plating of the cocktail (prior to inoculation of the feces) and the newly developed MPN/IMS methodology.

Study 4: Manure was sterilized and inoculated with a 10^4 streptomycin-resistant *E. coli* O157:H7/g cocktail and samples were directed-plated onto media containing streptomycin and subjected to the newly developed MPN/IMS methodology.

Study 5: Manure was sterilized and inoculated with a 10^2 streptomycin-resistant *E. coli* O157:H7/g cocktail and samples were directed-plated onto media containing streptomycin and subjected to the newly developed MPN/IMS methodology.

Sample collection

Approximately 1,000 g of bovine feces was collected for each of the three replications for all of the studies from the floor of a commercial feedlot pen. Each of the samples were stored in a cooler for transport from the feedlot to the microbiological laboratory (approximately 15 miles).

E. coli O157 Detection

Immunomagnetic separation (IMS) was initially performed on sample used in study 2 and 3 to qualitatively detect the presence of *E. coli* O157. The IMS detection methods used in these studies were adopted from Brashears et al.⁽²⁾ (2003) and slightly modified. Instead of manually washing the beads, an automatic IMS machine was used (Dynal, Lake Success, N.Y.). Another modification was in the biochemical tests that were performed in these studies. Instead of using indole and Voges-Proskauer tests, we used MacConkey broth (MACb), trypticase soy broth (TSB), and triple sugar iron slants (TSI). The above tube media cultures were then incubated at 37°C overnight. Colonies that fermented lactose (caused a purple to yellow media color change) in MACb, created turbidity in TSB, and were A/A (glucose and lactose and/or sucrose fermentation) or K/A (glucose fermentation only, peptone catabolized) plus gas in TSI were tested for O157 antigen with a latex agglutination kit (Remel, Lenexa, Kansas).

Cocktail preparation of E. coli O157:H7

For studies 1, 2 and 3, five different *E. coli* O157:H7 isolates originally isolated from bovine feces were cultivated in TSB broth at 37°C for 24 h. For studies 4 and 5, three different streptomycin-resistant *E. coli* O157:H7 isolates from bovine manure were used (University of Nebraska Department of Food Sciences and Technology). Each culture was sub-cultured at least 2 times before experimental use. Buffered-peptone water was inoculated with a portion of each culture to obtain a cocktail mixture for experimental use. Serial dilutions of the cocktails were performed

to obtain 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} dilutions to be inoculated into the manure for all of the studies.

Sample preparation

Feces were sterilized for studies 1, 4 and 5 by autoclaving at 121°C for 15 minutes. Sterile manure was used to facilitate both direct-plating of the *E. coli* O157 in the manure and MPN/IMS methodology analysis. An appropriate amount of the cocktail of *E. coli* O157:H7 was added to the manure to yield a 10^2 (study 1), 10^4 (study 4), and 10^2 (study 5) populations of *E. coli* O157:H7 in the sterile manure while a 10^3 (study 2) and 10^1 (study 3) target was obtained in the non-sterile manure. The pathogen cocktail was directly plated in the studies using non-sterile manure to verify the populations inoculated into the manure.

MPN/IMS methodology

Serial dilutions of the manure samples were made in 99 ml of BPW for each subsequent sample in all the studies. Based on the initial inoculation levels, 1 ml of the appropriate serial dilution was used to inoculate each of the first 3 MPN tubes, containing 9 ml of GN broth. The adjacent 3 MPN tubes were then inoculated with 1 ml of the next highest dilution and while the following 3 MPN tubes were then inoculated with 1 ml of the next highest dilution. These steps were then repeated for the next two highest dilutions providing a 3 X 5 MPN dilution scheme. The MPN tubes were incubated at 37°C for 6 h.

IMS was performed as previously described on each of the 15 MPN tubes for each of the samples after the incubation. After IMS, 50 µl of the bead-bacteria and PBS Tween mixture was plated onto CT-SMAC (studies 1, 2 and 3) or *E. coli* agar with Methylumbelliferyl-β-glucuronide and streptomycin (EC_{MUG}) (studies 4 and 5). The sorbitol-negative colonies (if present) on the CT-SMAC and any colonies on the EC_{MUG} were tested for O157 antigen with a latex agglutination kit (Remel, Lenexa, Kansas). Once a colony was found to be positive on each plate that MPN tube was considered to be positive.

Direct plating methodology

The pathogen cocktails for all the studies were enumerated by direct plating dilutions onto CT-SMAC (studies 1, 2 and 3) or EC_{MUG} (studies 4 and 5). This information was an important benchmark to determine the original population that was added to the feces. Manure samples were also direct-plated onto EC_{MUG} (studies 4 and 5 only) to determine the populations in the manure itself providing us with a double verification of the populations in the manure.

Data calculations and statistical analysis

All MPN calculations were conducted by using the Bacteriological Analytical Manual Online at: <http://www.cfsan.fda.gov/~ebam/bam-a2.html> (FDA/CFSAN). The MPN/g data was calculated to remove dilution factors and were log base-10 transformed to control statistical variance. All statistical analysis was performed using SAS proc GLM procedures (SAS Institute Inc., Cary, N. C.).

Results & Discussion

In studies 1, 2, and 3 there was no detectible statistical variation between direct plating of the cocktail and the IMS/MPN methodology (Fig. 1). The amount of the pathogen in the cocktail was adjusted to determine the amount of the pathogen actually added to the manure samples. In study one, the amount of *E. coli* O157:H7 inoculated into the feces as determined by direct plating was 8.88 log cfu/g while the MPN analyses determined the population to be 8.54 log MPN/g. Similarly, in studies 2 and 3, the amount of *E. coli* O157 determined by direct plating was 8.88 log cfu/g and 8.88 log cfu/g, while the amount determined by direct plating was 8.25 log MPN/g and 8.59 log MPN/g, respectively.

In studies 4 and 5 there was no detectible statistical variation between direct plating of the inoculated manure and the IMS/MPN methodology (Fig. 2). Direct plating of the manure in study 4 indicated that the population of *E. coli* O157 was 4.44 log cfu/g while MPN analysis indicated that it was 4.37 log MPN/g. In study 5, no differences were observed with direct plating yielding populations of 2.57 log cfu/g and MPN analysis indicating the populations were 2.42 log MPN/g.

Conclusions

The IMS/MPN methodology used in these studies is useful in enumeration of *E. coli* O157 in inoculated bovine fecal samples. Enumeration of *E. coli* O157 in bovine feces has been and will continue to be an important tool to the beef industry. In order to provide a safe and wholesome product to the consumer it is useful to know the quantity of *E. coli* O157 present in feces of cattle at the feedlot. If producers can provide cattle to the slaughter plants with lower fecal shedding and quantity of *E. coli* O157 then preventative measures at the plant will be more effective. *E. coli* O157 enumeration of bovine feces is imperative to the producer in order to investigate and monitor preventative measures at the feedlot.

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Tables and Figures

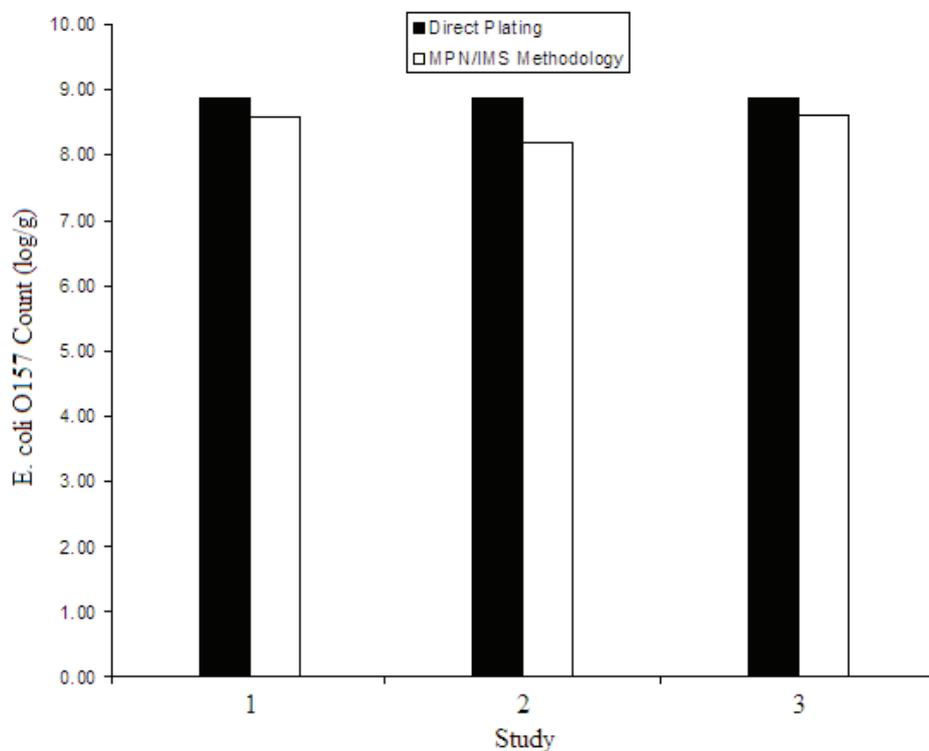


Fig. 1. *E. coli* O157 count in the manure adjusted from direct plating of the cocktail (Log cfu/g) and MPN/IMS methodology (Log MPN/g) for studies 1, 2, and 3. There was no detectable statistical variation between direct plating of the cocktail and the IMS/MPN methodology in each of these studies. Study 1 involved sterilized manure inoculated with a 10^2 *E. coli* O157:H7/g cocktail. Studies 2 and 3 involved non-sterilized manure inoculated with a 10^3 and 10^1 *E. coli* O157:H7/g cocktail, respectively.

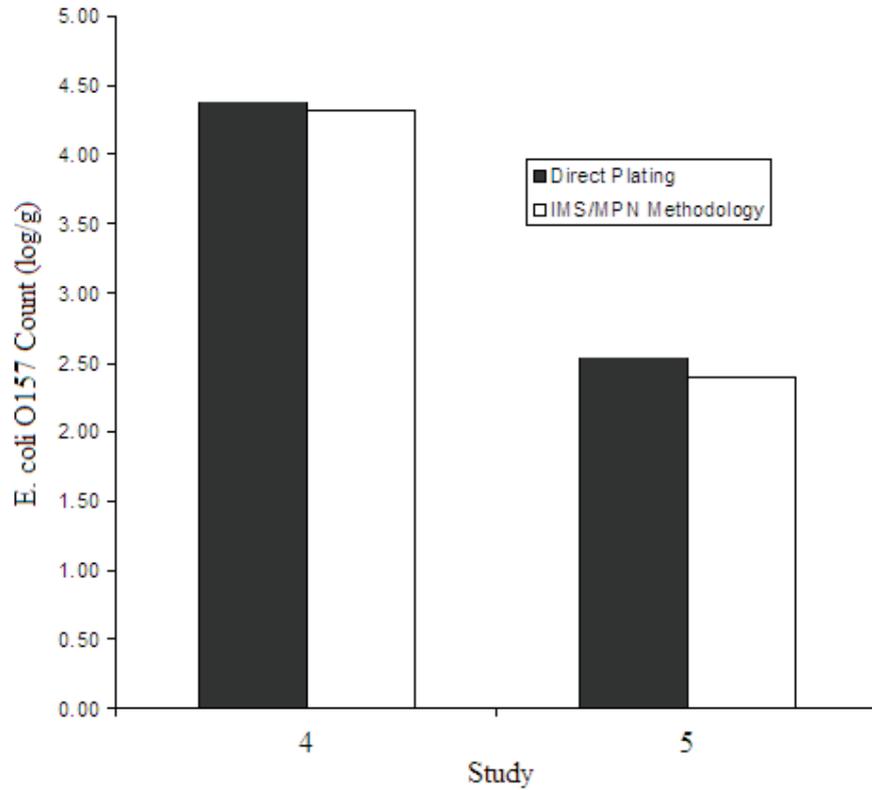


Fig. 2. *E. coli* count from direct plating of the inoculated manure (Log cfu/g) and MPN/IMS methodology (Log MPN/g) for studies 4 and 5. There was no detectable statistical variation between direct plating of the inoculated manure and the IMS/MPN methodology in both of these studies. Studies 4 and 5 involved sterilized manure inoculated with 10^4 and 10^2 streptomycin resistant *E. coli* O157:H7/g cocktail, respectively.

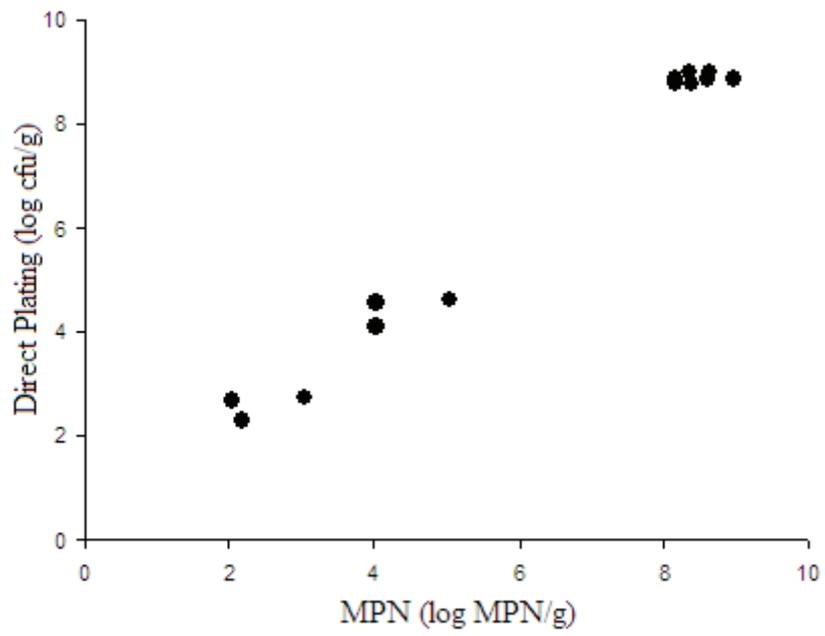


Fig. 3. MPN (log MPN/g) verse direct plating (log cfu/g) for the data points in all of the studies (5 studies x 3 replications per study = 15 data points).

EXPERIMENTAL EVALUATION OF NON-MICROBIOLOGICAL METHODS FOR PROCESS HYGIENE ASSESSMENT IN ABATTOIRS

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Key Words: process hygiene, red meat, chlorophyll, *Enterobacteriaceae*, TVC

Introduction

During slaughter and dressing of red meat animals, faecal contamination can be transferred to carcasses from a number of routes. These include the skin/hide of the animal, the gastrointestinal tract of the animal, and cross-contamination from environmental surfaces including processing equipment. In the UK there is a one in three chance that livestock-derived faecal material contains one of the five zoonotic agents (1) which cause over 80% of bacterial gastro-enteritis in humans.

To aid hygienic meat production in red meat slaughterhouses, the Meat (HACCP) Regulations, were implemented in the UK in 2002. These regulations require that carcasses and a proportion of the surfaces which come into contact with carcasses during dressing, must be sampled on a regular basis. Samples are examined for the total viable count (TVC) and numbers of *Enterobacteriaceae*, and the results give an indication of process hygiene. Analyses using conventional lab-based microbiological methods are currently exclusively used although these are costly. Another disadvantage of traditional microbiology is that the results take several days. If results were available in real time, and more rapid response to processing problems would be possible. The purpose of this study was to therefore evaluate rapid methods for their suitability in determining process hygiene in red meat slaughterhouses.

Several rapid methods are currently in use in the food industry. Commonly, such methods detect the presence of micro-organisms and/or food debris on food contact surfaces by detection of protein or ATP bioluminescence (2). Another rapid method being used extensively in the US in the meat industry is the detection of chlorophyll and its breakdown products on carcasses by measuring fluorescence when using the VerifEYE system (hand-held devices or a cabinet system). This detects emissions by chlorophyll a and its breakdown products at 675nm when excited by 420nm (3). Chlorophyll is broken down by the gut to fluorescent products which are present in the faeces of animals that have been fed a diet containing chlorophyll. Therefore any fluorescence detected on the carcasses indicates faecal, and hence possible bacterial, contamination.

In the UK rapid methods are not used extensively in red meat processing environments and the performance of non-traditional methods for the assessment of bacterial contamination is not known

Objectives

Two studies were carried out in a single low throughput abattoir in the South-West of England to evaluate the effectiveness of non-microbiological methods for the assessment of carcass and surface contamination during the dressing of cattle and pigs inoculated with chlorophyll, against the currently used microbiological methods

Methodology

Sample collection

Three cattle and three pigs were painted on the brisket and belly, respectively with a 50µg/ml solution containing chlorophyll a and b (50% of each), using a paint-brush method. Immediately after bleeding out, but before removal of the hide/entry into the scald tank, cattle and pigs were examined on the rump, flank, brisket and neck and the ham, back, belly and jowl, respectively using a hand-held version of the VerifEYE solo machine (Attec, UK) to detect the presence of chlorophyll. The same four sites on each animal species were sampled for *Enterobacteriaceae* and total viable count (TVC) by excising an area of hide from the cattle (10 x 10cm), and a piece of skin from the pigs (5cm²) and placing into sterile stomacher bags. The animals were dressed using conventional techniques and the carcasses tested on the same sites as above using the VerifEYE solo machine and the excision method

Six environmental surfaces were collected after the completion of dressing of each species, these being: the roll-out ramp, beef pram, flayer, knife, apron wash and the splitting saw, for cattle, and: the dehairing machine (inside and outside), the polishing table, knife, apron wash and splitting saw, for pigs. Surfaces were sampled using: a wet/dry swabbing method (20cm² area) to determine *Enterobacteriaceae* and TVC, two protein detection methods - Flash sticks (Biocontrol, UK), and Pro-TECT swabs (Biotrace Fred Baker, UK), Hygiena snapshot total ATP swabs (Hygiena International Ltd, UK), and the VerifEYE solo machine

After routine cleaning of the abattoir but before the start of slaughter the following day, the same six environmental surfaces for each species were examined, as outlined above

Laboratory analysis

From the excised pieces of hide a 5cm² area was aseptically excised and added to a sterile stomacher bag. To these and the excised pieces of carcass, 25ml of Maximum Recovery Diluent (MRD; Oxoid, UK) was added and the sample stomached for 2 min. The wet/dry swabs were added to 10ml MRD and vortexed for 1 min. All samples were further serially diluted in MRD and plated onto VRBG agar (Oxoid, UK) for enumeration of *Enterobacteriaceae*, and PCA (Oxoid, UK) for enumeration of TVC, using standard ISO methods

The Flash protein sticks and Pro-TECT swabs were examined for colour change according to the manufacturers instructions. The Hygiena snapshot total ATP swabs were further processed according to the manufacturers instructions and the light output read in a luminometer

Analysis of results

The counts of *Enterobacteriaceae* and TVC were calculated for the hide, carcass, and environmental swab samples, to determine Log_{10} CFU/cm². For the two protein detection methods a score of between 0-4 was assigned to each sample depending on the degree of colour change, with 4 being the strongest. A score of 0-4 was also assigned to each sample examined using the VerifEYE solo machine, depending on the degree and extent of fluorescence found on the area (with 4 being the highest degree of fluorescence over the maximum area; subjective). The reading obtained on the luminometer from the total ATP swabs taken was displayed as Relative Light Units (RLU)

Results & Discussion

The data obtained for the carcasses of both cattle and pigs were calculated to show the difference in both microbial counts and chlorophyll levels between the hide/skin and the carcass (Figure 1). The average TVC count (Log_{10} CFU/cm², from four sampled areas of each species) from cattle hide was similar to that obtained from the pigs skin (Log_{10} 5.2 and 5.3, respectively). However, the same count detected on the finished carcasses of pigs was higher than that obtained on the cattle carcasses. When the difference between the carcass TVC count and the hide/skin TVC count was calculated for each species, the difference was higher with the cattle (Figure 1). This indicates that the transfer of bacteria from the hide to the carcass of cattle was lower than with pigs. The difference between the average levels of *Enterobacteriaceae* on the hide/skin and the carcasses of both pigs and cattle was similar, indicating similar levels of bacterial transfer from hide/skin to carcass. The average level of chlorophyll detected on the hide/skin and carcasses of cattle and pigs varied greatly (cattle: 2.9 and 0.9, pigs: 1.1 and 0.2). The transfer of chlorophyll from the hide/skin to the carcass in both species also varied greatly (cattle: 2.0, pigs: 0.9), with the difference found to be greater in cattle (Figure 1). The difference in the level of chlorophyll detected on the hide/skin and carcasses from cattle showed a similar level as that obtained with the TVC count, and the corresponding difference in pigs, showed a similar level as obtained with the *Enterobacteriaceae* count.

The results obtained indicate that the transfer of contamination from the hide to the carcass was less with cattle dressing than with pig dressing, potentially indicating better process hygiene during the dressing of cattle in this abattoir. The results obtained also indicate that the detection of chlorophyll on carcasses may be a good indication of process hygiene, particularly in terms of the total bacterial load on cattle carcasses and bacteria from faecal origin on pig carcasses.

The environmental surfaces were examined pre- and post-cleaning to determine the effect of cleaning at reducing the levels of bacteria and visible contamination (Tables 1 & 2). With the cattle surfaces on average a 2 fold decrease in TVC count was observed which was also detected with the total ATP method and the Flash protein method (Table 1). Higher fold reductions were observed with the *Enterobacteriaceae* count (15 fold) and the Pro-tect protein method (8 fold). However, the chlorophyll levels detected using the VerifEYE solo machine showed no reduction between pre-and post-cleaning (Table 1). The reductions observed with the *Enterobacteriaceae* count from the surfaces sampled after pig slaughter were similar to those observed with the total ATP method (4 fold, Table 2). Both of the protein detection methods used showed a 2 fold reduction in contamination after the

surfaces had been cleaned (Table 2). The chlorophyll levels detected post-cleaning were higher than those detected pre-cleaning, and hence a reduction level was not obtained using this method (Table 2). The 15 fold reduction in TVC count observed post-cleaning was greater than the reduction detected with any of the other methods examined

The results obtained for the environmental surfaces indicate a potential correlation between measured total ATP, the Flash protein method, and TVC count, particularly with surfaces related to cattle slaughter. A similar correlation between the detection of microbial ATP and TVC from the surfaces of beef carcasses has already been found (4). Therefore, the detection of total ATP and protein may have a potential use to assess the effectiveness of surfaces cleaning in abattoirs

Conclusions

Overall, the results of the pilot study in an experimental abattoir indicate that the detection of chlorophyll on carcasses and methods to detect protein and total ATP on surfaces may have a potential for use in the assessment of abattoir process hygiene. However, these methods need to be further evaluated under commercial abattoir conditions

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Tables and Figures

Figure 1 – Difference between average detected *Enterobacteriaceae*, TVC and chlorophyll on hide/skin and carcass for cattle and pigs

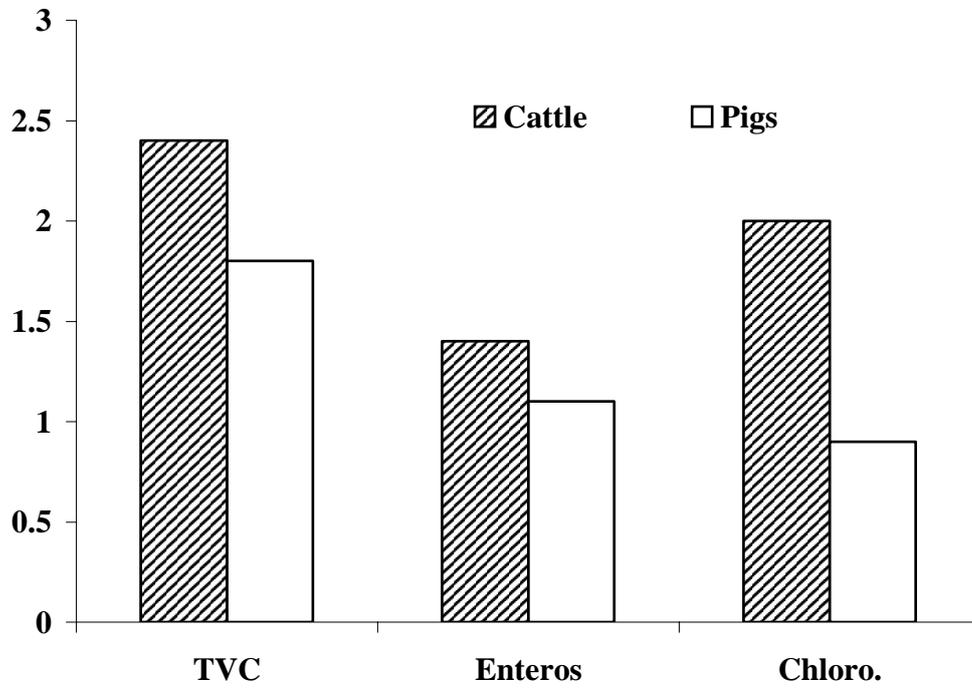


Table 1. Reduction in contamination parameters observed pre- and post-cleaning of surfaces in cattle slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Roll-out ramp	4.8	3.1	2.7	0	3	3	928	1558	3	0	1	0
Beef pram	4.8	3.6	3.5	0	2	2	1860	2044	5	1	3	2
Knife	0	3.7	0	0	0	0	195	337	0	0	1	1
Flayer	3.8	1.5	0.2	0	1	1	16361	8305	3	0	1	1
Apron wash	1.5	1.9	0	0	1	0	1011	2	1	0	2	1
Saw	4.4	3.1	1.8	1.1	0	1	4566	504	0	0	3	1
Mean	3.2	2.8	1.4	0.2	1.2	1.2	4153	2125	1.7	0.2	1.8	1.0
Reduction	2 fold		15 fold		0		2 fold		8 fold		2 fold	

Table 2. Reduction in contamination observed pre- and post-cleaning of surfaces in pig slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Dehairer (inside)	5.0	2.6	2.2	0	0	0	2760	903	3	3	3	1
Dehairer (outside)	1.1	1.2	0	0	0	0	120	542	0	0	1	1
Polishing table	4.6	0.2	2.2	0	0	0	1159	5696	1	0	4	3
Knife	2.9	4.2	0.2	0.7	0	0	48578	5724	3	3	3	0
Apron ash	0.8	1.0	0	0	0	0	2855	1547	0	0	0.5	2
Saw	1.9	0.5	0	0	0	1	8491	3791	2	0	2	1
Mean	2.7	1.6	0.8	0.1	0	0.3	10660	3034	1.5	1.0	2.2	1.3
Reduction	14 fold		4 fold		Increase		4 fold		2 fold		2 fold	

MEAT SAFETY GAINS BY DE-BONING OF INTACT CARCASSES

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Key Words: Intact carcasses, hot boning, bacteria, CNS, self-contamination

Introduction

Carcass contamination with CNS tissue has been an important issue since Bovine Spongiform Encephalopathy (BSE) was considered as a zoonosis. The significance of the carcass splitting saw in connection with contamination of carcasses by CNS tissue is documented (1). In addition, the possibility of contamination of the carcass by bacteria via the splitting saw also exists. De-boning implemented on intact carcasses with an intact spinal column would eliminate disruption of the spinal cord and its dissemination on the carcass. The food safety aspects of de-boning intact carcasses might also be considered as additional gains of hot boning in general. Such gains are reduction of the mass of carcass tissue requiring chilling and a potential to avoid weight loss from the meat during refrigeration since meat is vacuum- packed on removal from the carcass. Technically, hot boning also requires less manual labour than traditional de-boning of chilled meat. However, de-boning of intact carcasses needs more space and different facilities and may be more complicated than the working operations based on carcass halves or quarters. Accordingly, the food safety aspects of hot boning of intact carcasses have to be discussed in a cost benefit context.

Objectives

Our main objectives were to investigate whether de-boning of intact carcasses might have a positive influence on the degree of both bacteriological and CNS related contamination on carcass surfaces and also in meat from such carcasses.

Methodology

Sample collection from carcasses

The cattle were slaughtered in an abattoir with a capacity of slaughtering and hot boning of about 100 animals on a daily basis. Eighteen carcasses were kept intact during slaughtering and dressing (range 187.2 - 388.5 kg) and compared to forty-five carcasses (range 206,6 - 376.7 kg) split by an automatic circular saw on the same slaughter line (Landteknikk A/L, Oslo, Norway).

Both GFAP and S-100 β proteins have been used as indicators for contamination of carcasses with CNS tissue (2). Samples for detection of these two proteins from both split (n = 10) and intact carcasses (n = 10) were collected from five internal (no. 1 - 5) and five external regions (no. 6 - 10) on both sides (Figure 1). These surface samples were collected using synthetic sponges (Sydney Heath & Son, Stoke on Trent, UK), and placed into 20 ml PBS, 0.5 % Triton-X-100.

Collection of microbiological samples was performed by swabbing 100 cm² from leg, pelvic duct, belly, back, and elbow (letters a - e) (Figure 1) from both split (n = 25) and intact carcasses (n = 10).

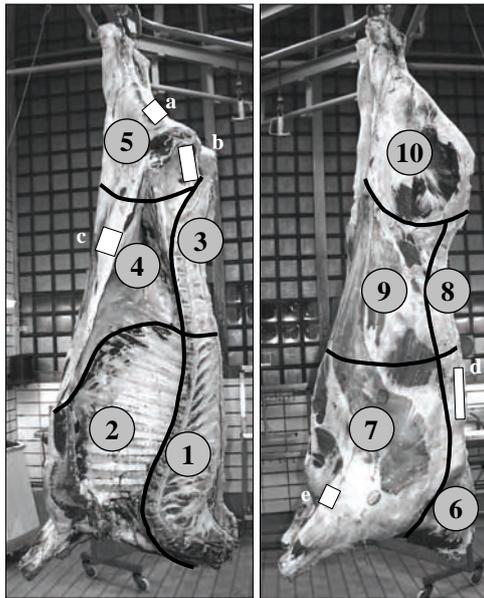


Figure 1. Schematic diagram of the ten areas (indicated by numbers) swabbed on the internal and the external surfaces of each half (left and right) of the split beef carcass. When swabbing intact carcasses, areas no. 1 and 3 on the internal surface were not relevant used. Microbiological sampling sites are indicated by letters.

Sample collection from minced meat

After meat inspection and trimming, the split carcasses were quartered with a manual circular saw and transported into the hot boning department and processed continuously. About 200 g of minced meat from split carcasses (n = 35) was sampled at random on the same days that the intact carcasses were processed. To avoid cross-contamination from split-carcasses, the intact carcasses (n = 16) were de-boned in a separate room. Minced meat from these carcasses was processed by a separate mincer (The Biro® Mfg Co, Marblehead, Ohio, USA). The mincer was washed and disinfected by hot water before use and between each carcass. About 200 g of minced meat from each of the intact carcasses was also sampled. For detection of CNS contamination and microbiological investigation, each sample was divided into two plastic bags.

Storage

Samples for detection of CNS contamination were stored at 4 °C for 48 hours and then frozen at -70 °C prior to analysis. Samples for microbiological analyses were stored at 4 °C for 24 hours before sample preparation and analysis.

Detection of CNS contamination

A modification of the capture ELISA (enzyme linked immunosorbent assay) for GFAP (3) and S-100 β (2) was used to detect CNS contamination of beef carcasses and minced meat.

Microbiological methods

The following parameters were investigated:

- Aerobic microorganisms (4),
- *Enterobacteriaceae* (5),
- *E. coli* (6).

Statistical analyses

t-test (Microsoft Excel, 2000)

Results & Discussion

Results are presented in Tables 1 and 2. In general, the contamination of split carcasses is caused by the carcass splitting saw, providing transport of CNS material and bacteria by direct contact, water sprays and via aerosols. Sample sites near the vertical incision of the splitting saw had higher levels of CNS indicators and bacteria than sites far from this incision.

Table 1. Average CNS contamination illustrated by the levels of GFAP and S-100 β on intact and split beef carcasses and in minced meat from these sources (Standard deviation). The results are expressed as ng/mg total protein. ND = not detectable. GFAP and S-100 β are considered to have a background level in “normal” meat of about 2 ng/mg

Parameter/source	Carcasses		Minced meat	
	Intact (n=10)	Split (n=10)	Intact (n=16)	Split (n=35)
GFAP	0.14 (0.62) ^a	88.40 (174.88)	ND (-)	0.00 (0.03)
S-100 β	0.95 (1.37) ^a	52.92 (134.76)	17.37 (8.90)	16.12 (9.37)

^ap < 0.01

Although the levels of CNS carcass contamination were significantly lower in intact carcasses compared to split carcasses, these differences were much reduced and were not significant in minced meat. In relation to bacteria, the same dilution effect was not seen in minced meat. The levels of bacteria were the same in both intact and split carcasses except for the sample sites along the back (*Enterobacteriaceae* and *E. coli*). However, the levels of Total viable count and *Enterobacteriaceae* were significantly lower in minced meat from intact carcasses.

Table 2. Average contamination illustrated by total viable count (TVC), *Enterobacteriaceae*, and *E. coli* on intact and split beef carcasses and in minced meat from these sources (Standard deviation). The results are expressed as log CFU/cm² from carcass surfaces and log CFU/g minced meat. ND = not detectable

Parameter/source	Carcass		Back		Minced meat	
	Intact (n=10)	Split (n=25)	Intact (n=10)	Split (n=25)	Intact (n=20)	Split (n=10)
TVC	4.27 (0.87)	4.16 (0.91)	3.61 (0.88)	3.87 (0.59)	3.20 (0.28) ^a	4.21 (0.59)
<i>Enterobacteriaceae</i>	0.87 (0.99)	1.20 (1.03)	0.14 (0.29) ^a	1.61 (0.90)	0.62 (1.04) ^a	3.01 (0.87)
<i>E. coli</i>	0.24 (0.71)	0.40 (0.70)	ND (-) ^a	0.62 (0.61)	0.11 (0.48)	0.31 (0.66)

^ap < 0.01

Conclusions

Both positive food safety aspects and additional general economic gains by hot boning of intact carcasses seem to over-shadow some negative practical and economic factors in a cost benefit context.

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QUANTIFICATION OF THE FIVE MOST FREQUENTLY FOUND HETEROCYCLIC AROMATIC AMINES IN MEAT SAMPLES WITH PLANAR CHROMATOGRAPHY

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Key Words: Heterocyclic Aromatic Amines, HAA, PhIP, MeIQ_x, 4,8-DiMeIQ_x, Norharman, Harman, meat, meat samples, Planar Chromatography, method development, quantification

Introduction

Heterocyclic Aromatic Amines (HAA) are among the most potent mutagenic substances [1]. Around 20 different HAA at low µg/kg level could be identified in food. In complex reactions at high temperatures they are formed of the precursors creatine, creatinine, amino acids and reducing carbohydrates. The concentrations depend amongst others on the way of preparation, heating time and temperature [2]. It could be ascertained that meat samples cooked under normal conditions contained PhIP, MeIQ_x, 4,8-Di-MeIQ_x, Norharman and Harman, which are the most widespread HAA in meat.

Objectives

HAA are typically quantified by column chromatography (HPLC, GC) with detection by UV-absorbance, fluorescence or mass spectrometry (MS) [3]. The aim of our research was the development of a new planar chromatographic method to identify and quantify the five most frequently found HAA in complex meat matrix more effectively.

Methodology

Chemicals

The standard substances MeIQ_x, 4,8-Di-MeIQ_x and PhIP were purchased from Toronto Research Chemicals, Ontario, Canada, Harman and Norharman from Sigma-Aldrich GmbH, Taufkirchen, Germany. All standards were dissolved in methanol containing small amounts of ammonia. All solvents used were of analytical grade; solvents of the mobile phase were chloroform (Sigma-Aldrich GmbH, Seelze, Germany), diethyl ether (Fluka, Buchs SG, Switzerland) and methanol (Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK).

Preparation of meat samples

Beef patties (60 g \pm 0.5 g) were purchased from Ranch Master, Wunstorf, Germany. The patties were coated with sunflower oil and put between two parts of tin foil and between two grill plates of a double contact grill (Nevada, Neumärker, Hemer, Germany). At a plate temperature of 230 °C the patties were grilled simultaneously on both sides for six minutes.

Sample Preparation

The extraction of HAA from the meat matrix was performed according to the solid phase extraction method of Gross and Grueter [4] with slight modifications [5].

Analysis of HAA

As stationary phase HPTLC plates silica gel 60 WRF_{254s} (20 x 10 cm) from Merck, Darmstadt, Germany were employed. For sample application, the dissolved substances were sprayed as bands by Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland). Afterwards, the plates were repeatedly developed in the same direction by the Automated Multiple Development System (AMD 2, CAMAG). The AMD 2 gradient scheme designed to separate the 5 HAA is shown in Table 1. Densitometry is performed via multi-wavelength scan with TLC Scanner 3 (CAMAG) at UV 262 nm, UV 316 nm and UV 366/>400 nm. For data acquisition and processing, winCATS software (CAMAG) was used.

Results & Discussion

After the determination of the most suitable plate material, different parameters of the mobile phase were optimized including solvents and solvent mixtures, the necessary number of gradient steps and migration distances. The gradient scheme in Table 1 shows that a seven-step development was necessary to separate the substances of interest. In step 1 the plate was developed with 100 % diethyl ether up to a migration distance of 30 mm. During the following steps the composition of the solvent mixture changed from 85 % chloroform/15 % methanol continuously to 87 % chloroform/13 % methanol with a migration distance of 60 mm each. Before each gradient step alkaline conditioning of the stationary phase was automatically performed.

After multiple development for about two hours the plate was scanned densitometrically. The optimized separation of the five HAA is shown in Figure 1. Detection was performed via multi-wavelength scan at 316 nm for PhIP (1), 262 nm for MeIQx (2) and 4,8-DiMeIQx (3), and at 366/>400 nm for Norharman (4) and Harman (5).

The conclusive next step was the investigation of the influence of meat matrix on the determination of HAA and the applicability of the developed method. Due to the matrix and the low levels of HAA in meat ($\mu\text{g}/\text{kg}$), accurate analysis is difficult and relatively high standard deviations are common [6].

In Figure 2 an overlay of two meat sample tracks is shown. One of them (blue analog curve) is a sample track spiked with standard solutions of Norharman and Harman, and the other (red analog curve) shows the track of a pure meat sample. The identity was confirmed by comparing these peaks with reference substances concerning retention time and spectral identity. As example, in Figure 3 a comparison

of the absorption spectra of MeIQx standard (1) and the corresponding peak from a meat sample (2) is shown. However, the complex meat matrix and the low levels of HAA in meat make a final identification unsure. In this context, i.e. for confirmation of positive results, the application of HPTLC-MS by a new online extractor is under investigation [7].

The quantification was carried out by external calibration with reference substances simultaneously on the same HPTLC plate and under idem chromatographic conditions. As example, the calibration curve of MeIQx which has a low relative standard deviation of $\pm 1.10\%$ is shown in Figure 4. The absolute amount of MeIQx on the plate was determined to be 12.7 ng in the pure and 33.9 ng in the spiked sample. With a recovery rate of 44 % the concentration of MeIQx in the meat sample was calculated to be 0.72 $\mu\text{g}/\text{kg}$. Thus besides screening, the presented method is suitable for quantification.

Modern planar chromatography shows a variety of advantages. The method is considered as very fast because of the possibility to applicate up to 20 tracks on one plate and to perform a simultaneous development. Ten tracks were needed for a five-point calibration (two tracks per concentration level for a repeat determination) whereas the residual ten tracks can be used for samples.

Before HPLC analysis, it is necessary to separate HAA from meat samples in polar and apolar fractions, because two different HPLC gradients for the fractions are necessary. Thus, one meat sample in duplicate analysis (= two pure and two spiked samples = 8 fractions) can be determined on one HPTLC plate. Comparing the retention time of about two hours in planar chromatography with the time in HPLC analysis (one hour per one fraction), it becomes obvious that planar chromatographic separation is four times faster than HPLC. In further studies it will be clarified whether it is also indispensable to divide the sample into two fractions for analysis by planar chromatography.

Furthermore, the new method is cost effective: only 60 mL solvent per plate are needed, less than 10 mL per fraction. The costs for HPTLC plates are less than 5 Euro (6 US\$) per plate.

Conclusions

In spite of the complex meat matrix and difficult determination involved, the planar chromatographic quantification of the five most widespread HAA is possible. The presented method is not only suitable for screening but even adequate for quantitative trace analysis. Further research will focus the validation of the developed method as well as the comparison between planar chromatography and the commonly used HPLC method. In the last few decades a crucial disadvantage of planar chromatography compared to HPLC and GC was the lack of coupling possibilities with MS as a sensitive and selective detector. To hyphenate both methods a new extraction device was developed by Luftmann [7]. The investigation of the appliance of this new device for HAA analysis will be the aim of further studies.

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supply of plate material and to Dr. Konstantinos Natsias, CAMAG, Berlin, Germany for support regarding equipment.

Abbreviations

MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
4,8-Di-MeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline
PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
Norharman: 9*H*-pyrido[3,4-*b*]indole
Harman: 1-methyl-9*H*-pyrido[4,3-*b*]indole

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Tables and Figures

Table 1: AMD-gradient scheme with solvent compositions and migration distances to separate the five most widespread HAA.

step number	chloroform [%]	methanol [%]	diethyl ether [%]	migration distance [mm]
1	0.0	0.0	100.0	30.0
2	85.0	15.0	0.0	60.0
3	85.4	14.6	0.0	60.0
4	85.8	14.2	0.0	60.0
5	86.2	13.8	0.0	60.0
6	86.6	13.4	0.0	60.0
7	87.0	13.0	0.0	60.0

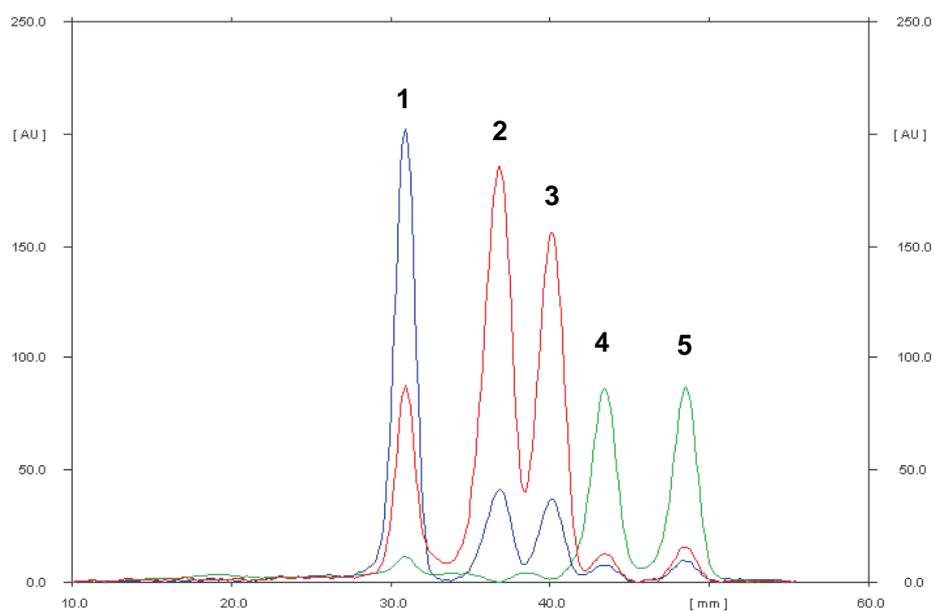


Figure 1: Optimized separation of the five HAA; detection via multi-wavelength scan was performed at 316 nm for PhIP (1), 262 nm for MeIQx (2) and 4,8-DiMeIQx (3), and at 366/>400 nm for Norharman (4) and Harman (5).

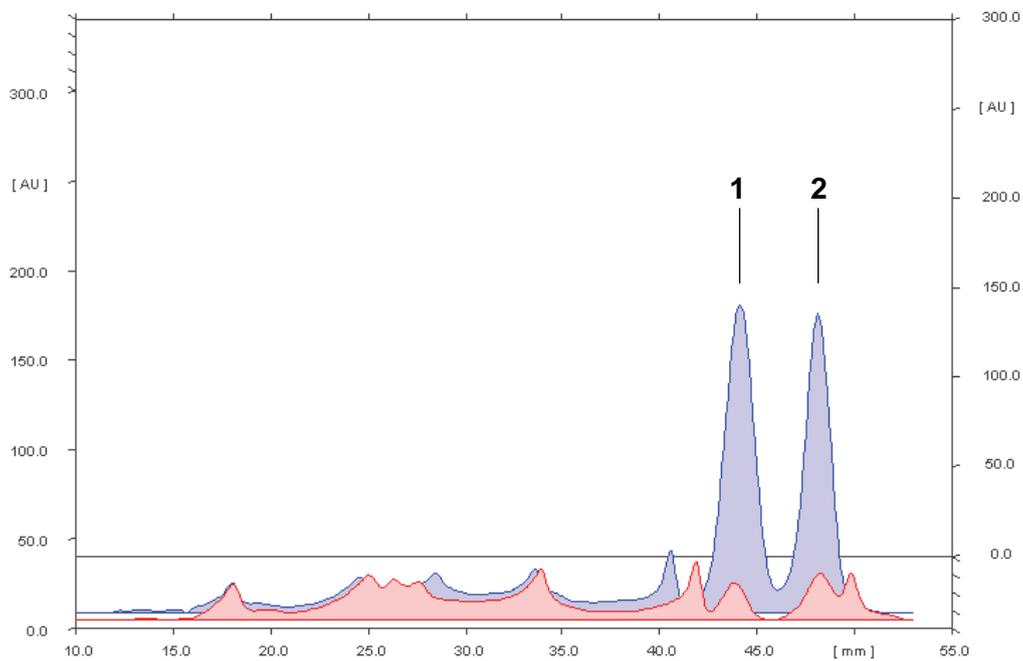


Figure 2: Track overlay of meat sample (red curve) and meat sample spiked (blue curve) with standard solutions of Norharman (1) and Harman (2).

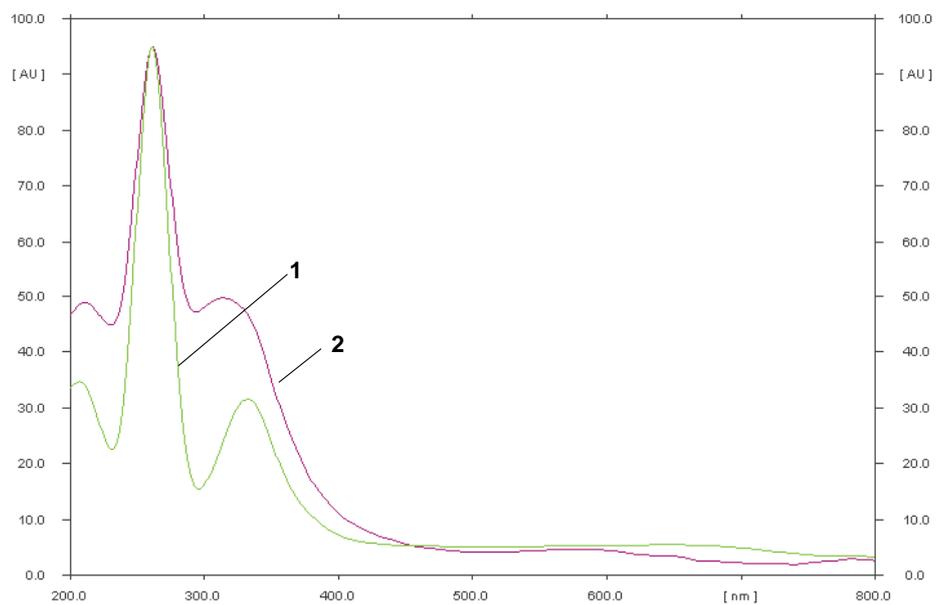


Figure 3: Comparison of UV/VIS absorbance spectra of MeIQx in standard solution (1) and in meat sample (2).

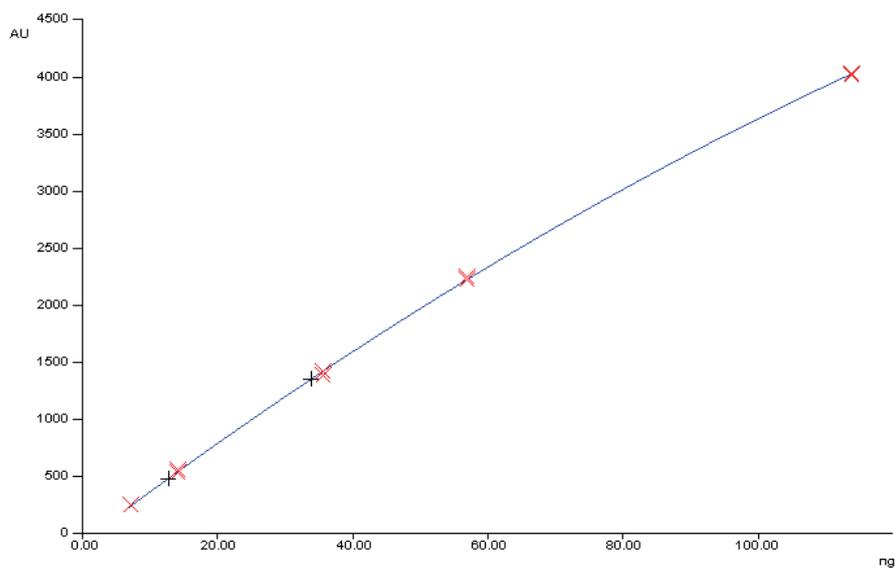


Figure 4: Polynomial calibration curve of MeIQx calculated via peak area ($y = -0.076 x^2 + 44.705 x - 75.741$, $r = 0.9992$, $sdv = \pm 1.10\%$).

METHOD FOR THE DETECTION OF DEXAMETHASONE IN CATTLE DRINKING WATER

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Key Words: Dexamethasone, water, cattle, residues

Introduction

Corticosteroids, such as dexamethasone, have many physiological roles. Dexamethasone (9- α -fluoro-16- α -methylprednisolone) is a synthetic glucocorticoid that is authorized for therapeutic use in veterinary medicine but its use as growth promoting agents is banned in the European Union. Low concentrations of glucocorticosteroids are known to increase weight gain, to improve feed conversion and to have a synergetic effect with other molecules like beta-agonists or anabolic steroids. Due to these effects, dexamethasone has been illegally used to obtain an economical benefit through an increased muscle development. But these substances remain in meat and have negative toxic consequences for consumers. In the inspection control, drinking water and feed given to the animals may be sampled at the farm.

A number of methods for the detection, determination and confirmation of dexamethasone in different biological matrices, like urine, faeces, liver, milk or feed, have been previously reported (Delahaut et al., 1997; Creaser et al., 1998; Stolker et al., 2000; Draisci et al., 2001; Cherlet et al., 2004). Recently, the performance of methods and the criteria for the interpretation of test results of official control laboratories within the European Union has been regulated in the Decision 2002/657/EC (EC, 2002). The presented method has been validated according to this Decision.

In this paper, a screening and confirmatory method for dexamethasone detection in drinking water in livestock has been developed and validated. This method is based on immunoaffinity chromatography followed by reverse-phase high performance liquid chromatography (IAC-RP-HPLC) with diode array detection at 242 nm.

Objectives

The goal of this work was the optimization and validation of a procedure for the detection of dexamethasone in water for cattle through immunoaffinity chromatography followed by reverse-phase high performance liquid chromatography (RP-HPLC).

Methodology

The chemical structure of dexamethasone (9- α -fluoro-16- α -methylprednisolone) and flumethasone are shown in figure 1. A scheme of the extraction protocol is shown in figure 2. Briefly, 5 mL of drinking water with added internal standard

(flumethasone) were placed in a test tube. The solution was loaded into an immunoaffinity column, containing specific antibodies for dexamethasone, and washed with 10 mL of diluted buffer and 5 mL of mili-Q water. Corticosteroids were eluted with 4 mL of ethanol:water (70:30), pH 5.0, and collected into test tube that was evaporated to dryness at 45°C under nitrogen stream. Then, the evaporated sample was resuspended in 200 µL of mobile phase consisting in acetonitrile:mili-Q water (30:70). 20 µL were injected into an Agilent series 1100 HPLC equipped with a diode-array detector. The column was a Synergi Max RP, 150 mm x 4.6 mm, from Phenomenex. The mobile phase, at a flow rate of 1 mL per min, was a solution consisting in acetonitrile:mili-Q water (30:70) and the eluent was monitored at 242 nm. In general, the following order of injections was followed for each set of samples: i) reagent blank, ii) compliant (water blank) control sample, iii) sample to be confirmed, iv) compliant control sample again and v) non-compliant (water fortified with 26 ng mL⁻¹ dexamethasone) control sample.

Results & Discussion

Stability: Dexamethasone solutions (10 ng mL⁻¹) were kept under frozen storage (-20°C) up to 6 months and showed good stability for the full period of time.

Specificity: A total of 20 drinking water samples were analyzed. Other water samples were fortified with flumethasone and betametasone, individually and altogether. The method discriminated very well between the analyte (dexamethasone) and closely related substances as can be appreciated in the chromatogram (see figure 2). As can be observed, potentially interfering substances (chemically related compounds) eluted at different retention times.

Recovery: The recovery was determined by experiments using a total of 24 fortified blank water samples. Three sets, 8 samples each, were added dexamethasone to a final concentration of 26, 39 and 52 ng mL⁻¹ for each set, respectively. The recoveries standard deviations and coefficients of variation were determined (see table 1). The mean recovery was 99.4 ± 1.3 % (mean ± SD).

Repeatability: A set of homogenized water blank samples were fortified with 26, 39 and 52 ng mL⁻¹ of dexamethasone. At each level, the analysis was performed with 6 replicates and the mean concentrations, standard deviations and coefficients of variation were determined (see table 2).

Decision limit (CC α): 22 blank water samples were analyzed and the signal to noise ratio calculated in the time frame where dexamethasone is expected. The decision limit was set as 3 times the signal to noise ratio. This gives a CC α = 26 ng mL⁻¹.

Detection capability (CC β): 20 blank water samples were fortified with 26 ng mL⁻¹ of dexamethasone, that corresponds to the decision limit, and the standard deviation was calculated. The value of the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the measured content equals the detection capability. The obtained CC β was 30 ng mL⁻¹.

Conclusions

The method based on immunoaffinity chromatography followed by RP-HPLC for the analysis of dexamethasone in cattle drinking water has been validated using water fortified at levels up to 150 ng mL⁻¹. The main recovery is 99.4 ± 1.3%. The decision

limit ($CC\alpha$) is 26 ng mL^{-1} and detection capability ($CC\beta$) is 30 ng mL^{-1} . Specificity, sensitivity and repeatability have been also validated using this protocol.

Acknowledgements

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Tables and Figures

Table 1.- Recovery

Level (ng mL^{-1})	Mean recovery (%)	Standard deviation	CV (%)
26	105.1	1.73	6.33
39	98.5	1.08	2.80
52	94.5	1.09	2.22
Mean	99.4	1.30	3.78

Table 2.- Repetability

Fortified level (ng mL ⁻¹)	Mean concentration (ng mL ⁻¹)	Standard deviation	CV (%)
26	30.4	0.70	2.32
39	45.5	0.61	1.34
52	63.0	2.42	3.84

Figure 1.- Structure of dexamethasone and flumethasone (internal standard)

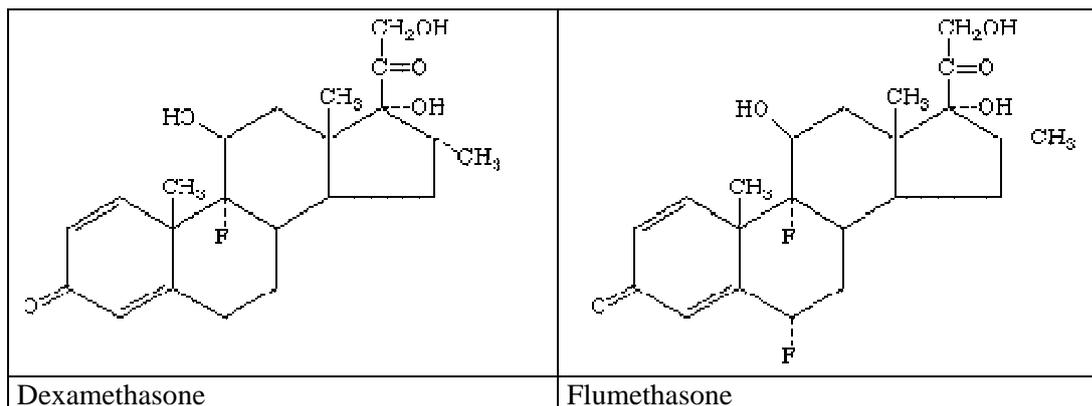


Figure 2.- Scheme of the extraction protocol

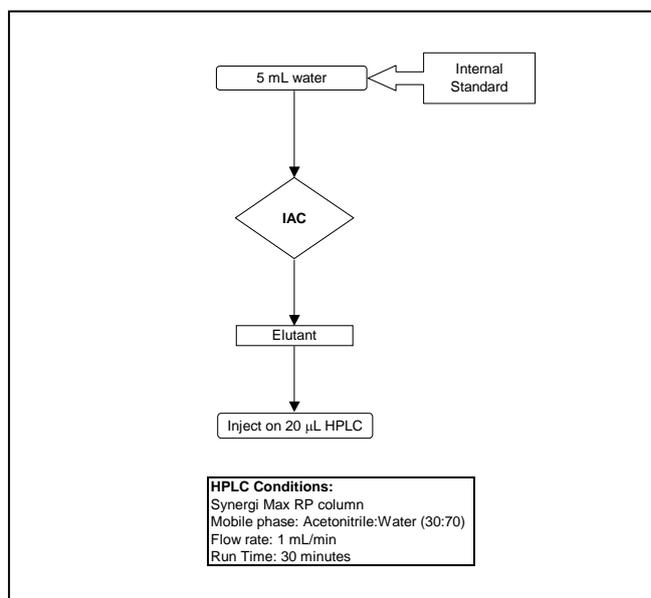
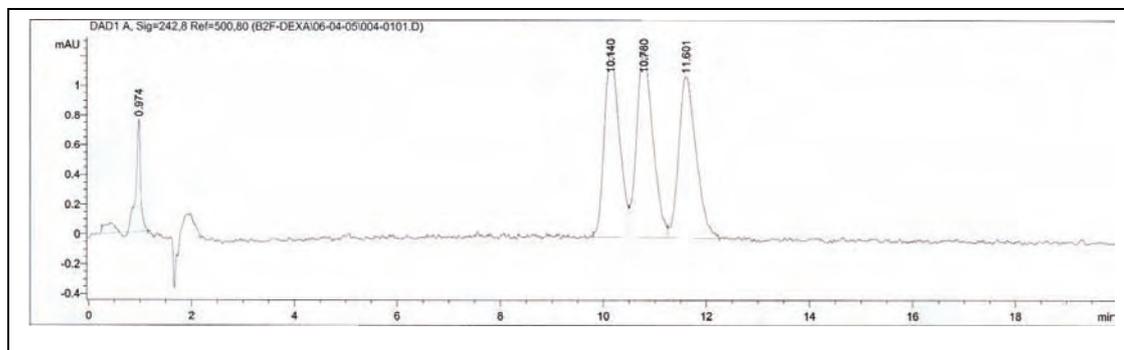


Figure 3.- Chromatogram of dexamethasone and closely related substances for the specificity study. Retention times of 10.14, 10.78 and 11.80 min were for betamethasone, dexamethasone and flumethasone, respectively.



A MODIFIED HPLC METHOD FOR THE DETECTION OF 6-METHYL-2-THIOURACIL IN CATTLE URINE

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Key Words: Methylthiouracil, thyreostatic agents, urine, cattle, residues

Introduction

Thyreostatic drugs have been applied illegally to farm animals to get an increased live weight gain. This gain is due to a higher water retention in edible tissues and this results in a fraudulent overweight of meat due to the higher water content. Furthermore, thiouracils remain in meat with potential toxic consequences for consumers.

Treatment of cattle with thyreostatics drugs can be detected through its analysis in residues from organs, excreta, plasma, meat and urine of the animals. In the inspection control, urine and faeces, as well as plasma, of the animals may be sampled at the farm (Courtheyn et al., 2002). A number of methods for the detection, determination and confirmation of thyreostatic drugs in cattle urine have been previously reported (Pochard et al., 1984; Moretti et al., 1993; Buick et al., 1998; De Wasch et al., 2001). Recently, the criteria for the interpretation of test results of official control laboratories within the European Union has been regulated in the Decision 2002/657/EC (EC, 2002). The presented method has been validated according to this Decision.

Objectives

The goal of this work was the optimization and validation of a procedure for the detection of thyreostatic agents, specifically 6-methyl-2-thiouracil (MTU), in cattle urine through reverse-phase high performance liquid chromatography (RP-HPLC).

Methodology

The methodology has been based on the work of Moretti et al. (1993) developed for plasma with some modifications. A scheme of the extraction protocol is shown in figure 1. Briefly, 1 mL of cattle urine with added internal standard (5,6-dimethyl-2-thiouracil) were placed in a test tube. 100 mg EDTA disodium salt, 3 mL ethyl acetate and 15 µL mercaptoethanol. After vortexing and mixing for 10 min, the tube was frozen for 10 min, at least. The organic layer was transferred to a clean tube and evaporated under nitrogen. After resuspension in methanol, it was transferred to an HPLC vial and evaporated again. Then, the evaporated sample was resuspended in 200 µL of mobile phase consisting in 25 mM phosphate buffer, pH 3.0, with 10% (v/v) methanol. 10 µL were injected into an Agilent series 1100 HPLC equipped with a diode-array detector. The column was a Kromasil C18, 150 mm x 4.6 mm, from

Scharlau (Barcelona, Spain) with a flow rate of 1 mL per min. The gradient ranged from 10% methanol in phosphate buffer to 26% at 8 min; then, it increased until 70% in 2 min and remained at that percentage for 7 min. Afterwards the initial conditions were recovered in 3 min. The eluent was monitored at 276 nm. In general, the following order of injections was followed for each set of samples: i) reagent blank, ii) compliant (urine blank) control sample, iii) sample to be confirmed, iv) compliant control sample again and v) non-compliant (urine fortified with 150 ng mL⁻¹ MTU) control sample.

Results & Discussion

Specificity: The method discriminated very well between the analyte (6-methyl-2-thiouracil) and closely related substances as can be appreciated in the chromatogram (see figure 2). As can be observed, potentially interfering substances (chemically related compounds) eluted at different retention times.

Recovery: The recovery was determined by experiments using a total of 90 fortified blank urine samples. Three sets, 30 samples each, were added 6-methyl-2-thiouracil to a final concentration of 100, 150 and 200 ng mL⁻¹ for each set, respectively. The recoveries are shown in table 1. The mean recovery was 64.9%, equivalent to a recovery factor of 1.54.

Repeatability: A set of homogenized blank urine samples were fortified with 100, 150 and 200 ng mL⁻¹ of 6-methyl-2-thiouracil. At each level, the analysis was performed with 6 replicates and the mean concentrations, standard deviations and coefficients of variation were determined (see table 2).

Within-laboratory reproducibility: A similar procedure as described above for repeatability was performed with different operators and under different environmental conditions. Results are shown in table 3.

Decision limit (CC α): 26 blank urine samples were analyzed and the signal to noise ratio calculated in the time frame where MTU is expected. The decision limit was set as 3 times the signal to noise ratio. This gives a CC α = 100 ng mL⁻¹.

Detection capability (CC β): 34 blank urine samples were fortified with 100 ng mL⁻¹ MTU, that corresponds to the decision limit, and the standard deviation was calculated. The value of the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the measured content equals the detection capability. The obtained CC β was 130 ng mL⁻¹.

Conclusions

The RP-HPLC method for the analysis of 6-methyl-2-thiouracil in cattle urine has been validated using urine fortified at levels up to 600 ng mL⁻¹. The main recovery is about 65% (recovery factor of 1.54). The decision limit (CC α) is 100 ng mL⁻¹ and detection capability (CC β) is 130 ng mL⁻¹. Specificity, sensitivity and repeatability have been also validated using this protocol.

Acknowledgements

Grant from Conselleria d'Agricultura, Pesca i Alimentació, Generalitat Valenciana (Valencia) is fully acknowledged.

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Tables and Figures

Table 1.- Recovery

Level (ng mL ⁻¹)	Mean recovery (%)	Standard deviation	CV (%)
100	60.5	14.6	24.1
150	64.4	8.8	13.6
200	69.8	16.1	23.1
Mean	64.9	14.0	21.6

Table 2.- Repeteability

Level (ng mL ⁻¹)	Mean recovery (%)	Standard deviation	CV (%)
100	66.1	13.5	20.4
150	105.6	6.8	6.5
200	166.5	28.1	16.9

Table 3.- Within-laboratory reproducibility

Level (ng mL ⁻¹)	Mean recovery (%)	Standard deviation	CV (%)
100	56.5	14.4	25.4
150	94.0	18.7	19.9
200	121.6	20.3	16.7

Figure 1.- Scheme of the extraction protocol

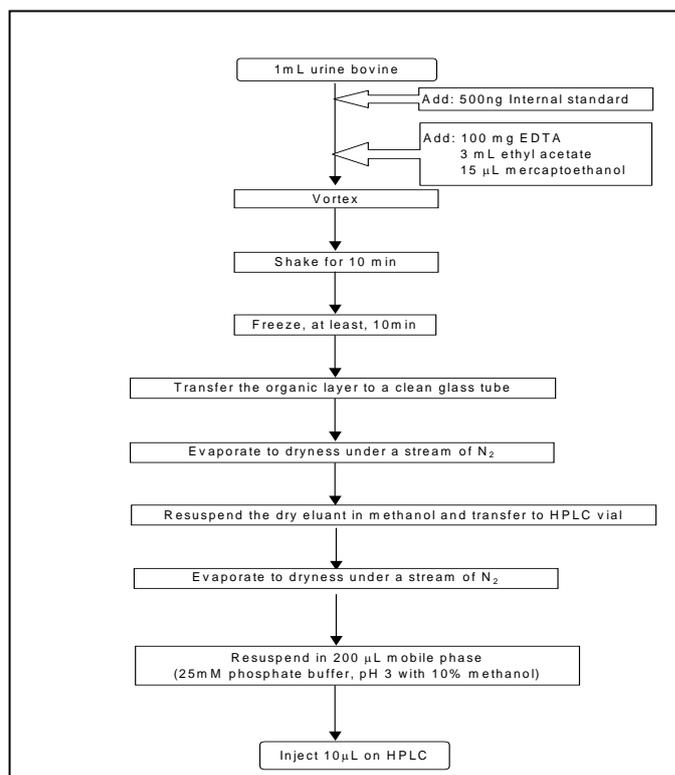
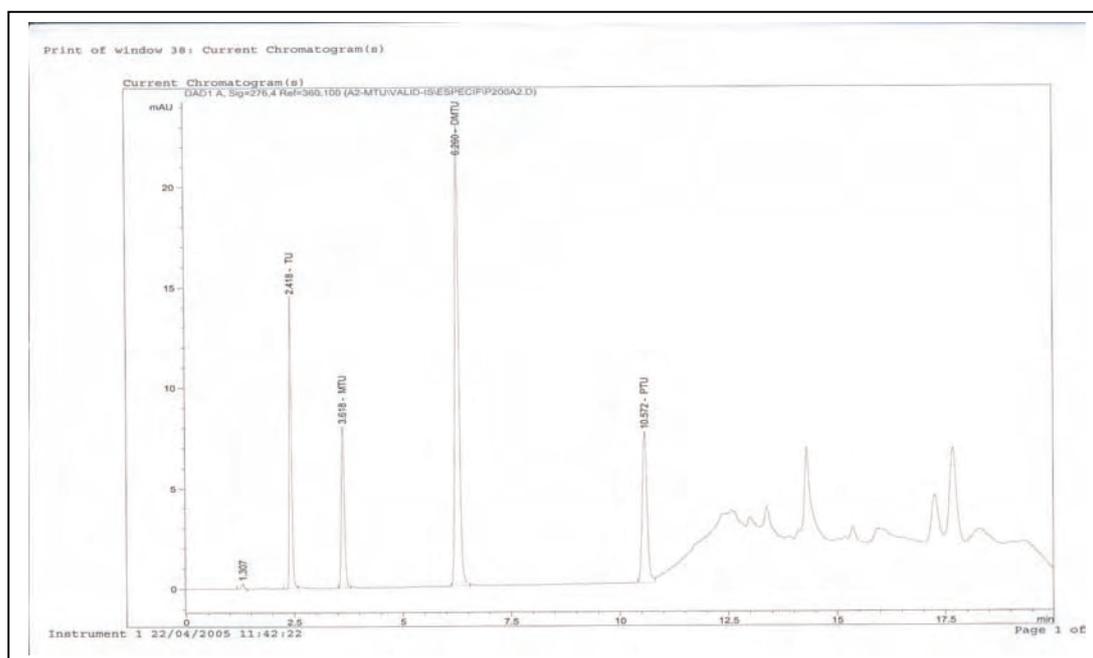


Figure 2.- Chromatogram of 6-methyl-2-thiouracil and closely related substances for the specificity study. TU: 2-thiouracil; MTU: 6-methyl-2-thiouracil; DMTU 5,6-dimethylthiouracil; PTU: 6-propyl-2-thiouracil.



DETERMINATION OF ADULTERATION OF MEAT RAW MATERIALS AND MEAT PRODUCTS

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According to official sources, Russia is swept over with the flow of poor quality and adulterated food products. These data are confirmed by the results of investigations of meat products quality in the Russian trade, which are regularly carried out at VNIIMP, by the initiative of controlling organizations and by ROSTEST-Moscow. Thus, up to 30% of the samples presented for the analysis don't meet regulations' requirements. 25% of meat products from this amount are adulterated by the composition of the used raw materials, primarily, by using plant proteins or carbohydrates instead of meat raw materials, and the remaining part by violation of the ratio protein/fat, deterioration of sensory characteristics, excessive use of connective tissue, etc. (Fig.1).

These data strongly suggest that dishonest producers, maintaining allowable levels of substances to be regulated, are using not agreed upon animal or plant components or their excessive amounts. Such products often not relevant to their name by their composition are allowed for marketing as being safe by microbiological and acceptable by sensory and chemical characteristics.

The Laws passed in RF about certification of products and services "About standardization", "About protection of consumers' rights"; "About quality and safety of foods" are intended for strict observation of the requirements concerning raw materials and contents of food products. The situation is generally favorable for the determination of product chemical composition. There are various methods available with adequate modern instrumentation, which allow to quick and accurate determination of product fat, protein, moisture, etc.

At the same time the techniques are known in scientific practice, that make it possible to answer the question about actual composition of food products and to identify the components of meat products subjected to different technological treatment.

Histological method of identification of the content of meat raw materials, used in the meat product

Using this method to study the histological preparations obtained from meat products, one can determine their following components of animal origin: muscle tissue of different species, connective tissue of different species, fat tissue, skin, vessels, nerves, by-products, plant additives (protein and carbohydrate in nature, including starch, wheat flour, and soy protein products (with differentiation into isolate, texturate, concentrate), carrageenan, and spices.

This standardized method covers meat products and comminuted meat products as follows: meat of all species of slaughter animals; mechanically deboned meat (including poultry meat); semi-finished meat products – natural, chopped, ground meat, pelmeni (those with poultry meat); pork products; sausage products (among

them those with poultry meat); canned meat and meat-plant products (among them those with poultry meat).

In analyzing the comminuted meat (ground meats), semi-finished meat products and final meat products, the most common situation is as follows: a) complete or partial substitution of mechanically deboned meat for ground meat; b) substitution of ground meat or product of mechanical deboning produced from poultry, for ground meat of slaughter animals. Studies of sausage products have shown that the content of ground meat had drastically excessive amount of connective tissue, and the added components not indicated in the normative documentation. The composition could contain trimmings, meat from heads, and meat from esophagus, by-products and plant components of carbohydrate or protein nature.

Using light microscopy soy protein products are reliably detected in meat products because they have specific microstructure. And the histological method can determine not only the fact of using soya, but also its technological form (isolate, concentrate, texturate), which is used in each specific case. Histological method can easily detect soya preparations in the amount of 1% in ground meat, semi-finished products of final meat products (based on the weight of the raw materials).

Use of mechanically deboned poultry meat is a special problem in adulterated meat products. In case of adulteration this kind of the raw material, apart from the components of animal origin can often contain non-meat animal components (for example, gizzards of poultry) or different plant additives, among them soy protein preparations or easily hydrated carbohydrates. Often, the content of these, having nothing to do with meat, preparations can be rather significant.

In studying the comminuted multi-component meat raw materials – mechanically deboned poultry meat as produced by different companies of the European Community and other European countries - a quantitative method of morphometric analysis for quality evaluation (composition of raw materials and tissue) was also used. The most differing versions of change of the components composition of mechanically deboned poultry meat and the adulterated product that came to sales channels under the same trade name are presented in Fig.2.

In the products of low quality the predominating components are connective tissue, which sometimes can significantly exceed half of the volume. Correspondingly, in poor raw materials the content of the component having the highest food value – muscle tissue – can be as low as 10 vol. % against 50 – 70 vol. % in high quality raw materials. If adulteration of mechanically deboned meat takes place, the histological analysis reveals various plant additives in the meat mass – plant, protein and carbohydrate components. In Fig. 2 they are 15 vol. %.

In the last years the share of imported products with adulterated composition has increased. Taking into consideration the opinion of experts predicting the increase of the share of meat products import following Russian joining WTO, the problem of carrying out identification of the composition of raw materials as used in the manufacture of meat products will cease to be just a scientific problem. The technique of quick determination of raw materials adulteration can at the same time become the means for preventing of penetration of poor quality imported products to the domestic food market.

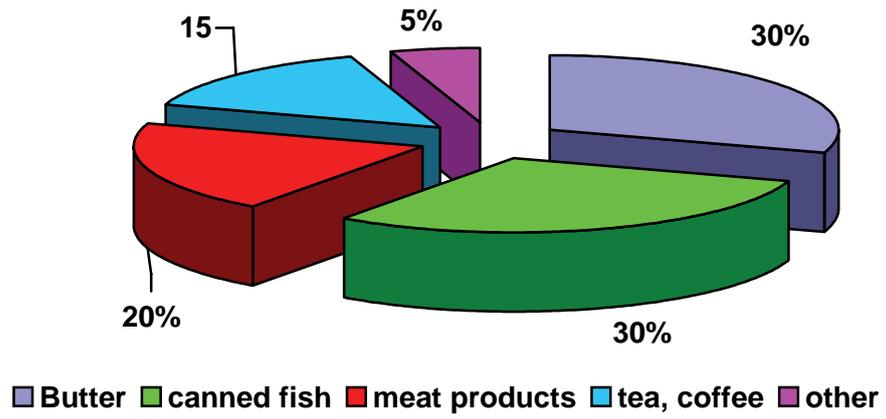


Fig.1 Adulterated products on Russian market in 2004. (Data of trade inspection of RF)

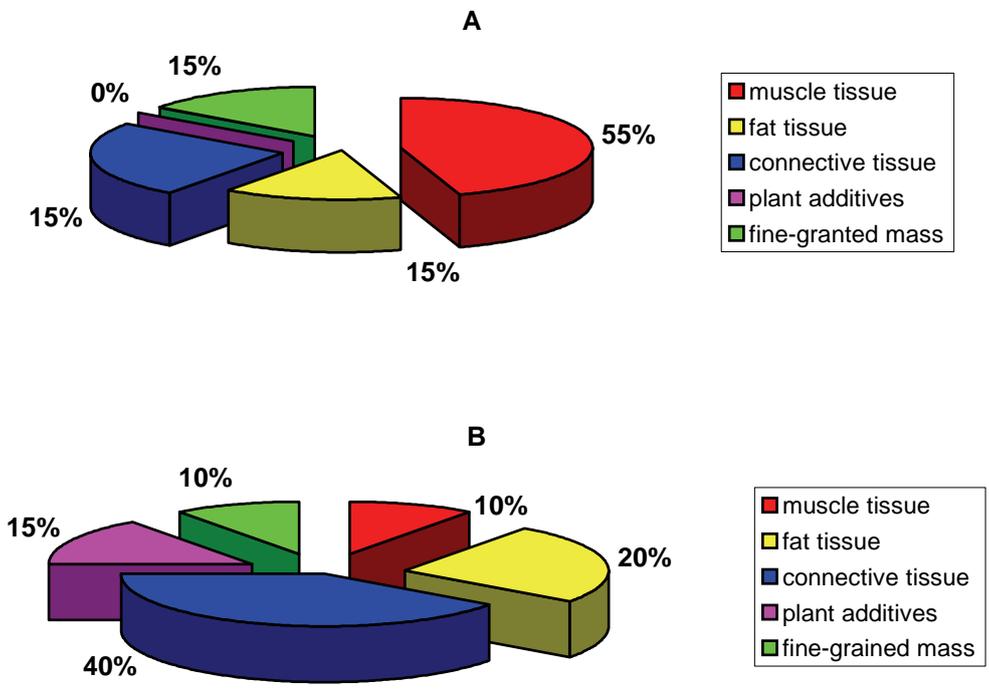


Fig. 2 Composition of raw materials in a quality and adulterated mechanically deboned poultry meat

DETERMINATION OF THE MAIN CONTAMINATION SOURCES OF ALHEIRA WITH STAPHYLOCOCCUS AUREUS USING A PCR BASE TYPING METHODOLOGY

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Key Words: Alheira, Staphylococcus aureus, contamination, RAPD typing

Introduction

Alheira is a traditional meat product with specific characteristics, both considering its raw materials and its manufacturing technology. It was verified in previous works the occurrence of a high contamination rate by *S. aureus* (Esteves et al, 2000).

The study of the occurrence of a microorganism throughout a production line, determines with some accuracy the main sources and ways used in the contamination of that food product (Samelis and Metaxopoulos, 1999).

Works by several authors (Aguado et al, 2001; Vogel et al, 2001; Peccio et al, 2003), have shown that the molecular typing methodologies may bring an important contribution in determining the food contamination source by specific microorganisms. The molecular typing methodology is applied with the purpose of identifying different strains (Struelens, 1998), making it possible to establish with greater accuracy the contamination sources and ways used by bacteria to the contamination of the final product.

Objectives

The aim of the present work was to identify the source(s) and the way(s) used by *S. aureus* in the Alheira's contamination, using different methodologies. The study of the occurrence of *S. aureus* throughout a food product's production line, and PCR based typing methodology applied to isolates obtained from different locations at the production line.

Methodology

Experimental design

It was followed the production of 16 lots of Alheira. *S. aureus* was isolated from various locations along the production line: samples of raw materials and condiments (poultry -P, pork -R; bread - B; gut - T; gut desanting water - WI; spices - E); swab of different contact surfaces (table - I; reservoir - Q; equipment - S; handler's hands - M); product in several stages of manufacturing (cooked meat - Z; batter before the

stuffing - L); water from final washing (WII) and finished product (H), in a total of 14 different sampling points. For each situation, four samples were considered. It was obtained 170 isolates for posterior molecular typing.

Randomly Amplified Polymorphic DNA (RAPD), and Repetitive Element PCR Typing (rep-PCR), were the PCR based methodologies used. A preliminary study was carried out, testing 5 primers in a sub-collection of 15 isolates of *S. aureus*, with the purpose of choosing the primer which offers the best results, considering the objectives of the current work.

S. aureus search

The search was made in 10 g, 10 ml or in 100 square cm of samples or the contact surfaces respectively. Samples were homogenised in peptone (0.3%) and NaCl (0.85%). After pre-enrichment in Chapman broth (Difco) presumptive *S. aureus* were isolated in Baird Parker Agar (Difco) supplemented with egg yolk tellurite and sulfametazine. Typical colonies were confirmed by Gram staining and the tube coagulase test was performed using coagulase plasma rabbit (BBL TM).

Molecular typing

DNA extraction from pure culture was made using Dneasy tissue kit (Quiagen), basically according to manufacturer indications. The lysis step was adapted to improve the final DNA yield. Lysosyme (5µg/µl), lysostaphine (0,03µg/µl), and mutanolysine (0,1 U/µl) (Sigma) were added to the lysis buffer. Samples were incubated (30 min, 37°C) to ensure an efficient lysis. Quantification and DNA purity evaluation were done by spectrophotometry using GeneQuant pro RNA/DNA Calculator (Amersham Pharmacia Biotech., 80-2109-99).

PCR mixture (25 µl) consisted of: 2.5 µl of Buffer (10x) (200mM Tris-Hcl, pH 8.4, 500m MKCl) (Invitrogen); 1.5 mM of MgCl₂ for the primer M13R2 and 3.5 mM for the others (Invitrogen); 200 µM dNTP (Roche Molecular Biochemical); 20 picomoles each primer (Tib Molbiol Syntheselabor); 1 U of Taq DNA polymerase (Invitrogen); 1 µg of genomic DNA.

PCR reaction was performed in a Thermal Cycler (Perkin-Elmer Corp. 2700) as follows: initial denaturation at 95°C for 5 min; 40 cycles with denaturation at 95°C for 0,30 s, primer annealing for 1 min (temperatures for each primer: M13R2- 38°C; ERIC1R- 65°C; ERIC2- 68°C; BOXA2R- 65°C; BOX1R- 68°C), and primer extension at 72°C for 1,30 min; followed by a final extension at 72°C for 5 min and cooling to 4°C.

PCR products were resolved by agarose gel (1.5%) electrophoresis (80 mA, 90 minutes), in 0.5x Tris-acetate- EDTA buffer stained with 0,1 µg/ml of ethidium bromide (Sigma). The profiles obtained were observed under UV light, and the image was captured by a video camera.

Data analysis

Band profiles were analysed using Bio Profile software. Reproducibility was tested on 10% of the isolates. The similarity of the DNA patterns was estimated by Dice coefficient and clustering was achieved by unweighted pair group method using arithmetic average linkage (UPGMA) using NtsysPC 2.1software. It was considered as a cluster formation limit a 95% similarity. A cluster was defined with at least a group of three isolates.

Results & Discussion

The number of samples with *S. aureus* is presented in table 1. We can point out the diversity of sampling point, including raw materials, contact surfaces and product in the various fabrication stages where *S. aureus* was isolated. This indicates the possibility of the existence of a continuous product contamination, throughout its manufacture, as well as the possibility of the occurrence of cross contaminations. The increase of the occurrence of *S. aureus* from the early stages of fabrication (cooked meat - Z) to the final product (Alheira - H) could be reflex of the contamination throughout the manufacturing operations, or the occurrence of environmental conditions favourable to the growth of the microorganisms eventually present.

Considering the spread occurrence of *Staphylococcus* in the processing environment, the simple search of the bacteria does not seem to be elucidative about the main contamination sources and ways used by *S. aureus* to contaminate the Alheira. Viewing the possibility of the simultaneous existence, along the production line, of more than one strain of *S. aureus*, the distinction between isolates in the final product and in several point along the production line, through molecular methodology, will allow to determine the used source and way of contamination (Khambaty et al.,1994). Between the five tested primers, M13R2 was chosen because it originated very clear and only moderately complex band profiles, with a good discriminative power.

Figure 1 represents the dendrogram resulting from the analysis of profiles obtained from the amplification of 170 isolates of *S. aureus*, using the M13R2 primer. The use of this primer lead to the formation of 42 different band profiles that resulted in the formation of 10 different clusters. 26% of the isolates were not included in any cluster. Clusters B and F, which include 46% of all the typed isolates, were the predominant band profiles.

Considering the elements of each clusters and the sampling points they come from, it was verified that the same pattern was observed in isolates from several locations of the production line, except for clusters E and J which include exclusively isolates from poultry (P) and pork (R), indicating that raw material presents characteristic patterns not found elsewhere in the production line, result in agreement with Lam and collaborators (1995).

Table 2 shows the several clusters, distributed by the 3 distinct areas throughout the Alheira's production line, as well as the different origins of the typed isolates.

Isolates from clusters H and I belong to raw materials reception area (samples of pork-R and poultry- P) and processing area (handler's hands -M and different stages of the Alheira's manufacturing, cooked meat - Z and batter before stuffing - L).

Fresh meat could never be a primary contamination vehicle, considering it is cooked before its addition in to the batter. The presence of isolates from these clusters in different production stages might be explained by the occurrence of cross contamination, probably throughout handlers, once the came RAPD pattern was also observed in their hands.

Clusters B, D and F present isolates from each one of the three areas. In the raw materials reception area, there are isolates originated from raw material (P and R), as well as in gut (T and WI). In the processing area they are found in the handler's hands (M), in some equipment's surfaces (Q) and in some of the analysed manufacturing stages (Z e L). The isolates from these clusters in the final product (H) might result both from cross contamination with raw material, where the handler seems to have an important role as a vehicle, or directly through contaminated gut.

Analysing the distribution of clusters A and C, we can verify that they are constituted only by isolates from processing and final product area. The fact that the occurrence of these patterns is not related to the raw materials means that its origin may be established specifically within the working environment. From the analysis of cluster C, we can say it is totally constituted by isolates from the handler's hands (M) and final product - Alheira (H), belonging to the same production lot (not presented data), which implies as primary origin of *S. aureus* the handler's hands.

Conclusions

The presence of *S. aureus* in Alheira might result both from cross contamination with raw material and gut. It was also shown that the handler have an important role has vehicle for cross contamination, and, it was also observed that they might be the primary source of these microorganisms.

Considering the objectives of this work, we may also conclude that the use of the RAPD molecular typing methodology, using the M13R2 primer, lead to a vast and extremely valid amount of information, concerning the sources and ways of contamination used by *S. aureus* in the contamination of Alheira, thus making it easier to establish strategies directed towards the control of this microorganism in this product.

Acknowledgment

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Tables and Figures

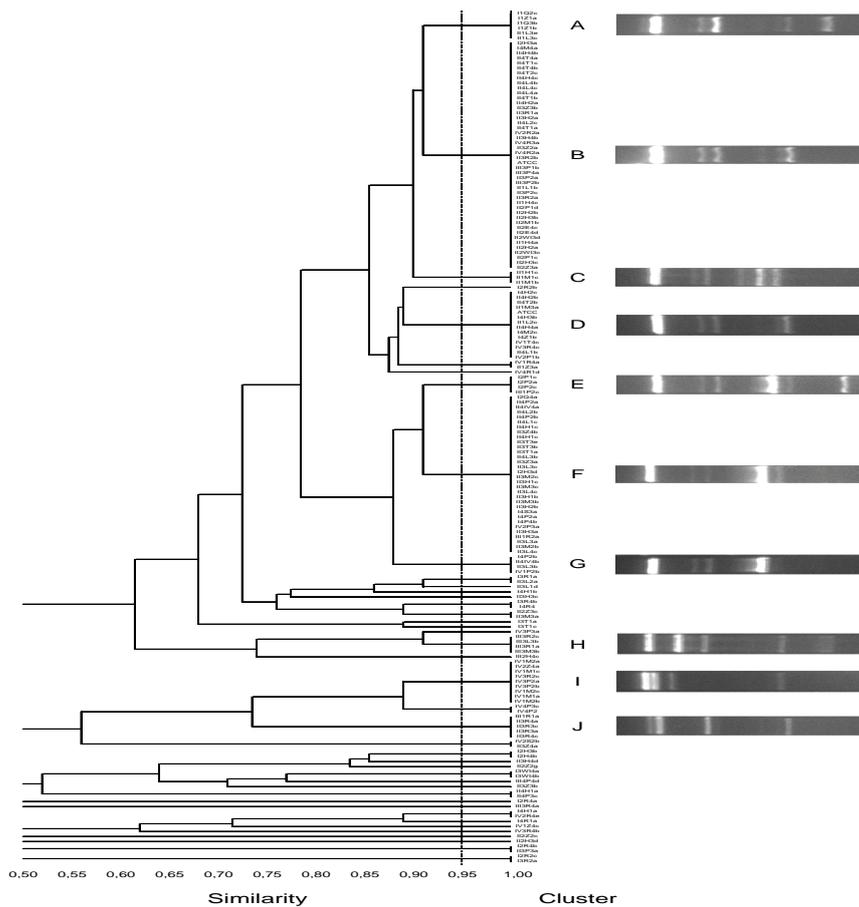
Table 1. Percentage (number) of samples with *S. aureus*

Sampling location (n=64)	Presence of <i>S. aureus</i> % (n)	
Water (WI)	6	(4)
Gut (T)	13	(8)
Bread (B)	5	(3)
Pork Meat (R)	50	(32)
Poultry Meat (P)	44	(28)
Spices (E)	2	(1)
Equip. Surf. (S)	3	(2)
Table Surf. (I)	-	-
Tank Surf. (Q)	6	(4)
Hand Surf. (M)	16	(10)
Cooked Meat (Z)	25	(16)
Dough (L)	22	(14)
Water (WII)	2	(1)
Alheira (H)	34	(22)
Total (n=896)		

Table 2. Clusters distributed by the 3 distinct areas throughout the Alheira's production line, as well as the different origins of the typed isolates.

Areas in production line	Isolates origin - Sampling location	Clusters
1 Raw materials and condiments reception area	WI; T;	B; D; F
	B; R; P; E;	B; D; E; F; G; H; I; J;
2 Processing area	S; Q;	A; F;
	M;	B; C; D; F; H; I;
	Z;	A; B; D; F; I;
	L;	A; B; D; F; G; H;
3 Finished product area	H;	B; C; D; F;

Figure 1. Dendrogram resulting from the analysis of profiles obtained from the amplification of 170 isolates of *S. aureus*, using the M13R2 primer.



DEVELOPMENT OF A METHOD TO MONITOR REAL-TIME THERMAL INACTIVATION OF PATHOGENS IN MEAT AND POULTRY PRODUCTS

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Introduction

This work is part of an overall research program, which is aimed at developing information and tools to ensure/improve the safety of meat and poultry products. Previous and current work by our research team (Warsow et al. 2003) has demonstrated the potential for contamination in marinated turkey breast when *Salmonella* penetrates intact, whole muscle during vacuum tumbling marination. Moreover, once *Salmonella* gained access to the inner portions of the muscle, we observed an enhanced thermal resistance as compared to the ground product (Orta-Ramirez et al. 2005). Although our previous work was designed to evaluate the degree of penetration and to quantify the effects of product structure (whole-muscle vs. ground) on thermal resistance of *Salmonella*, it did not evaluate “how” and “why” these phenomena occur. In order to develop the most effective intervention approach, we need to elucidate the mechanisms of pathogen penetration and enhanced resistance in these marinated whole-muscle products.

Pathogen migration into intact, whole-muscle products and the thermal resistance of these pathogens are clearly relevant to a wide and growing market sector of marinated products, ranging from value-added fresh retail products to ready-to-eat foodservice products. If pathogens are indeed able to migrate into vacuum marinated products and consequently exhibit enhanced thermal resistance, then the ramifications might be significant; the common assumption that intact products have pathogen-free interiors undoubtedly affects attitudes and behaviors associated with preparing these products for consumption. In fact, the Food Safety Inspection Service (FSIS) has distinguished between intact beef cuts (e.g., steaks, roasts) and non-intact products that have been “...injected with solutions, mechanically tenderized by needling, cubing, or pounding devices, or reconstructed into formed entrees,” when establishing policies regarding *E. coli* O157:H7 contamination (FSIS, 1999a). In doing so, FSIS also stated (FSIS, 1999a), “In these intact cuts the interior remains protected from pathogens that may exist on the exterior. It is highly unlikely that pathogens would migrate below the surface.” Although this is clearly a widely accepted assertion, our preliminary work has indicated that pathogens can indeed migrate into the interior of intact products, particularly if the product is subjected to vacuum tumbling.

With regards to thermal resistance of pathogens, FSIS recently established lethality performance standards for ready-to-eat (RTE), whole-muscle meat and poultry product (FSIS, 1999b), and has proposed to extend these standards to all RTE meat and poultry products (FSIS, 2001). These regulatory standards essentially state that a process must achieve a 6.5-log₁₀ or 7.0-log₁₀ reduction in *Salmonella* for meat and poultry products, respectively. Processors are no longer held to specific endpoint temperatures; however, they “must validate new or altered process schedules by

scientifically supportable means” (FSIS, 1999b). This new regulatory paradigm puts significant pressure on the industry to document process lethality for any new product or process.

This problem is also economically important, given that the meat and poultry industry is the largest component of U.S. agriculture, contributing over \$116 billion in annual sales to the GNP (AMI, 2003). Consumer trends for RTE products suggest continued rapid growth in this particular category (Russell, 2002), which includes a lot of marinated products. Therefore, given the economic importance of RTE products, there is an urgent need for information and tools that will enable the industry to design and operate value-added processes that ensure the safety of marinated, whole-muscle products.

Objectives

Based on previous work and preliminary results, our hypothesis is that *Salmonella* cells follow a non-random penetration pathway and exhibit preferential attachment within whole muscle after marination, which results in greater heat resistance than in ground products during thermal processing. To test this hypothesis, the overall goal of our research is to document, at the microscopic level, the penetration, attachment, location, and distribution of *Salmonella* within the muscle after marination as well as the thermal inactivation using confocal laser scanning microscopy (CLSM). The specific objectives are:

1. To determine the relationship between bacterial thermal inactivation and fluorescence loss using fluorescent-labeled bacterial strains
2. To test a custom-designed/built heating apparatus that can be operated under the confocal microscope to visualize thermal inactivation of bacteria using CLSM

The overall novelty of this project is our plan to visualize the destruction of pathogens in whole-muscle products, during thermal processing, by using a powerful microscopic technique. This project will make a unique contribution to the literature and to the safety of whole-muscle meat and poultry products, as this is the first project (to our knowledge) to study the thermal inactivation of bacteria on a cell-by-cell basis.

Methodology

Bacterial Cultures

Although the ultimate target of study is *Salmonella*, for the development of this technique we used Green Fluorescent Protein (GFP)-labeled *E. coli* O157:H7 that was readily available to us. GFP-transformed *E. coli* O157:H7 E318 (GFP-ECO157:H7) was obtained from Mansel Griffith, University of Guelph, Ontario, Canada. The stock culture was maintained at -80°C in water with 10% glycerol (Seo and Frank 1999). Cultures were activated by streaking on tryptic soy agar (TSA) containing 100 µL ampicillin per mL (TSA-amp) and incubation at 37°C for 24 h. To prepare the *E. coli* suspension for inoculation, 10-mL sterile deionized water was added to each of two TSA-amp plates and colonies were disrupted with a sterile bent glass rod. Culture controls for CSLM were prepared by building a round well of petroleum jelly on a clean microscopy slide with a syringe, adding 0.5 mL of bacterial suspension, then

positioning a coverslip with enough pressure to adhere to the petroleum jelly, therefore sealing the sample.

Meat Preparation

Turkey breasts and beef roasts were purchased from a local processor. Muscle-to-muscle variability should be minimal with these cuts and we assume animal-to-animal variability will not influence muscle structure. Immediately after receiving the meat, it was trimmed of excess fat, cut into appropriate sized roasts, vacuum packaged, and rapidly blast frozen (-28°C) at the Michigan State University pilot meat processing facility. A frozen roast or breast was partially thawed and one core was aseptically removed (cross section) using a sterile stainless steel corer (1 cm diameter). With a sterile knife, we cut slices (1-2 mm), mounted them on 2.5 cm diameter plastic dishes (Decagon, Pullman, WA) and dispensed petroleum jelly around the outer edges of the sample with a syringe. GFP-ECO157:H7 suspension (0.5. mL) was added to the slice and a coverslip was positioned to seal the sample. All mounted samples were placed in Petri dishes to provide additional containment while examined with CSLM.

Confocal Scanning Laser Microscopy

Samples were observed using a LSM 5 Pascal (Carl Zeiss, Inc., Thornwood, NY) under a 40x dry objective with an excitation wavelength of 488 nm. Random locations from each slice were sampled for penetration and attachment and to avoid photobleaching, no more than 5 fields per location were examined (Vodovotz et al. 1996, Prachaiyo and McLandsborough 2000).

Results & Discussion

Visualization of GFP-E. coliO157:H7 in Inoculated Whole-Muscle Turkey Breast and Beef Roast

GFP-expressing *E. coli*O157:H7 in deionized water (culture controls) were easily visualized as fluorescent rods (Fig. 1). CSLM images of inoculated turkey muscle before and after excitation at 488 nm (Fig. 2A and B, respectively) allowed visualization of certain muscle structures (muscle fibers, fat globules) while bacterial cells were easily recognizable.

However, in order to quantitatively describe the relationship between tissue structures and the location of bacteria within the muscle, it is necessary to more objectively analyze the images. Therefore, we applied several image enhancement tools to demonstrate that we could utilize these types of images to achieve the objectives of the proposed project. First, Gaussian and smoothing (3x3) filters were applied to the non-excited image (Fig. 2C), in order to highlight the image features associated with the interfaces between muscle fibers, and eliminate extraneous background information (such as the out-of-focus regions that show up as large black dots in Fig. 2A but are eliminated in Fig. 2C). The image processing results in Fig. 2C have not been optimized, in terms of achieving the maximum differentiation of image features; however, the preliminary results clearly show that the tissue features can be enhanced, relative to the original image. Subsequently, the excited image (Fig. 2B) was subjected to a basic thresh holding process in the green color band and then laid over the unexcited image to yield Fig. 2D, which shows that clusters of the bacteria

are clearly “aligned” near the tissue features highlighted in Fig. 2C, rather than being randomly distributed across the sample. The purpose of these images is to demonstrate that basic image processing techniques can be used to enhance the utility of the microscopy results. Once the image processing technique has been optimized, it will be possible to conduct statistical analyses of the image files (which are just matrices of numerical information) in order to determine a correlation between muscle fiber interfaces and location of the bacteria.

Thermal inactivation experiments

A unique, custom heating apparatus has been designed and built (and it is currently under testing) to hold the small sample “discs” in place on the microscopy stage (Fig. 3). The heating apparatus consist of a heating element (~3 cm diameter) placed on a 100 x 20 mm glass Petri dish. The electrodes connect to this heating pad through holes on the dish wall, and a fine-gauge thermocouple is also placed through the side of the Petri dish to maintain accurate readings of the sample temperature. The electrodes are attached to a power source via a PID electronic controller. The control is programmed as a variable power source, so that the temperature can be reached at different time periods and then can be held at the target temperature. When the sample is placed inside the Petri dish, the top can then be placed over to contain the inoculated sample. This apparatus enables precise control of sample temperature ($\pm 0.5^{\circ}\text{C}$), as the sample is heated and images are collected.

Because “macroscopic” isothermal inactivation tests are being conducted in a separate leveraging study, this heating unit is not designed to generate data for estimating thermal inactivation parameters. Rather, the primary goal is to observe, document, and quantify any effect of bacterial location on the time-to-inactivation. Therefore, the computer program is designed to produce non-isothermal heat treatments, consisting of a linear increase in temperature of $\sim 8^{\circ}\text{C}/\text{min}$ and three endpoint/holding temperatures (55, 60 and 65°C). This apparatus is currently fully operational, and experiments are being conducted to determine the relationship between bacterial thermal inactivation and fluorescence loss of GFP-labeled bacterial strains during cooking.

Conclusions

The results of this experiment confirm CSLM as a powerful technique to study the interaction of bacteria and muscle structural components during thermal inactivation. Only viable bacteria are visible on the microscopy images, thus, by heating an inoculated meat sample in place on the microscope stage, and collecting a time series of images without moving the sample, this method will be able to track when individual cells cease to fluoresce and are therefore inactivated. Overall, utilization of the new apparatus will provide fundamental information impacting pathogen penetration and enhanced thermal resistance in marinated meat and poultry products, with these findings eventually leading to a series of practical intervention strategies that can be applied in industry.

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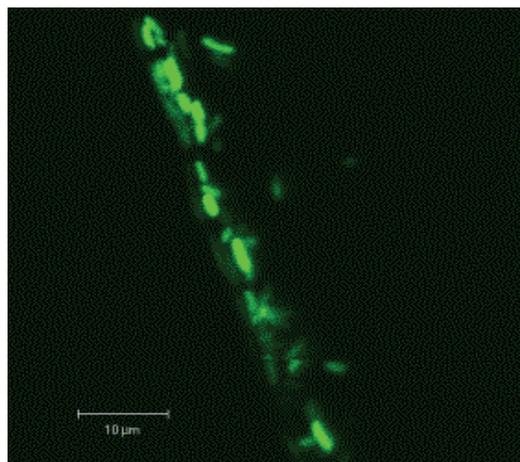
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Acknowledgements

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Tables and Figures



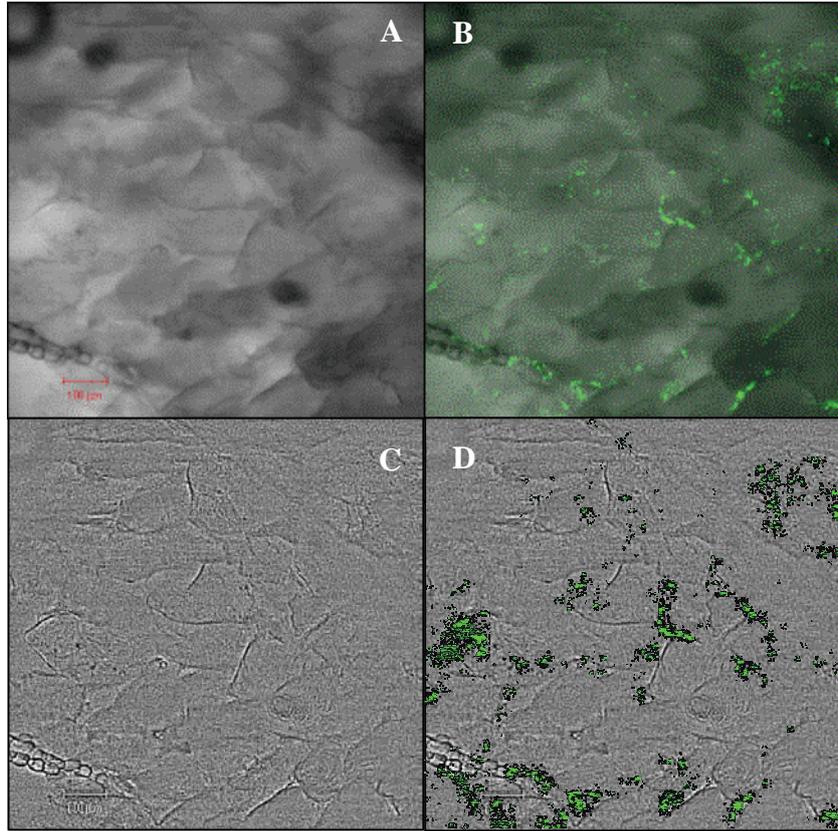


Figure 2. CSLM complementary images of surface-inoculated turkey breast before (A) and after (B) excitation at 488 nm, and after image enhancement of (C) the transmitted image and (D) the excited image. GFP-transformed *E. coli* O157H7 are colored fluorescent green.

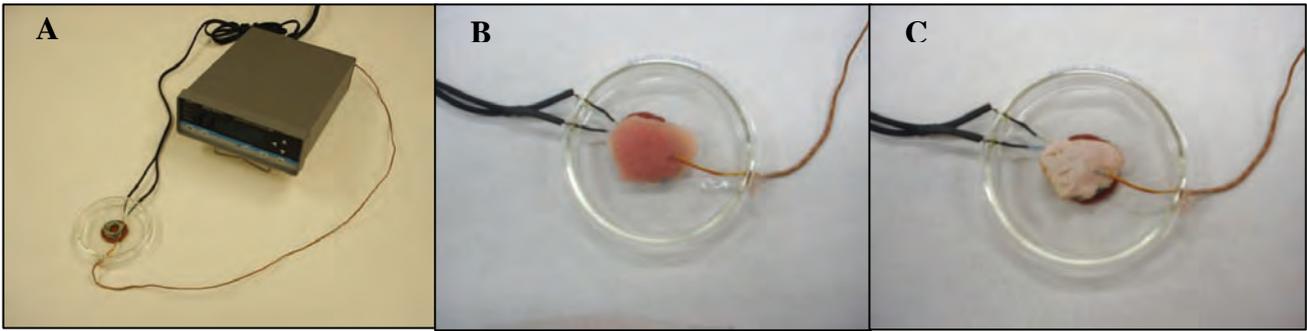


Figure 3. A custom-built apparatus allows cooking of meat samples under the microscope: (A) overview of heating unit and controller, (B) the raw meat is placed on the heating pad, and (C) the meat is cooked under contained conditions

PHOSPHOGLUCOMUTASE AS A NOVEL MEAT ALLERGEN

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Key Words: meat safety, meat allergy, phosphoglucomutase, serum albumin, cross-reactivity

Introduction

The prevalence of chicken allergy ranges from 0.6 to 5% in food allergic subjects (Table 1). Food allergy is triggered by allergic proteins (allergens) that bind to the patients IgE antibodies on mast cell surfaces. Although there are several allergens, chicken serum albumin has been implicated as the major chicken allergen. However, we detected unknown allergic band migrating at 58 kDa.

Objectives

The aim of this study was to identify the 58 kDa allergen and to clarify its properties. As a result, in this study, phosphoglucomutase (PGM) was identified as a novel chicken allergen. Thus, we evaluate whether bovine and porcine PGMs were also recognized by the patients IgE antibodies or not. The possible cross-reactivity between serum albumin and PGM was also investigated.

Materials & methods

Patients

Serum samples were obtained from 8 patients allergic to meat with atopic dermatitis. Meat allergy was proved by clinical symptoms and a positive radioallergosorbent test. Informed consent was obtained from all donors. Sera from two non-allergic adults were also used as negative controls. Serum samples were used after appropriate dilution with 0.1 % Tween 20 containing PBS (PBS-T).

Meat extract

Chicken, beef, and pork thigh meat were obtained from market source. Five grams of each meat was homogenized in 15 mL of 40 mM potassium phosphate buffer (pH 7.2) using a polytron homogenizer (Kinematica, Switzerland). The homogenate was centrifuged at 10,000 g for 20 min at 4 °C and the supernatant was filtered through filter paper (extract). Protein concentration of the extract was measured using a commercial kit (Bio-Rad, USA).

Western-blotting

Meat (chicken, beef, and pork) extract or fractions on DEAE-cellulose column chromatography (described below) were electrophoresed in 10 % polyacrylamide gels by the usual method and blotted onto polyvinylidenedifluoride (PVDF) membranes (Immobilon-P, Millipore, USA). Since patient IgE antibodies reacted strongly with BSA but not with human serum albumin (HSA), the membrane was soaked in a 1 % HSA (Sigma, USA)-PBS-T solution for blocking, instead of using BSA, which is frequently chosen. The membrane was then incubated with five-fold diluted pooled sera for 1 hr at room temperature and sequentially with biotinylated anti-human IgE (Kirkegaard & Perry Lab. Inc., USA) for 2 hrs at room temperature. The membrane was further incubated with avidin-DH and biotin-conjugated HRP (VECTORSTAIN ABC-PO kit, Vector Laboratories, USA) according to the manufacturer's protocol, and was finally stained with a diaminobenzidine substrate kit (Vector Laboratories). Separately, proteins were stained with coomassie brilliant blue (CBB; Bio-Rad).

For inhibition western-blotting analysis, the patient sera (2 ml) were preincubated with 2 mg of phosphoglucomutase (PGM, Sigma, USA) for 1 hr at room temperature, and then applied to the same analysis.

DEAE cellulose column chromatography

The chicken extract was put on a column (5x18 cm) of DEAE-cellulose (Wako Pure Chemical Industries, Japan) that had been equilibrated with 10 mM Tris-HCl buffer (pH 7.2). Elution with a linear gradient of 1 M NaCl in the same buffer was performed. Proteins were monitored by the UV absorbance at 280 nm. Fractions 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, and 36-40 were collected and applied to western-blotting as described above.

Determination of N-terminal amino acid sequence

The electroblotted proteins on PVDF membrane were stained with CBB. The bands of interest at 58 kDa were excised, and then the N-terminal amino acid sequences were analyzed by a G1005A Protein Sequencer (Hewlett Packard, USA).

ELISA

ELISAs were performed by the usual method. Briefly, 100 μ l of the meat extracts (0.025 mg/ml) or protein (PGM, BSA or GAPDH, Sigma) solutions (0.05-0.1 mg/ml) were coated on the well. After blocked with 1 % HSA in PBS, the appropriately (10-40 fold) diluted serum samples were applied to the well. For inhibition ELISA assay, proteins (BSA or PGM, 0.05-0.1 mg/ml) were added to serum samples as inhibitors prior to applying to the well. The binding of IgE-antibody to plate-coated antigen was determined by sequential incubations with biotinylated anti-human IgE, streptavidin-peroxidase conjugate (Boehringer Mannheim, Germany), and *o*-phenylenediamine (Wako). The values in Fig. 4 are expressed as relative ELISA values based on the absorption at 492 nm. Percent inhibition in Fig 3 and Table 2 was calculated as $(1-A/B)\times 100$, where A is the ELISA value of serum preincubated with inhibitor, and B is that of the control.

Results & Discussion

1. Phosphoglucosmutase (PGM) as a novel chicken allergen

Chicken extract was applied to SDS-PAGE, and the separated proteins were stained with CBB (Fig. 1A). Western-blotting analysis was also performed using patients IgE-antibodies (Fig. 1B). Several IgE-positive bands were detected; among them, previously-known allergens, such as chicken serum albumin (CSA), fructose-bisphosphate aldolase (FBPA), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), were identified according to their molecular masses. In addition, as shown in Fig. 1B, we detected unknown allergic band migrating at 58 kDa. Regretfully, we failed to reveal the N-terminal amino acid sequence by using a protein sequencer because of the low yield and/or the impurity of the sample.

Thus, chicken extract was first applied to DEAE-cellulose column chromatography as indicated in Fig. 2A. Fractions 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, and 36-40 on this chromatography were collected and applied to western-blotting. Since the 58 kDa protein was eluted mainly in fraction 11-15 (Fig. 2B, arrow), it was excised, and then the N-terminal amino acid sequence was analyzed again. As a result, the sequence was revealed to be VHIETVKTKA that is completely identical to the N-terminal amino acid sequence of chicken phosphoglucosmutase (PGM). Thus, we identified PGM as a novel chicken allergen.

2. Bovine, porcine and rabbit PGMs are also allergen for meat allergic patients

Rabbit PGM was also recognized by the patient IgE antibodies; PGM was judged to be a major allergen, since, among eight patients, six were reactive to rabbit PGM (Fig. 4, *left*). For further studies, we used rabbit PGM, since, only rabbit one was available from the market source.

By the same western-blotting analysis of beef and pork extract, we demonstrated that bovine and porcine 58kDa proteins (probably PGMs) were also allergic to meat allergic patients. In inhibition ELISA, the binding of IgE-antibody to beef, pork, and chicken was inhibited by the preincubation of serum with rabbit PGM (Fig. 3). Thus, we concluded that bovine and porcine PGMs were also allergen for meat allergic patients. This result also supported by the result of inhibition western-blotting analysis (*data not shown*).

3. The cross-reactivity between PGM and serum albumin

Next, we compared the IgE-binding abilities of PGM and previously-known allergens such as bovine serum albumin (BSA) and glyceraldehydes-3-phosphate dehydrogenase using eight individual serum samples (Fig. 4). The pattern of recognition by individual IgE antibodies was very similar between PGM and BSA; that is, patients who recognized PGM strongly, reacted also BSA strongly. Then, inhibition ELISA was performed to clarify whether there would exist the cross-reactivity between PGM and BSA. It was proven that the binding of IgE-antibody to PGM or BSA was inhibited by the preincubation of serum with BSA or PGM each other (Table 2). This fact clearly indicated that there surely exist the cross-reactivity between these two allergens.

Conclusions

We first identified phosphoglucomutase as a novel meat allergen and demonstrated its cross-reactivity with serum albumin, the major meat allergen. This finding would contribute greatly to the elucidation of meat allergy.

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Tables and Figures

Table 1. Prevalence of chicken allergy

Country (Subjects)	%	references
France (544 food allergic children)	0.6	Rance et al., 1999
USA (40 food allergic children with AD)	5	Sampson and Albergol 1984
South Africa (112 children with AD)	3.8	Steinman and Potter 1994
Switzerland (383 food allergic patients)	2.3	Etesamifar and Wuthrich 1998

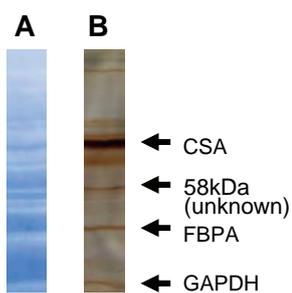


Fig. 1 SDS-PAGE analysis of chicken extract
(A) CBB staining, (B) immunostaining with patients IgE-antibodies

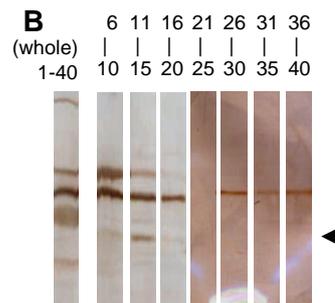
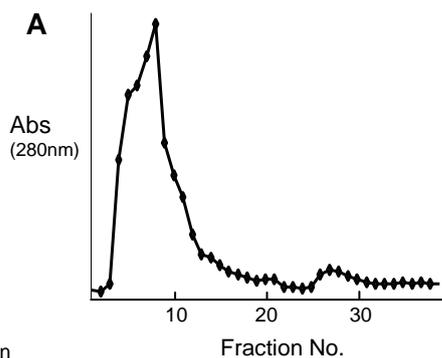


Fig. 2 DEAE-cellulose column chromatogram of extract and western-blotting analysis of its fractions using IgE-antibodies of chicken allergic patients

(A) Chicken extract was applied to DEAE cellulose column that had been equilibrated with 10 mM Tris-HCl buffer (pH 7.2). Elution with a linear gradient of 1 M NaCl in the same buffer was performed (Fr. No. 1-40). Proteins were monitored by the UV absorbance at 280 nm.

(B) The obtained fractions were applied to western-blotting. The 58 kDa band in Fr. 11-15 was excised, and then the N-terminal amino acid sequence was analyzed by a Protein Sequencer.

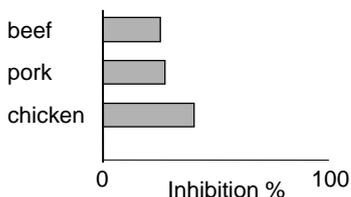


Fig. 3 Inhibition ELISA of beef, pork, and chicken extract using rabbit PGM as an inhibitor

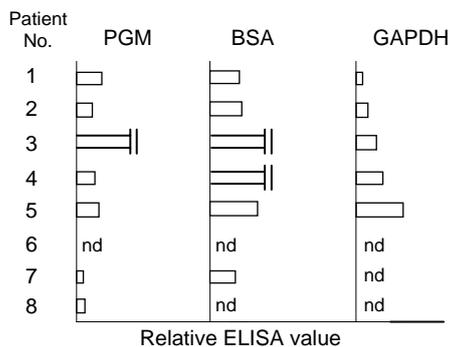


Fig. 4 IgE-binding abilities of PGM, BSA, and GAPDH
PGM, BSA, or GAPDH (10 µg/well) were coated on the well. After blocked with 1 % HSA in PBS, 10-fold diluted individual serum samples were applied to the well. The binding of IgE-antibody to coated antigen was determined.

Table 2 Crossreactivity between PGM and BSA

inhibitor	antigen coated on the well	
	PGM	BSA
PGM	96%	83%
BSA	92%	93%

PGM or BSA (0.1 mg/mL at a final concentration) was added to pooled serum samples diluted 10-fold in PBS-T. After 1 hr preincubation at 37°C, the PGM-serum or BSA-serum mixture was added to polystyrene microtiter plates coated with BSA or PGM (5 µg/well) and blocked with 1% HSA in PBS. Serum not treated with BSA or PGM was used as a control. The binding of IgE-antibody to PGM was determined.

Muscle Biology and Biochemistry

**PRENATAL DETERMINATION OF FAT AND COLLAGEN CONTENT
IN PORCINE MUSCLES**

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Key Words: Fetal origins; Connective tissue; Collagen; Fat; Pigs; *M. semitendinosus*.

Introduction

The early fetal environment plays a key role in development of skeletal muscle. Several studies have shown that intralitter variation, *in utero*, is reflected in the postnatal growth of skeletal muscle in the pig (Dwyer et al., 1994). It is well accepted that muscle growth is influenced by the number, size and type of the muscle fibres. Marked differences in fibre number have been demonstrated between the smallest and largest porcine littermates prenatally, with the smallest littermate having significantly fewer fibres than the largest littermate (Wigmore & Stickland, 1983).

Pilot studies carried out in our laboratory have indicated that the smallest littermate may also have a higher proportion of connective tissue in its muscle (Clelland, 2001). Muscle connective tissue provides structure to the muscle and is composed of ground substance, fibres and connective tissue cells (Purslow, 2002). A proportion of these elements of the connective tissue comprise collagen and fat deposits. These are important parameters to the meat industry as an increased amount of these components may impact on meat toughness and intramuscular fat respectively, and so influence the resultant meat quality.

Objectives

The primary objective of this study was to investigate the prenatal development of intralitter variation in content of fat, collagen and myosin (a major contractile protein found in skeletal muscle) of muscles. In the pig, it can be argued that differing levels of nutrition received, *in utero*, are a major cause of the observed intra-litter variation. The smallest and largest littermates were chosen and the content of fat, collagen I and myosin (embryonic) were analysed in the *M. semitendinosus* of both. This investigation will determine whether the differences in fat, collagen and myosin expression have a fetal origin.

Methodology

Fetal selection and preparation

A total of 23 pairs of porcine fetuses from a Large White-Landrace origin were used in this study, aged from 36-86 days gestation. This includes the timing of primary and secondary fibre formation and the ending of myogenesis (muscle formation) (Wigmore & Stickland, 1983). Runts were excluded from the study as outliers (Hegarty & Allen, 1978; Powell & Aberle 1980). The *M. semitendinosus* muscles were snap frozen in liquid nitrogen and stored at -80°C. Complete transverse sections (10µm) were taken from the mid-belly region of the *M. semitendinosus* using a cryostat (Bright, U.K) at -25°C. The sections were mounted on slides. Histochemistry and immunocytochemistry techniques were employed on the frozen transverse sections.

Histochemistry

The extent of fat deposition in the smallest and largest littermates was determined using Oil Red O stain and analysed using the Kontron Image Analysis software (Carl Zeiss, Germany). Analysis of fat deposition was made by measuring the area of stained fat within a unit area (mm²) of muscle. A representative portion (at least 2%) (Clelland, 2001) across the muscle from the deep part to the superficial part was used to perform the analysis.

Immunocytochemistry

An antibody to Collagen I (Sigma Chemical Co., UK) (1:100 dilution) was used to identify collagen fibres within the connective tissue matrix and an antibody to myosin (embryonic) (Developmental Studies Hybridoma Bank) was used to identify this abundant motor protein found in individual fibres of skeletal muscle. A standard antibody protocol was followed (Clelland & Stickland, 2001), using a biotinylated rabbit anti-mouse IgG secondary antibody (1:200 dilution) (Vector Laboratories, CA) common to both antibodies. Slides were analysed using the Kontron Image Analysis software. Analysis was performed by comparing stained component against background within a unit area (mm²) of muscle. Again, a representative portion (at least 2%) (Clelland, 2001) across the muscle from the deep part to the superficial part was used to perform the analysis.

Statistical Analysis

Paired t-Tests were performed on all results from the smallest and largest littermates analysed. Fetuses were grouped from day 36-60 (including the initiation of primary and secondary muscle fibre proliferation) and day 61-86 (including the period of differentiation of fibres and the ending of myogenesis). A P value of ≤ 0.05 was deemed to be of significance.

Results & Discussion

Histochemistry

Fig. 1 illustrates the transverse cryosections from the mid portion of the *M.semitendinosus* stained in Oil Red O solution for detection of lipid (fat) deposition. Qualitatively, more fat deposition is seen in the smallest fetus (Fig. 1a) compared to the largest littermate (Fig. 1b). Fat per mm² was determined for a representative portion of the section. A Paired t-Test showed the smallest littermate had significantly more fat per mm² present than the largest littermate ($P=0.01$) in the group of fetuses aged 36-60 days of gestation (Fig. 2). Also, in the group aged 61 – 86 days of gestation, the smallest littermate exhibited more fat per mm² than the largest littermate ($P=0.04$).

Immunocytochemistry I

An antibody against Collagen I was used (Fig. 3). Qualitatively it can be seen that in Fig. 3a the area of Collagen I in the smallest fetus is more densely packed than in the largest fetus (Fig. 3b). The endomysium is apparent around groups of fibres rather than individual fibres at this stage (60 days of gestation) (Fig. 3a & 3b).

Due to the acetone stage in the protocol only fetuses of 44-86 days of gestation could be analysed as this treatment proved to be too harsh for younger tissue sections. Fig. 4. illustrates Paired t-Test results demonstrating the smallest littermate had significantly more expression of Collagen I area per mm² than the largest littermate in the later stages of fetal life (61-86 days of gestation) ($P=0.008$).

Immunocytochemistry II

An antibody against myosin (embryonic) was used (Fig. 5a & b). Myosin per mm² was determined for a representative portion of the muscle section. A Paired t-Test showed the smallest littermate had significantly less myosin expression per mm² of muscle than the largest littermate ($P=0.04$) in the 36-60 gestational age group and the latter fetal age group (61-86 days of gestation) ($P=0.004$), Fig.6.

The results of the present study demonstrate that an increased proportion of fat and collagen I content was present in the muscles of the smallest littermate compared with the largest littermate, at least in the latter fetal stages. On the contrary, decreased expression of myosin was seen in the smallest littermate compared to the largest, indicating less muscle and more non-muscle may be present. This confirms the initial hypothesis.

The results presented here extend previous pilot studies carried out in our laboratory (Clelland, 2001). The increased non-muscle found in these pilot studies can now be confirmed as including increased fat and collagen I within the connective tissue composition of the smallest littermate. Decreased myosin expression confirmed the smallest littermate contains less muscle protein than the largest littermate.

In the present study it was demonstrated that significantly increased levels of fat per mm² were present in the smallest littermate compared with the largest littermate. The difference was seen at both the late embryonic to mid-fetal stages (36-60 days of gestation) and also the late fetal stages (61-86 days of gestation). A postnatal study by

Powell and Aberle (1981) showed that runts (pigs with severe intrauterine growth retardation) and small pigs have increased amount of intramuscular fat and perirenal fat compared to larger littermates. Fat deposition measured in the present study did not distinguish between intramuscular fat and fat found in adipocytes. However, it had been previously found that a higher number of small diameter adipocytes were present in runts (Powell & Aberle, 1981). Our results are further validated postnatally by Gondret et al, (2005). In their recent study the lightest and heaviest birthweight pigs were analysed at slaughter weight (mean weight of 111.8kg) and the lightest littermates contained higher levels of intramuscular lipid in *M. semitendinosus* compared with the heaviest littermate. Our result clearly demonstrates that the postnatal lipid differences have their origins prenatally.

Collagen is a major component of connective tissue. The fibrous material forms a continuous mesh within the extracellular matrix. Collagen is divided into seven different subtypes. The most important in fetal skeletal muscle is Collagen I (Listrat et al., 1999). In this present study elevated levels of Collagen I were found in the smallest littermate compared to the largest in later fetal life (61-86 days of gestation). Although debatable, collagen content has been linked with meat toughness (Fang et al, 1999). In the work discussed above (Gondret et al, 2005), low birth weight pigs at slaughter exhibited a low score for loin meat tenderness compared with the heaviest littermate (Gondret et al., 2005). These results may indicate a higher connective tissue in the less tender muscles. Our results indicate that differences in collagen content between littermates appear to have a prenatal origin.

Myosin is a major contractile protein found in muscle fibres. The results indicate that lower levels of myosin (expressed per mm²) in the smallest littermate is associated with higher levels of fat and collagen. From previous studies (Wigmore & Stickland, 1983) it is also known that the smallest littermates have fewer muscle fibres. Together these studies demonstrate an influence on muscle at both cell and protein level when littermates are compared.

Conclusions

Arguably, intralitter variation provides us with a good naturally occurring model of differing levels of nutrients reaching the offspring. A variation in blood flow and hence nutrients, as a consequence of good and restricted access from the placenta results in a wide range of littermate sizes within the entire litter (McClaren & Michie, 1960; Warwick, 1928; Perry & Rowell, 1969). Previous studies have investigated the influence of prenatal nutrition, either directly or by studying intralitter variation on muscle development and consequences for postnatal growth (Dwyer et al, 1994). These studies have shown that low nutrition (by either method) impedes fibre number development and correlates with future postnatal growth (Dwyer & Stickland, 1992; Dwyer et al, 1994; Ward & Stickland, 1991; Wigmore & Stickland, 1983). In this study we have shown a prenatal nutritional influence also on the non-muscle components of muscle and, from other work, this appears to continue postnatally. In this way prenatal programming is influencing the longer term postnatal phenotype of muscle.

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Tables and Figures

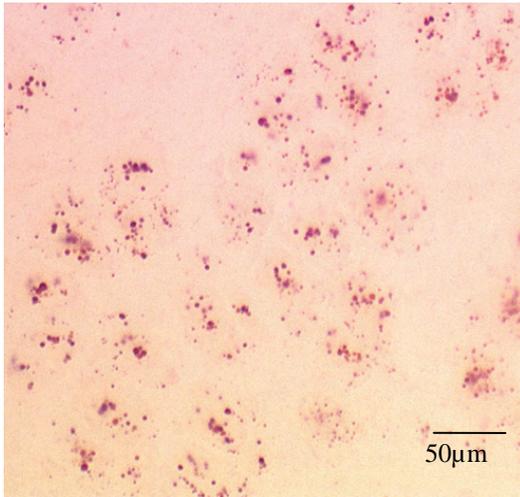


Fig. 1a

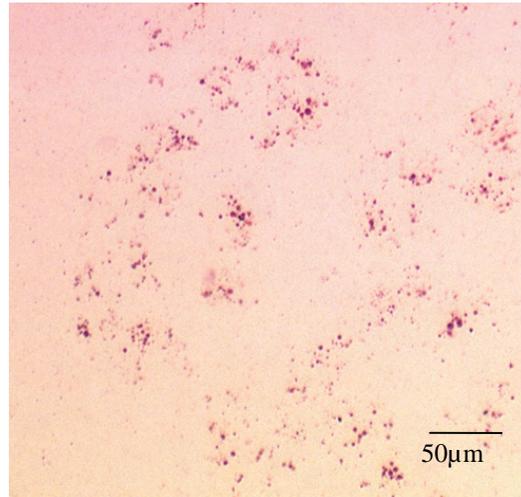


Fig. 1b

Fig. 1: Oil Red O stained sections from fetuses aged 60 days of gestation. a) Smallest littermate. b) Largest littermate.

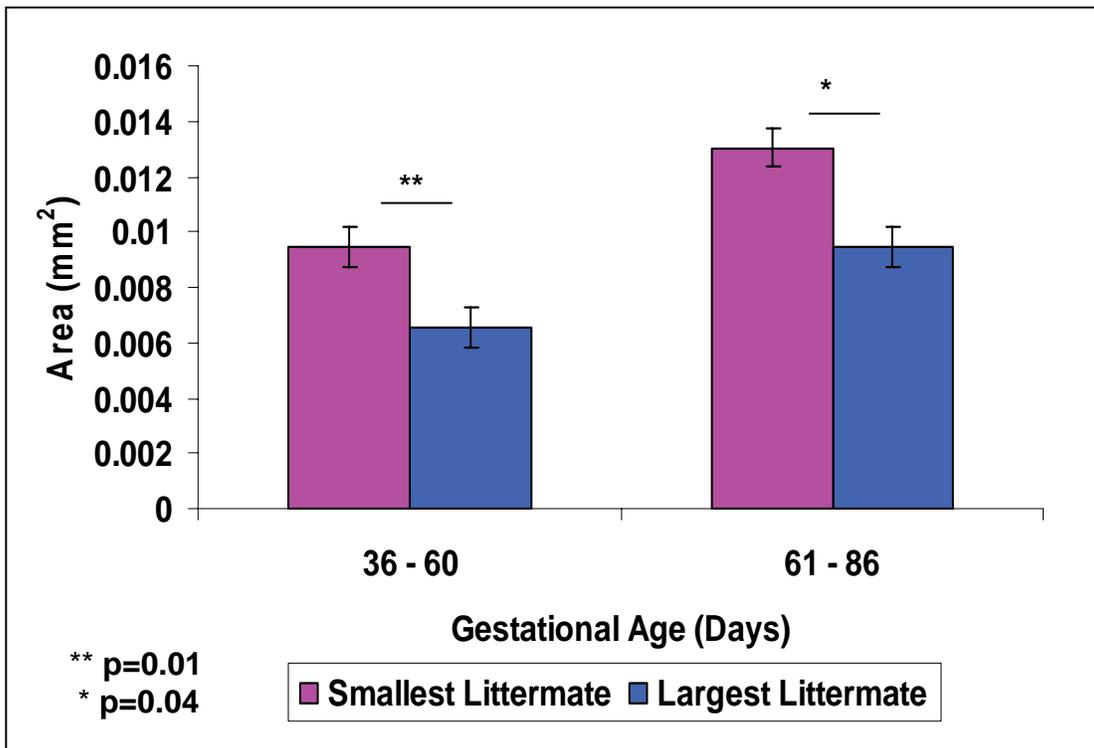


Fig. 2: Fat Area per mm² of Muscle for fetal samples aged 36-60 and 61-86 days of gestation. The smallest littermate has an increased amount of fat present compared to the largest littermate in the 36-60 days of gestation group (P=0.01) and the 61-86 days of gestation group (P=0.04).

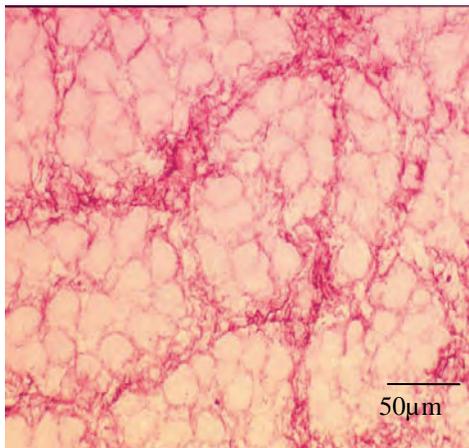


Fig. 3a

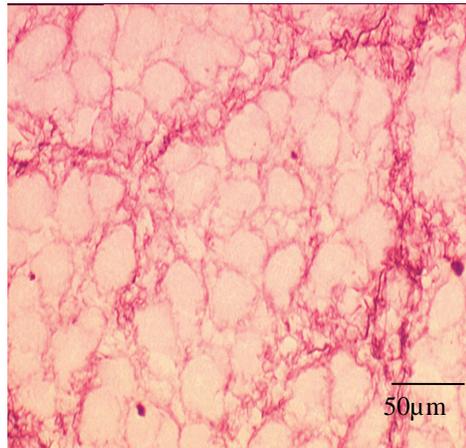


Fig. 3b

Fig. 3: Sections stained with Collagen I antibody, fetuses aged 60 days of gestation. a) Smallest littermate. b) Largest littermate.

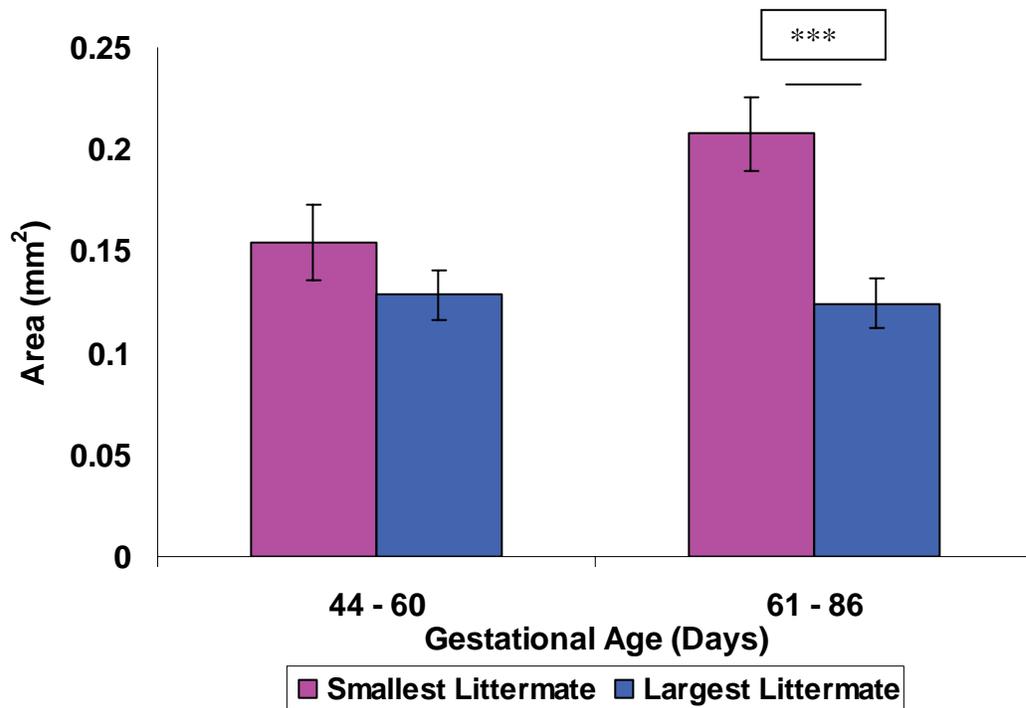


Fig. 4: Collagen I Area per mm² of Muscle for grouped fetal samples aged 36-60 and 61-86 days of gestation. The smallest littermate has a significantly increased amount of Collagen I present compared to the largest littermate ($P=0.008$) in the 61-86 days of gestation group.

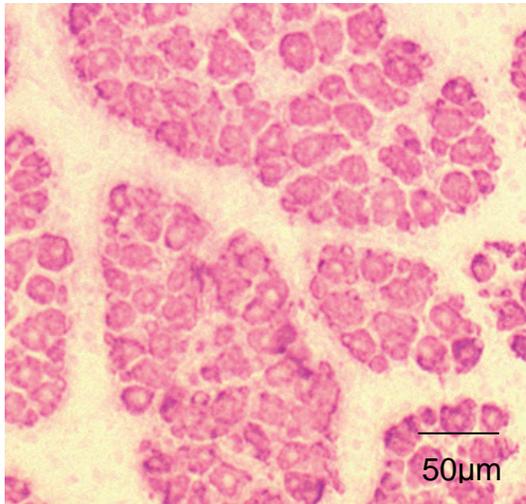


Fig. 5a

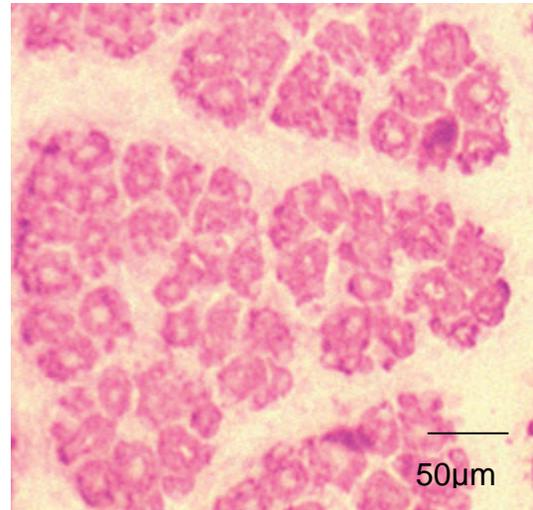
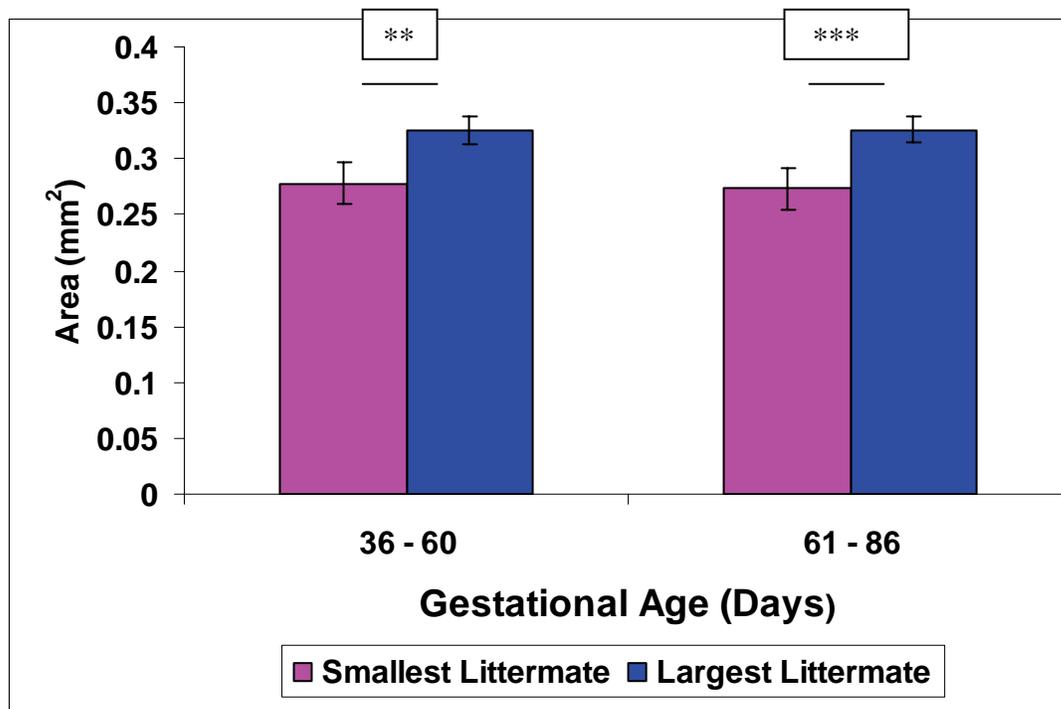


Fig. 5b

Fig. 5: Sections stained with Myosin (embryonic) antibody, fetuses aged 60 days of gestation. A) Smallest littermate. B) Largest littermate.



** p=0.04

*** p=0.005

Fig. 6: Myosin Area per mm² of Muscle for grouped fetal samples aged 36-60 and 61-86 days of gestation. The smallest littermate has an increased amount of myosin present compared to the largest littermate in the 36-60 days of gestation group (P=0.04) and the 61-86 days of gestation group (P=0.005).

THE EFFECT OF MECHANICAL STIMULI ON MUSCLE DEVELOPMENT IN VITRO

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Key Words: muscle development, calpains, signal transduction, mechanical stimulation

Introduction

The process of muscle growth is a central issue in the business of producing animals for meat. At the most fundamental level, the process of muscle development and growth is a complex sequence of events whereby muscle cells respond to a number of stimuli in order to form organised muscle tissue. Increase in muscle mass is greatly influenced by the rate of skeletal muscle protein synthesis, a process that can be altered by mechanical forces. Stretch- or load-induced signaling is now beginning to be understood as a factor which affects the mass and phenotype of muscles as well as the expression of a number of proteins within muscle cells (Carsen *et al.*, 1996; Winchester *et al.*, 1991). Use of magnetic field to produce mechanical forces to stimulate cell populations has been well documented (Glogauer *et al.*, 1998; Hagen *et al.*, 1996). Magnetic field stimulation has been shown to affect transcription of specific gene sequences, protein synthesis, the immune system and increase in Ca²⁺ influx (Michele *et al.*, 2001). Since the number of muscle fibers is fixed at birth and muscle mass is determined in part by fiber number, the mechanisms by which these numbers are determined are of interest to those involved in the study of muscle development.

Objectives

The purpose of this study was to investigate the role of mechanical signals in m-calpain induced muscle cell fusion. The specific goal of this work was to determine whether a mechanically stimulated cell population showed differences in the activity of m-calpain, an enzyme required for myotube formation in vitro.

Methodology

C2C12 cells from ATCC (American Type Culture Collection, Manassus, VA), were maintained under a 5% CO₂ stream at 37°C, plated at 7,500 cells/cm² on tissue culture surfaces and grown to 75% confluence in DMEM containing 10% foetal calf serum (FCS).

For stimulation experiments, cells were incubated with laminin coated 1 µm Encapsulated Super-Paramagnetic Microspheres (EMI- 100/40) for 30 minutes to allow

for bead attachment and then rinsed to remove unattached beads. Stimulated cultures were placed on a heating plate maintained at 37 °C under the electromagnet for a period of 6 hrs. Control cultures were kept in incubator at 37 °C during that period.

A magnetic field of 0.5 mT was generated by an electromagnet (Power Generator 0-30 Volts, 0.1-100 Hz; Elcanic A/S, Denmark). The magnet produced alternating MF at frequency of 1 Hz. Magnet was placed 10 mm over the monolayer of cells during the stimulation period. Cells lacking beads but placed under the magnetic field were used as an additional control.

For the determination of myoblast formation and size, cells were fixed with 4% paraformaldehyde, permeabilised with saponin and stained with Fluorescein isothiocyanate (FITC) (Sigma, St. Louis, MO), and 4',6-Diamidino-2-phenylindole (DAPI) (Sigma, St. Louis MO). Images were obtained using a Leica DmIRB inverted microscope (Leica, DK) couples to a Coolsnap digital camera (Roper Scientific, DE). Images were obtained and analysed using the Image Pro Plus system (Image House, DK). The results shown are an average of 5 different experiments performed on different days.

To determine calpain activity in the cell cultures, cells were plated at 75% confluence on microtiter plate and incubated for 40 min in the presence of 25 µM CMAC, *t*-BOC-Leu-Met, a membrane-permeable calpain specific fluorogenic substrate. The control cells were placed in an incubator at 37 °C, while the stimulated cells were incubated for 20 min with laminin coated microbeads assay, and then placed in a magnetic field for 5 h. The calpain activity was measured as a function of the rate of change in intracellular fluorescence using a fluorometer with 355-nm excitation and 460-nm emission filters.

For immunochemistry experiments, cells were cultured and stimulated as described above. The cells were removed from each dish by scraping, boiled in sample buffer for 5 minutes, and then centrifuged. Protein determination, using the BCA protein determination system (Pierce Scientific, Rockford, IL), was performed on each supernatant and the protein concentrations of each sample were adjusted to the same value. An aliquot containing 10 µg protein was added to each well of an 8-16% gradient gel and run for 90 min at 126 V. The proteins were transferred to a nitrocellulose filter and blocked for 1 h in 3% milk in Tris-buffered saline (TBS). The blots were incubated in a 1:500 dilution of the specified primary antibody in the 3% milk buffer overnight at 4°C, rinsed thoroughly with TBS and incubated in a 1:10,000 dilution of alkaline phosphatase conjugated secondary antibody in TBS for 1 h at room temperature. After thorough rinsing in TBS, the protein bands were visualized using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) and quantified using NIH Image

Results & Discussion

Mechanical stimulation increases myotube frequency but decreases the size of myotubes formed. C2C12 cells are able to fuse and form myotubes *in vitro* after switching the cells from media containing foetal calf serum to medium containing horse serum. The size and frequency of myotube formation can be visualized and quantified by staining cell membranes and nuclei. When cell cultures are mechanically stimulated through their laminin receptors, the myotubes formed contain significantly ($P < 0.001$) fewer nuclei than cultures of un-stimulated cells containing the coated beads (Fig 1a). Mechanical stimulation through laminin receptors significantly increases the number of

myotubes formed in culture ($P < 0.001$) (Figure 1b). Few if any myotubes can be seen in cultures stimulated with fibronectin coated beads. Therefore, mechanical stimulation via laminin but not fibronectin receptors has a significant effect on myotube formation *in vitro*.

Expression of m-calpain but not μ -calpain is up regulated in cells exposed to mechanical stimulation Protein extracts from control C2C12 cells and stimulated C2C12 cells were resolved in 8% polyacrylamide gel, transferred onto membrane and detected using antibodies against m- and μ -calpain (Fig 2). Mechanically stimulated cells show a 20-fold increase in m-calpain expression as compared to non-stimulated controls. Expression of μ -calpain is unaffected by mechanical stimulation. Similar controls were performed on cells stimulated with fibronectin coated microspheres without an increase in m-calpain expression.

Mechanical stimulation increases calpain activity in C2C12 cells. The level of fluorescence of a calpain specific substrate, CMAC, *t*-BOC-Leu-Met, was used to determine the level of calpain activity in the cells (Fig 3). The substrate becomes fluorescent only after specific cleavage by calpains. At time 0, before any mechanical stimulation was applied to the cultures, the level of calpain activity was not significantly different in the two cell populations. During the time course of the experiment, the control cells did not show any increase in calpain activity. The cells which had been stimulated with the laminin coated microspheres showed a sharp increase in calpain activity after 2.5h, after which time no further increases were observed.

Conclusions

We have shown that mechanical signals transmitted through the C2C12 cells interaction with laminin cause an increase in cellular differentiation. This signaling results in an increase in the number of myotubes formed in the cultures, with each individual myotube containing fewer nuclei. Mechanical stimulation increases not only the expression of m-calpain but also the overall activity of calpain in the cells.

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Tables and Figures

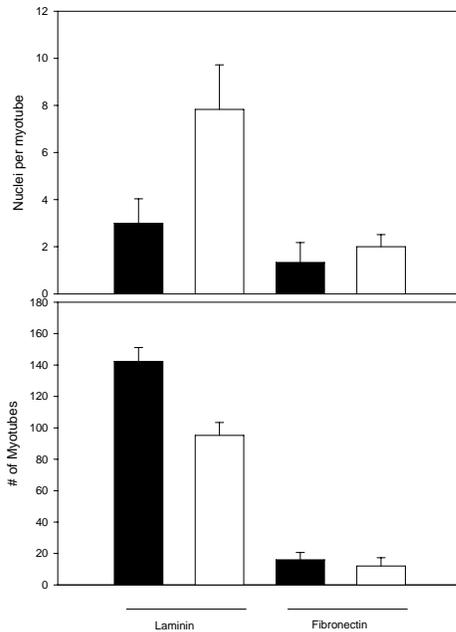


Figure 1. Frequency and size of myotubes formed by C2C12 cell populations in the presence and absence of mechanical stimulation. The number of nuclei per myotube (a) and the total number of myotubes (b) in cultures of cells stimulated with microspheres (black) as compared to un-stimulated controls. Stimulation with laminin coated microspheres causes an increase in the number of myotubes formed, but the resultant myotubes are significantly smaller. Few if any myotubes are seen when cultures are stimulated with fibronectin coated microspheres. Error bars - \pm SE.

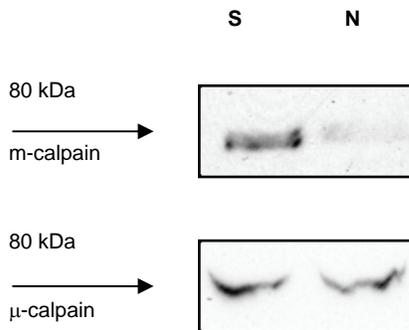
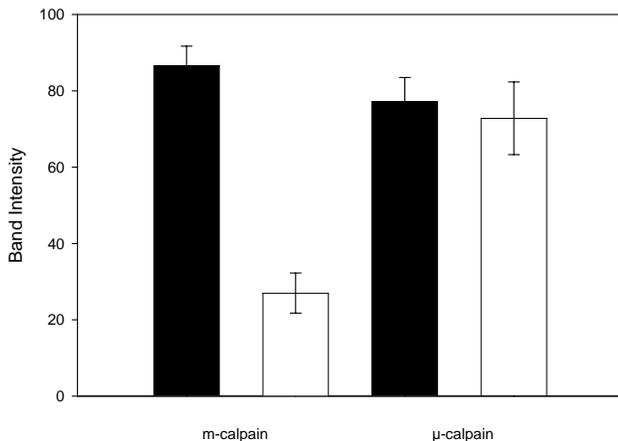


Figure 2. Protein extracts from control C2C12 cells (N) and stimulated C2C12 cells (S) were resolved in 8% polyacrylamide gel, transferred onto membrane and detected using antibodies against m- and μ -calpain. Mechanical stimulation increases m-calpain expression threefold but does not have an affect on μ -calpain expression. (Black-stimulated; White, control).



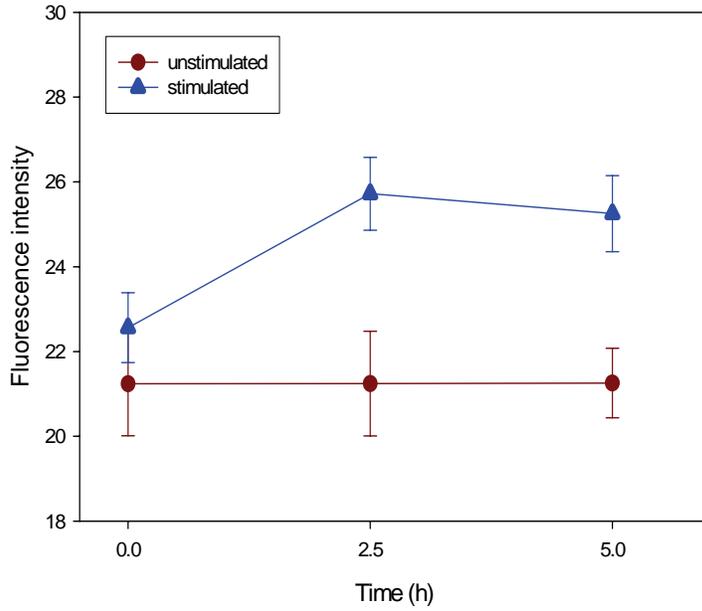


Figure 3. Quantitation of calpain activity using a fluorescent substrate probe. The control cells did not show an increase in calpain activity over the course of the experiment, while mechanically stimulated cells showed a marked increase in calpain activity by 2.5 h after which time no further increases were observed.

**EFFECT OF CREATINE SUPPLEMENTATION ON *LEPOMIS
MACROCHIRUS* (BLUEGILL SUNFISH)**

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Key Words: Creatine, Fish, Bluegill

Introduction

Creatine, a naturally occurring amino acid derivative, has long been used as a human muscle supplement. When supplemented, creatine draws water into muscle cells causing an increase in cell volume, total body water and total muscle volume (Hultman et al., 1996). Studies have been conducted on supplementing creatine to swine and horses (Berg and Allee, 2001, Berg et al., 2003, D'Angelis et al., 2005 and Stahl et al., 2001), but little has been done with supplementation to fish.

Bluegill (*Lepomis macrochirus*) were chosen for this study because of their popularity as food fish and sportfish. Bluegills are commonly raised by government and private producers for stocking into recreational ponds. Bluegills used for stocking are often small, fingerling (5-7 cm) sized fish. In the Midwest United States, research on using bluegill as a food fish is ongoing (Wang et al., 2000). Bluegill need to reach at least 227 g to be used in the food fish market (Brunson and Morris, 2000). When raised in ponds in the Midwest U.S., bluegills take more than two years to reach the 227 g market size (Hayward and Wang, 2001). Fish that have an extended grow-out period of more than two years are not ideal culture species. Since bluegill do not reach market weight within two years, it is necessary to find ways to increase gain in the same or a shorter amount of time.

Creatine was chosen as a supplement due to its ability to increase muscle mass by drawing more water into muscle cells. The increased muscle mass would increase the fish's overall gain, allowing fish to gain more in the same amount of time. Creatine was also chosen because it is a naturally occurring peptide, so foreign substances would not be introduced to the fish.

Objectives

Hypothesis: Fish supplemented creatine will weigh more than controls, will have more fat free tissue and moisture than controls, and also will have higher critical swimming speed.

Objective: To determine if creatine supplementation to bluegill would increase gain, increase the amount of fat free mass and moisture, and increase critical swimming speed in the fish.

Methodology

Live Animals

One hundred Bluegill sunfish (*Lepomis macrochirus*) of two different age groups were obtained from Osage Catfisheries (Lake Ozark, MO, USA) and held in the fisheries facilities at the University of Missouri-Columbia. Age group one (age-1) fish were approximately one year old and age group three (age-3) fish were approximately 3 years old. Fish were allowed to acclimate to the laboratory conditions before being sorted for size. Twenty four fish of the largest fish were selected from each age group to be used in the study. The starting mean weight of age-1 fish was $3.16 \text{ g} \pm 0.74$ (mean \pm SD) and the age-3 fish starting mean weight were $30.39 \text{ g} \pm 3.36$. Fish were randomly assigned to individual ten liter chambers (38 x 20 x 30 cm) within three different recirculating tanks. Sixteen chambers were held in each 1000 liter recirculating tank. Fish were acclimated to the chambers before the feeding trial began. Two balanced diets were formulated and manufactured by Purdue University. The treatment diet contained 2.5% creatine, and the control diet contained no creatine. Age-1 fish were fed three equal amounts daily, totaling 10% of their mean body weight. Age-3 fish were fed three equal amounts daily, totaling 4% of their mean body weight. Fish weights (g) were recorded the day before the feeding trial began. Twelve fish from each size group were randomly assigned to each diet. Fish were arranged in a randomized complete block design, with one fish from each size and treatment in each block and four blocks per tank. Fish were fed the experimental diets for 40 days, then measured and weighed on day 41. Feed was withheld for approximately 18 hours \pm 1, prior to being weighed on day 41. Feeding was resumed once fish were weighed. Relative amount of gain, which is grams of gain of per gram of fish, was figured using the following equation:

$$\text{Relative gain} = (\text{final weight} - \text{initial weight}) / [(\text{final weight} + \text{initial weight})/2]$$

Swim Testing

On day 42, swim capacity testing was started utilizing a Blazka flow through style swim tube. The tube is a cylindrical chamber with a propeller at one end, which can be programmed to create specific rates of water flow. The tube was used to obtain the bluegills' critical swimming speed (U_{crit}), or the highest water flow velocity that the fish can swim in before becoming exhausted. U_{crit} can be determined using the following equation:

$$U_{\text{crit}} = V_{\text{ls}} + (t_{\text{s}}/t_{\text{i}})V_{\text{i}},$$

where V_{ls} (cm/sec) is the velocity of the last swimming period prior to exhaustion, t_{s} is the time (min) spent swimming at the final velocity, t_{i} is the time increment of each swimming period, and V_{i} is the velocity increment (cm/sec). All velocity measurements were then converted to body lengths per second (BL/sec) to account for differences in

fish size. Fish were placed in the swimming tube and allowed to acclimate for twenty minutes at a minimal rate of flow (0.25 BL/sec). After acclimation the water velocity was increased 0.25 BL/sec (V_i) every five minutes (t_i) until the fish could no longer swim in the flow. Fish were considered exhausted when they became impinged against the rear panel of the swim tube. Once exhausted, fish were removed from the tube and replaced in their chamber. V_{1s} and t_s was recorded and U_{crit} for each fish was calculated.

Tissue Analysis

Fish were euthanized using a clove oil and water solution, then whole fish were grinded and homogenously mixed to allow for tissue analysis. Percent fat and moisture was obtained using a CEM Smart Trac System 5 (CEM Corporation, Matthews, NC, USA). Age-3 fish tissue was analyzed in duplicate with a sample size of $4.14 \text{ g} \pm 0.15$ (mean \pm SD). Age-1 fish did not provide enough tissue to analyze in duplicate, so single $4.02 \text{ g} \pm 0.12 \text{ g}$ tissue samples were analyzed. The following equation was used to obtain percent fat-free mass:

$$\text{Percent fat-free mass} = [\text{final weight (g)} - (\text{final weight} * \% \text{ fat})] / (\text{final weight})$$

Statistical Analysis

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was utilized to test for differences between relative gain of fish fed either treatment or control diets. The dependent variable in the model was treatment, which was creatine, with the independent variable being relative gain.

The GLM procedure was again used to test for differences between U_{crit} of treatment and control fish and also between the two age groups. The dependent variable was treatment, with the independent variable being U_{crit} .

The GLM procedure was also utilized to test for differences between percent fat and moisture of fish fed either the treatment or control diets. The dependent variable was treatment, which was creatine, and the independent variable was either fat or moisture.

Results & Discussion

40 Day Growth Results

Fish were weighed at the beginning of the feeding trial and again weighed at the end of the trial. Age-1 fish mean starting weight was $3.16 \text{ g} \pm 0.74$ and mean end weight was $30.39 \text{ g} \pm 3.36$. The mean relative gain for age-1 fish fed the treatment diet, containing creatine, was $1.03 \text{ g} \pm 0.52$, versus $1.21 \text{ g} \pm 0.11$ for age-1 fish fed the control diet, with no creatine (refer to Table 1). The mean relative gains for age-1 fish did not significantly differ. The lack of difference and also the large deviation for the creatine supplemented fish could be attributed to the same cause. Two of the creatine fed fish ceased to eat shortly after the trial began, resulting in loss of weight in one fish and nearly no gain in the second fish.

Age-3 fish mean starting weight was $30.39 \text{ g} \pm 3.36$ and the mean end weight was $73.89 \text{ g} \pm 4.47$. The mean relative gain for age-3 fish fed the treatment diet was $0.84 \text{ g} \pm$

0.04 compared to $0.84 \text{ g} \pm 0.08$ for age-3 fish fed the control diet. The mean relative gains are observably not significantly different. Additionally, the relative gain of age-1 fish was not significantly different from the relative gain of age-3 fish.

The relative gain results did not support the hypothesis of the experiment. The lack of increased gain due to creatine supplementation could be attributed to the environment the fish were housed in. The recirculating tanks allowed for water flow through among the chambers, but the circulation did not create a significant flow. The lack of flow or water movement resulted in a low activity rate in the fish. In addition to the lack of flow, there were also no predators to increase the bluegills' activity or swimming. Creatine functions to aid in reenergizing muscle after contraction. Due to environmental factors, the bluegills were not active, resulting in little muscle use. The lack of muscle use could explain the lack of effect from creatine.

U_{crit} Results

The mean Ucrit of age-3 fish (3.41 BL/sec) versus age-1 fish (4.04 BL/sec) differed significantly ($P < 0.0001$). Furthermore, the age-3 fish receiving creatine supplementation possessed a significantly lower Ucrit than age-1 fish supplemented creatine (3.46 vs. 3.98 BL/sec, respectively). However, the fish age group*creatine interaction was not significant ($P = 0.184$). The Ucrit results suggest that small fish may have a greater capacity to yield results from creatine supplementation.

Tissue Analysis Results

Tissue samples were analyzed for percent fat and moisture. The mean percent fat-free mass did not differ for age-1 treatment fish ($92.89\% \pm 1.22$) versus control fish ($92.76\% \pm 2.07$). The two means were not found to be significantly different. The mean percents fat-free mass for age-3 fish were $92.74\% \pm 0.59$ and $93.07\% \pm 0.62$, for treatment and control fish, respectively. The means were also not significantly different. Age-1 treatment fish had mean percent moisture of $71.39\% \pm 1.14$, and control fish percent moisture was $71.10\% \pm 2.63$. The mean treatment and control proportional moisture content for age-1 fish were not significantly different. The mean percent moisture did not differ for age-3 treatment fish ($70.84\% \pm 0.77$), versus control fish ($70.73\% \pm 0.87$).

The tissue sample analysis also did not support the hypothesis of the experiment. A characteristic of creatine is to increase total muscle volume by increasing intramuscular water. Based on this, it was hypothesized that percent moisture in the fish would increase. Additionally it was expected that percent fat-free mass would increase due to the increase in muscle volume due to creatine. The lack of significant differences in the percent fat-free mass and moisture could be attributed to the low activity level of the fish. The low activity level, explained by the aforementioned environmental effects, could have resulted in decreased intramuscular creatine uptake and storage.

Table 1. Relative gains, percent fat-free mass and moisture, and U_{crit} results

	Mean relative gain ^f	Mean % fat-free mass ^g	Mean % moisture ^h	U_{crit} ⁱ
Age-1 treatment fish	1.03 g ± 0.52 ^a	92.89% ± 1.22 ^b	71.39% ± 1.14 ^c	3.98 BL/sec ± 0.25 ^d
Age-1 control fish	1.21 g ± 0.11 ^a	92.76% ± 2.07 ^b	71.10% ± 2.63 ^c	4.09 BL/sec ± 0.19 ^d
Age-3 treatment fish	0.84 g ± 0.04 ^a	92.74% ± 0.59 ^b	70.84% ± 0.77 ^c	3.46 BL/sec ± 0.31 ^e
Age-3 control fish	0.84 g ± 0.08 ^a	93.07% ± 0.62 ^b	70.73% ± 0.87 ^c	3.36 BL/sec ± 0.22 ^e

^{a,b,c,d,e} Means with letters in common are not significantly different ($P < 0.05$).

^f Relative gain = grams of gain/gram of fish = (final weight – initial weight) / [(final weight + initial weight)/2]

^g % fat-free mass = [final weight (g) – (final weight * % fat)] / (final weight)

^h % moisture values are obtained by running samples in CEM machine

ⁱ $U_{crit} = V_{ls} + (t_s/t_i)V_i$, where V_{ls} (cm/sec) is the velocity of the last swimming period prior to exhaustion, t_s is the time (min) spent swimming at the final velocity, t_i is the time increment of each swimming period, and V_i is the velocity increment (cm/sec), all values are reported as BL/sec.

Conclusions

Despite the lack of significantly different results in relative gain and tissue samples, this experiment still provides useful information. The individual chambers the bluegills were confined to allowed for accurate gain measurements, but as noted, lowered activity level. Additionally, bluegills naturally inhabit freshwater lakes or ponds that do not have strong currents, resulting in an inherently lower activity level than fishes that live in flowing water (streams or rivers). The activity level of fish is an important aspect of creatine supplementation that offers opportunities for additional experiments. Bluegills could be used again in a similar experiment, but with an increased level of activity. An alternate option would be to use a species of fish that naturally occupy flowing water and house them in tanks that facilitate constant swimming.

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**SERUM HORMONE CONCENTRATIONS AS PREDICTORS OF CARCASS
COMPOSITION IN A RANDOM ALLOTMENT OF AMERICAN
FED BEEF CATTLE**

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Key Words: Leptin, IGF-I, Beef Cattle, Carcass, Fat

Introduction

The U. S. beef industry continues to struggle to better understand the latter part of the finishing phase and the changes that occur within the animals in the weeks prior to harvest. Feeding cattle to a desired United States Department of Agriculture (USDA) quality grade may appear to be quite easy, though maximizing performance (Feed/Gain) and minimizing excess 12th rib back-fat and subsequent increase in USDA yield grade (YG) during the last 60 days of feeding remains a challenge. The protein hormone leptin was discovered by Zhang et al. (1994) in the mouse model. Leptin is primarily secreted from white adipocytes with the task of regulating food intake, energy expenditure, and energy balance within the body (Houseknecht et al., 1998). Most important to the meat animal industry is the relationships that have been discovered between leptin and carcass merit. Serum leptin correlations have been reported by Minton et al. (1998) and Geary et al. (2003) with 12th rib back fat, USDA yield grade, marbling scores, kidney, pelvic and heart fat (KPH) and ultimately USDA quality grade in beef cattle. McFadin et al. (2003) reported positive correlations between serum leptin and 12th rib back fat, USDA yield grade and marbling score. The results of these trials tempt us to conclude that circulating serum leptin concentrations could be used as a means to predict beef carcass merit prior to harvest. At the same time, bovine growth hormone (bGH) and insulin-like growth factor-I (IGF-I) are well documented as endocrinological links to lean muscle deposition. Trenkle and Topel (1978) reported correlations between bGH, percent carcass fat and percent carcass muscle while Anderson et al. (1988) reported similar correlations between muscle and adipose tissue deposits with both bGH and IGF-I. This research has analyzed the correlations between leptin, bGH, IGF-I and carcass parameters in a random allotment of steers and heifers harvested through an American commercial harvest facility.

Objectives

The objectives of this project were to determine if correlations are present between leptin, insulin-like growth factor-I, bovine growth hormone and beef quality parameters in a random allotment of beef market animals.

Methodology

Animals were selected from the harvest line through the Emporia, Kansas Tyson Fresh Meats facility on 4 separate random collection (RC) days. Collection days are detailed in Table 1. The animals were from a variety of management strategies and were transported varying distances to the commercial harvest facility. Animals were randomly chosen from the bleed chain. No pre-harvest information was gathered prior to initiation on the project.

Table 1. Summary of days* in which random sampling occurred at the Emporia, Kansas Tyson Fresh Meats Facility

Variable	RCI	RCII	RCIII	RCIV
Date Sampled	3/30/2004	5/17/2004	8/17/2004	1/03/2005
Low Temperature, C	1.67	16.67	20.00	-1.67
High Temperature, C	11.67	27.22	32.78	1.11
Heifers	22	227	348	160
Steers	176	299	185	335

*Source: National Climatic Data Center, Washington, D.C. USA

Blood samples were collected at exsanguination and were allowed to clot for approximately 24 h at 4° C. Prior to centrifugation of the samples, caps were removed and tubes were reamed with wooden stir rods to aid in serum separation. Samples were centrifuged at 2,500 X g for 45 min. Serum was pipetted off, placed in 48 well plates (5 mL/well), and stored at -20° C until analysis. Leptin concentrations were determined by a double-antibody leptin radioimmunoassay as described by Delavaud et al. (2000). IGF-I and bovine growth hormone (bGH) concentrations were assayed as described by Lalman et al. (2000).

Hot carcass weight and packer number were recorded prior to being chilled at 2° C for 24 h. After 24 h chill, the carcasses were ribbed and the 12th/13th rib interface was exposed and allowed to bloom prior to obtaining skeletal maturity and marbling score. Carcasses were analyzed by trained evaluators from the University of Missouri. Ribeye area was determined using the reverse blot image technique described by Martin (1991). The reverse longissimus dorsi (LD) images obtained on the filter papers were analyzed at the University of Missouri by tracing the LD image outline with a pencil. The area was then determined through the use of a beef ribeye area dot grid (Martin, 1991).

Fat thickness was recorded on the bloom chain using a USDA preliminary yield grade ruler (USDA, 1997) at an anatomical location perpendicular to the vertebral column and ¾ the distance, caudal the ribeye muscle. The preliminary yield grades were adjusted, correcting for atypical fat distribution and/or defects. Percentage of kidney-pelvic-heart (KPH) fat was estimated. Marbling scores were determined by a trained evaluator from the University of Missouri. To minimize variation, the same evaluator determined marbling scores and maturity on RCII, III and IV animals. Animals within RCI were analyzed by a separate member of the University of Missouri carcass collection group. Both graders were standardized to marbling scores based on the USDA marbling standards (USDA, 1997; Abundant, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small, Slight, Traces, and Practically Devoid). Maturity scores were recorded if

identified outside of “A” maturity based on the USDA maturity classification standards (USDA, 1997). Dark cutters were also documented based on percentage of LD determined to be dark.

Statistics. The GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was utilized to test for differences between random collection days. Endocrine hormone and carcass parameters were used within the model as dependent variables while kill day and sex of the animals were independent variables. For the complete data set, relationships between serum endocrine concentrations (Leptin, IGF-I and bGH) and carcass traits were quantified by Pearson correlation coefficients and linear regression.

Results & Discussion

Table 2. Least squares means (SEM)^a for leptin, IGF-I, bGH and carcass traits in randomly selected cattle

Variable	<u>RCI</u>		<u>RCII</u>		<u>RCIII</u>		<u>RCIV</u>	
Leptin, ng/mL	12.71 ^{ef}	(0.59)	8.86 ^d	(0.23)	13.71 ^f	(0.24)	10.27 ^e	(0.25)
IGF-I, ng/mL	17.78 ^g	(0.58)	15.03 ^f	(0.22)	11.53 ^d	(0.23)	13.08 ^e	(0.24)
GH, ng/mL	33.65 ^e	(3.72)	41.78 ^f	(1.45)	24.85 ^d	(1.49)	54.52 ^g	(1.61)
Hot Carcass Weight, kg	330.49 ^d	(4.50)	329.31 ^d	(1.75)	348.05 ^e	(1.81)	360.37 ^f	(1.91)
Marbling Score ^b	42.07 ^{de}	(0.98)	41.00 ^d	(0.38)	42.87 ^{ef}	(0.39)	44.02 ^f	(0.42)
Fat depth, cm ^c	1.17 ^{de}	(0.06)	1.10 ^d	(0.02)	1.29 ^e	(0.02)	1.22 ^{ef}	(0.02)
Kidney-pelvic-heart fat %	2.22 ^d	(0.05)	2.77 ^f	(0.02)	2.62 ^e	(0.02)	2.26 ^d	(0.02)
Ribeye area, cm ²	85.67 ^{ef}	(1.26)	83.58 ^{de}	(0.49)	85.48 ^f	(0.51)	82.92 ^d	(0.54)
Calculated Yield Grade	2.61 ^d	(0.09)	2.76 ^d	(0.04)	2.98 ^e	(0.04)	3.07 ^e	(0.04)

^a (SEM) is the standard error of the least squares means.

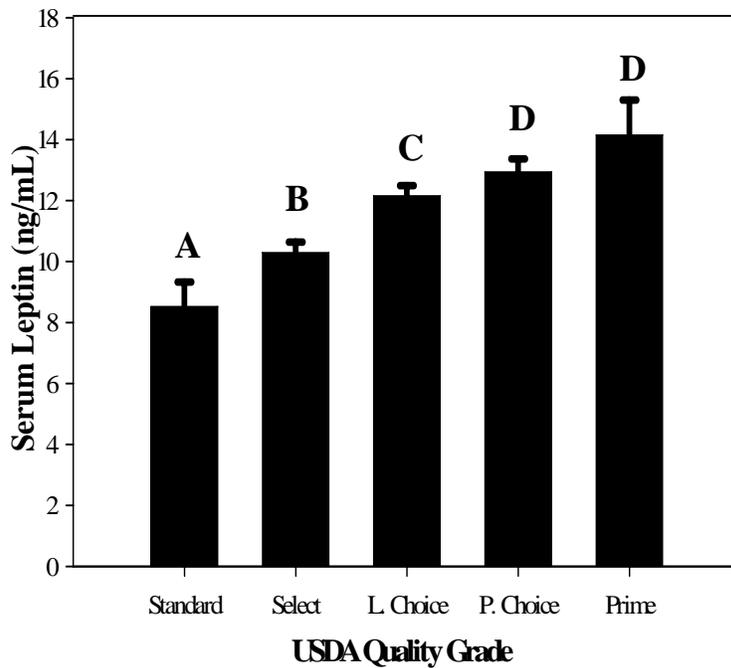
^b Mean marbling scores were derived from scores assigned based on a scale in which: 10 – 19 = Practically Devoid (PD), 20 – 29 = Traces, 30 – 39 = Slight, 40 – 49 = Small, 50 – 59 = Modest, 60 – 69 = Moderate, 70 – 79 = Slightly Abundant, 80 – 89 = Moderately Abundant and 90 – 99 = Abundant.

^c Back fat measurement taken at 12/13th rib interface.

^{d,e,f,g} Means within a row lacking a common superscript letter differ (P < 0.05).

Specifics about animals within each random collection are described in Table 2. When steers and heifers were separated by kill day, serum leptin concentrations were inconsistent. Within RCI and RCIV, heifers had higher (P < 0.05) leptin concentrations than steers, though within RC II and RCIII the opposite was true. Heifers had lower (P < 0.05) serum IGF-I concentrations, were fatter (P < 0.05) at the 12th rib and had lighter (P < 0.05) weight carcasses than their castrated male counterparts. Correlations between serum leptin and carcass parameters within this project agree with findings of Minton et al. (1998), Geary et al. (2003) and McFadin et al. (2003). In the present study serum leptin concentrations were correlated (P < 0.01) with 12th rib fat depth (r = 0.37), USDA yield grade (r = 0.32), marbling score (r = 0.28) and kidney-pelvic-heart fat (r = 0.23). Trenkle and Topel (1978) found correlations between bGH percentage of lean muscle mass while Anderson et al. (1988) reported correlations with both IGF-I and bGH with adipose and lean muscle percentages. Within this project, bGH was correlated (P < 0.01)

Figure 1. Least squares means for serum leptin by USDA Quality Grade



A, B, C, D Means lacking a common superscript differ ($P < 0.05$)

leptin concentrations when comparing USDA quality grade from lowest to highest; standard, select, low choice, premium choice, and prime carcasses, respectively. When analyzing the least squares means of serum leptin concentrations and USDA Yield Grade, yield grade 1 animals had the lowest serum leptin concentration (8.11 ng/mL) and differed from yield grade 2 animals ($P < 0.05$). Yield grade 2 carcasses were lower (9.80 ng/mL) than yield grade 3 animals (12.43 ng/mL) though yield grade 3, 4 and 5 animals did not differ ($P > 0.05$) in their serum leptin concentrations.

Conclusions

This project has shown that distinct, separable circulating leptin differences exist between USDA quality grades for a random allotment of young American beef cattle. Furthermore, endocrine hormone concentrations, including leptin, IGF-I and bGH, can be correlated with carcass parameters in randomly selected cattle despite varying management and nutritional regimes though leptin appears to be the best indicator of quality parameters. The ability to segregate live animals based on leptin concentrations and establish correlations with marbling score could be quite beneficial to producers marketing their animals on a carcass merit system based on higher USDA quality grades. This research shows that animals within quality grades up to the premium choice category can be segregated from each other through the use of serum leptin concentration. If determined pre-harvest, the premiums associated with choice and prime

to KPH ($r = - 0.24$), 12th rib back fat ($r = - 0.16$) and USDA yield grade ($r = - 0.11$). Insulin-like growth factor I was correlated ($P < 0.01$) with 12th rib back fat ($r = - 0.23$), KPH ($r = - 0.20$), marbling score ($r = - 0.20$), USDA yield grade ($r = - 0.17$), and sex of the animals surveyed ($r = 0.47$) with heifers consistently lower in serum concentration than steers.

Correlations existed between the endocrine hormones within the present study. Leptin was correlated to both IGF-I ($r = - 0.11$; $P < 0.01$) and bGH ($r = - 0.31$; $P < 0.01$) while IGF-I was correlated to bGH ($r = 0.16$; $P < 0.01$).

Figure 1 shows least squares means of serum

carcasses could have a substantial impact on beef producer's economic return. A rapid determinant of serum leptin concentration does not exist today, therefore future research must continue in an attempt to develop a producer friendly means of determination.

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**PREDICTIVE VALUE OF METABOLIC HORMONE CONCENTRATIONS
ASSESSED IN BEEF CALVES WEANED AT TWO DIFFERENT AGES ON
FINAL CARCASS QUALITY PARAMETERS**

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Key Words: leptin, IGF-1, growth hormone, insulin, carcass composition

Introduction

From a physiological perspective, the endocrine response of an animal may be viewed as a dynamic indicator of the interactive components of an animal's genetic capability in a given environment. We assert that if accurate predictions could be made at weaning or even 30 d prior to slaughter, by using physiological profiling in conjunction with a modeling system, producers would have more insight on individual animal needs. This has potential to maximize growth and development, thus allowing the producer to maximize their profit when the cattle are marketed.

The following study examines the possibility of incorporating four important metabolic hormones into such a system and their potential to predict end carcass United States (US) quality grade at the time of weaning. Leptin, insulin, insulin-like growth factor-1 (IGF-1), and growth hormone (GH) are all major determinants of protein and fat deposition in growing cattle. More specifically, leptin is a protein hormone produced by adipocytes and is transported to receptors located throughout the body (Houseknecht et al., 1998). In livestock, focus has been given to leptin for its ability to predict body fat mass. As livestock gain weight, and specifically fat mass, leptin levels increase in association with increased size and numbers of adipocytes. As a result of increased leptin levels, feed intake decreases (Friedman, 1998). Recent studies have focused on serum leptin levels and carcass composition of mature cattle at harvest (Geary et al., 2003; Buchanan et al., 2002; Wegner et al., 2001). These same studies found correlations between serum leptin levels and different fat depots (ex. Intramuscular, intermuscular fat) at the time of slaughter.

Insulin, like leptin, is a lipogenic hormone (Roemmich and Rogol, 1999), thus expectations are that a positive relationship would exist between serum insulin and body fat mass. Furthermore, insulin has been reported to stimulate leptin secretion (Houseknecht et al., 1998); thus leptin and insulin levels are expected to correlate well.

In contrast to leptin and insulin, GH plays a critical role in repartitioning nutrients from fat depots to increase protein accretion, especially in the earlier stages of growth. The influence of GH asserts itself soon after birth of the neonate and directly or indirectly influences the rate of cell multiplication and differentiation in several tissues and organs throughout the body. This cell growth continues up until sexual maturity when GH levels

decrease (Isaksson et al., 1985). Although earlier studies using GH levels alone to predict end carcass measurements were unsuccessful, using GH levels in conjunction with other developmental hormones in a model to predict final carcass measurements may prove to be a more feasible option (Connor et al., 1999; Connor et al., 2000).

In addition to GH, IGF-1 is also known to affect body composition (Maccario et al., 2000), as IGF-1 is regulated by GH. IGF-1 levels have been reported to increase congruent with protein accretion in cattle. By analyzing serum IGF-1, GH, and insulin concurrently with serum concentrations of leptin, at weaning (90 days of age vs. 205 d of age) a better understanding of calf body composition during growth may be determined (Maccario et al., 2000; Hornick et al., 2000), with the ultimate goal of establishing an equation useful in the prediction of animal performance and final carcass composition.

Objectives

The objective of this study was to determine the predictive value of serum concentrations of GH, IGF-I, insulin, and / or leptin, in beef calves weaned at two different ages and implanted or non-implanted with a growth promotant, on final carcass quality parameters.

Methodology

Cattle population

The use of animals in this study was approved by the Animal Care and Use Committee. One hundred-and-forty Angus X Gelbvieh and purebred Angus steers derived from two of the University of Missouri Beef Farms were randomly assigned to one of two weaning groups. Steers were EW (early weaned; averaged 90 d of age; n=70) or TW (traditional weaned; averaged 205 d of age; n=70). Steers were either implanted (EWI or TWI) with Synovex-S growth promoting implants at weaning and reimplanted 80 d after weaning or not implanted (EWN or TWN). Steers were randomized into treatments using sire and farm as variables. Blood samples were collected at d 0 (21 d after the acclimation period and weaning) for both groups and every 28 d thereafter until harvest.

Sample collection, handling and hormonal analysis

Blood samples were collected from the jugular vein and the final blood sample was taken at exsanguination. Serum was then harvested and stored frozen until analysis. Serum concentrations of leptin, GH, IGF-1 and insulin were assayed as described by Delavaud et al. (2000), Lalman et al. (2000), Chelikani et al. (2004), and Kolath et al. (2005).

Carcass data collection

Steers were harvested at a commercial packing plant at an average live weight of 523 ± 46 kg. Each animal was tagged with a sequence number following exsanguination, prior to hide removal. Hot carcass weights were documented for each animal.

After a 24-hour chill period, additional carcass measurements were collected. Ribeye areas were measured using the reverse blot image technique (Martin, 1991), which allows ribeye areas to be obtained at grading chain speeds.

Fat thicknesses were determined using a USDA preliminary yield grade ruler (USDA, 1997) at an anatomical location perpendicular to the vertebral column and ¾ the distance, caudal the ribeye muscle. To determine preliminary yield grades, the fat measurements were then adjusted, correcting for any atypical fat distribution.

Marbling scores were identified by an experienced USDA grader using the USDA marbling standards (USDA, 1997; Abundant, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small, Slight, Traces, and Practically Devoid). Maturity scores were also assessed using the USDA standards (USDA, 1997) for animals older than “A” maturity.

Statistical Analysis

Proc GLM and LSMEANS in SAS v9.1 system (Cary, North Carolina, USA) were used to analyze hormone levels and their relationship to US Quality Grade. In analyzing hormone data collected at weaning the following model was used: *hormone = quality grade / weaning status*. Analysis of the hormone data acquired 30 d prior to slaughter, with implant status as an additional treatment, the following model was used: *hormone = quality grade / weaning status / implant treatment*. US Quality Grades* were assigned to one of four levels for statistical analysis: Marbling scores assigned to 1 of 4 quality grade categories, Quality Grade 1 = Select (n = 30); quality grade 2 = Low Choice (n = 42); quality grade 3 = Average Choice (n = 36); quality grade 4 = Premium Choice or better (n = 24). [*Note USDA quality grades Select = low quantities of intramuscular fat, Premium Choice or better = Very high quantities of intramuscular fat

Figure 1. Mean insulin levels (ng/mL) at weaning and final quality grade.

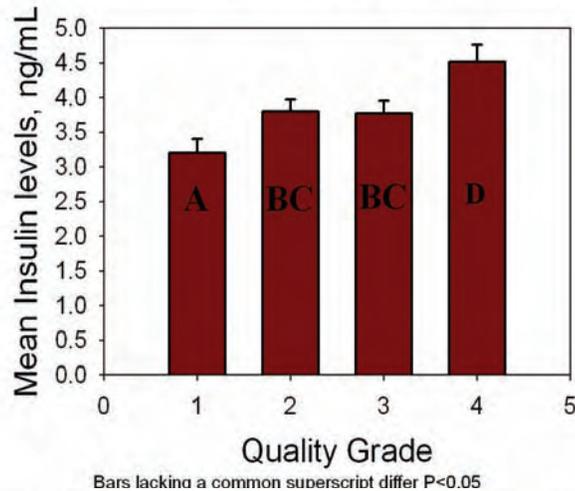
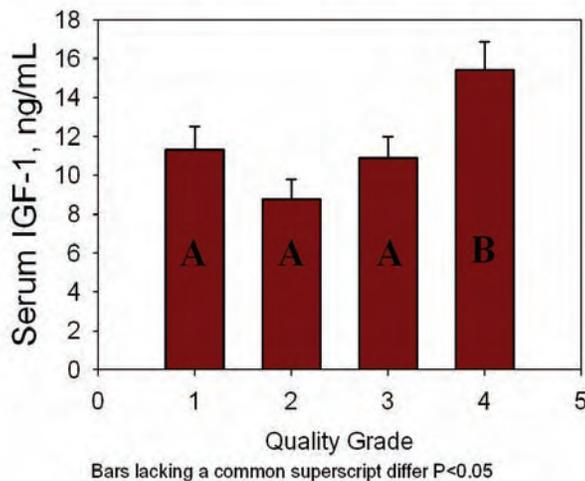


Figure 2. Mean IGF-1 levels (ng/mL) at weaning and final quality grade.



within the *longissimus dorsi* muscle at the 12/13th rib interface.]

Results & Discussion

At Weaning

Serum leptin levels at weaning had no relationship ($P > 0.49$) with final carcass quality grade in EW or TW steers. This may be due to the low amount of fat found in weaned calves, attributing to lower levels of leptin, making it more difficult to establish a relationship. Serum insulin levels, however, accounted for 18% of the variation in final carcass USDA quality grade and weaning status. There were main effects observed in quality grade ($P = 0.004$; Figure 1) and weaning status ($P = 0.001$) with no quality grade by weaning status interactions ($P > 0.05$).

Serum IGF-1 levels also had a significant relationship with final quality grade, accounting for 10% of the variation in quality grade. A quality grade main effect was observed ($P = 0.017$) with no quality grade by weaning status interaction (Figure 2).

Serum GH levels (ng/ml) at weaning had no main effect on quality grade ($P > 0.05$). This is not surprising considering the volatile nature of growth hormone, especially in growing calves. These results also support previous findings as mentioned in the introduction.

30 d Prior to Slaughter

Although serum leptin levels had no relationship with final quality grade at the time of weaning, they accounted for 18% of the variation in quality grade and weaning status 30 d prior to slaughter. Quality grade and weaning status main effects were significant ($P=0.0008$, Figure 3 and $P = 0.0053$, respectively), with no implant treatment effects. Because implant treatment had no effect, it was removed from the model.

These findings correlate with those observed by Brandt et al. (unpublished data), where final carcass quality grade was associated with serum leptin levels

Figure 3. Mean Leptin levels 30 d prior to slaughter and final quality grade.

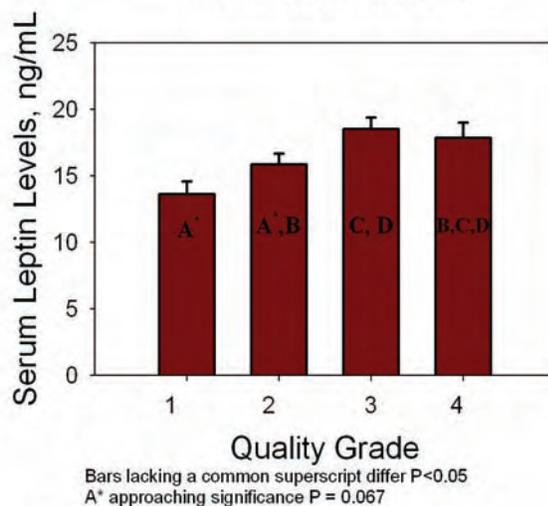
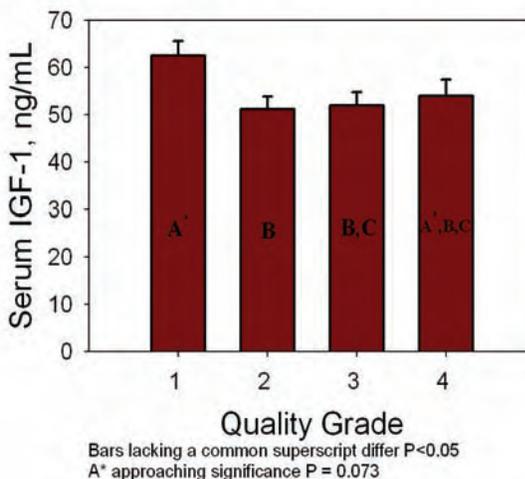


Figure 4. Mean IGF-1 levels 30 d prior to slaughter and final quality grade.



in beef animals. Serum insulin levels were found to have both treatment and weaning status main effects ($P=0.0065$ and 0.0009 , respectively); however, no quality grade main effects were found. This indicates that insulin may be a better predictor of final quality grade at the time of weaning rather than later in the feeding period. Alternatively, serum IGF-1 levels remained a strong predictor throughout the feeding period, having quality grade, weaning status and treatment main effects ($P = 0.0229$, Figure 4, $P = 0.0373$ and $P < 0.0001$, respectively) with no interactions at 30 d prior to slaughter. Once again, GH had no relationship with final carcass quality grade. GH can be easily influenced by season, physiological maturity as well as many other factors, so its inability to be a solid predictor is not surprising.

Conclusions

These data provide evidence that incorporating serum concentrations of insulin and IGF-1 into a modeling program for predicting final carcass quality grade at the time of weaning and incorporating serum concentrations of leptin and IGF-1 into a modeling program for predicting final carcass quality 30 d prior to slaughter may strengthen a prediction equation incorporating these variables along with physical measurements (e.g. body weight, ultrasound fat thickness) taken at the same time point.

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MEAT QUALITY AND MUSCLE FIBRE TRAITS IN RABBITS OF DIFFERENT GENETIC ORIGIN AND SEX

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Key Words: rabbit, genetic origin, sex, meat, fibre type distribution, enzymatic activity

Introduction

In some EU countries of Latin origin, such as Italy, France and Spain, the breeding of rabbits for meat production purpose is greatly diffuse. In these countries, the rabbit meat represents the fourth meat consumed, after pig, beef and poultry meats. Intensive rabbit meat production is based on the use of hybrid rabbits, extracted from a few breeds and strongly selected for production traits. The hybrid rabbits are nowadays showing poor resistance to some diseases, which increases the use of chemicals. On the other hand, the actual consumer is now looking for safer meats, obtained with the respect of the animal welfare and with good nutritional and sensory attributes. For these main reasons, the needs for the use of rabbit breeds, purebred or their crosses, showing a good resistance to diseases is emerging, with the aim to reduce or stop the use of chemicals for the benefit of the consumer. However, unselected breeds often show poor growth and carcass yield. Among the adoptable breeds, Vienna Blue and Burgundy Fawn ones seem to be adequate to be introduced for this purpose.

Objectives

The aim of this study was to compare the chemical composition of the hind leg meat, the pH_u and L*a*b colour of BF and LL muscles, the fibre type distribution and dimension, and the enzymatic activity of the LL muscle of rabbits of both sexes derived from 2 sire genetic origins, Vienna Blue and Burgundy Fawn, and the commercial hybrid rabbit. The study aimed also to investigate on the relationship between some muscle fibre traits and some indicators of the meat quality in rabbits of different genetic origin (GO). The meat quality analysis and the muscle fibre characteristics of B and F animals was performed in order to find that GO whom can give the best growth and meat performance, without renouncing at the viable attitudes of unselected rabbits.

Methodology

Forty-five rabbits of both sexes, derived from offspring of three GO (B = sire Vienna Blue, F = sire Burgundy Fawn and H = hybrid rabbits), were used. The mother origin of B and F GO was a mongrel. From weaning onward, all the animals were caged by pairs

indoor and fed ad libitum the same pelleted diet until the slaughter weight, fixed at 2.8 ± 0.11 kg. Within 10 minutes after death a sample of Longissimus lumborum (LL) muscle was removed from each rabbit, frozen in isopentane cooled by liquid nitrogen and stored at -80°C . The activities of the enzymes citrate synthase (CS, E.S 4.1.3.7) and lactate dehydrogenase (LDH, EC 1.1.1.27), were recorded. These enzyme were respectively characteristics of the oxidative and glycolytic metabolic pathways as described Bass et al. (1969). For histochemical determinations, 6 serial cross-sections were obtained with a cryostat at -20°C . One section was stained with azorubine (reference stain). Four sections were processed for the mATPase activity after acid or alkaline pre-incubation (Guth & Samaha, 1970). The sixth was stained for succino-dehydrogenase (SDH) activity according to Nachlas et al. (1957). Computerised image analysis (Buche, 1990) was used to classify the fibres as βR (red and slow twitch fibre), αR (red and fast twitch fibre) or αW (white and fast twitch fibre) according to Ashmore & Doerr (1971). The analysis combines the myofibrillar ATPase and SDH stain intensities of each cell. For each muscle fibre type the respective percentage and the cross-sectional area (CSA; μm^2) were determined. Twenty-four hours post mortem the pH (pHu) and the L^*a^*b colour values (CIE, 1976) were measured on Biceps femoris (BF) and LL muscles, while the chemical composition (water, lipids and ash) was analyzed on the hind leg meat (AOAC, 1984). Protein content was estimated by difference. ANOVA was performed using the GLM procedure of the SAS (1990) program. The model included the GO (H, B, F), the sex (S) and the GOxS interaction as fixed effects. LS means were calculated for all the effects involved in the model and the t test between means was calculated.

Results & Discussion

Table 1 summarises the effects of the rabbit's GO and sex (S) on the chemical composition of hind leg meat. At the fixed slaughter weight of 2.8 ± 0.11 kg, the rabbits of the 3 GO significantly differed in slaughter age (88 vs 109 vs 122 days for H, B and F GO, respectively; $P < 0.001$), indicating that B and F crossbred rabbits are less precocious and have lower growth performance than H rabbits. This difference in age did not produce a similar trend in the chemical composition of the hind leg meat because the meat of H rabbits had intermediate water and lipid content, while the highest water and the lowest lipid contents were found in B rabbits. The selection for increased growth rates in H rabbits has strongly increased their precocity and this could preclude their slaughter at eldest age, if the market conditions or specific production lines require eldest rabbits. The results obtained here show that rabbits from B group could be used for this purpose. The water content of the hind leg meat was found to be higher in males than in females (73.5 vs 72.9%; $P < 0.05$) while the lipid content showed the opposite trend (4.03 vs 3.06%, $P < 0.001$). However, the GOxS interaction evidenced that females have higher lipid content only in F ($P < 0.001$) and C GO ($P < 0.05$). Data reported in literature indicate that the chemical composition of the rabbit meat is not so influenced by the S either in hybrid (Dalle Zotte *et al.*, 1996) or in purebred rabbits (Prezioso *et al.*, 1996), when the age at slaughter is under 87 days. At older age the sexual dimorphism become important also in the rabbit, as demonstrated in the present work. The pHu value of the meat was not influenced by the experimental treatments (Table 2). It has been shown that the pHu

of meat did not differ among genetic lines of hybrid rabbits when the animals were slaughtered at the same live weight but within a difference of 15 days of age (Dalle Zotte & Ouhayoun, 1998). Nevertheless, the pHu value significantly decreases with rabbit's age, but this decrease stops at 42 days of age in predominant oxidative muscles, such as the *P. major* (Dalle Zotte & Ouhayoun, 1995), or at 70-77 days of age in predominant glycolytic muscles, such as the LL (Dalle Zotte & Ouhayoun, 1995) and the BF (Dalle Zotte *et al.*, 2005b). This is due to the lessening of the glycolytic activity (Dalle Zotte & Ouhayoun, 1995). The L* value of BF muscles was significantly modified by the GO (50.7 vs 52.9 vs 54.1 for H, B and F, respectively: $P < 0.05$) and was higher in B+F than in H ($P < 0.05$), indicating that crossbred rabbits could have paler meat than hybrid ones (Table 2). Since the age also increases from H to B and to F, this rise in L* value could also depend on the rabbits' age. In F animals, the females showed higher L* values than males (55.3 vs 53.0; $P < 0.05$). In literature it has been reported that L* value of BF muscle do not change with rabbit's age (Dalle Zotte *et al.*, 1996; Dalle Zotte *et al.*, 2005b; Preziuso *et al.*, 1996), but the considered ages were below 87 days. Preziuso *et al.* (1996) comparing the Burgundy Fawn and the New Zealand White sires did not observe difference in L*a*b* values of BF muscle; in the same study, L* value was higher and a* value was lower in females than males ($P < 0.05$) supporting the results found in the present work. The percentage of α W fibres in LL muscle differed between B and F animals (78.9 vs 84.3%; $P < 0.05$) while that of H rabbits was intermediate (83.2%; Table 3). Arnal & Lòpez (2001) did not find difference on fibre type distribution among 7 purebred and 1 synthetic line slaughtered at 77 or 84 days of age, without consideration of the respective weights. The increase with age of the percentage of α W fibres associated with an increase of the relative glycolytic energy metabolism, to the detriment of the percentage of oxidative and slow twitch fibres (β R), has already been reported (Dalle Zotte & Ouhayoun, 1995; Dalle Zotte *et al.*, 1996; Arnal & Lòpez, 2001; Dalle Zotte *et al.*, 2005a, 2005b). Since the variation of metabolic ratio between breeds result in some cases from differences in precocity (Ouhayoun & Dalle Zotte, 1993), in hybrid rabbits the α W fibres are predominant if compared with unselected rabbits. For these reasons, in the present study the elder age of F than H GO could determine an increase in the α W fibres percentage. Males showed higher mean CSA of α R fibres than females (1221 vs 952 μm^2 ; $P < 0.05$). The mean CSA of the three fibre types was the highest in B animals. This could indicate higher growth potentialities in B than in F animals. The CS and LDH activities in LL muscle were not affected by rabbits' GO and S.

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Conclusions

The results obtained in this study confirm that B and F crossbreds are less precocious than H rabbits. Furthermore, this study demonstrate that among B and F crossbreds, the B achieves earlier the fixed slaughter weight and produce leaner meat with a lower proportion of white muscle fibers but without change in the meat pHu and colour values. Compared to the H and F animals, the B ones evidenced a tendency towards higher CSA for all the fibre types in LL muscle. Further research need to be done in this area in order to clarify the relationship between the genetic origin and the fibre traits in rabbits slaughtered at advanced age for commercial purpose.

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Table 1. Performances at slaughter and chemical composition of hind leg meat (% of fresh weight)

	GENETIC ORIGIN (GO)			SEX (S)		<i>P-value</i> ^a				RMS E ^b
	H	B	F	FEMA LE	MA LE	G O	S	B+F vs H	GOx S	
N. rabbits	12	14	15	17	24					
Slaughter weight, g	2863	2792	2737	2810	2785	ns	ns	*	ns	113
Slaughter age, days	88 ^a	109.3 ^b	122.1 ^c	106.2	106.8	**	ns	***	ns	9.0
Water, %	73.0 ^{ab}	73.7 ^b	72.9 ^a	72.9	73.5	*	*	ns	ns	0.8
Protein, %	22.2	21.9	22.0	21.9	22.2	ns	ns	ns	ns	0.5
Lipids, %	3.56 ^{ab}	3.16 ^a	3.91 ^b	4.03	3.06	†	*	ns	* ^c	0.77
Ash, %	1.26	1.25	1.22	1.24	1.25	ns	ns	ns	ns	0.04

^a ns, not significant: $P>0.10$; †: $P<0.10$; *: $P<0.05$. ***: $P<0.001$. Within a row, means with different superscripts (a, b, c) are significantly different. ^bRMSE is the residual mean square error. ^c B =females 3.16% vs males 3.15% (ns); F= females 4.78% vs males 3.04% ($P<0.001$); H=females 4.14% vs males 2.98% ($P<0.05$)

Table 2. pH and L*a*b* colour values for *Biceps femoris* and *Longissimus lumborum* muscles

	GENETIC ORIGIN (GO)			SEX (S)		<i>P-value</i> ^a				RMSE ^b
	H	B	F	FEMAL E	MAL E	G O	S	B+F vs H	GOx S	
N. rabbits	12	14	15	17	24					
<i>Biceps femoris</i>										
pHu	5.83	5.80	5.75	5.77	5.81	ns	ns	ns	ns	0.08
L*	50.7 ^a	52.9 ^b	54.1 ^b	52.5	52.7	*	ns	*	** ^c	1.7
a*	4.41	4.02	3.88	4.51	3.71	ns	ns	ns	ns	1.76
b*	2.25	2.76	3.64	3.24	2.53	ns	ns	ns	ns	1.35
<i>Longissimus lumborum</i>										
pHu	5.66	5.64	5.65	5.63	5.67	ns	ns	ns	ns	0.07
L*	58.8	58.7	58.9	59.1	58.5	ns	ns	ns	ns	1.9
a*	2.05	2.65	1.93	2.13	2.29	ns	ns	ns	ns	1.25
b*	-2.09	-0.59	-0.08	-0.73	-1.12	ns	ns	ns	ns	1.44

^a ns, not significant: $P>0.10$; †: $P<0.10$; *: $P<0.05$ **: $P<0.01$. Within a row, means with different superscripts (a, b) are significantly different. ^bRMSE is the residual mean square error. ^c B=females 52.4 vs males 53.4 (ns); F= females 55.3 vs males 53.0 ($P<0.05$); H=females 49.7 vs males 51.8 (ns)

Table 3. Morphometric traits, fibre type distribution and enzymatic activity of the *Longissimus lumborum* muscle

	GENETIC ORIGIN (GO)			SEX (S)		<i>P</i> -value ^a				RMSE ^b
	H	B	F	FEMALE	MALE	GO	S	B+F vs H	GOxS	
N. rabbits	15	13	13	15	25					
<u>Fibre cross-sectional area (μm^2):</u>										
αR	915	1215	1130	952	1221	ns	*	†	ns	349
αW	1746	1947	1555	1704	1795	ns	ns	ns	ns	538
βR	758	1137	892	884	974	ns	ns	ns	ns	454
<u>Fibre type distribution (%)</u> :										
αR	12.9	15.4	13.2	14.1	13.5	ns	ns	ns	ns	4.2
αW	83.2 ^{ab}	78.9 ^a	84.3 ^b	82.3	82.0	†	ns	ns	ns	5.8
βR	3.9	5.6	2.5	3.6	4.5	ns	ns	ns	ns	4.3
<u>Enzymatic activity (IU)^c:</u>										
CS	5.72	5.89	6.10	6.02	5.79	ns	ns	ns	ns	1.35
LDH	909	912	837	893	879	ns	ns	ns	ns	131
LDH/CS	164	163	144	155	158	ns	ns	ns	ns	39

^a ns, not significant: $P > 0.10$; †: $P < 0.10$; *: $P < 0.05$. Within a row, means with different superscripts (a, b) are significantly different; ^bRMSE is the residual mean square error; ^cIU: moles of substrate degraded/min/g fresh meat

INTER-MUSCULAR DIFFERENCES IN RESPONSE TO LOW VOLTAGE ELECTRICAL STIMULATION OF LAMB

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Key Words: electrical stimulation, lamb, eating quality

Introduction

Traditional electrical stimulation (ES) increases the rate of post mortem glycolysis in red meat carcasses, hence facilitating rapid chilling pre-rigor and avoiding cold shortening (Chrystall and Devine 2000). However, extensive ES may inadvertently induce heat shortening, thus adversely affecting eating quality. Hence, most conventional ES procedures aim to decrease the pH of the *longissimus dorsi* muscle to no less than pH 6.3 1h post mortem. Conversely, Gault *et al.* (2000) demonstrated that low voltage electrical stimulation (LVES) of chicken carcasses could induce a rapid drop in the breast muscle to pH 6.0, inducing very early rigor onset, yet having no detrimental effect on eating quality. This was achieved using only 300 pulses of 100v LVES applied at various duty cycles (Li *et al.* 1993) to induce different rates of wing flapping, as opposed to the tetanus inducing duty cycles used in conventional red meat ES systems.

Unlike chicken breast muscle which comprises mainly α W (fast-twitch high glycolytic) muscle fibres (Sams and Janky 1990), the muscles of red meat animals consist of varying proportions of α W, α R (fast-twitch high oxidative) and β R (slow-twitch intermediate/high oxidative) muscle fibre types (Monin 1981). Thus their responsiveness to carcass LVES should reflect how the electrical parameters used affect the physiological processes that invoke muscle contraction and anaerobic energy utilization.

Objectives

To assess (a) the effectiveness of the glycolytic response of different muscles in lamb carcasses to low frequency LVES treatments which induce separate muscle contractions, thus avoiding complete tetanus, and (b) the effect of these treatments on the quality characteristics of muscles de-boned early and at 24h.

Methodology

Animals and treatments

This study used 40 (24 male; 16 female) Texel x Greyface crossbred lambs; mean live weight 44.76 kg (range 35-56kg). Groups of 4 lambs were randomly allocated to one of 9 treatments in which 300 pulses of LVES, at a constant 100v, were applied to each carcass 90s after captive bolt stunning and bleeding. The 3 x 3 + 1 split plot factorial design comprised the use of frequencies of 1, 3, and 5 Hz at pulse widths of 10, 50 and 100 ms. These equate to duty cycles of 1%, 3%, 5%, 10%, 15%, 25%, 30% and 50%. A control group of 4 lambs received no LVES treatment. All carcasses were dressed and split within 10 minutes of bleeding.

Muscles & de-boning

The muscles selected for assessment were the *semitendinosus* (ST); *longissimus dorsi* (LD); *vastus lateralis* (VL); *semimembranosus* (SM); *gluteus medius* (GM); *triceps brachii* (TB); *supraspinatus* (SS) and *infraspinatus* (IS). These represent a broad range of muscle types differentiated by their varying proportions of α W, α R and β R muscle fibre types respectively (Monin, 1981).

Muscles from alternate carcass sides within each treatment were removed for analysis immediately after splitting (early de-boned), while the matching sides were Achilles hung until 24h post-mortem (late de-boned). Chilling was carried out at 9°C for the first 24h post-slaughter. Muscles were then stored aerobically for one week at 2°C when the remaining analyses were completed.

Meat quality measurements

Muscle pH decline was monitored at 1h, 2h and 24h post mortem in each of the early de-boned muscles, and at 24h in the late de-boned muscles. Approximately 1g samples of muscle were frozen in liquid nitrogen until analyzed by the iodoacetate method of Bendall (1975). Sarcomere lengths were measured in samples of early and late de-boned muscles at 48h post-mortem by the method of Koolmees *et al* (1986), and cooking loss and shear force assessed (Gault *et al*, 2000) at 48h and 7 days post mortem. Data were analyzed by ANOVA (Genstat, 2003).

Results & Discussion

Intermuscular and treatment effects on quality parameters - whole carcasses

The primary findings of this experiment were in identifying significant ($P < 0.001$) intermuscular differences amongst all of the quality traits measured for all treatments combined (Table 1), namely pH after 1h, 2h and 24h; and sarcomere length, cooking loss and shear force values after 48h and 7 days storage. This simply confirms the wide range

in metabolic activity, chemical composition and impact of anatomical location of the muscles chosen.

Likewise, a comparison of the quality traits measured between the control treatment and those of the combined LVES treatments for all muscles combined (Table 2) shows that the LVES treatments gave significantly lower ($P < 0.001$) mean pH values at 1h and 2h post-slaughter. In contrast, LVES, taken as a combined treatment, had no significant effect ($p > 0.05$) on the other quality parameters compared to the control, the only exception being the greater cooking losses at 48h ($P < 0.01$) due to LVES.

Pulse width and frequency of LVES were also analyzed as independent treatments against the control treatment for all muscles combined. In general, neither frequency nor pulse width had a significant effect ($P > 0.05$; data not shown) on any quality parameter. The only significant interaction found was that between pulse width and cooking loss at 48h ($P < 0.01$; data not shown), similar to that found between the combined LVES treatments and the control in Table 2. Consequently, although the combined LVES treatment significantly increased the glycolytic response of all muscles (Table 2), the combinations of pulse width and frequency of LVES chosen had little specific influence on any quality parameter. This strongly suggests that the combinations of pulse width and frequency used were equally effective in enhancing a glycolytic response without adversely affecting other quality characteristics.

The effects of control and combined LVES treatments on the pH values of individual muscle groups are shown in Table 3. In the control carcasses, the order of mean pH fall 1h after slaughter followed the expected trend in glycolytic response related to predominant muscle fibre type in lamb muscles (Monin, 1981). The predominantly α W fibre-type ST gave the greatest decrease, the α R LD and SM an intermediate response, and the β R SS and IS the least response. Likewise, for the ES carcasses, a similar trend was observed, the main exception being the unexpectedly large pH fall in the TB, a predominantly β R metabolic type muscle. The low pH_{1h} values of the ST and TB suggest that these muscles were very close to entering rigor. In contrast, when using pH decline as a measure of glycolytic responsiveness to LVES, the other muscles were much less responsive, especially the SS, VL and LD. Intermuscular pH differences at 2h reflected those at 1h, whereas those at 24h reflected normal intermuscular biochemical differences attributable to glycogen reserves and fibre type distribution (Table 3).

In contrast to the intermuscular treatment interactions shown in Table 3, no significant interactions ($P > 0.05$; data not shown) were found for any of the other quality parameters measured.

Pulse width and frequency of LVES were also analyzed as independent treatments against control values in relation to individual muscle groups (data not shown). The only significant interactions found were those between pulse width and pH_{1h} ($P < 0.05$), pulse width and cooking loss at 48h ($P < 0.01$), and pulse width and shear force value at 48h ($P < 0.05$). Frequency of LVES gave no significant interaction ($P > 0.05$) between any of the meat quality parameters measured and individual muscle groups.

Intermuscular and treatment effects on quality parameters - early and late de-boning

Early de-boning for all treatments and muscles combined (Table 4) resulted in significantly shorter sarcomeres and higher shear force values at both 48h and 7d (all $P < 0.001$). There was no significant effect of de-boning time on cooking losses.

Likewise, early de-boning for all treatments combined brought about similar intermuscular effects (Table 5), sarcomeres being significantly shorter ($P < 0.001$) and shear forces at both 48h and 7d significantly higher ($P < 0.001$) than the comparable muscles de-boned after 24h. There were no significant intermuscular effects or interactions on cooking losses ($P > 0.05$; data not shown).

Interestingly, a significant combined muscle \times LVES \times control treatment interaction ($P < 0.05$) was found for shear force values at 48h (Table 6). Although early de-boning clearly induced an increase in shear force, this was generally significantly less for the LVES treatments. As expected, there was generally no difference in shear force values between the control and LVES treatments for the 24h de-boned muscles. The effect of the interactions between pulse width and frequency of LVES are difficult to interpret, although there would appear to be a trend suggesting that the lowest shear forces may be found with higher frequency \times pulse width combinations, i.e. at higher duty cycles.

Conclusions

This study has confirmed that important intermuscular differences exist in the glycolytic response of lamb carcasses to pulsed LVES treatments. The greatest response was seen with predominantly α W fibre-type muscles, and the least with predominantly β R fibre-types, some muscles reaching pH 6.0 within 1h of slaughter. While significant intermuscular differences in all other quality parameters were found, the LVES treatments used had no significant effect on these. In contrast, early de-boning resulted in significantly higher shear force values after 48h for all muscles combined. However, these were significantly lower for those from the LVES treated carcasses, even though some of these muscles would have entered rigor early. This merits further study on the use of extended LVES to minimize the adverse effects of early de-boning of lamb carcasses.

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Tables and Figures

Table 1: Intermuscular differences in the quality attributes of lamb.

Muscle	pH _{1h}	pH _{2h}	pH _{24h}	SL _{48h} μm	CL _{48h} %	CL _{7d} %	SF _{48h} kgcm ⁻²	SF _{7d} kgcm ⁻²
ST	6.10	6.01	5.80	2.11	33.0	33.5	7.51	5.02
LD	6.37	6.29	5.60	1.87	25.4	27.0	5.44	4.05
SM	6.32	6.20	5.68	1.91	30.0	31.7	6.11	4.58
VL	6.41	6.30	5.80	1.93	30.3	31.0	6.11	3.91
GM	6.35	6.27	5.66	1.86	27.3	29.0	5.80	4.15
TB	6.22	6.13	5.79	2.15	26.3	27.6	5.34	3.45
SS	6.56	6.41	5.91	1.86	32.5	32.3	5.46	3.94
IS	6.42	6.31	5.90	1.84	25.3	24.4	5.09	3.56
mean	6.34	6.24	5.77	1.94	28.8	29.6	5.86	4.08
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
n	40	40	80	80	80	80	80	80
l.s.d. (P<0.05)	0.05	0.05	0.02	0.06	0.8	0.7	0.32	0.21

Table 2: Effect of combined LVES v control treatments on the quality attributes of lamb for all muscles combined.

Treatment	pH _{1h}	pH _{2h}	pH _{24h}	SL _{48h} μm	CL _{48h} %	CL _{7d} %	SF _{48h} kgcm ⁻²	SF _{7d} kgcm ⁻²
Control	6.59	6.48	5.73	1.92	26.62	28.29	6.06	4.41
LVES	6.32	6.21	5.77	1.94	28.99	29.70	5.84	4.04
P	<0.001	<0.001	NS	NS	<0.01	NS	NS	NS
n	32/288	32/288	64/576	64/576	64/576	64/576	64/576	64/576
l.s.d. (P<0.05)	0.11	0.10	0.09	0.07	1.51	1.96	0.76	0.52

Table 3: Effect of combined LVES treatments on inter-muscular pH values compared to control values.

Muscle	pH _{1h} control	pH _{1h} LVES	pH _{2h} control	pH _{2h} LVES	pH _{24h} control	pH _{24h} LVES
ST	6.46	6.06	6.38	5.97	5.85	5.80
LD	6.54	6.35	6.42	6.28	5.56	5.60
SM	6.55	6.29	6.41	6.18	5.61	5.68
VL	6.58	6.39	6.43	6.29	5.80	5.80
GM	6.59	6.32	6.45	6.26	5.62	5.67
TB	6.61	6.18	6.53	6.08	5.71	5.80
SS	6.66	6.54	6.63	6.38	5.87	5.92
IS	6.72	6.39	6.62	6.27	5.56	5.60
mean	6.59	6.32	6.48	6.21	5.73	5.77
P		<0.01		<0.001		<0.01
n	4	36	4	36	8	72
l.s.d. ^a	0.16	0.05	0.15	0.05	0.07	0.02
l.s.d. ^b		0.16		0.15		0.10

^a least significant difference (P<0.05) for means within a column.

^b least significant difference (P<0.05) for means across each pair of columns.

Table 4: Effect of de-boning time on quality attributes of all muscles combined.

De-boning time	pH _{1h}	pH _{2h}	pH _{24h}	SL _{48h} µm	CL _{48h} %	CL _{7d} %	SF _{48h} kgcm ⁻²	SF _{7d} kgcm ⁻²
early	-	-	5.76	1.81	29.13	29.54	6.46	4.51
24h	-	-	5.77	2.07	28.37	29.56	5.26	3.66
P	-	-	<0.05	<0.001	NS	NS	<0.001	<0.001
n	-	-	320	320	320	320	320	320
l.s.d. (P<0.05)	-	-	0.01	0.06	0.81	0.68	0.14	0.11

Table 5: Effect of de-boning time on quality attributes of individual muscles.

Muscle	pH _{24h} early	pH _{24h} 24h	SL _{48h} early	SL _{48h} 24h	SF _{48h} early	SF _{48h} 24h	SF _{7d} early	SF _{7d} 24h
ST	5.79	5.81	1.92	2.31	8.36	6.66	5.79	4.25
LD	5.61	5.58	1.82	1.91	5.97	4.92	4.38	3.72
SM	5.68	5.67	1.87	1.94	6.25	5.96	4.62	4.55
VL	5.81	5.80	1.85	2.02	6.93	5.29	4.37	3.44
GM	5.67	5.67	1.81	1.89	6.29	5.32	4.41	3.89
TB	5.79	5.79	1.81	2.49	5.99	4.70	3.92	2.98
SS	5.89	5.94	1.73	1.98	5.95	4.96	4.29	3.59
IS	5.85	5.94	1.70	1.99	5.94	4.24	4.28	2.83
mean	5.76	5.77	1.81	2.07	6.46	5.26	4.51	3.66
P	<0.001		<0.001		<0.001		<0.001	
n	40	40	40	40	40	40	40	40
l.s.d. ^a	0.03	0.03	0.08	0.08	0.45	0.45	0.30	0.30
l.s.d. ^b	0.03		0.08		0.44		0.30	

^a least significant difference (P<0.05) for means within a column.

^b least significant difference (P<0.05) for means across each pair of columns.

Table 6: Effect of de-boning time on combined muscle shear force values at 48h as a function of individual LVES and control treatments.

De-boned	PW(ms)	Frequency (Hz)			mean (PW)	
		1Hz	3Hz	5Hz		
early ^a	control	6.90				
	10ms		7.12	7.17	6.07	6.79
	50ms		6.24	5.85	6.48	6.19
	100ms		6.13	6.55	6.10	6.26
	mean (F)		6.50	6.52	6.22	6.41
24h ^a	control	5.22				
	10ms		5.62	5.50	5.29	5.47
	50ms		5.46	5.20	5.24	5.30
	100ms		4.85	5.27	4.92	5.01
	mean (F)		5.31	5.32	5.15	5.26
P		<0.01	<0.01	<0.01	<0.01	
reps		32	32	32	32	
l.s.d. ^b		1.06	1.06	1.06	1.06	

^a least significant difference (P<0.05) = 0.44 for all means within each de-boning period.

^b least significant difference (P<0.05) for all treatment means.

YIELDS OF EASILY RELEASABLE MYOFILAMENTS AND MYOFIBRILLAR PROTEOLYSIS IN DENERVATED MUSCLE.

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Introduction

A small sub-population of myofilaments, termed easily releasable myofilaments (ERM) is readily released from the surface of myofibrils under relaxing conditions (i.e. 3mM MgATP in 0.1mKCl, 3mM EGTA, 1mM DTT, 20mM tris-maleate, pH7.0 – Low Salt Buffer [LSB]). It has been proposed that ERM are early intermediates in the turnover of myofibrils (1). If this hypothesis is correct it would follow that ERM yields would be increased in conditions where proteolysis of myofibrils is elevated, and in several instances this has been shown to be the case. Increased yields of ERM have been found in post-mortem bovine chicken and porcine muscle (2) and in Vitamin-E deficient muscle (3).

In many cases the detection of myofibrillar proteolysis is a rather subtle matter. The conventional method of examination of myofibrillar protein subunit composition by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE), although quite sensitive, usually detects but a weak signal of proteolysis even when proteolysis is known to be elevated, e.g. as evidenced by SDS-PAGE examination of myofibrils during the post-mortem ageing of beef. However, if much of the activity during myofibrillar proteolysis takes place in the small ERM sub-population of myofilaments, then closer examination of the ERM fraction by SDS-PAGE should more easily detect evidence of proteolysis in conditions where proteolysis is enhanced.

Myofibrillar proteolysis is elevated in denervated muscle. In this present study ERM yields were measured in denervated rat skeletal muscle and the ERM fraction from denervated muscle was carefully examined by SDS-PAGE for evidence of proteolysis.

Objectives

To determine if ERM yields are elevated in denervated muscle and to examine ERM by SDS-PAGE for a strong signal of proteolysis.

Methodology

Myofibrils were prepared from the gastrocnemius muscle by the rigor method from groups of mature male Wistar rats (360-399g). Denervation was effected by the surgical removal of a 1 cm section of the sciatic nerve from the upper right hind limb. The muscles of the untouched contra-lateral limb served as the control samples. Because the

individual gastrocnemius muscles are small, 4-5 animals were used for each control/denervated sample pair in order to allow sufficient ERM protein to be prepared to calculate ERM yields. Data from ten such paired samples were statistically analysed using a paired t-test. The animals were denervated 14 days prior to sacrifice. ERM were prepared from myofibrils as previously described (2) and myofibrils and ERM were subjected to SDS-PAGE, with separation achieved on an 8-18% linear gradient slab gel.

Results & Discussion

Denervation for 14 days caused a marked decrease in the size of the gastrocnemius and the soleus muscles. Control gastrocnemius weighed 1846 ± 107 mg, denervated gastrocnemius weighed 1110 ± 161 mg, control soleus weighed 146 ± 12 mg, and denervated soleus weighed 80 ± 8 mg (means \pm 1s n=4 animals).

ERM yields from control and denervated muscles are illustrated in Figure 1. ERM yield from denervated muscle, at $3.69 \pm 0.75\%$ of total myofibrillar protein, was 56% greater than ERM yield from control muscle at $2.37 \pm 0.45\%$ (means \pm 1s, n=10, $P \leq 0.001$). Protein release measured in the absence of Mg-ATP i.e. in presence of LSB alone, was very low but the means here were also significantly different – $0.54 \pm 0.18\%$ total myofibrillar protein in denervated muscle vs $0.38 \pm 0.17\%$ in control muscle (means \pm 1s, n=10, $P \leq 0.001$).

Figure 2 illustrates the myofibril and ERM protein subunit profiles as separated on SDS-PAGE from control and denervated gastrocnemius muscle. The main protein bands are readily identified as myosin, α -actinin, actin, thin myofilament regulatory proteins and thick myofilament associated proteins. Little difference is discernible between control and denervated myofibrils (lanes 2 and 3, control and denervated muscle respectively, on SDS-PAGE), despite the considerable wasting of the denervated muscle.

In consonance with the increased ERM yields from denervated muscle, the ERM protein subunit profiles on SDS-PAGE differ markedly from each other between control and denervated muscle (Figure 2, lanes 4 and 5 – control and denervated muscle respectively). An extensive ladder of bands running from approximately 33kDa to 14kDa was apparent in the denervated ERM fraction, but not in the control ERM fraction. There was also a noticeable decrease in the myosin to actin ratio in the ERM denervated fraction compared to control ERM.

The extensive ladder of lower molecular weight bands that appears in denervated ERM presumably reflects proteolytic breakdown products of higher molecular weight proteins. This indicates that myofibrillar proteolysis is concentrated in the ERM fraction, i.e. the loosely attached peripheral myofilaments of the myofibril, because these breakdown products are not at all obvious when the whole myofibril is examined. Since ERM protein constitutes only 2 to 4 percent of total myofibrillar protein, the apparent effects of proteolysis, if concentrated in this ERM fraction, would be massively diluted when the whole myofibril is examined by SDS-PAGE.

Other work in this laboratory has shown that ERM from post-mortem bovine muscle also display the progressive appearance, as ageing of the muscle proceeds, of protein subunit in the molecular weight range 22kDa to 45kDa, and these bands are much more readily apparent in ERM than in preparations of whole myofibrils examined by SDS-PAGE.

ERM in post-mortem muscle also show a decreased myosin to actin ratio compared to whole myofibrils, similar to that seen in denervated muscle, and the decreasing ratio is progressive with extent of post-mortem ageing. This is mostly likely explained by Z line weakening due to proteolysis leading to an easier release of thin myofilaments thereby enhancing the ratio of thin to thick myofilaments in ERM preparations.

Many investigations of myofibrillar changes in muscle in conditions of increased protein turnover or studies of the post-mortem ageing of meat have concentrated on whole muscle samples or on myofibril preparations (4, 5). Very few studies of changes in myofibrils under conditions of enhanced proteolysis have concentrated on ERM. Future studies in this area might more fruitfully concentrate on ERM where the signal of proteolysis is more easily detected.

Conclusions

ERM yields are significantly increased in rat skeletal muscle after 14 days of denervation compared to undenervated control muscle. ERM prepared from myofibrils from denervated muscle show clear evidence of proteolysis on examination by SDS-PAGE. Since this evidence is not readily apparent in whole myofibrils prepared from denervated muscle, it seems that the signal of myofibrillar proteolysis is much more readily detectable in the ERM fraction. This has significance for future studies of myofibrillar proteolysis in various conditions.

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Tables and Figures

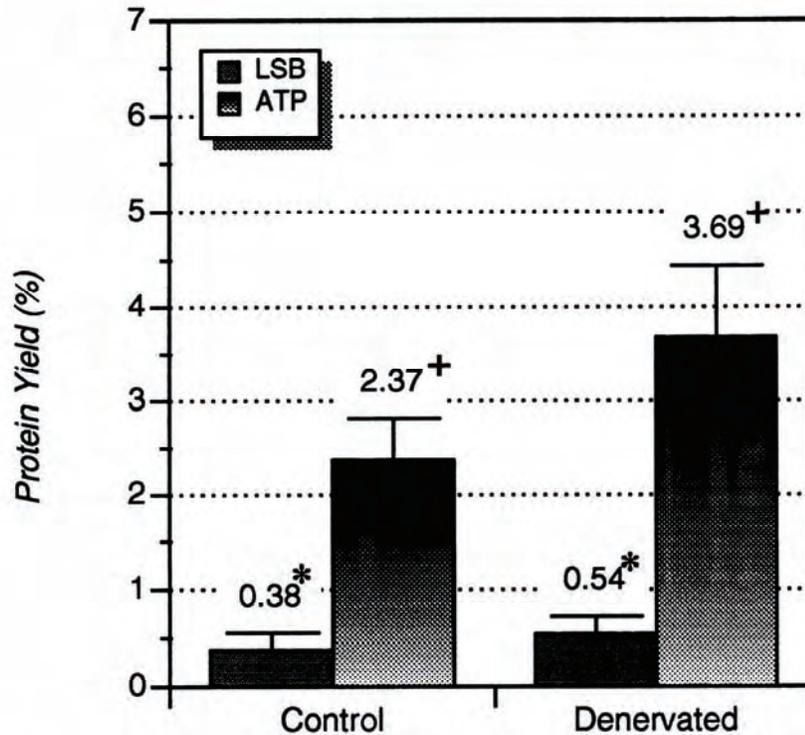


Figure 1. ERM yields (in presence of Mg-ATP and Low Salt Buffer [LSB]) and protein released from myofibrils in the absence of Mg-ATP (in presence of LSB alone) from rat gastrocnemius muscle after 14 days denervation and from control contra-lateral gastrocnemius muscle that was not denervated. Yield are expressed as percentage of total myofibrillar protein. Bars represent means \pm 1s, n=10 paired preparations of control and denervated samples (4-5 animals were required for each control/denervated sample pair). Significant differences were observed between LSB control vs LBS denervated and between ERM (ATP) control vs ERM (ATP) denervated as assessed by paired t-test ($P\leq 0.001$).

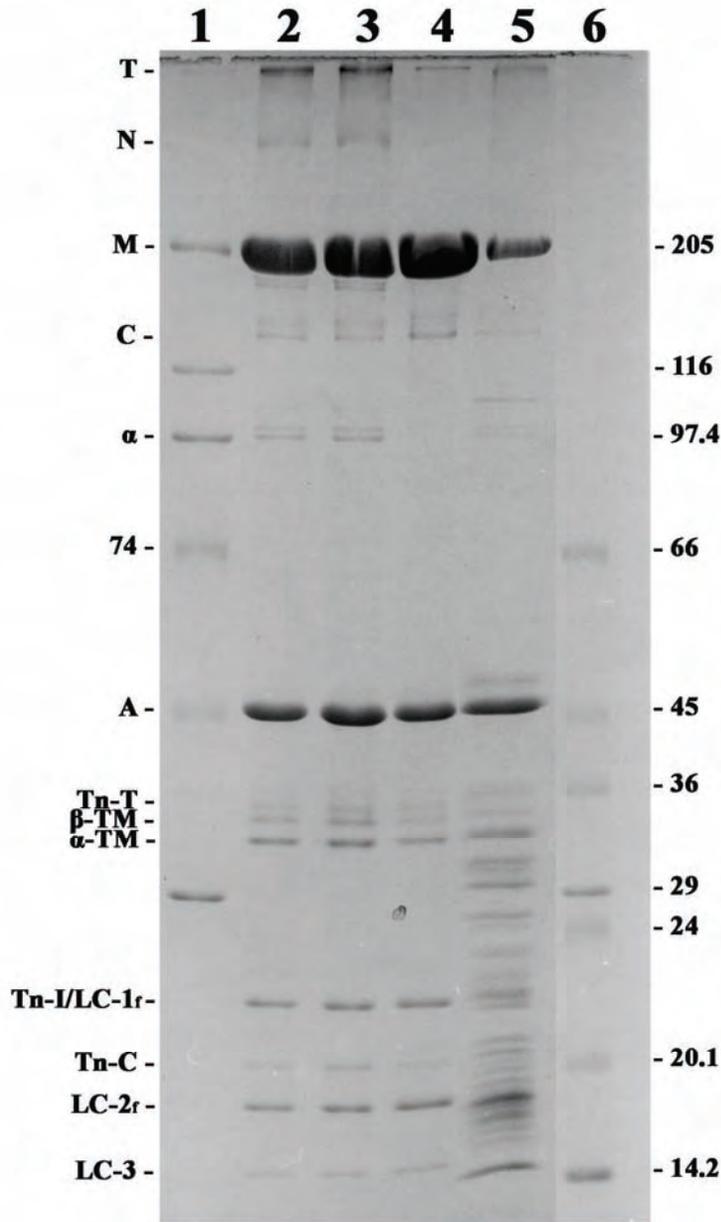


Figure 2. SDS-PAGE profiles of myofibrils and ERM from rat gastrocnemius muscle from control and 2 week denervated animals. Lanes 1 and 6 correspond to high and low range molecular weight markers respectively. Numerical values are quoted as kDa. Lanes 2 and 3 are myofibrils from control and denervated muscle respectively. Lanes 4 and 5 are ERM from control and denervated muscle respectively. 30 μ g total protein was applied per well and separation was achieved on an 8-18% linear gradient slab gel. T: titin; N: nebulin; M: myosin; C: c-protein; α : α -actinin, A: actin; TnT: troponin-T; TM: α , β -tropomyosin; LC1_f: fast isoform of myosin light chain-1; Tn-1: troponin-1; TnC: troponin C; LC2_f: fast isoform of myosin light chain-2; LC3: myosin light chain-3.

PREDICTION OF FRESH PORK QUALITY USING INDICATORS OF PROTEIN DEGRADATION AND CALPAIN ACTIVATION

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Key Words: calpain, desmin, tenderness, water holding capacity

Introduction

Variation in pork quality attributes like water holding capacity and tenderness continues to reduce value of fresh pork. While it is clear that pH and temperature decline influence fresh pork quality, identification of undefined sources of variation contributing to poor quality is required to provide opportunities to improve pork quality. Postmortem changes in meat protein contribute to fresh meat quality. Myofibrillar fragmentation has long been associated with improved tenderness of meat. A current hypothesis explored by several laboratories (Melody et al., 2004; Morrison et al. 1998) proposes that proteolysis of key myofibrillar proteins minimizes the loss of water holding capacity caused by lateral shrinkage of myofibrils in postmortem muscle (Diesbourg et al., 1988). The proteolysis of meat proteins that contribute to these fresh pork characteristics is most frequently attributed to μ -calpain. It is therefore proposed that activation of μ -calpain is expected to predict variation in fresh pork water holding capacity and tenderness.

Objectives

The objectives of the current study were to 1) Determine the relationship and predictive value of pH, temperature and indicators of proteolysis and calpain activity at various postmortem times on quality in the pork loin; and 2) Determine the specific time points most important for solving problems with water holding capacity and tenderness.

Methodology

Sixty-four Duroc x Yorkshire gilts (n = 32) and barrows (n = 32) were harvested using humane practices at the Iowa State University Meat Laboratory, Ames, IA. All pigs were harvested at approximately 113 kg. All pigs originated from the same farm and were held in lairage overnight without feed. Four groups (16 pigs per group) over a 4 wk period were used. Barrow and gilt carcasses were randomly assigned to a 2x2 treatment arrangement, varying in the interval between sticking and scalding (5 or 10 min) and duration of scalding (5 or 8 min).

Core temperature and pH of the LD (at the last rib) on the right side of the carcass were measured at 45 min, 2 h, 4 h, 6 h and 24 h postmortem. At 24 h postmortem samples from the LD were excised from the left side of the carcass. Two 2.54 cm thick LD chops were removed approximately 2.54 cm anterior to the hipbone pocket. These

samples were used for drip loss. From a point immediately anterior to the previously mentioned LD chops, four 2.54 cm thick chops were removed for Warner-Bratzler Shear force analysis (Gardner et al., 2005).

Drip loss was measured in duplicate using the previously mentioned LD chops during 1 d of storage at 1 °C in a plastic bag under atmospheric conditions. Purge loss was determined on LD samples from the sirloin portion of the loin held in a vacuum package for 7 d. Drip loss and purge loss were calculated as a percentage of initial weight of the product lost during storage (Melody et al., 2004). Two 2.54 cm LD chops were removed and stored in a vacuum bag at 1 °C for 3 or 5 d postmortem. After aging, chops were frozen (-20 °C) until Warner Bratzler Shear force analysis (Lonergan et al., 2001).

To monitor protein degradation, desmin was selected as an indicator of overall proteolysis. Samples were removed from the LD immediately anterior to the corresponding chops used for WBS. Desmin degradation was determined on samples aged 1, 5 and 7 d postmortem using immunoblotting (Melody et al., 2004) and recorded as a ratio of each sample to intact desmin in a reference sample run on all gels. μ -Calpain autolysis was determined on samples aged 1 d postmortem with immunoblotting and reported as percentage of the large subunit present as 80, 78 or 76 kDa polypeptide.

All data were analyzed using SAS Version 8.2 (Cary, NC) and significant correlations are reported at the $P < 0.05$ level. Stepwise regression analysis was used to determine the utility of biochemical characteristics in prediction of pork quality. Variables were allowed to enter the model when $P > F$ values were significant at the $P < 0.05$ level. Model R^2 are given to determine the predictive ability of the variables on quality measurements. R-square regression was used to determine the effect individual variables had on the variation in pork quality attributes.

Results & Discussion

Drip loss was correlated to pH at all points measured, most notably at 4 and 6 h postmortem (Table 1). While drip loss was not correlated to temperature at 2, 4, 6 or 24 h postmortem, purge loss was positively correlated to temperature at these time points. This suggests that drip loss and purge may be influenced by independent events in early postmortem muscle.

μ -Calpain autolysis, as determined by a decrease in the percent 80 kDa (intact) large subunit and an increase in the percent 76 kDa autolysis product, appears to be hindered by low pH early postmortem. Because calpain autolysis is considered to be the hallmark of calpain activation, the data suggest that low pH early postmortem will delay activation of μ -calpain. Desmin degradation is more closely correlated to variation in temperature decline, while μ -calpain autolysis is correlated with variation in pH decline (Table 1).

Use of pH, temperature decline, desmin degradation and μ -calpain autolysis to predict water holding capacity demonstrates that pH at 6 h postmortem predicts approximately 34 % of the variation in drip loss (Table 2). Inclusion of 24 h pH and percent of μ -calpain large subunit present as the 76 kDa autolysis product (%76) improves the model R^2 to 0.483. A negative coefficient for %76 indicates that less autolysis within the first 24 h postmortem tends to predict greater drip loss, even after considering pH at two distinct time points. The first variable to enter the model to predict purge loss was the ratio of intact desmin present at 1 d postmortem. A high proportion of intact desmin predicts

greater purge loss. This result also points to proteolysis exerting an influence on water holding capacity independent of pH and temperature decline.

Proteolysis of meat proteins is known to contribute to a decrease in shear force. The proportion of the μ -calpain large subunit present as the 76 kDa autolysis product was the first variable to enter the model predicting WBS at 3 d postmortem (Table 2). A negative coefficient indicates that progression of autolysis, and presumably activation of μ -calpain, decreases WBS. The final model predicting WBS at 3 d postmortem includes pH at 45 min, pH at 4 h, pH at 6 h, %76 at day 1, and intact desmin ratio at 5 d postmortem. Interestingly, coefficients for 45 min and 4 h pH were negative, indicating a low pH at these times will contribute to higher WBS. In contrast, the coefficient for 6 h pH was positive, indicating that a low pH at 6 h postmortem contributes to a lower WBS. These data point out that an intermediate rate of pH decline may create conditions necessary to ensure a decline in WBS with postmortem aging. This is consistent with a recent report demonstrating that μ -calpain activity was greater at pH 6.5 than pH 7.5 or 6.0 (Maddock et al., 2005).

Prediction of WBS at 5 d postmortem suggests that temperature decline, autolysis of μ -calpain and degradation of desmin all independently contribute to variation in WBS. Negative coefficients for 24 h and 6 h temperature suggest that low temperature at these time points contributes to higher WBS. Lower temperature at these time points could contribute to slower rates of autolysis and activation of calpain. Alternatively, lower temperature could contribute in some way to cold shortening. Current data cannot exclude either possibility.

Table 3 demonstrates that temperature decline and calpain autolysis contribute to the rate of desmin degradation, especially after 1 d postmortem. The predictive power of pH, temperature, and calpain autolysis is strongest at days 1 and 3. The only variable that enters the model to predict desmin degradation at 7 d postmortem is the percent of the large μ -calpain subunit present as the 76 kDa autolysis product. In all cases, a negative coefficient indicates that less autolysis by 1 d postmortem predicts less desmin degradation at 1, 5 and 7 d postmortem.

Conclusions

This study advances efforts to define mechanisms underlying variation in fresh pork quality by demonstrating a relationship between activation of μ -calpain and degradation of desmin. Moreover, it is apparent that the rates of μ -calpain autolysis and degradation of desmin are correlated with water holding capacity and shear force of fresh pork. Inclusion of calpain autolysis and desmin degradation measurements in prediction models suggests that these measurements are not simply indicators of pH decline, but may have a direct influence on meat water holding capacity and tenderness.

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Tables and Figures

Table 1 Pearson correlations between pH and temperature of the longissimus dorsi (X) and dependent variables (Y).

Dependent Variable	LD pH _{0.75} ^a	LDpH ₂ ^a	LDpH ₄ ^a	LD pH ₆ ^a	LD pH _u ^a	LD T _{0.75} ^b	LD T ₂ ^a	LD T ₄ ^b	LD T ₆ ^b	LD T _u ^b
Drip ₁ ^c	-0.524**	-0.467**	-0.597**	-0.597**	-0.447**	0.035	0.013	0.063	0.066	-0.000
Drip ₅ ^c	-0.398**	-0.395**	-0.499**	-0.539**	-0.423**	0.040	-0.000	0.171	0.189	0.201
Sirloin Purge ^d	-0.155	-0.231	-0.256*	-0.318**	-0.263*	0.148	0.005	0.346**	0.426**	0.361**
WBS ₃ ^e	-0.205	-0.052	-0.031	0.169	-0.267*	-0.023	-0.052	-0.095	-0.209	-0.082
WBS ₅ ^e	-0.229	-0.170	-0.188	0.053	-0.131	-0.038	-0.131	-0.336**	-0.411**	-0.301*
Desmin ₁ ^f	-0.034	-0.069	-0.081	-0.177	-0.239	-0.075	0.090	0.543**	0.457**	0.493**
Desmin ₅ ^f	-0.078	0.131	-0.148	-0.122	-0.307*	-0.047	0.011	0.167	0.164	0.197
% 80 kDa ^g	-0.465**	-0.297*	-0.294*	-0.173	-0.292*	-0.124	-0.152	-0.078	-0.173	-0.050
% 78 kDa ^g	-0.273	-0.292*	-0.287*	-0.285*	-0.011	0.002	-0.112	0.120	0.285*	0.062
% 76 kDa ^g	0.467**	0.371**	0.366**	0.287*	0.195	0.079	0.167	-0.023	0.287*	-0.005

* Significant correlation between X and Y variables at the P < 0.05 level.

** Significant correlation between X and Y variables at the P < 0.01 level.

^aPostmortem pH at 45 min (pH_{0.75}), 2 (pH₂), 4 (pH₄), 6 (pH₆), and 24 (pH_u) h.

^bTemperature measured at 45 min (T_{0.75}), 2 (T₂), 4 (T₄), 6 (T₆), and 24 (T_u) h postmortem.

^cDrip loss measured on LD chops after 1 and 5 d of storage.

^dPurge loss measured on the sirloin after 7 d of storage.

^eWarner-Bratzler Shear force values determined at 3 and 5 d postmortem.

^fMeasurement of the degradation of desmin at 1 and 5 d postmortem

^gPercent of μ -calpain large subunit present as 80 (intact), or 78 and 76 kDa autolysis products 1 d postmortem

Table 2. Prediction models for drip loss, purge, and WBS. Independent variables used include longissimus pH, longissimus temperature, ratio of intact desmin and % μ -calpain large subunit present as intact (%80), 78 kDa autolysis product (%78) and 76 kDa autolysis product (%76) 1 d postmortem.

Dependent Variable	Independent Variable	Coefficient	Model R ²
Day 1 Drip loss	1 6 h pH	-3.263	.343
	2 6 h pH 24 h pH	-2.818 -4.215	.425
	3 6 h pH 24 h pH %76	-2.404 -3.842 -0.013	.483
Sirloin Purge	1 Intact Desmin Ratio (day 1)	2.577	.250
	2. Intact Desmin Ratio (day 1) 6 h pH	2.350 -1.677	.306
D3 WBS	1 %76 kDa	-0.011	.152
	2. %76 kDa 6 h pH	-0.014 1.095	.262
	3. % 76 6 h pH 4 h pH	-0.012 3.435 -1.990	.376
	4 % 76 6 h pH 4 h pH Intact Desmin Ratio (day 5)	-0.008 3.437 -2.094 .564	.433
	5 % 76* 6 h pH 4 h pH* Intact Desmin Ratio (day 5) 45 min pH	-0.005 3.361 -1.187 .724 -1.435	.501
Day 5 WBS	1 Loin Temp 24 h	-.48	.152
	2 Loin Temp 24 h % 76	-.468 -0.010	.278
	3 Loin Temp 24 h % 76 Loin temp 6 h	-.342 -.010 -0.068	.345
	4 Loin Temp 24 h % 76 Loin temp 6 h Intact Desmin Ratio (day 5)	-.369 -.071 -.007 .533	.397

Table 3 Prediction models for intact desmin ratio. WBS. Independent variables used include longissimus pH, longissimus temperature, and % μ -calpain large subunit present as intact (%80), 78 kDa autolysis product (%78) and 76 kDa autolysis product (%76) 1 d postmortem.

Dependent Variable		Independent Variable	Coefficient	Model R²
Desmin Day 1	1	Temp 4 h	.075	.318
	2	Temp 4 h % 76	.074 -.004	.44
	3	Temp 4 h % 76 Temp 24 h	.060 -.004 .168	.529
	4	Temp 4 h % 76 Temp 24 h Temp 45 min	.066 -.004 .175 -.038	.557
Desmin Day 5	1	% 76	-.005	.172
	2	% 76 45 min pH	-.007 .402	.242
	3	% 76 45 min pH 24 h pH	-.007 .459 -1.068	.319
Desmin Day 7	1	% 76	-.004	.155

THE EFFECT OF OXIDATION ON THE INTERACTION OF CALPASTATIN WITH μ -CALPAIN

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Key Words: calpain, calpastatin, oxidation, H₂O₂, proteolysis, autolysis

Introduction

Postmortem proteolysis of muscle proteins is known to influence functional characteristics in meat including tenderness and water-holding capacity. The calpain proteinases, particularly μ -calpain, play a major role in the proteolysis of key proteins in postmortem muscle. Because of the cysteine residue found in the active site of calpains, the oxidative state of the environment can affect calpain activity. Previous research (Maddock et al., 2004) has shown oxidation by H₂O₂ significantly limits activity of μ -calpain *in vitro*; indicating that oxidation of calpain could be a source of variation in postmortem proteolysis. Rowe et al. (2004) determined that irradiation of beef steaks early postmortem inactivated μ -calpain and decreased postmortem proteolysis.

The calpain inhibitor, calpastatin binds to the calpain heterodimer in the presence of calcium at three sites, domain VI on the 28-kDa subunit and domain IV on the 80-kDa subunit (Nishimura and Goll, 1991) and also to or near the active site of calpain (Goll et al., 1992). The influence of oxidation on ability of calpastatin to inhibit calpain has been described (Maddock et al., 2004), but a specific mechanism is not yet defined. Previous research found that a) calpastatin decreased μ -calpain degradation of desmin and b) oxidation clearly decreased desmin degradation by μ -calpain (Figure 1; Maddock et al., 2004). However, increased degradation of desmin was observed in the presence of H₂O₂ as increased amounts of calpastatin were added to the digests. It is notable that oxidation of the μ -calpain/calpastatin complex resulted in greater desmin degradation (Maddock et al., 2004).

Objectives

These observations lead to the hypothesis that calpastatin, when bound to μ -calpain, can protect μ -calpain from being oxidized. Therefore, the objective of this study was to determine if, in the presence of calpastatin, oxidative conditions allowed for activation of μ -calpain.

Methodology

Purified porcine μ -calpain (0.6 units) was incubated in 165 mM NaCl, 50 mM HEPES, pH 6.5, on ice for 1 h. Treatment groups consisted of 1) 100 μ M CaCl₂; 2) 4 mM N-ethylmaleimide (NEM) or 0.170 μ M H₂O₂; 3) either 4 mM NEM or 0.170 μ M H₂O₂

with 100 μM CaCl_2 ; 4) 1.2 units purified porcine calpastatin and 100 μM CaCl_2 ; and 5) 1.2 units calpastatin, 100 μM CaCl_2 , then either 4 mM NEM or 0.170 μM H_2O_2 ; or 6) 4 mM NEM or 0.170 μM H_2O_2 . A control of μ -calpain incubated in 50 mM HEPES, pH 6.5 on ice for 1 h was used. After incubation, reactions were stopped with 20 mM EDTA. Samples were aliquoted for casein zymography and SDS-PAGE analysis. Comparisons were made between treatment groups on μ -calpain activity and autolysis.

Casein zymography and native gels. Casein zymography was used to determine remaining μ -calpain activity after incubation. Native gels were used to confirm μ -calpain was present in the sample. Gel samples were made for casein zymography and nondenaturing polyacrylamide gels by diluting the sample 70:30 (sample: electrophoresis sample buffer [20% glycerol, 0.1% bromphenol blue, 0.75% 2-mercaptoethanol (MCE), 150 mM Tris-HCl, pH 6.8]). Samples were loaded onto nondenaturing acrylamide gels containing casein and identical nondenaturing gels that did not contain casein. Gels were run at a constant voltage of 75 V for approximately 18 hours. Casein gels were incubated in 5 mM CaCl_2 , 0.1 % MCE, 50 mM Tris-HCl, pH 7.5 solution overnight at room temperature to activate any potentially active μ -calpain. Both casein and native gels were stained in 0.1 % Coomassie brilliant-blue R-250, 40% methanol, and 7% acetic acid solution and then destained in a 40% methanol, 7% acetic acid solution. Clear zones on the casein zymograms indicated activation of μ -calpain. Stained protein on the native gels confirmed the presence of μ -calpain in the gel sample that was used in the casein zymograms when no active μ -calpain was detected.

SDS-PAGE. Gel samples were prepared for SDS-PAGE by diluting samples 70:30 (sample: buffer tracking dye solution [3 mM EDTA, 3% SDS, 20% glycerol, 0.003% pyronin Y, and 30 mM Tris-HCl, pH 8.0]). Samples were run on a 10% polyacrylamide separating gel at a constant voltage of 120 V for approximately 3.5 hours. Gels were stained in 0.1% Coomassie brilliant blue R-250, 40% methanol, and 7% acetic acid solution, and destained in a 40% methanol, 7% acetic acid solution. Differences in autolysis of the 80 kDa subunit of μ -calpain to a 78-kD and a 76-kDa autolysis product were evaluated.

Results & Discussion

Previous research (Maddock et al., 2004), using purified porcine myofibrils as a substrate, determined that μ -calpain proteolytic activity was inhibited by calpastatin and oxidation (Figure 1) as determined by degradation of desmin. Calpastatin inhibited μ -calpain activity in the samples that had not been oxidized with H_2O_2 , as shown by an increase in intact desmin. Oxidation with H_2O_2 clearly inhibited desmin degradation by μ -calpain, particularly when no calpastatin was used in the experiment. However, inclusion of the oxidant with μ -calpain and calpastatin proved to stimulate proteolysis of desmin, indicating increased μ -calpain activity. This led to the hypothesis that calpastatin, when bound to μ -calpain, can prevent μ -calpain from being oxidized.

After incubation, casein zymograms, native gels, and SDS-PAGE were run on each sample. Clear zones on the casein zymograms indicate that active μ -calpain was present in the samples, which demonstrates that μ -calpain in the experiments was not inactivated, either by autolysis or irreversible oxidation. The stained native gels (Figure 2A) correspond to the casein gels and indicate the presence of μ -calpain in gel samples loaded

onto the casein zymograms. SDS-PAGE gels (Figure 2A) indicate activation of μ -calpain based on autolysis of the large 80 kDa subunit degrading to a 78 kDa and 76 kDa subunit. Autolysis is often used as an indicator of μ -calpain activation and can also directly cause inactivation of μ -calpain (as reviewed by Croall and DeMartino, 1991) and as shown in Figure 2A (Lane 10). Incubation of μ -calpain with NEM resulted in loss of μ -calpain proteolytic activity (Figure 2B). NEM binds irreversibly to reduced cysteine residues on proteins (Riordan and Vallee, 1972), essentially acting as an oxidizer. Therefore NEM may bind the active site cysteine residue of μ -calpain, preventing activation. When NEM was incubated with μ -calpain in the presence of calcium, autolysis of μ -calpain did not occur (Figure 2B), indicating that μ -calpain was likely not activated. Calpastatin inhibited μ -calpain autolysis. μ -Calpain activity was observed on casein zymograms (Figure 2A [Lane 1] and 2B) after incubation with calpastatin and calcium. When NEM was added after the formation of the μ -calpain/calpastatin complex (Figure 2A [Lane 3] and 2B), autolysis of μ -calpain did occur. Therefore, calpastatin alone inhibited μ -calpain autolysis, but the addition of NEM to the reaction after the formation of the μ -calpain/calpastatin complex allowed for autolysis and inactivation of μ -calpain. This is an interesting observation in that NEM caused complete loss of μ -calpain activity and prevented autolysis, calpastatin prevented autolysis, but together calpastatin and NEM permitted autolysis of μ -calpain to occur.

Incubation of μ -calpain with H_2O_2 resulted in no loss of proteolytic activity (Figure 2A [Lane 7] and 2B) as observed in the casein zymograms. When calcium was used in the incubation of μ -calpain with H_2O_2 , autolysis did occur (Figure 2 [Lane 2] and 2B) and autolytic inactivation of μ -calpain activity was observed. As previously observed with NEM, when H_2O_2 was added to the reactions after the calpastatin/ μ -calpain complex formed, autolysis of μ -calpain occurred (Figure 2 [Lanes 5 and 9] and 2B) and inactivation of μ -calpain also was observed as indicated by the casein zymograms. Conversely, when H_2O_2 was added to μ -calpain before the addition of calpastatin and calcium (Figure 2 [Lanes 6 and 8] and 2B), no autolysis of μ -calpain was observed and proteolytic activity was still apparent on the casein zymograms, indicating that μ -calpain had not been activated. Collectively, the results indicated that oxidation of the μ -calpain/calpastatin complex promotes autolysis, activation, and autolytic inactivation of μ -calpain to occur. This conclusion is consistent with the preliminary data (Figure 1) that indicated greater proteolytic activity of μ -calpain occurred in the presence of calpastatin and H_2O_2 . Conversely, exposure of μ -calpain to an oxidant (NEM or H_2O_2) before exposure to calpastatin inhibits autolysis of calpain. Interestingly, the reagents used in the study appeared to affect the μ -calpain/calpastatin complex similarly, but when used alone, NEM causes complete and irreversible loss of proteolytic activity, whereas H_2O_2 inactivation of μ -calpain is reversible and proteolytic activity is recovered if reducing conditions are introduced.

Conclusions

Because oxidative conditions do occur in meat during the storage period, they can have an effect the activity of μ -calpain and its effects on protein proteolysis. Further research must be conducted to understand the interaction of μ -calpain with calpastatin, particularly in postmortem muscle. The data from this study make strides toward

understanding the mechanisms of activation of μ -calpain and how specific inherent factors can affect calpain activity.

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Figures

Figure 1. Relative intensity of intact desmin after incubation with μ -calpain for 60 min at pH 6.5 (n=4).

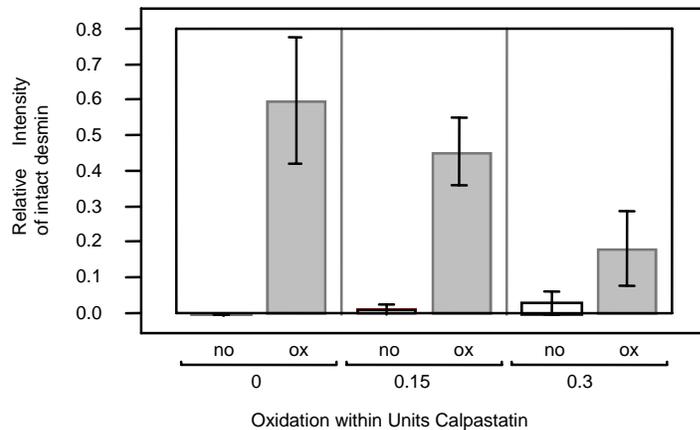
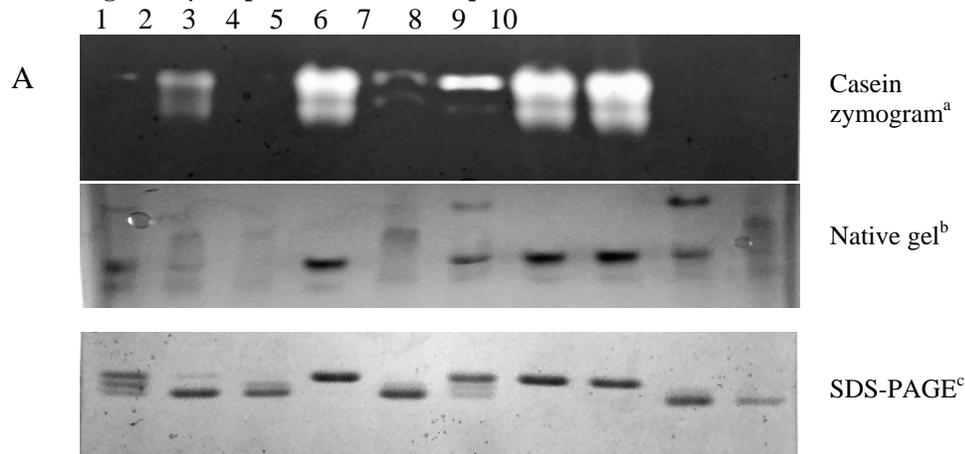


Figure 1. Relative intensity of intact desmin based on densitometry measured and standardized with a control sample from western blots of purified myofibrils digested with μ -calpain for 60 min. The x-axis depicts presence of H_2O_2 in digests within calpastatin treatments.

Figure 2. μ -calpain incubations at pH 6.5



B

Treatment (Lane on gels)	Proteolytic activity	Autolysis
C) μ -calpain (4)	+++	---
1) μ -calpain + CaCl_2 (10)	-	+++
2a) μ -calpain + NEM (NA ^d)	---	---
2b) μ -calpain + H_2O_2 (7)	+++	---
3a) μ -calpain + NEM + CaCl_2 (NA ^d)	---	---
3b) μ -calpain + H_2O_2 + CaCl_2 (2)	+	+++
4) μ -calpain + calpastatin + CaCl_2 (1)	+	+
5a) μ -calpain + calpastatin + CaCl_2 + NEM (3)	--	++
5b) μ -calpain + calpastatin + CaCl_2 + H_2O_2 (5, 9)	+-	+++
6) μ -calpain + H_2O_2 + calpastatin + CaCl_2 (6,8)	+++	-

^{a,b,c} Proteolytic activity of μ -calpain is depicted by casein zymogram and autolysis of μ -calpain is depicted by SDS-PAGE gel. Native gel is used to show location of protein on casein zymograms.

^d NA (Not shown on gels)

Figure 2. A) Casein zymograms gel depicting available μ -calpain activity as shown by a clear zone after incubation under conditions described in Figure 2B. Native gel depicting presence of μ -calpain in sample to give indication as to state of μ -calpain loaded in each sample on casein zymograms. SDS-PAGE depicting autolysis of the 80 kDa band to a 78 kDa and 76 kDa band indicate activation of μ -calpain during the incubations. B) Description of different treatments used with μ -calpain and description of observable proteolytic activity in casein zymograms and autolytic activity based on SDS-PAGE. All treatments are described in order of addition to incubation buffer. The number in parentheses behind treatment description corresponds to the lane on Figure 2A.

IDENTIFICATION AND CLASSIFICATION OF PROTEINS RELATED TO HUNTER L* VALUE AND DRIP LOSS IN PORK LONGISSIMUS MUSCLE

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Key Words: Hunter L*, Proteolysis, Pig

Introduction

Meat color and water-holding capacity (WHC) are the most important pork traits determining consumers' preference (Rosenvold et al. 2003) because their relation to pale, soft, and exudative (PSE) characteristicse (Offer et al., 1989). Until recent years, biological mechanisms related to PSE meat has been determined largely by one-dimension electrophoresis and/or western blotting for a particular protein (Warner et al., 1997), myofibril fragmentation index (Rees et al., 2002), and peptide and amino acid profiles (Moy et al., 2001). However, recent development of a commercial high throughput proteome analysis equipment opened the possibility to characterize whole muscle proteins simultaneously and determine their relation to PSE meat.

Objectives

As a model study, the current study was conducted to identify proteins related to hunter L* value and drip loss in pig *m. longissimus dorsi* during chiller ageing.

Methodology

Six male landraces (100 ± 11 kg) were sampled from the National Livestock Research Institute (NLRI) breeding program, and slaughtered conventionally at the NLRI abattoir. To generate various meat qualities during chiller ageing, both feeding and physical stress treatments were enforced prior to slaughter(Hwang et al., 2005). The day following slaughter, *m. longissimus dorsi* muscles (from the 7th thoracic vertebrate to the last lumber vertebrate) were removed, cut into three potions, vacuum-packed, and randomly assigned to one of three ageing periods (1, 3, and 7 day) for the objective quality measurements of meat color and drip loss. The samples were held at 1°C for the relevant ageing period. Hunter L* value (lightness) was determined by a Minolta Chromameter (CR300, Minolta, Marunouchi, Japan) other materials and methods including two-dimension electrophoresis and identification procedure were reported by Hwang et al. (2005). Discriminant analysis was performed by Systat (version 10.2) using a backward stepwise method with alpha to enter = 0.15.

Results & Discussion

To examine the relationship between changes in semi-quantitative spot density and meat quality traits, 2DE profiles were examined at 0, 1, 3, and 7 day postmortem in triplicates. Animal variations including state of health, growth path, and animal handling prior to slaughter were removed by calculating the levels of spot density at 1, 3, and 7 day postmortem as a percentage of 0 hr (i.e., biopsy tissue). Figure 1 illustrates a 2DE reference map and identified spots. There were more spots altered during ageing in terms of density and electrophoretic properties, but only those identified proteins were presented in this study. Density of each protein and its relationship with meat quality varied to a large extent depending on meat quality. However, based on average value, nine spots (spots, 11, 22, 27, 32, 34, 46, 69, 77, and 94) were consistently reduced, while five spots (spots 42, 73, 84, 88, and 96) fluctuated during ageing (Data not shown). The result was likely related to the limited number of animals in this study. In addition, this could be due to the inherent limitation of 2DE-based proteome analysis in terms of risk of co-migration of intact proteins and/or intermediate degradation products (Lametsch *et al.*, 2001). Table 1 shows consensus protein identities and their electrophoretic properties and their relationship with objective meat quality. A large number of spots were significantly matched by other species such as human, rat, and bovine. This could be due to the limited number of public accessible porcine protein information. Twelve proteins were related to hunter L* value, which included contractile apparatus and related proteins such as alpha actin, myosin light chain 1, cofilin 2 and troponin T, and chaperone proteins of alpha-b crystalline. Four proteins (troponin T, adenylate kinase, ATP-dependent proteinase SP-22, and DJ-1 protein) were related to drip loss. Lametsch *et al.* (2003) found that 26 proteins were related to pork tenderness. These proteins included myosin heavy chain, titin, myosin light chain I, myosin light II, CapZ, and cofilin. The current result was largely in agreement with the previous observation in a sense that most proteins were contractile proteins. This suggested that objective meat qualities of tenderness, meat color, and drip loss were closely associated with postmortem proteolysis.

It is true that objective assessment of pork quality is not a direct reflection of PSE meat, but in the current study Hunter L* value was used to classify pork quality based on Warner *et al.* (1997), who defined hunter L* value higher than 50 as PSE, between 50 and 42 as normal, and lower than 42 as dry, firm, and dark (DFD). Regardless of the fact, to examine whether these proteins were able to classify objective pork qualities (i.e., PSE, normal, DFD), a discriminant analysis was performed by a backward stepwise selection. As meat color and drip loss were concomitant phenomena, all proteins related to drip and hunter L* value were incorporated into the model. Table 2 and Fig. 1 show canonical discriminant functions and their scores on biplot. By the analysis, nine proteins were retained in the final model, in which 69% of samples were classified correctly (PSE: 100%, Normal: 83%, DFD: 33%, data not shown). The final model excluded myosin light chain 1, substrate protein proteinase SP-22, adenylate kinase, and actin fragments. The model classified 100% of PSE meat, while four out of six DFD samples were classified into PSE (two samples) and normal (two samples). Given the fact that PSE meat is the most notorious single factor causing economic loss for the pork industry, while DFD meat is rarely observed in practice, the current model meets the industrial needs. The cumulative proportion indicated that the first canonical variable accounted for 65.3% of the dispersion, where Chain A deoxyribonuclease I (SP77), Actin alpha 1

(SP27), and Troponin T (SP73) were main drivers to separate normal and PSE meats. The results implied that PSE meat was largely characterized by both lower densities of Actin alpha 1 and Troponin T, and high density of Chain A deoxyribonuclease I.

Conclusions

The current study demonstrated that the 2DE-based proteome analysis could be a feasible approach in exploring proteins related to postmortem meat quality traits. However, a larger scale study is necessary to ensure the current results, as the number of animals and identified proteins in this model study were very limited.

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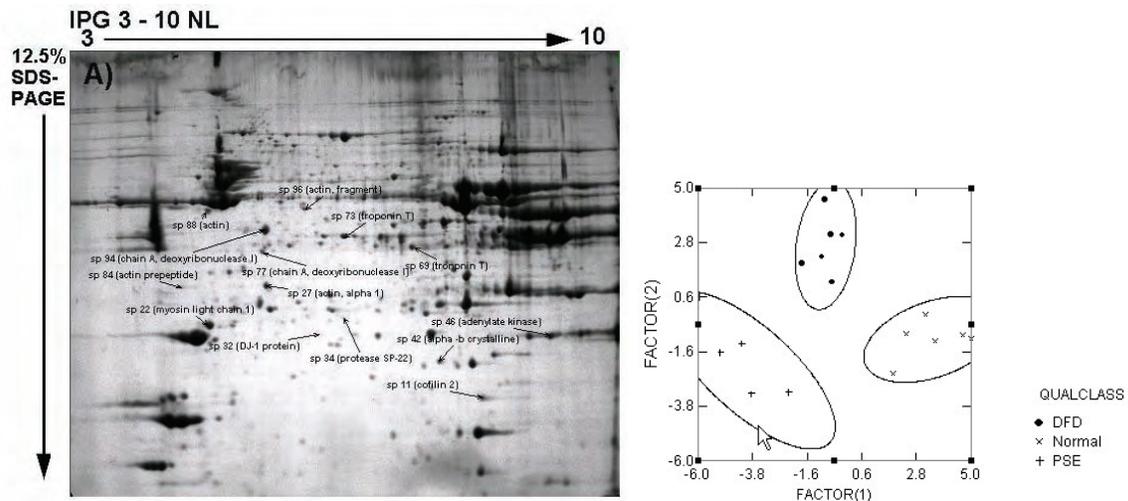


Fig. 1. Reference 2DE protein map and identified proteins related to Hunter L* and drip loss during ageing, and canonical score plot of discriminant analysis at Table 2(PSE: 100%, Normal: 83%, DFD: 33%).

Table 1. List of spot number (SP No.), consensus protein identity, NCBI gi number, method of identification (MI), theoretical pI and molecular($\times 10^3$ Da) on Fig. 1 gel

SP No.	Consensus protein identity	NCBI gi No.	MI ¹⁾	pI/MW	Correlation (r)	
					Drip loss	Hunter L*
SP11	Cofilin 2	gi 6671746	QT	7.66/18.8	NS ²⁾	-0.49*
SP22	Myosin light chain 1	gi 127151	MT	5.0/22.2	NS	-0.64**
SP27	Actin, alpha 1	gi 27819614	MT	5.31/42.5	NS	-0.53*
SP32	DJ-1 protein	gi 7429593	QT	6.33/20.1	-0.54†	NS
SP34	Substrate of mitochondrial ATP-dependent proteinase SP-22	gi 627764	QT	5.73/21.7	-0.53†	-0.43†
SP42	Alpha-b crystalline	gi 7441290	MT	6.76/20.1	NS	-0.56*
SP46	Adenylate kinase	gi 230801	MT	8.37/21.9	-0.77**	NS
SP69	Troponin T slow type isoform sTnT2	gi 34393192	MT	6.41/30.0	NS	-0.61*
SP73	Troponin T slow type isoform sTnT1	gi 34393190	MT	5.92/31.2	-0.59*	-0.65**
SP77	Chain A, deoxyribonuclease I complex with actin	gi 229690	MT	5.09/41.8	NS	-0.62**
SP84	Actin prepeptide	gi 178067	MT	5.12/37.2	NS	-0.48*
SP88	Actin, fetal skeletal (fragment)	gi 90263	MT	5.83/39.5	NS	-0.49*
SP94	Chain A, deoxyribonuclease I complex with actin	gi 229690	MT	5.09/41.8	NS	-0.73***
SP96	Actin, fetal skeletal (fragment)	gi 90263	MT	5.83/39.5	NS	-0.50*

¹⁾ MT: MALDI-ToF peptide mass fingerprint, QT: ESI/MS/MS fragmentation sequencing

Table 3. Canonical discriminant functions of discriminant analysis, and canonical scores of group means determined by a backward stepwise method with alpha to enter = 0.15. The model included 14 spots having a significant correlation with hunter L* or drip loss

Canonical discriminant function	Factor	
	Factor 1	Factor 2
Constant	-12.389	-6.158
Chain A, deoxyribonuclease I (SP77)	-0.228	0.001
Troponin T (SP69)	-0.081	-0.03
Alpha-b crystalline (SP42)	-0.025	0.007
Actin prepeptide (SP84)	-0.011	-0.055
DJ-1 protein (SP32)	0.064	0.033
Cofilin 2 (SP11)	0.074	0.026
Chain a, deoxyribonuclease I (SP94)	0.084	0.038
Troponin T (SP73)	0.132	0.066
Actin, alpha 1 (SP27)	0.192	0.008
Myosin light chain 1 (SP22)	.	.
Substrate protein proteinase SP-22 (SP34)	.	.
Adenylate dinase (SP46)	.	.
Actin, fragment (SP88)	.	.
Actin, fragment (SP96)	.	.
Cumulative proportion of total dispersion		
	0.653	1

**INFUSION OF NITRIC OXIDE DONORS AND INHIBITORS INTO LAMBS
INFLUENCES PLASMA METABOLITES, POSTMORTEM MUSCLE
METABOLITES AND MEAT QUALITY**

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Introduction

Nitric oxide (NO) is a signalling molecule that regulates processes such as force production, blood flow, glucose metabolism, calcium homeostasis, and proteolysis in skeletal muscle (Balon and Nadler, 1997; Zhang et al., 2004). The generation of NO by nitric oxide synthase (NOS) activity in animal skeletal muscle is directly related to the degree of relaxation (stretching), leading to an increased provision of blood/nutrient supply (Rubinstein et al., 1998) and swelling of muscle cells. Due to the multi-faceted effects of NO in skeletal muscle, it is postulated that altered levels of NO pre-slaughter may affect muscle contraction and glycogen depletion in skeletal muscle pre-slaughter which may impact on meat quality. Infusion of the NOS inhibitor, L-NAME 2 h prior to slaughter has been reported to increase post-slaughter glycolysis and improve tenderness of muscle *longissimus thoracis* (LT) but not the *semimembranosus* (SM) muscle in lambs (Cottrell et al., 2002).

Objectives

To investigate the effect of infusion of a NOs inhibitor (L-NAME) or a NO donor (L-Arginine) or both on plasma and muscle metabolites and subsequent effects on meat quality.

Methodology

Forty second cross ((Merino x Border Leicester) x Poll Dorset) wether lambs (12 months old) weighing 43-45 kg were blocked into four groups on body weight. The groups were brought into a shed, acclimatised for 2 weeks and then randomly divided into one of four treatments in a 2 x 2 factorial design. At 24 h prior to slaughter, lambs were catheterised via the jugular vein and on the day of slaughter, at 190 min pre-slaughter, the lambs were treated with either (1) Control (30 mL 0.9% NaCl), (2) L-Arginine.HCl = Arginine (ARG, 500 mg/kg body weight), (3) L-NAME = L-NAME (30 mg/kg body weight) or (4) L-Arginine plus L-NAME = BOTH. Blood samples were collected from all lambs at regular intervals before and after the infusion time. Plasma was centrifuged and separated, then stored frozen until analysis for glucose, lactate and NOx (nitrate and nitrite) as described in Cottrell et al. (2004). At 190 min post infusion, lambs were slaughtered, samples of LT

and SM were collected at 15 min, 1h, 3h and 24h post-slaughter for muscle glycogen and lactate determination and muscle pH recorded at 1 (initial) and 24 h (ultimate) post-mortem. Samples of LT and SM were removed at 24 h post-slaughter and vacuum packaged for the measurement of Warner-Bratzler shear force (WBSF), Myofibrillar Fragmentation Index (MFI), sarcomere length and surface colour ($L^*a^*b^*$) after a 30 min bloom, after 0 and 3 days of ageing at 2°C. Muscle metabolites and meat quality characteristics were determined as described by Cottrell et al. (2002;2004). Data were subjected to ANOVA with a 2x2 factorial design using day of infusion and lamb as blocks. Main treatment effects of ARG, L-NAME and their interaction on plasma metabolites, muscle glycogen and muscle lactate was compared with sampling time relative to time of infusion (ARG x L-NAME x Time). Meat quality aspects were reported for arginine, L-NAME main effects and their interaction (ARG x L-NAME) as there was no treatment x time effect.

Results & Discussion

Lambs receiving either arginine alone or both arginine and L-NAME had higher plasma glucose ($P < 0.001$) compared with control or L-NAME treatment (Figure 1a). There was an ARG x Time interaction ($P < 0.001$) such that ARG caused an increase in plasma glucose up to 120 min post-infusion compared to those lambs receiving control or ARG. Plasma lactate concentration was reduced ($P < 0.001$) by ARG compared to L-NAME (Figure 1b). However, there was an interaction (Arg x L-NAME; $P < 0.03$) such that ARG and L-NAME together caused a prolonged reduction in lactate. There was no effect of arginine or L-NAME or interactions on plasma NO_x concentration measured at 120 min post-infusion ($P > 0.05$; Table 1). Over the 15 min to 24 h post-mortem, L-NAME treatment tended to have higher ($P = 0.09$; 0.98 vs 1.04 g/100g muscle) LT glycogen while ARG ($P = 0.08$; 1.27 vs 1.42 g/100g muscle) treatment tended to have higher SM glycogen compared to control lambs. Arginine increased (0.39 vs 0.41 g/100g muscle; $P < 0.041$) and L-NAME decreased (0.42 vs 0.38 g/100g muscle; $P < 0.039$) SM lactate content over the 15 min to 24 h period, respectively.

L-NAME treated lambs had a higher 1 h pH post-slaughter in the SM ($P < 0.01$) while ARG treated lambs had a lower 1 h pH in the LT ($P < 0.08$) and SM ($P < 0.05$) muscles but there were no differences in 24 h pH (Table 1). Neither surface colour of the LT and SM muscle measured as lightness (L^*), redness (a^*) and yellowness (b^*) after 0 and 3 days of ageing nor meat quality characteristics evaluated as sarcomere length, purge, MFI or cook loss was affected by any treatment ($P > 0.05$, data not presented). L-NAME decreased the WBSF of the SM for 0 day aged muscle (4.38 vs 4.73, $P < 0.03$) particularly when arginine was simultaneously infused (L-NAME x Arg; $P < 0.05$).

In summary, infusion of a NO donor (L-Arginine.HCl) at 500 mg/kg body weight significantly altered blood glucose and lactate concentration in lambs over the 120 min post-infusion relative to control and L-NAME. Infusion of a NOS inhibitor (L-NAME) at 30 mg/kg body weight did not change blood glucose or lactate levels compared with control lambs. There was a tendency for increased glycogen in the LT and SM with L-NAME treatment over the post-slaughter period. Post-slaughter muscle lactic acid production was significantly increased by ARG and decreased by L-NAME only in the SM but not in the LT. These were attributed to a decrease and increase in muscle pH in

the SM 1h post-mortem with ARG and L-NAME treatment, respectively. Tenderness, as measured by WBSF, was reduced in the SM at 1 day post-slaughter by infusion of the NOS inhibitor, particularly when combined with infusion of the NO donor. It is not clear whether the lower lactate levels and higher pH in muscle SM with NOS inhibition pre-slaughter was causative in the significant increase in toughness of the SM at one day post-slaughter.

Conclusions

Manipulation of endogenous nitric oxide levels *in vivo* through the infusion of nitric oxide donors and nitric oxide synthase inhibitors pre-slaughter influenced plasma metabolites and post-slaughter muscle metabolites and the tenderness of a leg muscle.

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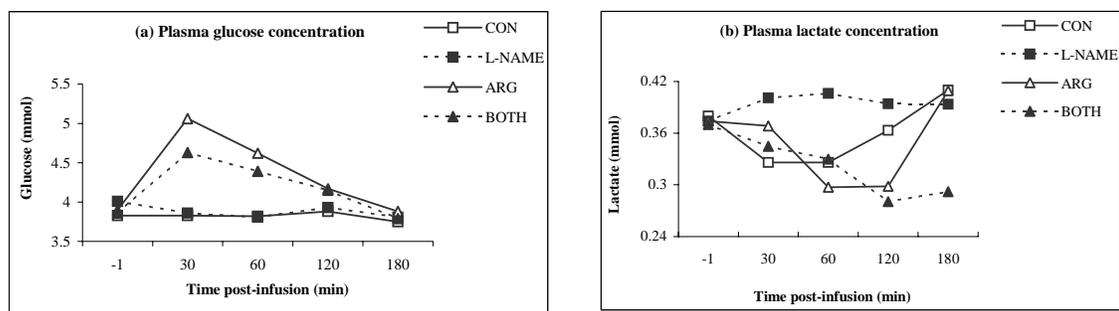
Tables and Figures

Table 1: Plasma NOx (nitrate and nitrite) concentration, initial (1 h) and ultimate pH (24 h) and Warner-Bratzler shear force (WBSF) values of muscle *longissimus lumborum* (LT) and *semimembranosus* (SM) measured after 0 (0d) and 3 days (3d) of ageing from lambs treated with L-arginine methyl ester hydrochloride (L-NAME, 0 or 30 mg/kg body weight) or arginine (0 or 500 mg/kg body weight)

L-NAME (L)	0		30		S.E.D.	P-Value			
	Arginine (A)	0	500	0		500	A	L	L x A
Plasma NOx (μmol)		11.10	13.38	12.38	11.02	2.0	0.75	0.71	0.21
Initial pH-LT		6.89	6.84	6.93	6.83	0.06	0.08	0.68	0.65
Ultimate pH-LT		5.70	5.71	5.71	5.72	0.02	0.31	0.61	0.88
Initial pH-SM		6.42	6.36	6.64	6.48	0.08	0.05	0.01	0.36
Ultimate pH-SM		5.62	5.62	5.63	5.64	0.02	0.31	0.20	0.69
0d WBSF LT (kg)		6.40	6.28	6.23	5.75	0.44	0.35	0.28	0.58
3d WBSF LT (kg)		5.71	5.29	5.49	5.41	0.43	0.42	0.86	0.59
0d WBSF SM (kg)		4.56	4.20	4.57	4.89	0.22	0.88	0.03	0.04
3d WBSF SM (kg)		4.22	3.87	3.88	4.16	0.31	0.86	0.92	0.16

Means of plasma NOx, muscle pH, and WBSF are average of 10 lambs, 10 lambs x 2 and 10 lambs x 6 measurements, respectively.

Figure 1: Plasma (a) glucose and (b) lactate concentration of lambs infused with saline (control, CON), L-NAME, arginine (ARG) or arginine and L-NAME (BOTH) in relation to time of infusion. P



= 0.001, 0.340, 0.116, 0.800 for arginine, L-NAME, ARG x L-NAME and ARG x L-NAME x Time, respectively for glucose and P = 0.001, 0.737, 0.030, 0.220 for arginine, L-NAME, ARG x L-NAME and ARG x L-NAME x Time, respectively for lactate concentration.

**REGRESSION ANALYSIS OF DESMIN AND TROPONIN-T DEGRADATION
AND FEEDING REGIMES TO TENDERNESS OF THE LONGISSIMUS
OF CULL COWS**

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Key Words: Desmin, Troponin-T, Cull Cow, Shear force

Introduction

Last year 2.7 million cull beef cows and 2.3 million cull dairy cows (USDA-NASS, 2005) were harvested, which accounts for 16.7% of the total cattle slaughtered in the United States. This aged cow population represents an area of the meat industry that is under utilized. Commonly, meats from these animals are used in lower quality products such as ground beef. As the cow ages, physiological changes occur that decrease the tenderness of the meat. Including, changes in the collagen content (Bosselmann et al., 1995) and possibly the endogenous protease population as well. Postmortem (PM) proteolysis of muscle proteins also plays an important role in meat tenderness. Degradation of Desmin, located on the periphery of the myofibrillar Z-disk (Price and Schweigert, 1987), is involved with PM tenderization (Hwan and Bandman, 1989; Whipple and Koohmaraie, 1991), as well as Troponin-T degradation, which is associated with the thin filament (Price and Schweigert, 1987). The extent of degradation of these structural and peripheral proteins could affect the progression of PM tenderization (Huff-Lonergan, 1996). In purchasing meat consumers place tenderness as one of the most important factors (Miller et al., 1995), affecting acceptability and how much they are willing to pay for the product (Lusk et al., 2001). To help improve the value of cull cow carcasses, cows are finished on high energy diets. This practice increases muscling and steak tenderness (Schnell et al., 1997). Another potential method to increase muscling is with the addition of Optaflexx™ (ractopamine HCl).

Objectives

Determine the correlation between percent shear force decrease and Troponin-T and Desmin degradation in non-dairy type cull cows finished on forage maintenance diet, high energy concentrate diet, and high energy plus Optaflexx™.

Methodology

Live Phase

Animals were raised per approved University of Illinois protocols. Cows were allotted to pens (twelve pens of five animals) based on body weight, hip height, body condition score and ultrasonic backfat thickness. Dietary treatments consisted of a forage maintenance diet (CON), high energy concentrate diet (FED), and high energy plus Optaflexx™ (OPTA). Cows were harvested in four groups (1 pen per treatment, 15 animals total) over four consecutive weeks. Therefore, after the first harvest group started on trial, each following week another group of three pens was placed on trial. All cows were fed for 57d, with the OPTA group receiving Optaflexx™ at 200 mg/head/d for the last 35d (fed according to label directions). FED and OPTA groups were adapted to the final diet over a 3 week period.

Harvest

After 57d on trial, each harvest group of cows was transported to the University of Illinois Meat Science Abattoir the night before harvest. During lairage, cows had full access to water, but were held without feed. The following morning, all cows in the group were harvested in random order.

Each cow was weighed to obtain a live weight, immobilized with a captive bolt stunner, exsanguinated, and head removed. The hide was removed and the carcass was eviscerated. After evisceration, carcasses were split, washed, weighed, and placed into a 4°C cooler. Observations were made and recorded regarding any cows that were pregnant or any carcasses that were condemned due to sickness.

Ageing Samples

At 2d post-mortem, the ribeye from both sides of the carcass had a 2.5cm section removed to expose a fresh surface. Two 2.5cm steaks were cut from each ribeye. These steaks were randomly assigned to ageing periods of 2, 7, 14 and 21d post-mortem. Steaks within the same ageing period were vacuum packaged together, boxed and stored at 4°C until the appropriate ageing time, at which point they were frozen at -30°C until shear force analysis could be completed.

Shear Force Determination

Steaks used for Warner-Bratzler shear force determination were thawed overnight at 4°C, trimmed to a uniform size and cooked on a Farberware open-hearth grill (Model 455N, Walter Kidde, Bronx, NY). Internal temperature was monitored using copper-constantan thermocouples (Type T, Omega Engineering, Stamford, CT) connected to a digital scanning thermometer (Model 92000-00 Barnant Co., Barington, IL). Steaks were cooked on one side to an internal temperature of 35°C, turned over and cooked to a final internal temperature of 70°C. After cooking, steaks were allowed to cool to 25°C before four 1.3cm cores were removed parallel to the orientation of the muscle fibers. Cores were sheared on an Instron® universal testing machine (Model 112) set with a 10kg load

cell and a 200mm per minute chart drive and crosshead speed. Shear force was determined for each core, and these values were averaged for each sample.

Tissue Preparation

Proteomic analysis was performed on 51 *Longissimus* muscle samples. Day 0 samples were obtained by biopsy from the 9-10th rib region at 1.5h post-mortem, snap frozen in liquid nitrogen and stored at -80°C. Aged samples were collected from steaks aged for 13d post mortem and then frozen at -80°C until analysis. Myofibrillar proteins were extracted as described by Kent and coworkers (2004) with some modifications. In brief, frozen muscle portions were powdered in liquid nitrogen and a 200mg sample was transferred to a 1.5ml microcentrifuge tube. Five volumes (v:w) of extraction buffer (100mM Tris-HCL [pH 8.3] and 5 mM EDTA) were added to the tube and homogenized with an Ultra-Turrax (IKA Werke, Germany) set to high for 15sec, vortexed, then homogenized again for 15sec. This was all completed on ice. Two-hundred microliters of the fraction were prepared for SDS-PAGE with the addition of an equal volume of treatment buffer (.125 M Tris-HCl [pH 8.6], 4% SDS, and 20% glycerol). The suspension was then heated at 70°C for 20min and centrifuged at 15,000 x g for 30min. Supernatant was collected and stored at -80°C for further analysis. Protein concentrations were determined with the Pierce BCA (Pierce Biotechnologies, USA) microplate protein assay according to manufacturer's protocol. Fractions were diluted 15-fold with distilled water so that the concentration would fall within a readable range. Bovine serum albumin was used to generate a standard with 7 points between 2mg/ml and 0.1mg/ml. Standards were made fresh daily from a 2mg/ml stock and each assayed plate contained its own standard curve. Protein concentrations from the fractions were adjusted to 5mg/ml for SDS-PAGE.

SDS PAGE

Protein electrophoresis was carried out using precast NuPAGE 10% Bis-Tris gels with MOPS running buffer (Invitrogen, USA). Loaded samples contained 20ug protein per lane and were prepared per NuPAGE protocol (Invitrogen, USA). Magicmark XP (Invitrogen, USA) was loaded for a chemiluminescent molecular weight standard. Gels were run on a water-cooled Hoefer SE260 (Hoefer, USA) at a constant voltage of 180v for 90min.

Western Blotting

After electrophoresis gels were transferred onto PVDF 0.45um membranes (Invitrogen, USA) in a Trans-Blot Cell (Bio-Rad, USA) for 3h at a constant voltage of 30v. Membranes were processed for chemiluminescent detection using the Western Breeze Immunodetection kit (Invitrogen, USA). Membranes were blocked for 30min and then probed with Desmin antibody D1033 at 1:500 (Sigma, USA) and Troponin-T antibody T6277 at 1:1000 (Sigma, USA) for 60min. Membranes were washed for 45min, probed with alkaline phosphatase goat anti-mouse secondary antibody for 30min and washed again for 45min. Membranes were incubated with substrate for 5min and visualized using the ChemiGenius² Imaging System (Syngene, UK). Images were analyzed using GeneTools (Syngene, UK). Changes in Desmin and Troponin-T protein

profiles were determined by the percent decrease in intact protein from 0 to 13d per animal and correlated to percent decrease in shear value from 2 to 13d.

Statistical Analysis

Data were analyzed with the MIXED procedure in SAS (1999) using the lsmeans statement for means and standard error calculation. The pdiff option was used for determining significance. When determining significance for the effect of dietary treatment, only the comparisons of Control vs. Fed and Fed vs. Fed + Optaflexx™ were evaluated. Significance was determined at $P > 0.10 = \text{NS}$, $P \leq 0.10 = \dagger$, $P \leq 0.05 = *$, $P \leq 0.01 = **$, $P \leq 0.001 = ***$. For all data, Pen (5 cows/pen) served as the experimental unit. Regression analysis was performed with REG procedure in SAS (1999).

Results & Discussion

Initial shear force values were lower with the FED and OPTA compared to CON ($P < 0.05$), however, there were not any differences in the final shear force values ($P > 0.10$; Table 1). This suggests that meat from forage fed cows can be of equal tenderness when aged for a sufficient period of time. Day 7 and 14 shear values were numerically lower in the FED and OPTA treatments indicating that high energy diets can potentially decrease the time needed for ageing.

SDS-PAGE of 20ug of 0 and 13d *Longissimus* total protein preparations yielded similar protein profiles (Fig. 1). Visualization of the Desmin Western blot displayed an undegraded band at 55 kDa at 0d and three proteolytic fragments of 49, 47 and 40 kDa (Fig. 2) and decreased undegraded band intensity (Fig. 3). Enzymatic degradation of Desmin from 0 to 13d was not affected by the three feeding regimes ($P > 0.10$; Table 2). Desmin degradation was chosen as an indicator of PM proteolysis because it is a specific substrate for the calpain system (Taylor et al., 1995). Also, other proteolytic systems do not have access to, or capabilities to degrade Desmin (Goll et al., 1992; Koohmaraie, 1992). Analysis of the Troponin-T Western blot yielded an intact doublet band of 37 kDa at 0d, and proteolytic fragments of 35 and 32 kDa (Fig. 4), and decreased intact band intensity on 13d (Fig. 5). When comparing Troponin-T degradation from 0 to 13d in the CON group to the FED group, the CON cows had a higher percent difference ($P = 0.07$) (Table 2). Given the high coefficient of determination, Troponin-T may serve as a good indicator of PM proteolysis. Taylor and co-workers (1995) demonstrated that during PM proteolysis myofibrils are broken at their I-bands. Because of this, it is possible that when Troponin-T, a regulatory component in the actin-myosin complex breaks down, the thick and thin filament interactions are altered, which can be a indicator of tenderness (Huff-Lonergan et al., 1996).

The coefficient of determination (R^2) between Desmin and Troponin-T degradation from 0 to 13d and percent shear force decrease from 2 to 14d were 0.68 and 0.59 respectively. Coefficient of determination with combined Desmin and Troponin-T degradation against percent shear force decrease was 0.75 (Table 3).

Conclusions

Overall, Desmin demonstrated a greater coefficient of determination (R^2) than Troponin-T, yet both demonstrated an additive effect when combined and plotted against percent shear force decrease. Initial shear force values were lower for FED and OPTA groups compared to CON, but feeding regimes did not affect the final shear force value, nor the percent Desmin degradation. However, percent Troponin-T degradation was higher in the CON cows compared to the FED cows ($P=0.07$). FED and OPTA treatments showed the potential to decrease the time needed to age samples.

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Tables and Figures

Table 1. Cull Cow Ageing Samples: ¹ Warner-Bratzler shear force

Time	CON (C)	FED (F)	OPTA (O)	C vs. F <i>P</i> -value	F vs. O <i>P</i> -value	SEM
Pens, n	4	4	4	--	--	--
2-d Ageing	7.11	6.30	5.91	*	NS	0.50
7-d Ageing	5.90	5.73	5.40	NS	NS	0.55
14-d Ageing	5.14	4.72	4.78	NS	NS	0.63
21-d Ageing	4.73	4.36	4.42	NS	NS	0.56

NS > 0.10, † ≤ 0.10, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001

¹ Steaks for ageing were cut from the ribeye from both sides of the carcass and randomly assigned to the appropriate times

Table 2. Cull Cow Ageing Samples: % Protein Degradation From 0d to 13d in the *Longissimus*

Protein	CON (C)	FED (F)	OPTA (O)	C vs. F <i>P</i> -value	F vs. O <i>P</i> -value	SEM
Desmin	62%	56%	53%	NS	NS	0.07
Troponin-T	49%	33%	43%	†	NS	0.07

NS > 0.10, † ≤ 0.10, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001

Table 3. Cull Cow Ageing Samples: Regression Analysis in *Longissimus* samples^A

Protein	Equation ^B	R ²
Desmin	$y = .433d + .019$	0.68
Troponin-T	$y = .3902t + .103$	0.59
Desmin & Troponin-T	$y = .297d + .180t + .021$	0.75

^A % Protein degradation to % reduction in shear force

^B y = % decrease in shear force, d = % Desmin degradation, t = % Troponin-T degradation

Fig 1. Representative SDS-PAGE of total protein isolates from 0d and 13d *Longissimus* samples stained with coomassie blue.

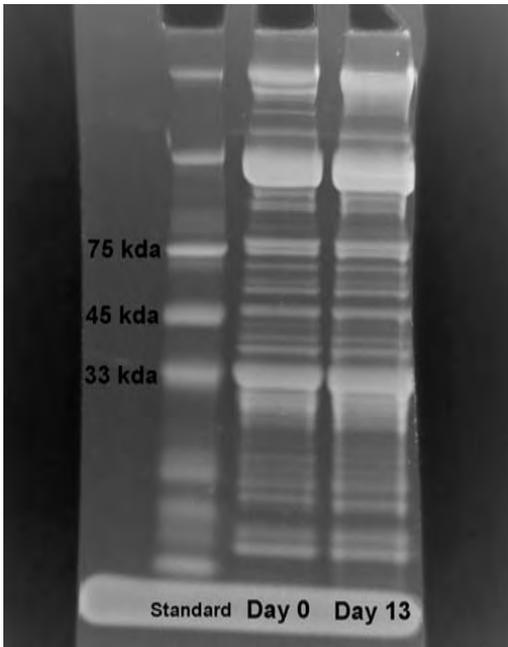


Fig 2. Western Blot with Desmin antibody at 1:500 dilution

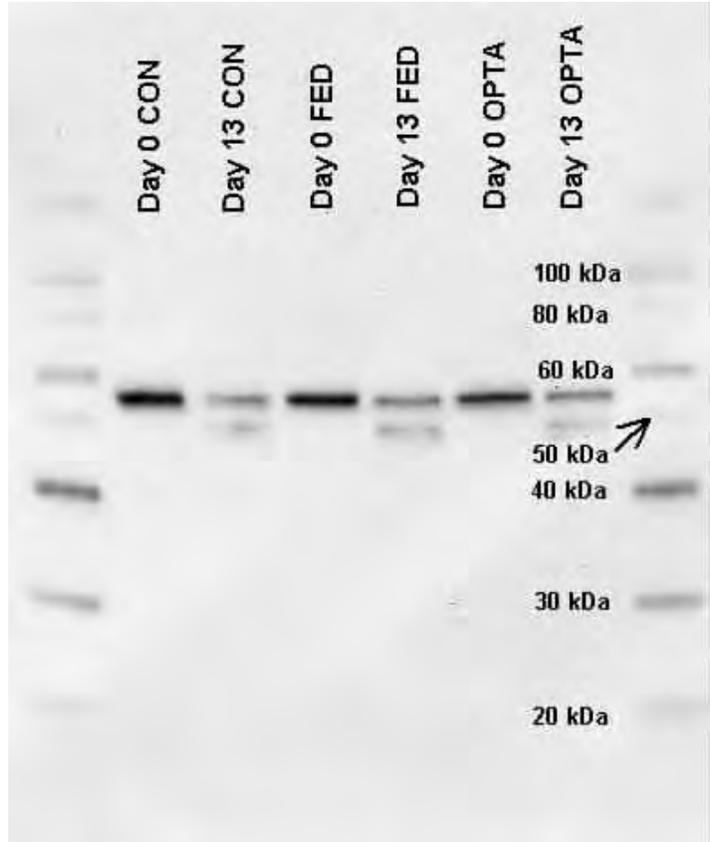


Fig 3. Three-Dimensional image of Desmin Western blot depicting band intensity.

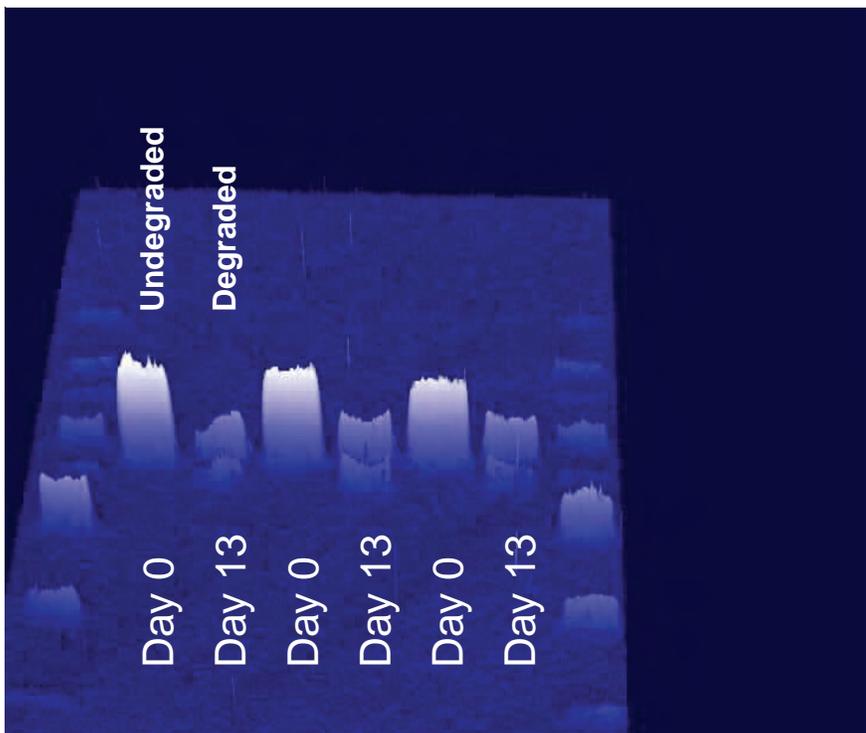


Fig 4. Western Blot with Troponin-T antibody at 1:1,000 dilution

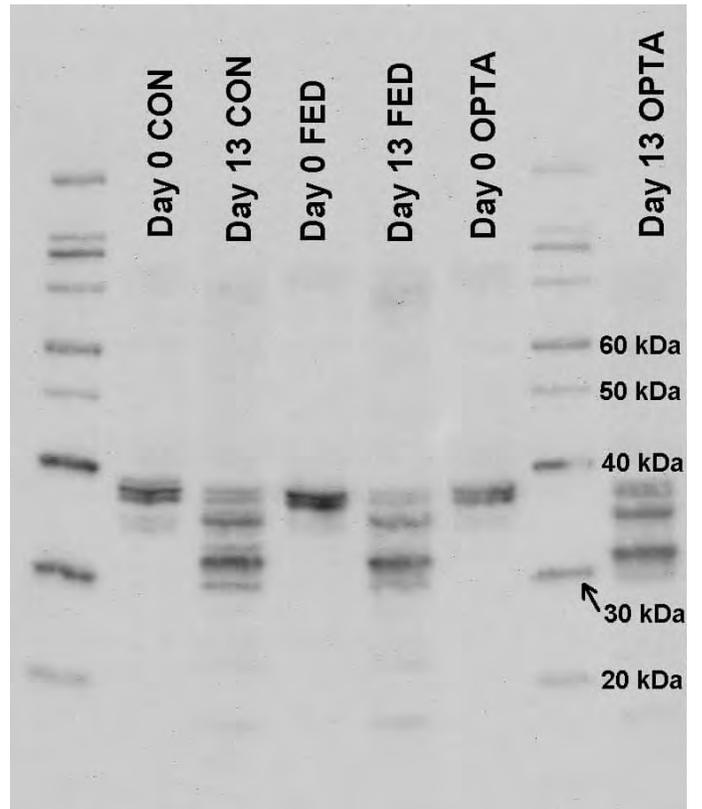
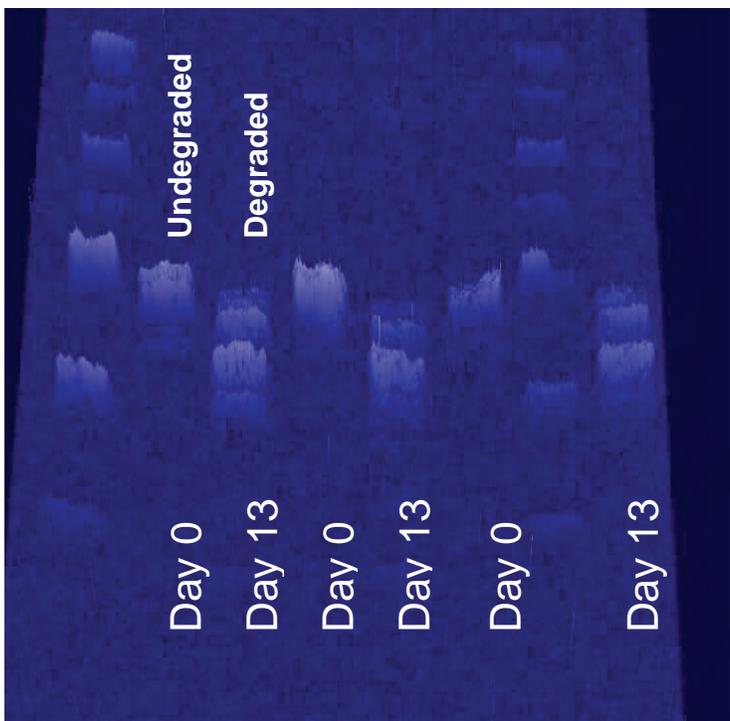


Fig 5. Three-Dimensional image of Troponin-T Western Blot depicting band intensity.



PROTEOLYTIC FRAGMENTS IN BOVINE EXUDATE AS POTENTIAL TENDERISATION MARKERS

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Key Words: Beef, drip loss, exudate, tenderisation, longissimus dorsi.

Introduction

The variability in meat quality, particularly in tenderness has become a significant consumer concern and is becoming an increasing problem for the meat industry. However, even with the best available controls during the breeding, slaughtering and storing, the tenderness of meat still varies, preventing meat producers from marketing their produce on the basis of consistent quality (Maher *et al.*, 2004). These difficulties could be reduced if carcasses showing inferior quality could be identified on the slaughterline, thus enabling them to be handled and marketed separately from high quality carcasses.

The appearance of proteolytic fragments is related to the rate and extent of tenderness in beef (Ouali, 1990; Koohmaraie., *et al* 1991). Much work concerning the proteolysis of ageing beef has focused on degradation as observed within the myofibrillar fraction (Hopkins & Thompson, 2002). It is likely that products of myofibrillar proteolysis may appear in other muscle fractions. Similarly it may be that proteolysis of sarcoplasmic proteins may provide some information regarding the rate or extent of proteolysis with in a muscle. Previous research at The National Food Centre has focused on TCA soluble extracts (O'Reilly *et al.*, 2004). This research focuses bovine exudate and investigates if information regarding postmortem proteolysis can be obtained through 1D gel electrophoresis studies of this exudate over the postmortem ageing period.

Objectives

The first objective of this study was to optimise methods to study products of proteolysis in bovine exudate. The second objective was to determine if potential markers of beef tenderness were present in the exudate.

Methodology

Forty steers were slaughtered according to standard commercial procedures in an Irish abattoir. pH, conductivity and temperature were recorded up to 24hrs postmortem in the factory and thereafter at The National Food Centre. *M. longissimus dorsi* (LD) was removed and steaks (2.54 cm thick) prepared for quality analysis over the 14 days post-

mortem ageing period. In order to minimize variation, each steak was taken from the LD at the same area on each carcass. Attributes measured included sarcomere length (day 2 post-mortem), colour (day 14 post-mortem), warner-bratzler shear force (day 2, 7 and 14 post-mortem), water holding capacity, intramuscular fat and moisture. Data were analysed to categorised the samples on the basis of toughness/tenderness. Potentially confounding factors such as extreme values of sarcomere length and intermuscular fat (IMF) were considered when catagorising samples.

Different methods for the collection of exudate were evaluated: (1) collection directly from the carcass shortly after slaughter, (2) collection at 2, 7 and 14 days using the protocol of Honickel (1987) (gravitational) and (3) collection after centrifugation of a 9g core at 37000g (4°C) for 20 minutes (centrifugal method). Various sample preparation methods were also considered. The optimal collection/preparation procedure was shown to be a simple centrifugation step combined with dissolving the sample in SDS sample buffer. SDS-PAGE (Laemmeli *et al.*, 1970) was conducted using different acrylamide concentrations (7%, 12% and gradient (8-18%) acrylamide gels). Based on visual assessment bands of interest were identified based on differences between tough and tender meat. Repeatability studies (n=10) were carried out to ensure reproducibility of these results. Gels were scanned using a colour image scanner (Epson perfection 3200) and optical density (OD) values obtained using Labworks 4 proteolysis analyser package. Coefficient of variation (CV) was calculated on these values (expressed as 'OD of band of interest' and 'OD relative to OD for total lane') to aid future semi-quantitative analysis of results. Bands of interest were selected and qualitative analysis carried out by MALDI-ToF following trypsin digest.

Results & Discussion

From the comparison of the three different methods for retrieving drip loss it was found that the optimal method was the centrifugal method (Figure 1). The gravitational one produced similar qualitative results but was more time consuming and did not always provide sufficient volume of exudate.

Two samples from the tough and tender groups were selected for analysis by SDS-PAGE. Visual assessment of these gels indicated some differences in banding patterns between the two. In particular a fragment around the 37 kDa region was present in both groups early postmortem but disappeared totally in the tender group (Figure 2). Repeatability studies were successful as CVs of approximately 2-6% were obtained for 'OD of band of interest' and 'OD relative to OD for total lane'.

In order to characterise the protein fragments that could be isolated from the exudate by 1D SDS-gels a MALDI-ToF was made finding that all the proteolytic fragments identified were products of regulatory enzymes from the muscle metabolism such as amylo-1,6-glucosidase, pyruvate kinase, glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase, carbonic anhydrase and phosphoglycerate mutase (Figure 3). The lactate dehydrogenase fragment corresponds to the 36.9kDa. Previous studies (Stoeva *et al.*, 2000, Nakai *et al.*, 1995) on TCA soluble fragments from beef and in drip loss from pork (Lametch. *et al.*, 2003) had reported some peptides originated from degradation of sarcoplasmic and myofibrillar proteins.

Initial semi-quantitative analysis has been carried out on a larger number of samples and results indicate that the density of this band may be a useful indicator of tenderness in conjunction with other measurements. Further research is on-going to verify this.

Conclusions

A method was developed to enable visualisation of products of proteolysis in bovine muscle exudate. Bands of interest were analysed qualitatively by MS MALDI ToF. Disappearance of lactate dehydrogenase in the bovine exudate may be indicative of the proteolysis of muscle proteins and hence may have potential as an indicator for tenderness.

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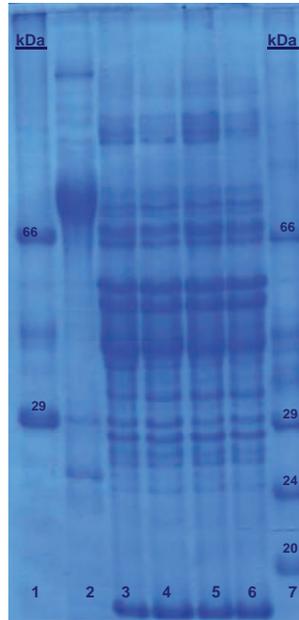


Figure 1: 12% acrylamide SDS-PAGE gel comparing three collection methods of drip loss. The drip collected directly from the carcass did not have a clear band pattern.

Lanes:

- 1. High molecular standard marker (kDa)**
- 2. 0 days drip collected directly from the carcass**
- 3. 2 days gravitational**
- 4. 2 days centrifugal**
- 5. 7 days gravitational.**
- 6. 7days centrifugal**
- 7. Low molecular standard (kDa)**

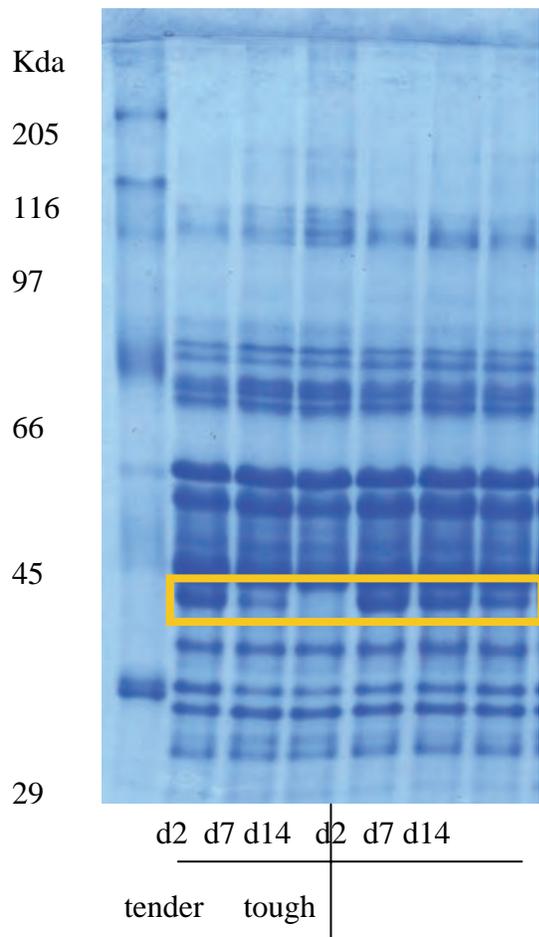


Figure 2: 12% acrylamide SDS-PAGE gel comparing centrifugal exudate from samples classified as tender and tough. We can see how the 36.9 kDa band is disappearing with time

KDa

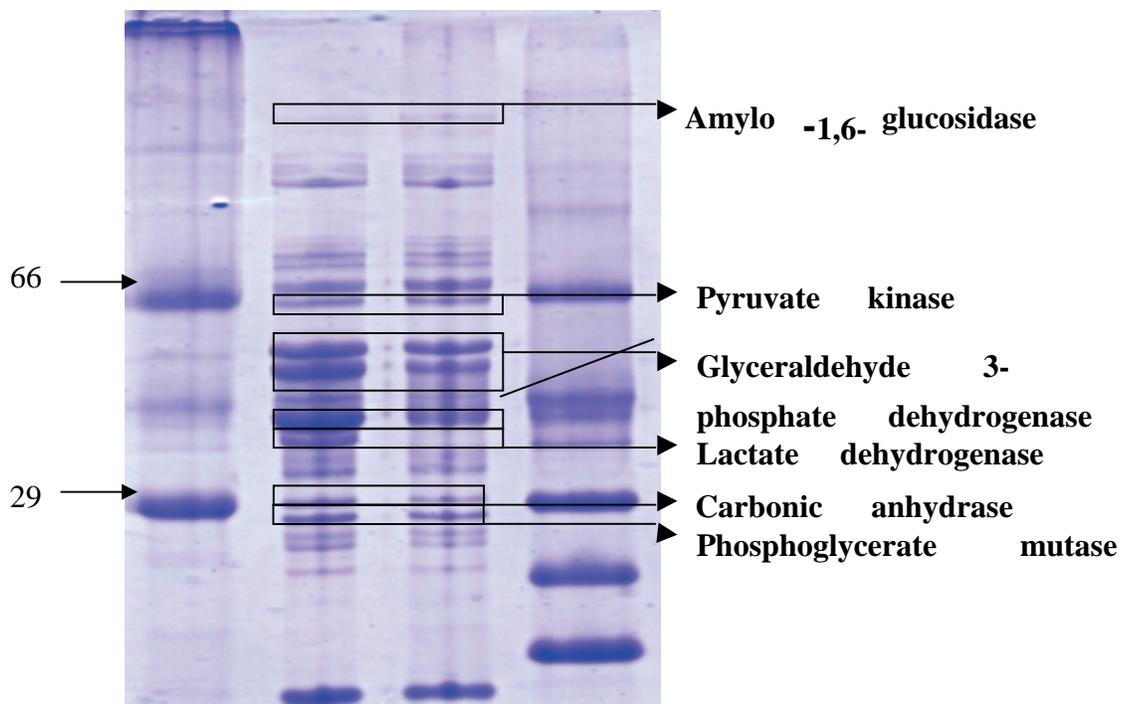


Figure 3: 12% acrylamide SDS-PAGE minigel. Lanes 1,4 : molecular weight markers 2 : tough sample and 3: tender sample. This minigel was used for isolating the samples for identification of fragments using MADI-TOF

**VARIATION IN MUSCLE MICROSTRUCTURE AND BEEF TENDERNESS
IN AGED *M. LONGISSIMUS DORSI* OF NORWEGIAN RED CATTLE (NRF)
- PRELIMINARY RESULTS**

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Key words: beef, tenderness, microstructure, calpastatin

Introduction

Muscle is a composite structure comprised of contractile myofibres attached by connective tissue. Post mortem storage of meat is known to increase tenderness, i.e. being related to the degree of alterations of the structural components of the muscle and associated proteins during rigor and subsequent post rigor storage. Structural changes which occur post mortem are fibre contraction, breaks in the cytoskeleton proteins and thereby weakening of I-band near the z-disk with subsequent breaks in the sarcomeres (Taylor et al., 1995). Furthermore, detachment of the endomysium occurs, probably due to degradation of the proteoglycans in the extracellular matrix (Hannesson et al., 2003). PH fall and calpain mediated proteolyses are furthermore regarded as important contributors to the structural alterations (Koochmariaie, 1996).

Less clear is how the variability of muscle morphology and composition affect the post mortem structural alterations and thereby quality. Shackelford et al. (1994) found that variation in calpastatin not always explain all tenderness phenomena. Sometimes other factors such as connective tissue characteristics and muscle contraction also play a role in tenderness of the aged meat. Frylinck and Heinze (2003) showed that genotypic meat quality differed among some African breeds due to genetically determined interacting biochemical and physiological factors. Fibre contraction, fibre-fibre attachment, and fibre breaks were significantly effected by breed-ageing, and also correlated to shear force and protease activity (Taylor and Frylinck, 2003). It has been shown that part of the variation in tenderness is inherited in Norwegian Red Cattle (NRF). In a pilot study with two breeding lines of NRF bulls, Aass (1996) found a heritability of 40% for tenderness. A follow up study on genetic causes of variation in beef tenderness support these results (Aass et al., 2005). As a part of this study microscopic structural changes in the muscle during storage was quantified and related to the WB tenderness in 28 randomly selected animals. This was done in order to elucidate individual variation in post mortem structural changes and to identify main structural features affecting WB tenderness.

Objectives

The aim of the present study is to investigate the relative importance of structural changes in myofibers and connective tissue for variability in beef tenderness, including

studies of the post mortem glycolysis and calpastatin activity that may be related to inherent causes of tenderness in Norwegian Red Cattle.

Methodology

The 28 bulls used in this study were slaughtered and handled as described by Aass et al. (2005). Muscle tissue samples for enzyme and inhibitor activities were collected together with a hot-boned sample of the loin (*M. Logissimus dorsi*; 10th thoracic to 2nd lumbar vertebrae). The loin was immediately vacuum-packed and conditioned at 12 °C for 10 h before ageing at 4 °C for 7 days, followed by measurements of Warner-Bratzler shear force (WB).

For WB analyses, meat slices (3.5 cm thick) were vacuum packed, heated in a water bath at 70°C for 50 min and chilled in ice water for 45 min. Slices (1cm thick) were cut twice in the fibre direction to give samples (ten replicates) of 2x1x1cm³ which were cut using a WB force device (triangular version) in an Instron Materials Testing Machine.

pH were determined 1, 6, 10 and 48 hours pm. The pH were measured with a Knick Mikroprocessor pH Meter Portamess 752, Electrode Mettler Toledo InLab 427

The enzyme analyses include the activity of m-calpain, μ -calpain and calpastatin, according to the procedures described by Shackelford et al. (1994) with some modifications. Only the calpastatin results will be reported here. The calpastatin assay as described by Aass et al (2005) was performed 24-31hrs pm.

Muscle samples for microscopy were taken 7 days pm from the adjacent slices to those used for WB measurements. Muscle blocks of 2 x 2 x 3 mm³ were fixed in 2.5% glutaraldehyd in cacodylate buffer and embedded in plastic resin as previously described by (Ofstad et al., 1993). For all samples, 3 μ m thick plastic sections were cut both perpendicular and longitudinal to the fibres. Optical microscopy observations were of sections stained in 0.1 g/100 ml toluidine blue dissolved in 0,1Msodium acetate solution. At the light microscopic level the evident changes included fibre contraction, fibre detachment, and partial and full break in the fibres. Optical microscopy with a 20x objective was used to measure myofibre to myofibre detachment and myofibre to perimysium detachment. Quantification of structural changes was performed as previously described by Taylor and Frylinck (2003). This involved counting the numbers of myofibres attached and partly or completely detached from the neighbouring myofibre for a minimum of 200 myofibre-to-myofibre attachments on transversally sectioned myofibres. In addition, myofibre-to-perimysium detachments were determined in a similar manner by counting the number of the myofibres attached and partly or completely detached from the perimysium, for a minimum of 30 myofibre-to-perimysium attachments for each sample. Contracted and broken fibres, partly and totally, were counted on a minimum of 30 fibres on longitudinally sectioned muscle fibres.

In order to estimate correlation between design variables (X) myofibre-myofibre (F-Fd), myofibre-perimysium (F-Pd) detachments, pH, calpastatin and the Y-variable tenderness (WB) we used partial least squares regression (PLSR). The data analysis was performed by using Unscrambler[®] 9.1 (Camo AS, Oslo, Norway, 2004).

Results & Discussion

The WB values for the 28 bulls are presented in Figure 1. The values represent typical variation in tenderness for LD of NFR bulls. Genetic factors account for a significant part of the variation observed in WB tenderness (Aass et al., 2005).

The light micrographs in Figure 2 illustrate structural changes in 7 days pm LD muscle with high and low WB-values (white columns). Myofibre-myofibre and myofibre-perimysium detachments are indicated with arrows in Figures 2a and c. Fibre contraction (asterisk) is evident as waves and contraction bands as shown in Figure 2b. Fully and partly broken fibres are marked with arrows in Figure 2d. The mean values of the muscle structure changes are given in Table 1. The mean values of the structural changes are in accordance of those reported for some African breeds by Taylor and Frylinck (2003). The very large standard deviations however indicate large individual variations among the 28 bulls examined in this study.

In the tough muscle some gaps can be seen between the myofibres, whereas the perimysium seems quite intact (Fig 2a). In the tender muscle loss of myofibre-myofibre attachments as well as loss of myofibre-perimysium attachments are clearly evident (Fig 2b). In average for all the samples 65% of the myofibres were detached and 38% of the myofibre were detached from the perimysium (Table 1). Previous studies have shown that this is due to costamere degradation by calpain (Taylor et al., 1995; Koochmaraie, 1996). Recently, Hannesson et al (2004) showed that in bovine LD muscle widening of the endomysial sheaths, being evident after 1 day of storage, were also caused by break down of the matrix components, i.e. large proteoglycans and hyaluronic acid. Our results indicate that the myofibre-perimysium attachments are more stable than the myofibre-myofibre attachments.

It is well established that proteolysis of myofibrillar proteins leads to increased fragmentation of myofibrils during post mortem storage (Taylor and Koochmaraie, 1998; Ho et al, 1996). However, most of the quantitative changes described have been at the ultrastructural level; i.e. fractured myofibrils. Minimum size of structures which can be sensory perceived is estimated to 100 μm (Hatae, 1990). This corresponds to the size of two fibres, and would include breaks of the whole fibres and myofibre to myofibre adhesion (Taylor and Frylinck, 2003). Figure 2d shows that in the sample with the lowest WB value the myofibres are severe fractured both partly and in the entire width of the myofibre. The tough sample (Fig 2b) possessed more contracted fibres than the tender one.

Table 2 shows mean values of calpastatin and pH at 1, 6, 10 and 48 hrs pm. The pH fall over this period was app. 1 unit, and there were only minor differences in ultimate pH among the animals. The pH will thus not be included in the regression model.

Figure 3 shows the correlation plot for Factor 1 versus Factor 2 from a PLS regression with structural changes and calpastatin as design parameters (X) on WB values as Y-variables. Both, the X variables and the WB values were weighted by their standard deviations prior to PLSR. The inner and outer circles indicate 50% and 100% explained variance in the model, respectively. The first factor explains 23% of the total variance of the design variables, which models 37% of the variance in the measured WB values. Factor 2 explains 24% of the variance in the design variables modelling 7 % of the Y variance. The X-loading shows that fibre breaks govern most the variation in the first factor and is negatively correlated to the WB values. Myofibre-perimysium detachment

and calpastatin activity, being opposite correlated are the main factors explaining most of the variation in the second factor. Myofibre-myofibre detachment and fibre contraction, being opposite correlated, have less effect on the 7 days measured WB values. However, the myofibre-myofibre detachment may have an impact on the degree of fibre contraction which also influence meat quality (Herring, 1965). Previous studies have shown that the myofibre-myofibre detachments occur within 24 hrs pm (Hannesson, 2003, Taylor and Frylinck, 2003). Such processes may have an impact on the post mortem structural changes and should be further studied.

The correlation plot in Figure 3 indicates that myofibre breaks are the main structural factors responsible for the variation in WB values. Frylinck and Heinze (2003) reported that WB shear force was significantly associated with fiber breaks at day 21. The myofibre detachment or contraction had no effect on the WB values. However, it is possible that this effect had been larger if texture had been measured on uncooked samples. In fish connective tissue degradation was associated with loss of texture hardness (Taylor et al., 2002).

The breaks in the fibres are probably due to reduced calpastatin activity, which allows higher calpain activity (Taylor and Koochmarai, 1998). In this study, the correlation between calpastatin activity and breaks were -0.45 (22 animals). Aass et al (2005) reported that the WB was positively related to an increase in calpastatin activity ($r_p = 0.44$) when analysed on 98 bulls. Both WB and calpastatin activity showed some heritability, i.e. 0.20 and 0.22, respectively. This study clearly demonstrated that structural variations influence variation in meat quality, which may be due to genetic differences within a breed.

Conclusion

This study shows that there is considerable individual variation in both post mortem myofibre detachments and breaks in the myofibres in LD muscle in NFR bulls. Fibre breaks are probably the main cause for the variation in WB tenderness measured 7 days p.m. However, due to the large individual variation in connective tissue decomposition between the bulls, the importance of such processes for the meat quality can not be ignored. In general the myofibre-perimysium attachments were more stable than the myofibre attachments. In addition the preliminary results of this study imply that structural changes and WB tenderness are closely related to the calpastatin activity. In a parallel study, with a larger number of bulls, it was shown that genetic factors accounted for a significant part of the variation observed in calpastatin activity and WB tenderness.

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Table 1. Structural changes 7 days pm. Values are the mean \pm standard deviation of 100 fibres of each of 28 animals.

Myofibre – myofibre detachments	Myofibre- perimysium detachments	Broken Myofibres	Contracted Myofibres
65 \pm 31	38 \pm 22	27 \pm 32	18 \pm 20

Table 2 Values are the mean \pm standard deviation of 22 animals.

pH 1h	pH 6h	pH 10h	pH 48h	Calpastatin (Units/gr)
6.6 \pm 0.2	6.1 \pm 0.2	5.8 \pm 0.2	5.5 \pm 0.1	2.5 \pm 0.6

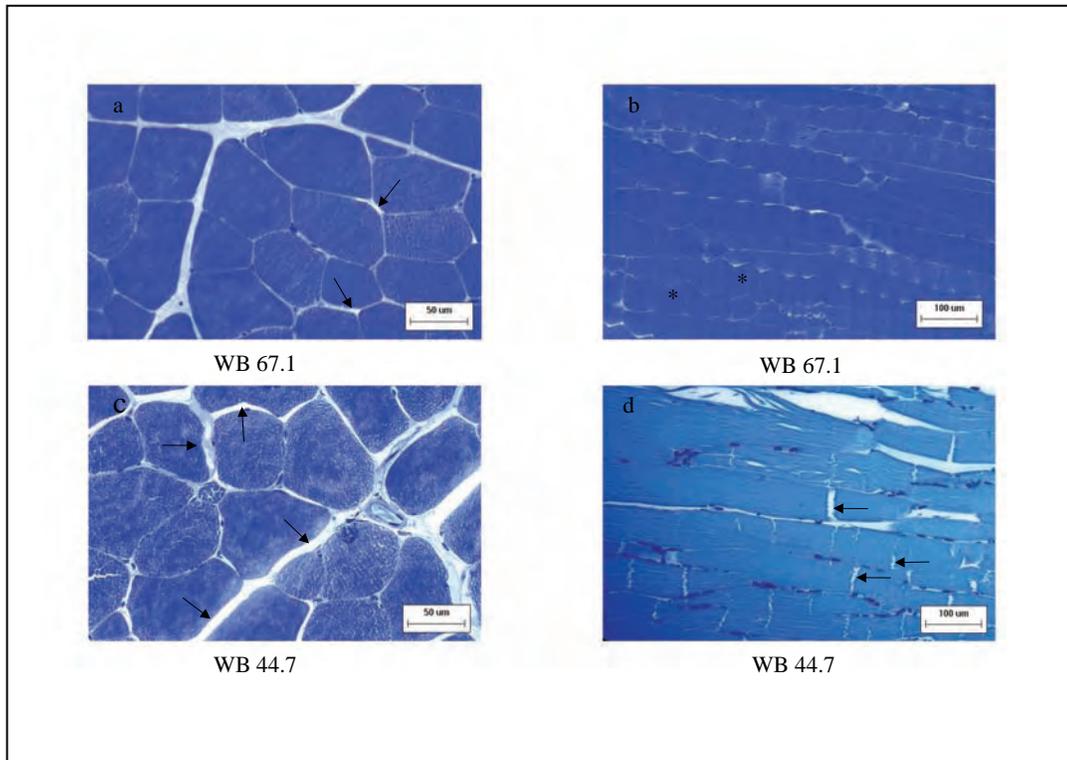


Figure 1. Light micrographs of LD muscle with high (a-b) and low (c-d) WB values at 7 days pm. Myofibre-myofibre and myofibre-perimysium detachments are indicated with arrows in (a and c). Fibre contraction (asterisk) is evident as waves and contraction bands in (b). Fully and partly broken fibres are marked with arrows in (d).

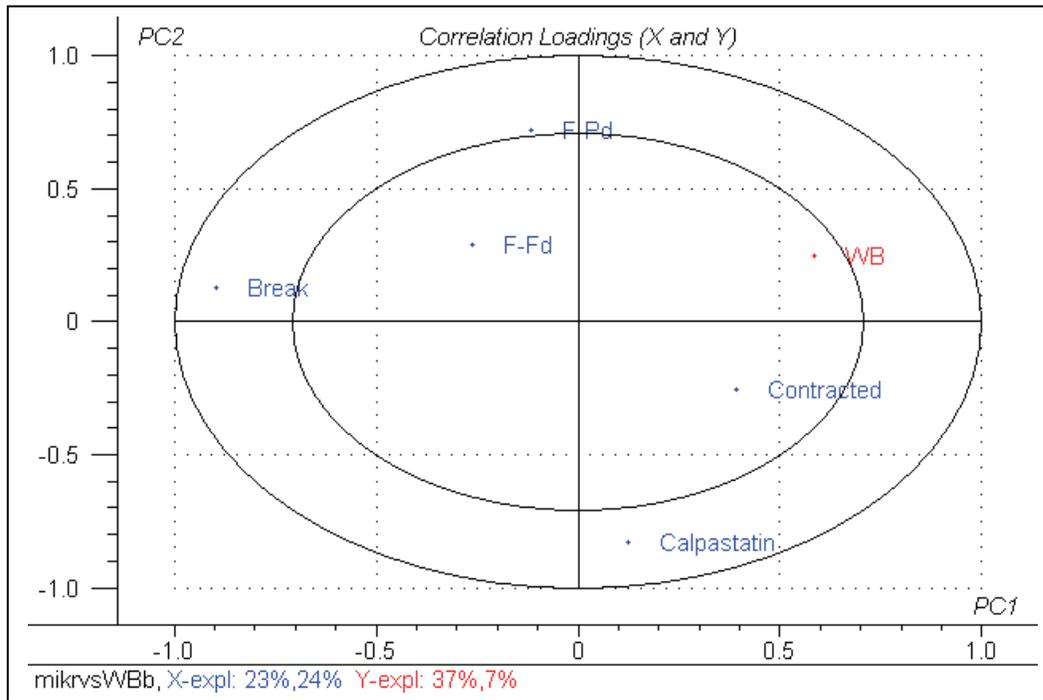


Figure 2. Correlation plot for Factor 1 versus Factor 2 from a PLS regression with structural changes and calpastatin as design parameters (X) on WB values as variables (Y).

**BLADE TENDERIZATION AND HYDRODYNAMIC PRESSURE PROCESSING
EFFECTS ON PROTEIN CHARACTERISTICS IN TOP ROUNDS
FROM BRAHMAN CATTLE**

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Key Words: Hydrodynamic Pressure Processing, Blade Tenderization, Brahman

Introduction

Inconsistent beef tenderness is a major problem in the meat industry. Due to the high consumer demand for lean yet tender cuts of beef and the high degree of tenderness variability between muscles and animals, it is necessary for the meat industry to develop methodologies to effectively tenderize tough cuts of beef. Technologies such as blade tenderization (BT) and hydrodynamic pressure processing (HDP) can be utilized to improve the tenderness of inherently tough cuts of meat, such as those from Brahman cattle. BT physically tenderizes muscle by inserting blades or needle probes into the muscle tissue to sever connective tissue and the myofibrillar structure. HDP has been shown to reduce shear force values by 59% in cold shortened beef semimembranosus muscles (Solomon et al., 1997), however, the mechanism by which HDP shock waves tenderize meat is not fully understood. HDP tenderization is thought to occur through the physical disruption of the myofibrillar structure (Zuckerman and Solomon, 1998). It was hypothesized that combining BT and HDP would greatly enhance the tenderness of tough beef cuts compared to either treatment alone due to BT disrupting the intramuscular connective tissue and allowing more extensive HDP tenderization (Liu et al., 2004). Although previous work has established that BT and HDP technologies effectively tenderize meat, there is a lack of data characterizing the effects that these technologies have on muscle proteins as they relate to muscle ultrastructure and the mechanism of tenderization.

Objectives

The objective of this study was to investigate the effects of HDP, BT, and BT followed by HDP (BT+HDP) on protein characteristics related to tenderness and protein functionality of inherently tough top rounds taken from Brahman cattle.

Methodology

Top rounds from Brahman cattle (Sub Tropical Agriculture Research Station, Brookville, FL) were frozen 7 days postmortem. Tenderness prescreening was conducted and twelve of the toughest top rounds (shear force range: 6.80 – 9.75 kgf) were selected for this study. Top rounds were thawed (2°C for 96-120 h) and subdivided parallel to the

long axis of the cut into two sections. Each section was randomly assigned to one of three treatments: BT, HDP, or BT+HDP in a balanced incomplete block design. Prior to treatment a subsection was removed perpendicular to the fiber direction of the muscles within each section to serve as a paired control. BT and BT+HDP samples were passed once through a blade tenderizer unit (Model MT-M5, Lumar Ideal Inc., Montreal, Quebec, Canada) parallel to the muscle fibers (2.3 penetrations/cm²). HDP and BT+HDP samples were vacuum packaged, heat shrunk, and placed on a flat steel reflector plate inside a 98-L suspended plastic container filled with water. A 100 g binary explosive, detonated 31cm above the meat, was used to generate the hydrodynamic pressure.

Following the tenderization treatments, 2.5 cm thick steaks were cut from each subsection (both treated and control) and cooked to 71°C according to the AMSA (1995) guidelines on an electric George Foreman Indoor/Outdoor grill (Model GGR50B, Salton, Mount Prospect, IL). Warner-Bratzler shear force (kgf) measurements were obtained using the Universal Instron Testing Machine (Model 1122, Instron Corporation, Canton, MA).

In addition to removing steaks for tenderness evaluation following the tenderization treatments, samples were also removed for protein analysis. These samples were vacuum packaged, stored at -20°C, and thawed overnight at 4°C prior to processing and analysis. From these samples, the sarcoplasmic and myofibrillar protein fractions were isolated by homogenization/centrifugation according to the procedure of Goll et al. (1974) with modifications. Isolated protein fractions were separated electrophoretically using the SDS-PAGE technique of Laemmli (1970) with modifications. Myofibrillar proteins were separated on 4-20% acrylamide gradient gels and sarcoplasmic proteins were separated on 10-20% acrylamide gradient gels. Samples were dissolved in sample buffer (8.0 M Urea, 2.0 M Thiourea, 0.05 M Tris (pH 6.8), 75 mM DTT, 3 % SDS, 0.05 % Bromophenol blue) in boiling water for 5 minutes. Bands were visualized by Coomassie brilliant blue R-250 staining and the intensity of the bands was measured using a KODAK Gel Logic 200 imaging system and KODAK 1D Image Analysis software (Eastman KODAK Co., Rochester, NY). Myofibrillar fragmentation index (MFI) was measured for each sample according to the procedure of Hopkins et al. (2000). Sarcoplasmic protein solubility was determined in 0.025 M potassium phosphate (pH 7.2) buffer and myofibrillar protein solubility was similarly determined in 1.1 M KI/0.1 M potassium phosphate (pH 7.2) buffer according to the procedures of Schilling et al. (2002). All protein concentrations were measured using the biuret method (Total protein reagent, Sigma-Aldrich Inc., St. Louis, MO).

MFI, protein solubility, and SDS-PAGE band intensity data were analyzed using the Proc MIXED procedure in SAS® (version 9.1, SAS Institute Inc., Cary, NC). The model included treatment (BT, HDP, BT+HDP) and type (control, treated) as class independent variables and round as a random effect.

Results & Discussion

A previous study on these samples demonstrated that BT and HDP improved tenderness 18% and that BT+HDP improved tenderness 14% compared to paired controls (Liu et al., 2004). Table 1 shows the impact tenderization treatments had on MFI, protein solubility, and SDS-PAGE banding patterns. The MFI value, a measure of the degree of

fragmentation of myofibrils upon homogenization, is often used as an indicator of the degradation of myofibrillar proteins under postmortem conditions. Overall, MFI values were negatively correlated to shear force measurements ($r = -0.53$). BT, HDP, and BT+HDP treated samples had 35% higher ($p < 0.01$) MFI values than paired controls. MFI values did not differ among the three treatments. Thus, MFI data suggest that part of the tenderization effect of BT and HDP treatments is the result of a breakdown of intra- and intermyofibril linkages.

Past research has shown that high pressure treatments can influence the solubility characteristics of various muscle proteins (Macfarlane and McKenzie, 1976). This phenomenon, however, was not readily apparent with the HDP and BT+HDP treatments. Neither sarcoplasmic nor myofibrillar protein solubility differed statistically ($p > 0.05$) between treated and control samples. Sarcoplasmic and myofibrillar protein solubility did not significantly differ among treatments. It should be noted, however, that HDP and BT+HDP treated samples had higher myofibrillar protein solubility than paired controls in all but two of the rounds (data without these samples not shown). Due to the small number of samples, a large decrease in the myofibrillar protein solubility between the control and treated samples taken from these two rounds contributed to the high degree of variability in the myofibrillar solubility measurements and masked overall treatment effects. Overall, solubility data did not significantly correlate with tenderness data.

To investigate shifts in protein profiles and proteolytic differences between samples, SDS-PAGE analysis was performed. Sarcoplasmic electrophoretic protein gels exhibited banding pattern differences between top rounds (particularly 185, 145, 90, 42, and 23.5 kDa fragments), but minimal differences between paired control and treatment samples. Likewise, few differences were observed among BT, HDP, and BT+HDP treatments with respect to sarcoplasmic protein banding patterns. Myofibrillar protein banding patterns also varied significantly between top round samples. Compared to paired controls, HDP and BT+HDP samples had significantly higher ($p < 0.05$) ~100-110 kDa to actin band intensity ratios (Table 1), whereas BT and control samples did not differ. The identity of this band has not been positively determined but it is hypothesized to be part of the C-protein (~140 kDa) which helps stabilize myosin molecules in the thick filament. This hypothesis is based on the findings of O'Halloran et al (1997) that during aging of bovine muscle a 110 kDa fragment appeared in the myofibrillar fraction which was later shown by sequence analysis to have strong homology with C-protein (Casserly et al., 1998). The myofibrillar electrophoretic protein gels also showed a considerable amount of variation in the intensity of the 30 kDa protein fragment from sample to sample, but no treatment differences were detectable.

Conclusions

BT and HDP treatments are both effective methodologies for tenderizing top rounds from Brahman cattle. The results from this study suggest that the mechanism of tenderization is different between these techniques. Overall, no synergistic or additive effects were observed for any of the measured parameters with the combination BT+HDP treatment. The fact that BT samples had an increased MFI and few if any protein solubility or SDS-PAGE banding pattern differences compared to controls confirms that BT likely tenderizes muscle by physically disrupting and severing the connective tissue

and myofibrillar structure. Slight shifts in the SDS-PAGE banding patterns of myofibrillar proteins and increased MFI with HDP and BT+HDP treatment indicate that in addition to the physical disruption to the myofibrillar structure, HDP treatment may also influence tenderization through direct alterations of muscle proteins.

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Tables and Figures

Table 1. Least square means \pm standard errors of MFI, protein solubility, and SDS-PAGE myofibrillar protein band intensity measurements of blade tenderization (BT), hydrodynamic pressure processing (HDP), BT followed by HDP (BT+HDP), and control samples from Brahman top rounds.

Treatment	Type		Trt ¹	Significance	
	Control	Treated		Type ²	Trt \times Type
Myofibrillar Fragmentation Index					
BT	45.8 \pm 6.3 ^b	62.2 \pm 8.2 ^a	0.9844	0.0020	0.9934
HDP	46.5 \pm 7.0 ^b	62.7 \pm 6.9 ^a			
BT+HDP	45.8 \pm 6.7 ^b	61.4 \pm 5.9 ^a			
Sarcoplasmic Protein Solubility (mg protein/g muscle tissue)					
BT	41.2 \pm 2.9	38.3 \pm 2.1	0.6631	0.1796	0.7714
HDP	42.2 \pm 3.9	41.9 \pm 2.1			
BT+HDP	44.3 \pm 2.9	40.6 \pm 1.8			
Myofibrillar Protein Solubility (mg protein/g muscle tissue)					
BT	110.9 \pm 13.6	109.9 \pm 13.5	0.4737	0.8239	0.1917
HDP	134.3 \pm 13.6	127.4 \pm 12.8			
BT+HDP	111.3 \pm 9.1	127.1 \pm 11.1			
Myofibrillar protein SDS-PAGE staining intensity (100-110 kDa : actin band intensity ratio)					
BT	0.181 \pm 0.015 ^b	0.191 \pm 0.022 ^{ab}	0.0863	0.0216	0.4631
HDP	0.185 \pm 0.010 ^b	0.229 \pm 0.015 ^a			
BT+HDP	0.176 \pm 0.007 ^b	0.209 \pm 0.008 ^a			

¹ Trt factor compares BT, HDP, and BT+HDP

² Type factor compares Control to Treated samples

^{a,b} Values with different superscripts differ significantly (p<0.05)

ANALYSIS OF CHANGES OF THE PROTEIN ISOELECTRIC POINT OF PORK AS A FACTOR AFFECTING ITS TENDERNESS AND WATER HOLDING CAPACITY

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Key Words: pork, proteins, IEF, meat properties

Introduction

One of the phenomena typical of after slaughter changes is the advancing acidification of tissue directly after slaughter followed by its alkalization. It is believed that the observed drop in the pH value is the result of meat anaerobic glycolysis, whereas the alkalization is the consequence of protein decomposition. Modern meat packaging and storage methods slow down alkalization processes but protein degradation, including, in particular, cytoskeleton proteins, continues to take place and contributes to the improvement of meat tenderness. Information characterizing changes in the protein isoelectric point is scarce, especially with regard to the products of protein degradation.

Objectives

The aim of the performed investigations was to analyze protein changes in meat stored in a cold room with regard to the protein isoelectric point (IP) and to ascertain whether the above changes are associated with changes of selected meat properties, especially tenderness and water holding capacity.

Methodology

The experimental material comprised musculus longissimus dorsi (LD), its lumbar and thoracic parts, from 34 pigs. Their origin, nutrition as well as rearing conditions were controlled and their pre-slaughter weight was about 105 kg.

The studies included both normal quality muscles as well as muscles with watery (PSE) and acid (ASE) properties. The quality classification was based on measurements of the pH value taken 45 minutes (pH₁) and 24 hours (pH₂) after slaughter and of electrical conductivity measured 90 minutes (EC₁) and 24 hours (EC₂) after slaughter. Normal quality meat (RFN) required the following characteristics: pH₁ above 5.8, whereas EC₁ and EC₂ - below 8 mS/cm. In the case of acid meat, the EC₁ limiting value was below 8 mS/cm, while meat with PSE defects was characterized by the pH₁ value below 5.8. After 24 hours, in the case of both defects, the EC₂ was above 8. The entire

muscle was divided 24 hr post mortem into portions to be analyzed 48, 120, 168 and 336hr after slaughter which were vacuum-packaged in polyamide-polyethylene bags and stored in refrigerated conditions. At each of the above-mentioned four terms, the pH value was determined and the isoelectrofocusing analysis (IEF) was performed on the proteins from centrifugal drip obtained as the result of tissue centrifugation (25 000 g; 20 min., 2°C). Instrumental measurements of meat tenderness and water holding capacity as well as sensory assessment were performed after 48 and 168 hours of storage. IEF of the proteins of centrifugal drip was carried out using agarose gels (1.2%) at the pH 3-10 gradient (Pospiech et al., 2001). Before separation, samples were desalted using Sephadex G-10. The identification of titin, myosin and troponin T (Tn-T) in the centrifugal drip was confirmed using Western blotting analysis according to the method of Fritz and Greaser (1991) with one modification referring to the type of the used membrane. Samples subjected to the IEF were blotted onto nitrocellulose membrane instead of Immobilon (Fritz and Greaser 1991). Anti-myosin monoclonal (A4335 from Sigma), anti-titin (9D10) and anti-Tn-T (9D) were used as the primary antibodies. The secondary antibody was goat anti-mouse IgG (H&L) conjugated to the alkaline phosphatase from Organon Teknika. Titin 9D10 and troponin T –D10 antibodies were obtained from the Muscle Biology Laboratory University of Wisconsin.

The water binding capacity was determined by measuring the amount of weight losses of the meat samples (around 500g) stored for 48 or 168 hr at 2°C. The sensory assessment was performed according to the method of linear scaling and comprised the evaluation of taste, flavor, juiciness and tenderness. It was carried out on meat heated to the temperature of 70°C. Cooked samples measuring 10 x 10 mm were also used to assess tenderness with the assistance of the Instron type 1140 apparatus.

Results & Discussion

The statistical analysis of the pH values measured 45 minutes after slaughter revealed significant variations in the obtained results, depending on meat quality. The obtained mean pH_i value at the level of 5.37 for PSE muscles was considerably lower than values observed in the case of the RFN (6.5) and ASE (6.25) muscles. On the other hand, statistically non-significant pH value relationships were found between the muscles of different quality at later terms, i.e. after 24, 48, 120, 168 and 336 hours of storage. Mean pH values ranged from 5.32 to 5.44, irrespective of the meat quality. The ASE muscles showed a tendency towards the lowest pH value, especially after 24 h storage. The results recorded at the first two terms of measurements correspond with literature data which characterize properties of the LD muscle of varying quality (Feldhusen et al. 1987, Borzuta and Pospiech 1999, Lee et al. 2000,).

It was observed that the value of the electrical conductivity, when measurements were taken 90 minutes after slaughter, was significantly diverse between the PSE muscles (14.06) and the ASE (3.75) and RFN (3.58) muscles. After 24 h of storage, differences between all quality groups were statistically significant and those values amounted to: 11.43 for the PSE meat, 9.31 – for the ASE meat and 4.57 – for the RFN meat.

Weight losses during storage were affected significantly both by the quality and time factors. In the case of the watery meat, the size of the drip, both at the first (48hr) (3.46%) as well as the second (168hr) (6.79%) term of measurement was significantly higher in

comparison with the ASE and RFN samples (respectively: 1.97% and 4.66% for the ASE and 1.34% and 3.54% - for the RFN). After 168 hr of storage, a significant increase of the juice loss from the meat tissue was observed in comparison with the first date of measurements, irrespective of the quality of the raw material.

In comparison with the normal and watery meat, acid meat, after 48hr of storage, was characterised by a slightly better tenderness (41.58N/cm^2) and its tenderness was found improved only slightly (39.51N/cm^2) after 168hr of storage. A significantly greater shear force was found for normal muscles (44.09N/cm^2) and PSE muscles (45.86N/cm^2) at the first date of measurements in comparison with the results obtained after 168hr of storage (36.32N/cm^2 and 36.04N/cm^2 , respectively).

The separated protein samples were analyzed within the following 5 IP ranges, which values were adopted arbitrarily: 1 - IP<4.69; 2 - IP 4.7÷5.59; 3 - IP 5.6÷7.69; 4 - IP 7.7÷8.79; 5 - IP>8.8. Within the implemented IP ranges, either the greatest concentrations of protein bands and/or significant changes in their quantities found in the course of meat storage were observed.

It was then found that the amount of proteins from the first range increased gradually during the storage period from the value of 13.16% 48 hr after slaughter to 14.82% - 336 hours after slaughter. The statistical evaluation of the percentage share of proteins from the second IP range (4.7÷5.59) revealed significant differences between the storage times of 48 and 336 hr. In the case of the 48 hr, the mean value of the share was 24.34% and increased to the value of 27.91% at the last date of analysis. After 120 and 168 hr of storage, the mean values amounted to 24.95% and 25.62%, respectively. As regards the third range (IP 5.6÷7.69), a significant decrease in the proportion of the separated bands after 336hr storage were recorded, from 17.44% to 12.94%. In the case of two intermediate terms of analyses, identical proportions (15.94%) of these proteins were found. Proteins of the fourth IP range were characterized by the highest proportion, on average 37.6%, of that after 48hr, and it increased by about 1.8% at the last term of analyses. Bands of the fifth range with IP>8.8 constituted the lowest proportion, on average from 7.14 to 7.65% at the analyzed terms. The above remarks indicate that in the course of meat storage the greatest changes took place in the case of proteins found in the second and third range, i.e. those which were characterized by the isoelectric point at the 4.7÷7.69 interval.

In order to achieve a more comprehensive identification of the selected proteins (titin, myosin and Tn-T), western blotting was performed. It was found that these proteins were characterized by the IP of a wide range of pH values. In the case of titin, it was observed that, with the passage of meat storage time, both the number and intensity of bands with higher IP values increased. The proportion of titin bands of IP<4.69 decreased from 8.55% after 48hr storage to 1.17% at the last date of analyses. The greatest, almost double, increase in the proportion of this protein, together with the advancing process of proteolysis, was observed in the fourth IP range (7.70÷8.79). The mean share of this band amounted to 18.84% after 48hr and reached the value of 37.35% after 336hr. Western blotting with the antibody against myosin was characterized by the smallest changes in the proportions of the separated bands. The amount of proteins from the first IP range (<4.69) increased from 8.26% to 15.14% after 336hr. An increasing trend was also recorded in the second IP range (4.7÷5.59). In the case of the IP>7.7, the proportion of proteins derived from myosin was the least diversified and, on average, amounted to

41.47%. In the case of the Tn-T antibody, its bands were found a little shifted towards higher IP values during storage but this shift was somewhat different from that observed in the case of the titin. The proportion of bands with $IP < 4.69$ decreased from 12.11% after 48hr to 2.73% after 336hr of storage. The proportion of proteins from the last range ($IP > 8.80$) increased, in relation to their amount at the first date of analyses, by 9.5% after 120hr and by 7.22% and 7.27% at the consecutive storage periods.

The variations concerning the meat of different quality for the three proteins were relatively small. However, the above observations indicate that in the case of each of the analyzed proteins, changes in their proportions were observed within specific ranges and they were probably the result of the on-going proteolysis. It is worth noting that, in the case of titin and Tn-T, major proteins associated with the meat tenderization process, the storage process led to the decrease in the proportion of proteins with low IP and increase of those with high IP. In the case of myosin, the observed changes were, generally speaking, reverse but changes in its proportions were more restricted. The meat pH value is a resultant of actions of many factors (Pösö & Puolanne 2004). After slaughter special role may be probably played by proteins of the drip. Since the tissue pH value underwent relatively small changes in the course of the 2-week long cold storage, it can be assumed that changes associated with the protein degradation resulting in the disappearance of some and the development of others – as products of these changes – counterbalanced changes in the pH value of the tissue and, consequently, contributed to the described phenomenon. However, it did not mean that some meat properties could not undergo changes. The increased amount of the drip obtained from it could have resulted from the decreased share of myosin, or its structural sub-units, characterized by a higher IP since water holding capacity is strongly related with IP and increases as the pH value moves away from the IP of the given protein. The observed increased proportions of titin and Tn-T bands of higher IP may, in turn, indicate that tenderization leads to the liberation from the muscle structure of these proteins characterized by high IP. Perhaps these changes are associated with the changes of the PEVK region of titin, the protein responsible for actin-myosin interactions and meat tenderization (Niederlander et al. 2004, Boyer-Beri & Greaser 1998, Greaser et al., 2002). It can be presumed that their more comprehensive characterisation, both as regards the moment of their appearance as well as the determination of relationships between the occurrence of given proteins and meat properties, especially tenderness could lead to a better understanding of mechanisms affecting them.

Conclusions

IEF separations of the proteins from centrifugal drip of the pig LD muscle indicated that they were dominated by proteins with the IP ranging from $7.7 \div 8.79$ (about 37.6% on average) and the range $4.7 \div 5.59$ (on average about 25.7%). The smallest proportion of proteins derived from IP over 8.8 (7.4%).

In the course of the two-week long storage of meat, the greatest changes in the amount of proteins bands were observed in the range $4.7 \div 5.59$ (increase) and $5.6 \div 7.69$ (decrease).

Myosin immunoblotting indicated the smallest changes from among the three analyzed with regard to IP. The proportion of this protein increased for the range of IP less than 5.59 which could have been associated with the increasing drip from the tissue.

In the case of titin and troponin T, the proportion of bands with higher IP (>7.7) increased. Since meat aging is associated with fractures in the structure of these proteins, it can be presumed that a more comprehensive characterization of relationships between their appearance and meat tenderization and water holding capacity could lead to the development of markers of these processes.

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**EFFICACY OF A REFRIGERATION STORAGE TECHNOLOGY
TO IMPROVE TENDERNESS OF BROILER BREAST MEAT
AT AN INDUSTRIAL PROCESSING PLANT**

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Key Words: texture, ultrastructural evaluation, myofibrillar fragmentation index

Introduction

Consumer satisfaction with meat tenderness will determine if the purchase is repeated in the future (Morgan et al., 1991). Therefore it is critical for food industries and meat processors to consistently produce a tender meat product that meets or exceeds consumer expectations. In order to have a consistency in tenderness is necessary to standardize the procedures of ante- and post- mortem in particular handlings of carcasses. Several factors are known in order to explain this meat tenderness variation including the amount of intramuscularly fat, water holding capacity and actomyosin complex (Avery et al. 1996). Collagen and its crosslinking are factors involved as the birds advance in aging (Coro et al., 2002) and also the changes that occur during postmortem storage such as aging, type of rigor, sarcomere length, skeletal restraint and proteolytic activity (Pearson, 1987). Koohmaraie (1996) reported that prevention of sarcomere length shortening could prevent meat toughening. In poultry, cold shortening is not normally a cause of toughening in normal industrial processing providing the breast meat is not pre-maturely excised or otherwise altered (Papinaho and Fletcher, 1996). Papinaho and Fletcher (1996) described temperature induced shortening in broiler breast muscle sampled as intact, and excised muscle. The postmortem tenderization by proteolysis has been studied extensively and the calpain system has been known to initiate the myofibrillar framework enzymatic digestion (Goll, et al. 1997). Koohmarie (1996) pointed out that the breakdown of key proteins which maintains the sarcomere structure cause its weakening and the meat tenderness and the Z-line degradation was one of the main changes occurred during postmortem tenderization (Taylor et al., 1995)

Objectives

The objective of this work was to investigate a refrigeration technology of a broiler breast meat by measuring the myofibrillar fragmentation index, tenderness measurement, ultrastructural evaluation and sensory acceptance of intact and excised samples at an industrial processing plant.

Methodology

Samples

Seventy two chicken of Cobb lineage of 42 days were slaughtered according to the industry plant routine practice consisting essentially in sequence of electrical stunning, bleeding, defeathering, evisceration, carcass water cooling, deboning and refrigeration (Guarnieri et al., 2004). Samples were divided into two experimental groups in relation to breast meat (*Pectoralis major*), intact breast (n=36) and excised breast (n=36).

Refrigeration techniques

Both samples were stored in plastic boxes at $2^{\circ}\text{C} \pm 2$. Six samples of each experimental groups were taken for analysis of MFI and shear force measurement at 0, 8, 12, 24, 48 and 72h post mortem.

Myofibrillar Fragmentation Index (MFI)

MFI was determined as indirect measurement of calpain activity according to Culler et al (1978).

Shear force measurement

Samples were packed in plastic bags and submitted for cooking in a water bath until the samples internal temperature reached the value of 75°C . After refrigeration at $2^{\circ}\text{C} \pm 2$ for 12 h, samples were cut at the size of 1cm^3 , analyzed on a texturometer TATX-2i and results were expressed in Newtons.

Sensory Analysis

Intact and excised samples taken at 0, 24 and 72h from refrigeration conditions were submitted to acceptance test. Fillets were vacuum packed and submitted to cooking process in an oven at 80°C until the internal temperature reached the value of 75°C . Five samples for each intact samples and excised fillets were refrigerated down to 28°C and randomly served to 28 untrained panelists. Structured hedonic scale of 9 points, 1= dislike very much to 9= like very much, was used in order to the panelists evaluate the samples acceptance (Meilgraad et al., 1999).

Electron Microscopy

Seventy-two hours excised samples were fixed in 2% glutaraldehyde in 0.14M sodium cacodylate buffer, pH 7.4 and 0.18M sucrose. After being washed in phosphate buffer, samples were postfixed in 1% osmium tetroxide in phosphate buffer for 1 h, followed by dehydration in acetone and embedded in Araldite resin. Ultrathin sections (50 nm) were stained with saturated uranyl acetate in 50% ethanol and lead citrate for 1 h. The ultrastructure was observed with an electron microscope Philips CM 100 (Guarnieri et al. 2004).

Statistical Analysis

The experiment was entirely randomized and experimental treatments were intact and excised meat samples and evaluated at the refrigeration period of 0, 8, 12, 24, 28 and 72h post mortem. Statistical analysis was carried out using the Statistica Program version 5.0 (Oklahoma, 1995). Student test t was also applied to determine the significance level between two treatments: intact and excised samples in each period of in relation to MFI and meat tenderness. Variance analysis and Tukey test were used for samples acceptance comparison.

Results & Discussion

Myofibrillar Fragmentation Index

Figure 1 shows the MFI of intact and excised broiler breast meat. As it can be seen, MFI increased from the beginning to 24h period of refrigeration becoming virtually constant up to 72h of storage for both samples. These results indicated that calpain enzyme system reached the maximum activity in conditions of $2^{\circ}\text{C} \pm 2$ around 24h of refrigeration process. However, intact breast presented MFI higher ($p \leq 0.05$) in relation to deboned samples throughout treatment and this difference was 13.2% from 24h onwards.

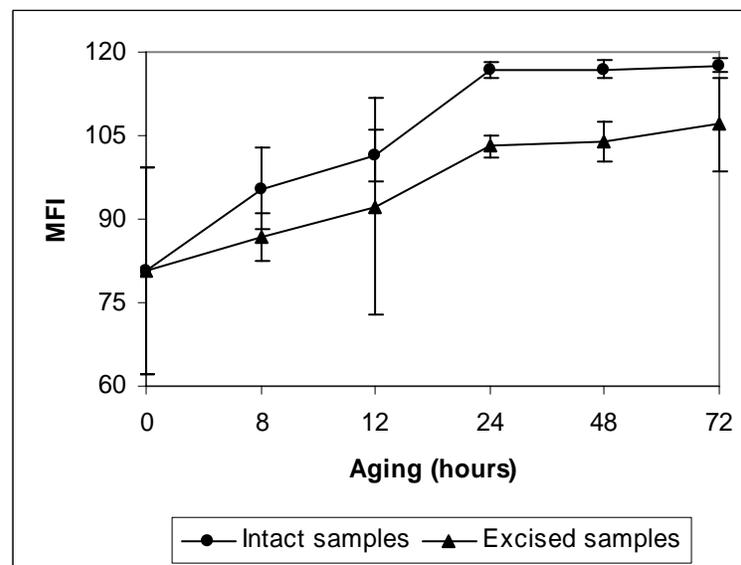


Figure 1. Myofibrillar fragmentation index of broiler breast fillet. A gradual increase of MFI occurred with storage being more pronounced in intact samples in relation to excised samples.

Breast meat tenderness measurement

Figure 2 shows the decrease of shear force value for both samples being more noticeable in samples kept for 24h becoming virtually constant throughout experiment.

Again the tenderness was higher in intact in comparison to excised samples ($p \leq 0.05$). A negative significant correlation ($R = -0.93$) was observed between MFI and shear force values indicating higher the proteases activity lower was the breast meat texture. Calpains enzymes digested the sarcomere structure particularly Z-lines improving the meat tenderness and synergistically samples attached to bones were protected against muscles restrain avoiding further the rigor shortening and the intact fillets became more tender.

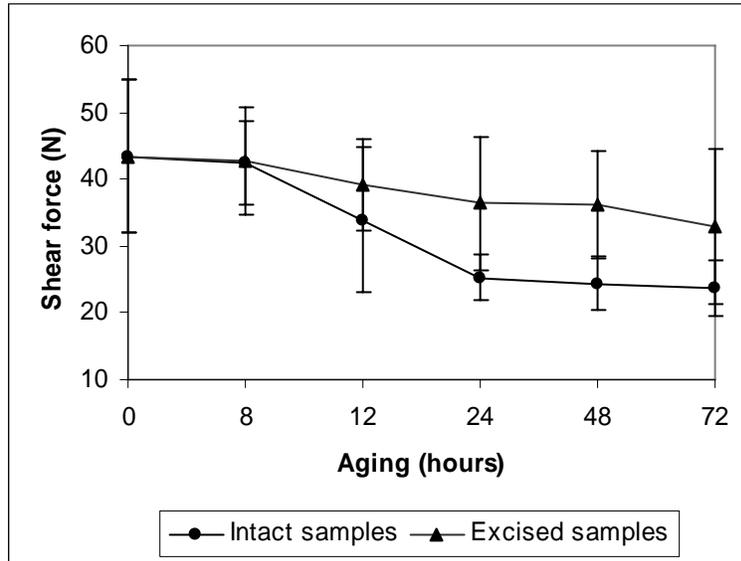


Figure 2. Shear force values of broiler breast fillet. A gradual decrease during storage occurred for shear force being more pronounced in intact samples in relation to excised samples.

Sensory analysis

Table 1 presents the results of acceptance test for both samples. The best scores were attributed to intact samples kept refrigerated for 24h and 72h and the acceptance index were 75.0 and 77.0%, respectively. These results of panelists acceptance were in accordance to the results of MFI and shear force analysis.

Table 1 –Average and acceptance index applying structured hedonic scale of 9 points, 1= dislike very much to 9= like very much, intact and excised samples of broiler breast meat refrigerated at 2°C in different periods of storage.

Refrigerated samples stored at different period of time	Score	Acceptance index (%)
Breast fillet 0h	4.18 ^b ± 1.85	46.4
Intact breast fillet 24h	6.75 ^a ± 1.97	75.0
Excised breast fillet 24h	5.36 ^b ± 1.93	59.6
Intact breast fillet 72h	6.93 ^a ± 1.41	77.0
Excised breast fillet 72h	5.32 ^b ± 2.25	59.1

Means followed by different letters differed significantly by Tukey test of 5% of probability (n=28).

Electron Microscopy

Figure 3 shows a picture of longitudinal muscle section from excised samples stored for 72h and was characterized by a normal miofilaments structure and showed a regular transverse striation with evident bands of I and A, zone H and lines Z and M. The sarcomeres were of equal length and width but the faint Z-lines indicated that there were some protease activities within the muscle.

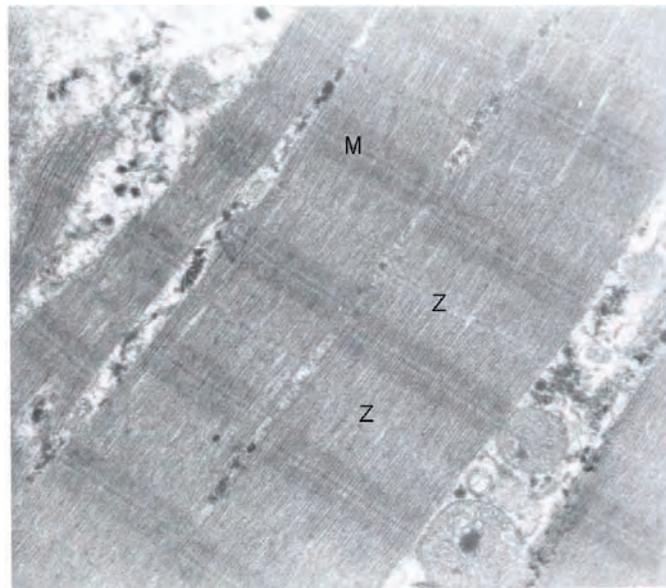


Figure 3. Electron micrograph of *Pectoralis major m.* stored for 72h at $2^{\circ}\text{C} \pm 2$. Note a faint Z-disk digested by calpain (Z- Z-line, M-M-line). Magnification 23 000 x.

Conclusions

Our results of myofibrillar fragmentation index, shear force measurement, taste panel analysis and ultrastructural evaluation demonstrated that the technology of storing intact broiler breast fillet refrigerated at $2^{\circ}\text{C} \pm 2$ for a minimum of 24h was an excellent procedure to obtain a very tender meat.

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**A LIGHT MICROSCOPY STUDY ON CONNECTIVE TISSUE
DECOMPOSITION IN BOVINE *M. LONGISSIMUS DORSI* DURING
THE FIRST WEEK *POST MORTEM***

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Key Words: Connective tissue, decomposition, C0S and C6S, perlecan

Introduction

Tenderness is an important factor for the consumer's acceptance of meat, and postmortem storage of meat is known to increase tenderness. Tenderness is determined by the structural properties of myofibers and connective tissue and the processes going on in the tissue. In a recent light microscopy study, splitting of bovine *M. longissimus dorsi* was reported to occur inside the myofibers as well as in the connective tissue between the myofibers and between the myofiber-perimysium during the first 7 days after slaughter (Ofstad et al. 2005). Degradation of meat is reported to occur in the Z-lines of the myofibres (Taylor et al, 1995). Ruptures and splitting in the connective tissue is considered a result of tissue decomposition. In a recent study on bovine *M. longissimus dorsi* we reported that decomposition of connective tissue could be detected as early as 24 hours after slaughter by light microscopy on Haematoxylin eosin stained cryo-sections (Hannesson et al. 2003). Furthermore, proteoglycan aggregates of high molecular size carrying chondroitin/dermatan sulfate glycosaminoglycans (GAGs) and hyaluronic acid, were involved in the process as judged by gel chromatography and PAGE. Ruptures and splitting in the connective tissue is considered as a parameter for tissue decomposition. In the present study we have focused on the splits occurring in the connective tissue; in the myofibre/myofibre attachment as well as in the myofibre/perimysium attachment. The amount of splitting was calculated and the expression and localization of sulfated glycosaminoglycan (GAG) structures carried by the large chondroitin sulfate proteoglycans (CSPGs) studied by immunohistochemistry using monoclonal antibodies (mAbs). MAb against the large heparan sulfate proteoglycan of basement membranes, perlecan were also included. The Warner Bratzler values for the different meat samples are included.

Objectives

To study components involved in myofiber/ connective tissue detachments in bovine skeletal muscle aged for 7 days. The focus is on the expression and distribution of unsulfated and 6-sulfated chondroitin sulfate and the large basement membran heparan sulfate PG, perlecan, and possible changes in the expression of these epitopes after 7 days storage.

Methodology

Animals: Specification and handling of the animals and the method used for WB-measurements were the same as described by Aass et al.(2005).

Preparation of samples for microscopy: Plastic and cryo-sections were taken on muscle samples collected from the medial central part of bovine *M.longissimus dorsi* adjacent to the pieces used for WB after removal of the epimysium. Pieces of approximately 4 x 4 x 3 mm were cut at different locations.

Plastic-sections. Fixation was carried out in 2.5% glutaraldehyde in cacodylate buffer and embedded in plastic resin, Histo-resin (Ofstad et al.1993). Cross-sections (3µm) were then stained in 0.1% Toluidine Blue O (Sigma/Aldrich) dissolved in aqueous sodium acetate (1g/100ml)

Splitting was calculated after measuring the number of splits between the myofibers and myofiber/perimysium (Ofstad et al. 2005). No efforts were done to evaluate the length or with of the splits.

Cryosections: The pieces were embedded in O.C.T. compound (Tissue Tek 4583, Miles Inc., Diagnostic Division, Elkhart, USA) for 30 minutes and finally immersed in liquid nitrogen. The frozen samples were stored at -80 C

Immunohistology

Enzyme treatment: To generate the antigenic epitopes for detection of the different sulfate structures present in the CS/DS chains, the samples were digested with a drop of chondroitinase ABC lyase from *Proteus vulgaris* (0.5 units/ml) (EC 4.2.2.4, Sigma Chemical Comp. St. Louis, MO, USA) in 0.1 M Tris-HCl buffer pH 8 for 4 h at 37C. (Yamagata *et al.* 1968). This method produces a terminal disaccharide, consisting of an unsaturated uronic or iduronic acid residue adjacent to the N-acetylgalactosamine that may be unsulfated or sulfated in the C4 or C6 positions (Couchman et al. 1984)

Antibodies: For detection of N-acetylgalactosamine sulfated in C6 (C6S), the mAb 3B3 was used. For detection of unsulfated N-acetylgalactosamine (C0S) the mAb 1B5 was used (Cateron et al.1985).

For detection of perlecan mAb A7L6 (Chemicon International LTD, Hofheim, Germany) was used. Perlecan clone A7L6 reacts with perlecan core domain IV (Couchman and Ljubimov, 1989).

For immunostaining, an immunoperoxidase system Vectastain Universal Elita ABC kit (Vector Laboratories, Inc., Burlingame, CA) was used according to the manufacturers recommendations. Before immunostaining, the 5 µm cross-sections were fixed in 8% formaldehyde in phosphate buffered saline (PBS, pH 7.4) for 5 min. Unspecific binding sites were blocked using 5% bovine serum albumin (Sigma-Aldrich Chemie) in PBS added normal serum from horse (Vectastain Universal Elita ABC kit. The sections were then incubated over night at 4 °C with the following mAbs; 1B5 (1:200), 3B3 (1:200) and A7L6 (diluted 1:100).The mAbs were diluted in PBS added 5% BSA and 0.005 % Tween-20 (Sigma-Aldrich). The cover slips were photographed in a Leica DMLB microscope (Leica Microsystems Nussloch GmbH, Germany) by a Spot RT Color Camera (Diagnostic Instruments inc. Burroughs Sterling Heights, Michigan).

Results & Discussion

The results obtained on plastic sections from *M.longissimus dorsi* after 7 days storage after slaughter, using toluidine blue, are shown in figure 1 a and b. Figure 1a represents the tougher muscle with a Warner Bratzler (WB) value of 44.7, whereas figure 1b represents the tender muscle with a WB value of 90.1. An extensive breakdown of the connective tissue was seen in the tender muscle (Fig 1b). In the tough muscle the decomposition was hardly visible (Fig 1a). The decomposition appeared in the endomysial area, between the individual myofibers as well as between the myofibers and the perimysium as illustrated with arrows in figure 1b. The present result shows a tough and a tender muscle selected from a population of 28 animals, consisting of animals with a wide range in tenderness scores (Ofstad et al. 2005). In this population 65% of the counted myofibers showed detachment with one or more of the adjacent myofibers whereas 38 % of the myofibers lining the perimysia showed detachment. The results indicate that the myofiber-perimysium attachment is more resistant to decomposition compared to the myofibre-endomysium attachments. Large individual variations in the ability to split were observed between the animals.

To examine possible components involved in the detachments, proteoglycans (PGs) carrying chondroitin/dermatan glycosaminoglycan (GAG) chains with either unsulfated (C0S) or 6-sulfated (C6S) N-acetylgalactosamine residues were selected. The large PG-HA aggregates shown to decompose in a previous study after gelfiltration and PAGE of tissue extracts (Hannesson et al.2003) carry C0S and C6S epitopes (results not shown).

The muscle samples in the present study were collected from an animal exhibiting a WB score decreasing from 90 to 48 after 7 days of storage. Samples taken some hours after slaughter (day 0) and stored for 7 days were compared. The results obtained using the mAb against unsulfated PG epitopes are illustrated in figure 2a and b. At day 0 a distinct and consistent staining is observed in the endomysia (E) (Fig.2a). A somewhat weaker staining is observed between the myofibers and the perimysium. After 7 days the staining is no longer consistent and several splits are seen between the myofibers, on both sides of the structure carrying the unsulfated GAG epitopes (Fig. 2b). Some detachments are furthermore evident between the myofiber and the perimysium, the latter showing almost no stain except for the nerve (N).

Using the antibody against the C6S epitopes a similar staining pattern as with C0S is observed with a distinct and consistent staining of the endomysia at day 0 (Fig.3a) After storage splitting of myofibers is evident. Furthermore, some endomysia still exhibit color whereas in others expression of 6-sulfated epitopes cannot be detected. The change in the staining pattern and the localization of the splitting in the sections after storage support the previous biochemical analysis that CS/DSPGs may be involved in post mortem tenderization of beef.

The staining pattern obtained using the basement membrane heparan sulfate PG, perlecan, is shown in figure 4 a and b. In the samples collected at day 0 a distinct and consistent color is lining all myofibers. After 7 days a weaker staining is evident in the areas with intermyofibrillar ruptures compared to more intact areas (see asterisks Fig. 4b). In the area without visible detachments a borderline of strong stain is present between myofiber and perimysium (see arrow Fig.4b). Furthermore, a strong coloration is seen in the walls of the blood vessels (arteries (A) and veins (V)). The importance of perlecan for basement membrane integrity is clearly demonstrated in perlecan knock out

mice (Costell et al., 1999). The perlecan null mutations were embryonic lethal due to an abnormal development of the heart and deterioration of the basement membranes in regions with increased mechanical stress, such as the contracting myocardium and the expanding brain vesicle. In addition, a defective collagenous network was present in the cartilage of perlecan null mutants (Arikawa-Hirasawa et al 1999).

In addition to the ruptures between the myofibers and between the myofibers and the perimysium, ruptures (R) are visible in the perimysium proper (Fig.4b). Ofstad et al. (2005) have reported using transmission electron microscopy an extensive breakdown of the extracellular matrix between the collagen fibers in fish white muscle. This was most evident in fish species with gaping problems as Atlantic cod. Nishimura et al. (1995) demonstrated that a breakdown had occurred in between the collagen fibers in beef conditioned for 21 days using microscopy and maceration techniques.

According to the different localizations of the ruptures, several components and processes may be involved in meat tenderization. Further studies are necessary to clarify the processes involved.

Conclusions

Decomposition of connective tissue in bovine meat occurs during the first 7 days of post mortem storage. Splitting occurs in several locations; between the myofibers, between the myofibers and the perimysium and in the perimysium proper.

The area between the myofibers appeared more vulnerable for splitting than the area between the myofibers and the perimysium.

A change in the expression pattern of unsulfated- and 6-sulfated CS/DS PGs and perlecan were evident after 7 days storage, indicating a role for the proteoglycans in the loss of adhesion.

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Tables and Figures

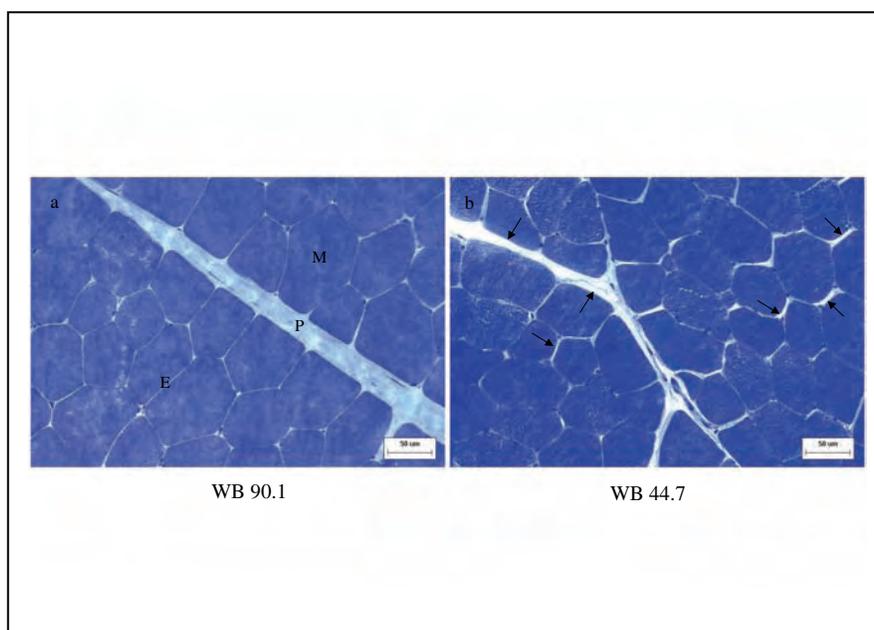


Figure 1a and b show the plastic embedded sections from bovine *M. longissimus dorsi* obtained after aging for 7 days. The sections were stained by toluidine blue. 1a represents the tougher beef, whereas 1b represents the tender beef (WB 90.1 and 44.7). M, P and E indicate the myofiber, peri- and endomysium, respectively. The arrows show the ruptures between the myofibers and between the myofibers and the perimysium.

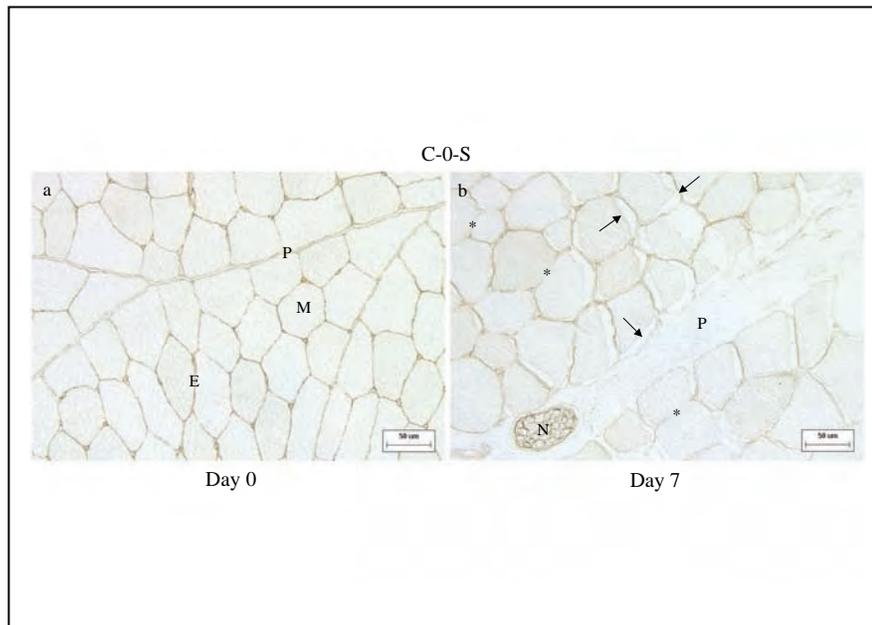


Figure 2a and b show the cryo-sections stained with mAbs against unsulfated GAG epitopes (C-0-S). 2a represents beef obtained day 0 showing a distinct and consistent staining around all the myofibers. 2b represents beef after 7 days aging and splitting is evident in different areas indicated by arrows. The asterisk shows the endomysial areas with no visible C0S epitopes. N represents a nerve, P the unstained perimysium.

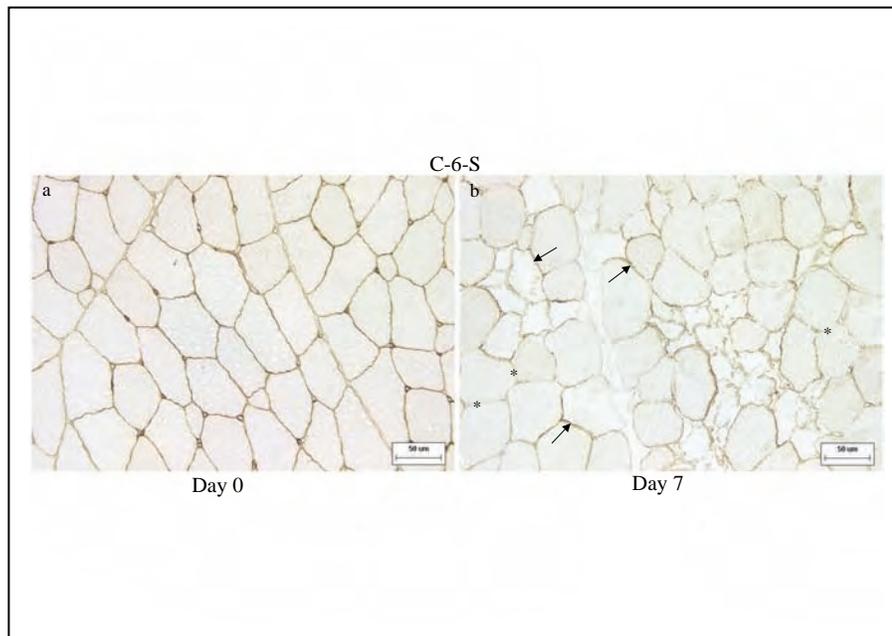


Figure 3a and b show the cryo-sections stained with mAbs against 6- sulfated GAG epitopes (C-6-S). 3a represents beef obtained day 0 whereas 2b represent beef after 7 days aging. In 3a a distinct and consisting stain is lining the myofibers. At day 7 the expression of the epitopes is still strong in some areas (arrows) but lacking in others (asterix).

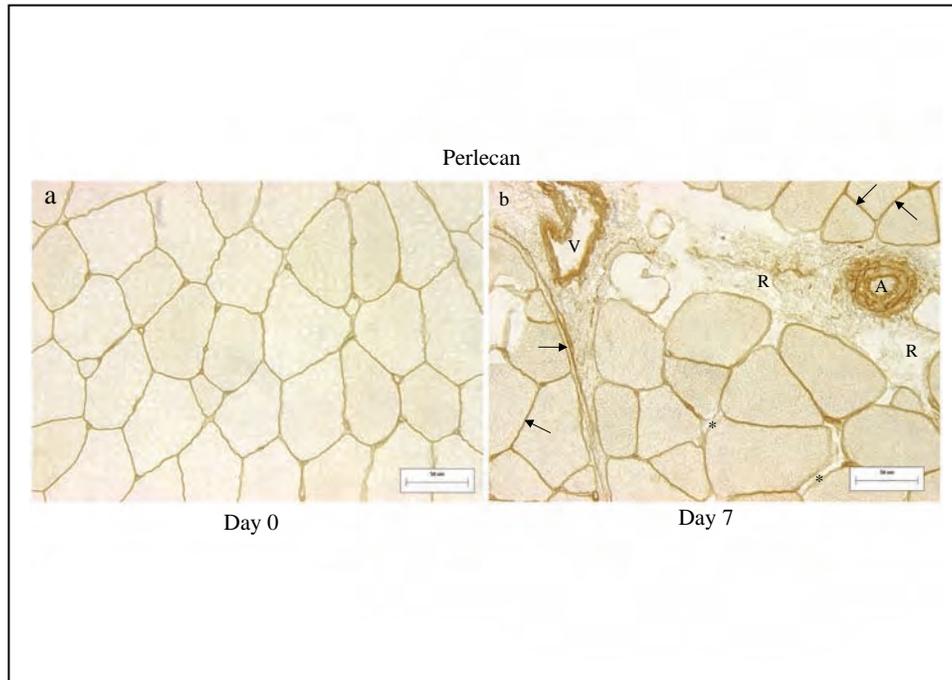


Figure 4a and b show the sections obtained at day 0 and day 7 using the mAb against perlecan. The areas without visible detachments exhibit still strong and consistent and V represent arteriolar and veinole, respectively. In addition ruptures are visible in the connective tissue proper.

PROTEOMIC ANALYSIS OF POST MORTEM CHANGES IN BOVINE MUSCLE

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Key Words: Bovine/ Muscle proteome/ Post mortem/ Meat quality

Introduction

Consumers rate tenderness as the major quality trait of beef [1], thus understanding factors involved in tenderness development is a major concern for the beef producing industry. Meat tenderness is a complex trait which is closely related to the biological traits of the live animal, hence biological sciences, including genetics, physiology, cell biology and biochemistry has been widely employed for decades to characterize the biological mechanism behind major variability of meat tenderness [2]. Especially, the post mortem glycolysis and proteolysis in muscle tissues have a major influence on tenderness and quality of meat [3, 4]. Proteolytic enzymes, including: calpains, cathepsins and proteasomes [5] have been proposed to participate in the post mortem protein degradation,. However, the biochemical mechanisms of the meat tenderization process are not yet well described.

2-DE based proteome analysis is a potent tool for characterizing post mortem changes in muscles, and recent studies of the post mortem proteomes of porcine [6-8] and fish [9] muscles have been used to identify altered patterns of myofibril proteins that occur during storage of meat. In particular, a number of metabolic proteins were found to change during post mortem storage of porcine meat [7]. Although the in vivo roles of these metabolic proteins are well known, their influence on post mortem changes, and the relations to meat tenderness remains unclear.

Objectives

The objective of the present work was to characterize proteome patterns of post mortem bovine muscle, with special attention to the changes in metabolic proteins during early post mortem storage of beef carcasses. The post mortem protein patterns of *M. Longissimus dorsi* (LD) and red part of *M. semitendinosus* (ST) were chosen for this study because the metabolic phenotypes of these two muscles are well known to differ.

Methodology

The experiment included 12 *Holstein Friesian* bull calves. The calves were slaughtered at the experimental house and samples were removed 15 min post mortem and 24 h post mortem from the red part of the ST and the LD. Soluble fraction of muscles was extracted in TES buffer by using ultraturax. The running of two-dimensional gel electrophoresis (2DE) was performed according to the method described previously [6]. The second dimension was run vertically and the proteins were separated in 12% SDS-PAGE gels. Analytical 2DE gels were silver stained as previously described [6], while preparative gels for mass spectrometry (MS) were silver stained according to Shevchenko [10]. The 2DE gels were analysed using the ImageMaster™ 2D Platinum software Version 5.0 (Amersham Biosciences). The spots were automatically matched with the spots of a reference gel. The spot report was imported into Unscrambler version 9.0 (CAMO A/S, Norway) and 50-50 MANOVA [11] (<http://www.matforsk.no/ola/ffmanova.htm>) for statistical analyses. An Ultraflex MALDI-TOF/TOF mass spectrometer with LIFT module (Bruker Daltonics) was used for protein identification.

Results & Discussion

Fig. 1 shows representative 2DE patterns of the TES-soluble protein fractions extracted from LD and ST muscles. The proteins in the molecular mass region of 10 kDa to 75 kDa, and the pH range between 4 and 7, were included in comparative analyses. Image analyses allowed matching and relative quantitation of 923 spots from the LD data set and 630 spots from the ST data set. The complete data set of spot intensities from comparative image analyses was analysed by PCA, and resulting score plots are presented in Fig. 2. The PCA score plots for LD and ST reveal that two clusters related to sampling time, i.e. at slaughter (T₀) versus 24 h post mortem are formed. The results (data not shown) from rotation tests [11] indicate that 13 spots from the LD data set and 18 spots from the ST data set were significantly changed ($p < 0.05$), and these spots were subjected to identification by MS. Among the identified spots, 5 were observed to change in similar patterns both in LD and ST muscles, namely cofilin, lactoyglutathion lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27 and HSP20, while altered expression patterns of 15 spots were unique for either LD or ST (data not shown).

Expression patterns from comparative 2DE analyses contain complex information that is partly hidden if only classic statistical methods are used; hence increasing awareness of the advantage and usefulness of multivariate statistical methods has emerged [12]. In this study we have used PCA and rotation testing to explore the variation in the comparative 2DE patterns (Fig. 2). Furthermore, traditional significance testing for marker selection is not adequate for analysis of many variables, as the false discovery rates will be unacceptably high, as is often seen in the analysis of microarray and proteome data [13]. For example, 50 significant markers will always be selected by a 5% significant level from 1000 spots when using a traditional t-test [11]. We have therefore used a rotation test for adjusting the p-values, and have thereby reduced the false discovery rate.

The observed decreases in cofilin, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27 and HSP20 as seen in both in LD and ST muscles indicate that these changes are immediate post mortem happenings, and has also been observed to occur in porcine muscles, that have much faster rigor development than cattle muscle. A metabolic enzyme, lactoylglutathione lyase, was increased in both muscles during early post mortem storage. This enzyme catalyses the first step of the glyoxal pathway, in which lactic acid is the end product [14]. Several altered spots were identified only in LD muscle. This may be due to different solubilisation properties of myofibril fragments of the two different muscles, and may be related to the rate of the tenderisation process in these muscles. Several subunits of 20S proteasomes and 26S proteasomes were identified to decrease in intensity post mortem in ST muscle. Several authors have previously suggested that proteasome complexes play a role in post mortem proteolysis and meat quality [5].

Biochemical and ultrastructural differences between the various fibre types in the two muscles may explain the differences observed in the protein pattern. These differences reflect distinct metabolic and physiological functions of the different muscles [15]. The energy metabolism, which is responsible for the rate and extent of the pH decrease post mortem, occurs at different rates in different types of muscles (red and white) [16]. Several studies have shown that the metabolic properties of muscles are related to variation in glycogen content and ultimate pH of meat [17, 18].

Conclusions

In this study, we have compared the protein patterns of two different bovine muscles (LD and ST) at two time-points post slaughter, in order to study changes in their post mortem proteomes. Five proteins, namely cofilin, lactoylglutathion lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27 and HSP20, were changed in both ST and LD muscles during post mortem storage. Fifteen additional protein changes were observed in either ST or LD muscles. Further study is required to reveal how the reported changes are related to meat quality traits of cattle.

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Tables and Figures

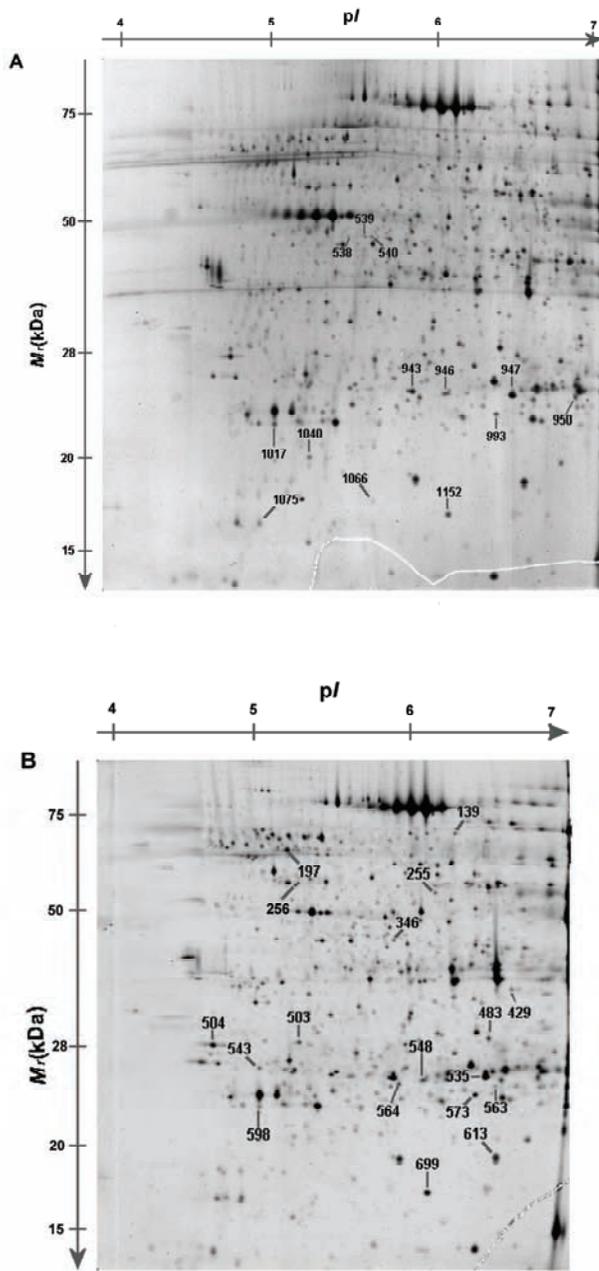


Figure 1. Silver-stained 2DE master gel of A) bovine *M. longissimus dorsi* (LD) muscles and B) bovine *M. Semitendinosus* (ST) muscles. Marked spots were identified by mass spectrometry.

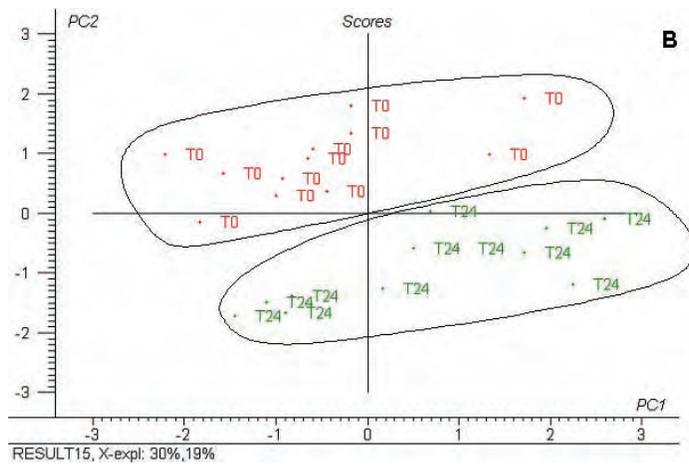
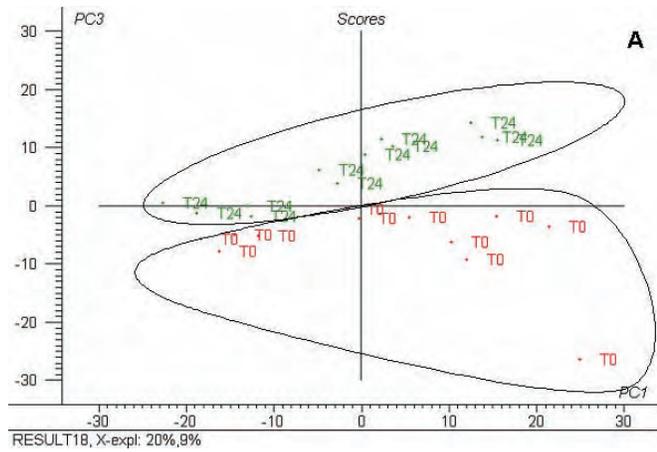


Figure 2. Spot intensities from comparative image analysis were analysed by principal component analysis (PCA). A) PCA score plot of 923 matching spots from bovine *M. longissimus dorsi* (LD) muscles related to post mortem storage time. B) PCA score plot of 630 matching spots from bovine *M. Semitendinosus* (ST) muscles related to post mortem storage time. T0: samples taken at slaughter time, T24: samples removed from the carcass 24 h after slaughter. Circles are drawn to show the two clusters relating to sampling time.

OVINE DOUBLE-MUSCLING: MUSCLE STRUCTURE & PROTEIN PATTERN

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Key Words: Sheep, QTL Texel, Hypertrophy, Muscle, 2D-electrophoresis.

Introduction

To improve carcass muscularity, breeds with hypertrophied muscle can be used. Belgian strains of Texel sheep harbor a QTL (Quantitative Trait Locus) with considerable effect on muscle development^(1,2). This effect does not seem to be associated with sensory qualities degradation. Improvement of muscularity is often accompanied, in many species, by a decrease in meat sensory quality in relation to changes in muscle contractile and metabolic properties and to structural modifications as fibre area⁽³⁾.

Proteins are the main constituents of the muscular cell. They determine structural and functional characteristics of the cell and to a certain extent the sensorial quality of meat. 2-D electrophoresis (2-DE) is of a great interest since it allows the concomitant separation of hundred or even thousand of proteins. Thus, 2-DE is a powerful tool for studying protein expression in relation with different factors.

Until now the study of sarcoplasmic proteins, constituting approximately 30% of total proteins was little developed. However this fraction contains the majority of metabolism proteins and of signal transduction pathways.

The current study aims at determining the effect of the Belgian Texel QTL on structural traits and protein composition of two leg muscles.

The comparative study of two muscle types, hyper- vs normo-developed, originating from double-muscled vs not double-muscled haplotype gives us the ability to identify sarcoplasmic proteins implicated in muscular development.

Objectives

The current study aims at determining the effect of the Belgian Texel QTL on structural traits and protein composition of two leg muscles.

Methodology

Animals and muscular samples

We used crossbred lambs originating from F2 crossing between Romanov ewes and double-musced Belgian Texel rams. Animals were genotyped on "Texel" Locus for the QTL associated to the double-musced phenotype(2). 15 homozygote Texel (TT) and 17 homozygote Romanov (RR) lambs were selected for analysis. Animals were slaughtered at fixed weight of 33 kg for females and 39 kg for males. After slaughter, carcasses were dressed according to commercial practices and were weighted. Carcasses were split down the vertebral column in the sagittal plane and through the pubic symphysis. Hind legs were split down transversally between the last lumbar vertebra. The left hind leg was weighted. Semimembranosus (Sm) and Vastus medialis (Vm) muscles were removed and weighted. Sm was cut transversally to fibre direction in midbelly muscle region. The transversal area of Sm was recorded. The two muscles were sampled in the middle part for histochemical and 2D-electrophoresis analyses. Muscle samples were taken from Sm and Vm within 30 min after slaughter, frozen in liquid nitrogen. Then samples for electrophoresis were reduced to a fine powder under liquid nitrogen using a mortar and a mechanical pestle and stored at -80°C until protein extraction was performed. Samples for histology were kept at -80°C .

Histochemistry

Transverse cryo-sections (10 μm) from Sm (15 TT and 17 RR) and Vm (4 TT and 9 RR) muscle samples were incubated with amylase and stained with PAS^(4,5). According to this method, basal lamina were stained, showing muscle fibre and capillaries contours. Height light microscope images per section, corresponding to the total area of 0.5 mm^2 , were analysed. Numbers of fibres and capillaries were recorded. The transversal section of fibres was measured by computer image analysis. In Sm muscle, an extrapolated total number of capillaries and fibres was calculated using the muscle transversal section area. GLM procedure was applied for mean comparison between the two haplotypes (TT, RR). Means were adjusted to carcass weight. The sex effect was removed.

Sarcoplasmic protein extraction

2-DE gels were performed on 5 TT and 5 RR Sm muscles and 4 TT and 4 RR Vm muscles. Gels were produced in triplicate. The sarcoplasmic fraction was obtained using a method inspired from the subcellular fractionation of Pietrzak⁽⁶⁾. The extraction buffer consisted of 50mM KCl, 4mM MgCl_2 , 20mM Tris, 2mM EDTA, 1% (w/v) DTT and 5mM Pefabloc at pH7. 150mg of muscle were added to 1.5mL of extraction buffer in an Eppendorf containing a glass bead. Homogenisation was performed in Retsch MM2 agitator (Retsch, Haan, Germany) for 1h at 4°C . Extracts were centrifuged at 10,000g for 15min at 6°C and the supernatant was collected. Samples were frozen in liquid nitrogen and stored at -80°C .

2-dimensional electrophoresis

Immobilised pH gradient (IPG) isoelectric focusing (IEF) was carried out in a Protean IEF cell (Bio-Rad), using Bio-Rad ReadyStrip, 17cm, pH5–8. 110 or 300µg of protein were loaded onto the strips for analytical or preparative gels, respectively. Proteins were loaded by inclusion of an adequate volume of extract in a buffer consisting of 7M urea, 2M thiourea, 2% (w/v) CHAPS, 5mM Pefabloc, 0.2% (w/v) DTT and 0.2% carrier ampholytes. Strips were rehydrated overnight. For the subsequent IEF, voltage was increased gradually to 8,000V until a total of 80,000Vh. Strips were immediately frozen and stored at -20°C until further use. Prior to SDS–PAGE, strips were equilibrated for 15min followed by 20min in a solution of 6M urea, 30% (v/v) glycerol, 2% (w/v) SDS and 50mM Tris, supplemented successively with 1% (w/v) DTT or 2.5% (w/v) iodoacetamide and bromophenol blue as a dye. SDS–PAGE was performed in a protean Ixi cell (Bio- Rad) on 12% polyacrylamide gels at 15mA per gel, until the dye track reached the end of the gels. Analytical and preparative gels were silver stained following the protocol of Yan *et al.*⁽⁷⁾.

Image analysis

Gels images were acquired through a GS-800 densitometer and analysed using the PDQuest software (Bio-Rad). After automated detection and matching, highly saturated or ill-defined spots were manually removed and matching across gels was inspected and corrected when necessary. Intensity was expressed in ppm and data were analysed using the software SASx8.1 and a One-Way ANOVA test was used to study genotype effect on protein expression.

Protein isolation and identification by MS

Spots were excised from preparative gels using pipette tips. Gel pieces were placed into a 1.5mL Eppendorf and destained for 10min with a solution containing 30mM KFe and 100mM Sodium thiosulfate, then the gel pieces were washed three times in milliQ Water for 10min. The trypsin digestion and the desalting were done according to manufacturer's protocol (Montage In Gel DigestZP Kit, Millipore).

Resulting peptides mixtures were loaded directly onto the MALDI target. The matrix solution (5mg/mL α -cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid) was added immediately and allowed to dry at room temperature. A Voyager DE-Pro model of MALDI-ToF mass spectrometer (Perseptive BioSystems, Farmingham, MA, USA) was used in positive-ion reflector mode for peptide mass fingerprinting. External calibration was performed with a standard peptide solution (Proteomix, LaserBio Labs, Sophia-Antipolis, France). Internal calibration was performed using peptides resulting from auto-digestion of porcine trypsin. Monoisotopic peptide masses were assigned and used from NCBI database searches with the "Mascot" and "Profound" softwares (<http://www.matrixscience.com> and <http://prowl.rockefeller.edu>).

Results & Discussion

Muscle structure

Results of dissection and histochemistry are presented in table 1. The Sm muscle was heavier and thicker in TT haplotype than in RR. Conversely, the Vm weight was not different between the two haplotypes. These observations showed that hypertrophy was differentially expressed according to the muscle. This is in accordance with previous research on double-musced cattle⁽⁸⁾. In both muscles, fibre area was not different between haplotypes. The extrapolated total number of fibres of Sm was higher in TT than in RR. It can be concluded that the Sm hypertrophy is not due to fibre hypertrophy but to hyperplasia. These characteristics are comparable to those described in double-musced cattle presenting a mutation on the gene coding for myostatin^(8,9). The Belgian Texel QTL for muscle hypertrophy has also been identified in the chromosomal region of the myostatin encoding gene⁽¹⁾. However, sequencing of the gene did not reveal mutation. The ratio of capillaries to fibre number was lower in Sm muscles of TT lambs. This ratio was not different among genotypes in Vm. These results mean that the Sm hypertrophy is not associated to a proportional increase of blood supply.

2-DE and mass spectrometry

On over 800 proteic spots detected on gel, we have determined 102 spots which varied statistically significantly between the 2 genotypes on Sm. Among these 102 spots, 87 were over expressed in TT genotype. 35 of the 102 spots were picked off and analysed for identification by MALDI-ToF mass spectrometry, and 19 were identified (Table 2). In the Vm 74 spots were defined as varying with genotype. Among them, 29 were over expressed in TT genotype. 16 of the 74 spots were analysed by mass spectrometry and we succeeded to identify 5 proteins (Table 2). Three of the identified proteins were common to both muscles.

In Sm, some of the identified spots corresponded to same proteins. Among those redundant proteins, Creatine kinase (CK) was observed at different molecular weights (Mw), only one spot corresponded to the theoretical Mw. The five others CK spots were probably fragments of the whole protein. According to their observed Mw, the Glycogen myophosphorylase (GP) spots also corresponded to fragments. Due to its high Mw and basic pI, the whole protein can not be separated on our 2-D gels. Moreover there is an exposed serine in the 3D structure of ovine GP that could explain a specific clivage creating a 35 kDa fragment. Concerning Pyruvate Kinase (PK), two spots were also evidenced at a lower Mw than their theoretical weight.

In a previous study, protein fragments were also observed very early after slaughter⁽¹⁰⁾. We may suppose that the presence of fragments was due to early proteolysis. In this case, we hypothesize that the fragments expression is more a reflect of initial rate of entire protein than a mark of more acute proteolysis in hypertrophied Sm.

The two other spots of PK were positioned at their theoretical Mw. They probably corresponded to the entire protein, but they varied by their pI. It is also the case for the enolase proteins. These different pI locations of a same protein onto the gel map could correspond to different isoforms due to post translational modifications.

All of these proteins as well as the lactate dehydrogenase were over expressed in the Sm of TT and are enzymes related to glycolytic metabolism. Enolase and PK are enzymes of distal glycolysis, as for CK it is an enzyme essential to the refilling of cellular ATP stock, in fast glycolytic muscles it is essential to ensure fast spatio-temporal energy buffering. Moreover, it is noticeable that CK Knock Out mice exhibit a marked decrease in skeletal muscle weights⁽¹¹⁾ which support the link between CK over expression and Sm hypertrophy in TT.

This over expression was in accordance with a previous study showing a higher level of the fast isoform, associated to glycolytic metabolism, of the myosin heavy chain in Sm muscle of the TT genotype⁽²⁾. It also can be related to the lower level of capillary supply. These particularities suggested an evolution of this hypertrophied muscle metabolism to a more glycolytic pattern.

Two spots were chaperone proteins: the small heat shock protein 27kDa (HSP-27) and the Glutathione S-transferase Pi (GST-Pi). They were over expressed in Sm of TT genotype. HSP-27 has been reported to play a central role in the structural and functional organization of the three-dimensional intermediate filament and the actin microfilament system⁽¹²⁾. Moreover, HSP-27 has been found to be over expressed in hypertrophied cultured cells⁽¹³⁾. HSP-27 exist in many isoforms⁽¹⁴⁾ and Kim⁽¹⁵⁾ reports that one isoform is more expressed in glycolytic muscle which agrees with the switch in fiber's type towards a more glycolytic one in TT animals muscles⁽²⁾. Thompson proposed that HSP-27 could be involved in long term skeletal muscle adaptation to hypertrophy⁽¹⁶⁾.

The GST-Pi is involved in the mechanisms of cellular detoxification and cellular resistance to oxidative stress⁽¹⁷⁾; a lot of oxidative and cytotoxic molecules are produced during muscle metabolism so GST-Pi over expression agrees with higher metabolism in Sm of TT.

The transferrin was also over expressed in TT genotype. It is a protein usually synthesised in liver which binds iron and delivers it to cells. It has been suggested that it could be synthesised by growing cells when vasculature is insufficient, and that transferrin enhances action of neural tissue on growth and development of cultured skeletal myoblasts. The Sm from TT genotype has a less developed vasculature than Sm from RR genotype (Table 1) and given its increased metabolism, its iron needs are elevated so local expression of transferrin could be augmented to supply this need.

As for the α 1-antitrypsin, it is a member of the serine protease inhibitor protein family (serpins) and is the principal serum inhibitor of proteolytic enzymes such as elastase, serpins are also able to inhibit cystein proteinase like cathepsin K, L and S in muscle⁽¹⁸⁾. Here we show that α 1-antitrypsin protein was under expressed in both muscles of the TT genotype. So in this study α 1-antitrypsin seems to be a marker of TT genotype.

Most of varying proteins in the Vm were under expressed in TT genotype (Table 2), where hypertrophy seemed to be repressed. Markers of glycolytic metabolism were under expressed like the triose phosphate isomerase and GP.

The only identified over expressed spot was the Antiquitin, a member of aldehyde dehydrogenase superfamily, which is supposed to be involved in osmotic stress regulation⁽¹⁹⁾.

As for GST-Pi and GP, conversely to their expressions in Sm, they were under expressed in Vm of TT genotype. So we found them more expressed in the TT genotype

in the most developed muscle (Sm). Moreover GST-Pi over expression is associated to cancerous proliferation⁽²⁰⁾, i.e. an uncontrolled hyperplasia. So GST-Pi could play a role in the maintenance of the hyperplasia in Sm muscle of TT animals.

Conclusions

To conclude we have shown that some proteins can be linked to a TT genotype, but it is impossible to generalize results from one muscle to others. With regard to the GP and GST-Pi, their expressions seem to be linked to hypertrophy. Additional studies are needed for a better comprehension of muscular development mechanisms and the maintenance of muscularity.

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Tables and Figures

Table 1. Means of dissection and histochemical measurements and genetic effects

Measurements	Mean	SD	QTL effect	
			Test _a	TT – RR ^b
Leg weight. g	2312.5	115.6	*	89.64
Muscle weight. g				
Sm	230.4	23.7	***	43.67
Vm	39.8	6.8	NS	-1.3
Transversal area of Sm. cm ²	25.4	4.7	***	2.93
Fiber transversal area. μm ²				
Sm	1756	507.1	NS	-232.95
Vm	3123	649.8	NS	-31.2
Capillary/Fiber Number				
Sm	0.81	0.17	*	-0.17
Vm	1.06	0.12	NS	-0.035
Fiber total number in Sm	1 593 664	54 9318	*	525 334
Capillary total number in Sm	1 235 259	360 499	NS	186290

^aNS = P > 0.10 ; *P < 0.05 ; ***P < 0.001

^bA positive value for TT – RR corresponds to an additive increase due to the Belgian Texel QTL. T = Texel. R = Romanov.

Table 2. Mass spectrometry identified proteins and their expression levels in TT genotype vs RR genotype.

<i>Semimembranosus</i>			
Acc N°	Protein ID	Expression TT/RR	p
gi 6013379	Glutathione S-transferase Pi (<i>Capra hircus</i>)	2.5	<0.001
gi 61553385	Heat Shock Protein 27 kDa (<i>Bos taurus</i>)	2.4	<0.01
gi 57163939	Glycogen myophosphorylase (<i>Ovis aries</i>) Fgt	3.9	<0.0001
gi 57163939	Glycogen myophosphorylase (<i>Ovis aries</i>) Fgt	1.9	<0.0001
gi 27806645	Enolase (<i>Bos taurus</i>)	2.5	<0.01
gi 27806645	Enolase (<i>Bos taurus</i>)	2.4	<0.01
gi 33286422	Pyruvate kinase (<i>Homo sapiens</i>)	2	<0.001
gi 33286422	Pyruvate kinase (<i>Homo sapiens</i>)	1.9	<0.01
gi 478822	Pyruvate kinase (<i>Homo sapiens</i>) Fgt	2.2	<0.001
gi 478822	Pyruvate kinase (<i>Homo sapiens</i>) Fgt	2	<0.001
gi 6729828	Creatine kinase (<i>Oryctolagus cuniculus</i>) Fgt	1.9	<0.01
gi 6729828	Creatine kinase (<i>Oryctolagus cuniculus</i>) Fgt	1.9	<0.01
gi 6729828	Creatine kinase (<i>Oryctolagus cuniculus</i>) Fgt	1.9	<0.01
gi 4838363	Creatine kinase (<i>Bos taurus</i>) Fgt	2.8	<0.05
gi 4838363	Creatine kinase (<i>Bos taurus</i>) Fgt	2.3	<0.05
gi 4838363	Creatine kinase (<i>Bos taurus</i>)	2	<0.001
gi 59858383	Lactate dehydrogenase (<i>Bos taurus</i>)	2	<0.01
gi 29135265	Transferrin (<i>Bos taurus</i>)	3.6	<0.001
gi 57526646	α1-antitrypsin (<i>Ovis aries</i>)	0.4	<0.0001
<i>Vastus medialis</i>			
Acc N°	Protein ID	Expression TT/RR	p
gi 25108887	Antiquitin (<i>Homo sapiens</i>)	2	<0.05
gi 59858493	Triose phosphate isomerase (<i>Bos taurus</i>)	0.3	<0.05
gi 57526646	α1-antitrypsin (<i>Ovis aries</i>)	0.5	<0.05
gi 57163939	Glycogen myophosphorylase (<i>Ovis aries</i>) Fgt	0.4	<0.05
gi 6013379	Glutathione S-transferase Pi (<i>Capra hircus</i>)	0.5	<0.05

Fgt: fragment of protein

In bold: proteins identified in Sm and also in Vm muscle

AVERAGE MUSCLE FIBRE CROSS SECTIONAL AREA VARIES CONSIDERABLY IN PORK LOINS

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Key Words: pig; muscle fibre size; pork loin

Introduction

Porcine *M. longissimus dorsi* contains about 80 per cent of glycolytic type IIB fibres calculated based on the fibre number and about 90 per cent calculated based on the fibre area (Oksbjerg et al. 1995, Ruusunen et al. 1996). Therefore, the size of these fibres has a great effect on the average muscle fibre cross sectional area in this muscle. When a muscle grows, the diameter of type IIB fibres increases faster than that of type I. In a newborn pig the sizes of all muscle fibres are the same, but at the slaughter weight, the size of type IIB fibres are twice as large as the size of type I and type IIA fibres (Bader 1983). Chrystall and coworkers (1969) found that muscle fibre diameter increased 100% from birth to 25 days, whereas from 100 to 125 days of age, muscle fibre diameter increased only 10 per cent. The rate of the muscle fibre growth in porcine longissimus dorsi after 150 days of age is very slow.

When a muscle grows both the cross sectional area and the length of the fibres increase. Muscle size can also increase by satellite cell proliferation but this is not a very important way. Carcass fat content affects muscle fibre size so that in fat carcasses the muscle fibre size is smaller than in lean carcasses (Seideman et al. (1989).

Objectives

The aim of the present study was to investigate how much does the average muscle fibre cross sectional area range in pork loins at the live weight of 95-100 kg.

Methodology

The study consisted of 27 Finnish Landrace and 28 Yorkshire pigs slaughtered at the live weight of 95-100 kg. Average muscle fibre cross sectional area in *M. longissimus dorsi* was measured with Image Analyzer from sections stained with myosin ATPase method (Brooke and Kaiser, 1970). Pork loin cross sectional area was measured one day after slaughter.

Results & Discussion

Gilts had a larger loin area ($p < 0.05$) and a larger average muscle fibre cross sectional area ($p < 0.05$) than the barrows. Karlsson et al. (1994), Ruusunen et al. (1996) and Nürnberg and Ender (1990) have also shown that both the muscle fibre cross sectional area and the loin area are larger in gilts than in barrows. The differences in the loin area and in the average fibre cross sectional area between the breeds were not significant ($p > 0.05$) (Table 1).

According to Chrystall and coworkers (1969), the growth of muscle fibres in *M. longissimus dorsi* is most rapid early in life declining later to a near stationary level. One reason for this is that the fat starts to accumulate in the carcass at that stage. Carcass fat content affects muscle fibre size so that in fat carcasses the average muscle fibre cross sectional area is smaller than in lean carcasses (Seideman et al. (1989). Therefore, it is important to take both the live weight of the pig and carcass lean fat/content into account when studying muscle fibre cross sectional area. In Figures 1 and 2, the average fibre cross sectional area is presented in relation to the cross sectional area of pork loin at the live weight of 95-100 kg. The average muscle fibre cross sectional area varies considerably in pork loin with the same cross sectional area regardless of breed or sex. The pork loin area ranged from 30 to 35 cm² in three pigs, from 35.1 to 40 cm² in 18 pigs, from 40.1 to 45 cm² in 22 pigs and from 45.1 to 50 cm² in 12 pigs. The relationships between the largest average and the smallest average muscle fibre cross sectional area in these groups were 1.5, 1.4, 1.7 and 1.7, respectively.

Muscle fibres are oxidative at the early stages of growth due to the dense capillarization and the small cross sectional area of muscle fibres, but when the fibres grow, they become more anaerobic. Thus, selection based on small fibre cross sectional area could result in pigs with more oxidative muscle. It has been found that the more oxidative the muscles are, the better the sensory quality of meat is (Essen-Gustavsson and Fjelkner-Modig, 1985).

A high number of muscle fibres with small fibre cross sectional area per loin area permits more growth potential to the muscle without a decrease of the oxidativity (Ashmore and Vigneron 1988). Miller et al., (1975) and Søholm Petersen et al., (1999) found that carcass meat content can be increased by choosing animals with the biggest number of muscle fibres although Staun (1963), Seideman et al. (1989) and Larzul et al. (1997) have shown the opposite that the muscle fibre thickness correlates positively with lean meat content. It is, however, not yet known which muscle fibre cross sectional area at a certain live weight leads to (i) a fast growth, (ii) oxidative muscles, (iii) pigs whose carcasses contain a lot of lean meat and (iv) also meat with good technological and sensory quality.

Conclusions

The muscle fibre cross sectional area can vary considerably in pork loin with the same cross sectional area at the live weight of 95-100 kg regardless of breed or sex.

Table 1. Live weight (kg), loin area (cm²) and average muscle fibre cross sectional area of *M. longissimus dorsi* (µm²).

	Barrows (N=28)	Gilts (N=27)	p	Landrace (N=27)	Yorkshire (N=28)	p
Live weight (kg)	98.2	97.7	0.310	98.3	97.6	0.125
Loin area (cm ²)	40.4	43.1	0.0087	42.7	40.8	0.072
Muscle fibre area x 10 ³ (µm ²)	3.9	4.5	0.0012	4.2	4.1	0.464

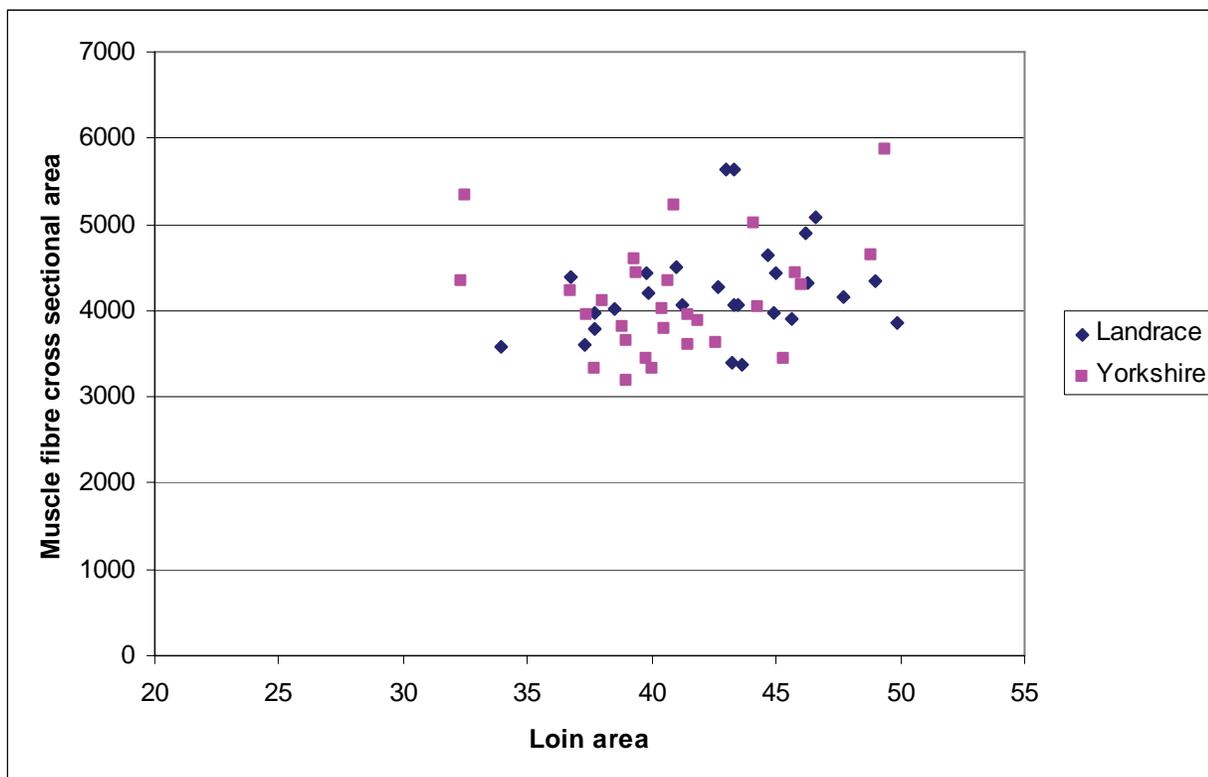


Figure 1. Average muscle fibre cross sectional area (µm²) of *M. longissimus dorsi* in relation to loin area (cm²) in Landrace and Yorkshire pigs.

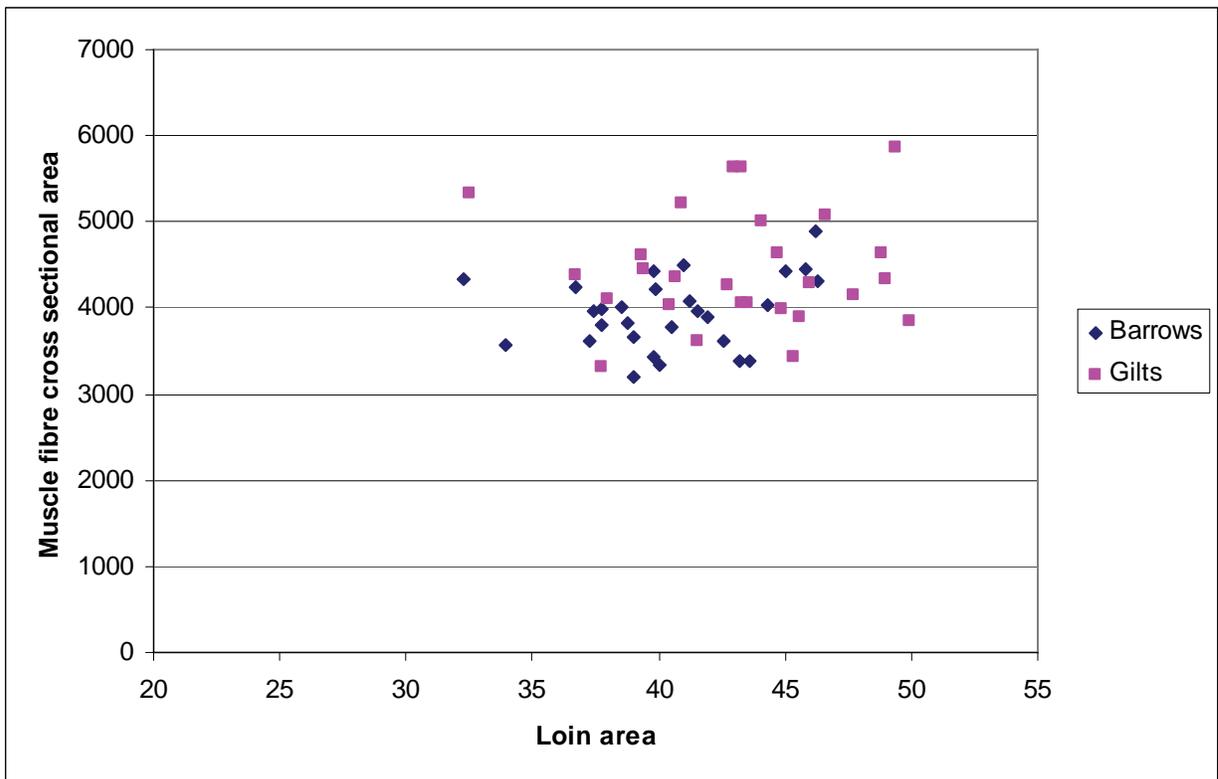


Figure 2. Average muscle fibre cross sectional area (μm^2) of *M. longissimus dorsi* in relation to loin area (cm^2) in barrows and gilts.

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QUANTIFICATION OF INTRAMUSCULAR FAT IN THE *LONGISSIMUS DORSI* OF FED BEEF CATTLE

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Key Words: *longissimus dorsi*, Intramuscular fat, Beef

Introduction

Approximately 26% of the U.S. fed cattle population is fed and harvested in the Texas Panhandle annually (SPS, 2000). Beef carcasses are assessed a dollar value per carcass weight based on a combination of quality grade (marbling score of the loin muscle and estimated physiological maturity) and yield grade (estimation of fat to muscle ratio). These estimates are determined by evaluating the exposed *longissimus dorsi* muscle between the 12th and 13th ribs.

The value assigned to a beef carcass greatly depends on the amount and distribution of the marbling observed at a single point, by a USDA grader. However, determining marbling at one given point may not represent the entire *longissimus dorsi* muscle. Previous research, (Blumer et al. 1962; Cross et al., 1975) suggest that marbling varies throughout the *longissimus* muscle.

Objectives

To illustrate the variation of intramuscular fat throughout the *longissimus dorsi* muscle using subjective visual evaluation and crude fat analysis.

To develop predictive equations to estimate percent crude fat in beef *longissimus dorsi* muscle from subjective marbling.

Methodology

Beef *longissimus dorsi* muscles (n = 40) were selected at a commercial beef processing facility in the Texas Panhandle. Two trained personnel subjectively evaluated marbling at the 12th/13th rib interface. *Longissimus dorsi* muscles were selected based on specific criteria of the following: 1) quality grades (USDA Select (n=10); USDA Low Choice (n=10); USDA High Choice (n=10); and USDA Prime (n=10), 2) external fat ranged from 2.5-3.7 mm, 3) ribeye area ranged from 81.93 cm² to 88.38 cm² and 4) hot carcass weights ranged from 334 kg to 378 kg. The muscles were transported under refrigerated conditions to the West Texas A&M University meat laboratory and stored overnight at 1.6°C.

Fabrication

Seventy-two hours after harvest, muscles were fabricated into 3.175 cm transverse slices. After slicing, steaks were allowed to bloom for 30 min. (forming a bright cherry red color) to allow oxygenation of myoglobin.

Subjective evaluations

Two trained panelists subjectively evaluated visual marbling scores on each sliced steak using USDA marbling cards as a reference. Individual partitions were provided to separate individual slices during the evaluation process. The posterior surface of the wholesale rib slices and anterior surface of the wholesale loin slices were used for evaluation under incandescent lighting (1,304 lux).

Crude fat analysis

Transverse slices (0.635 cm thick) were removed from the evaluated side of each slice. External fat and connective tissue were removed and the muscle tissue was cut into 2.5 cm X 2.5 cm cubes. Cubes were kept in a -70°C freezer (Revco Upright Elite) for at least 24 h. Once frozen, the muscle was pulverized using a Waring blender. Samples from each carcass were analyzed for percentage of fat by microwave oven and nuclear magnetic resonance analysis following AOAC (PVM – 1:2003) procedures. Briefly, two glass fiber square sample pads were used to spread a 3-5 g meat sample. Each meat sample was evenly spread across the pad then covered using another pad sandwiching the sample. Meat samples were dried by a moisture/solid analyzer microwave chamber revealing percent moisture after dried to a constant weight. Pads were removed and rolled in a Trac film and compressed in a plastic sleeve and placed in a NMR (Nuclear Magnetic Resonance) chamber for analysis.

Statistical Analysis

A one-way treatment structure was used in a complete block experimental design structure. Carcass was the blocking factor, anatomical position within the loin was the treatment, and a slice of loin was the experimental unit. Data were analyzed using the MIXED procedure of SAS (SAS Institute; Cary, NC). The model included the fixed effect of position within the *longissimus* muscle and the random effect of carcass. Treatment means were generated using the LSMEANS (also known as adjusted means) option and separated when significant ($P < .05$) using the PDIFF option.

Crude fat and visual intramuscular fat data were analyzed using the Proc REG procedure of SAS (SAS Institute, Cary, NC) to develop a linear regression model to predict crude fat.

Results & Discussion

Visual marbling score evaluations

Visual evaluations revealed that significant differences ($P < .05$) of intramuscular fat were detected among anatomical positions throughout the *longissimus dorsi* muscle for all USDA quality grades except USDA Select. USDA Prime marbling scores (Figure 1) illustrate a slight tendency for marbling scores to increase toward the posterior *longissimus* region. Box plots (Figure 3) for USDA Premium Choice marbling scores illustrate a tendency for marbling to decrease at extremities as compared to the 12th/13th rib interface. USDA Low Choice (Figure 3) and USDA Select (Figure 7) show no trend, indicating somewhat homogenous marbling scores throughout the *longissimus dorsi* for lower quality grades. In agreement with Blumer et al. (1962), Cook et al. (1964) and Cross et al. (1975), the current data revealed that marbling is randomly deposited throughout the *longissimus* muscle and varied among anatomical position. Furthermore, variability of marbling score increased as quality grade increased.

Crude fat determination

Crude fat analysis revealed that significant differences ($P < .05$) of crude fat percentages were detected among anatomical positions for all USDA quality grades. Box plots for all quality grades illustrate high variability for crude fat throughout the *longissimus dorsi* muscle, suggesting that marbling is independent among anatomical positions.

Regression equation

The regression equation developed to predict the percentage of crude fat from a known marbling score is as follows: crude fat = (- 0.58) + 0.17 * marbling score ($R^2 = 0.55$). Unaccounted variability may be due to experimental errors from laboratory procedures, handling and processing of samples.

Conclusions

This study investigated the variation of intramuscular fat within the *longissimus dorsi* muscle. Visual (subjective) evaluations suggested that estimating marbling at one given point (12th/13th rib interface) was representative of the marbling average of the entire loin. Crude fat values suggested that intramuscular fat increases from the midpoint of the loin to the extremities.

A predictive equation can be utilized to determine a crude fat percentage based on visual marbling evaluation. Coefficient of determination ($R^2 = .55$) indicate that just over 50% of the variation in crude fat is accounted for by visual marbling. A predictive equation of crude fat percentage can be utilized to communicate on a common scale with everyday consumers. Branded beef programs or retailers could purchase and sell products based on a fat percentage basis rather than a quality grade basis. Additionally, crude fat

percentage could be used to determine if a beef product were eligible to be sold according to the American Heart Association guidelines.

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Tables and Figures

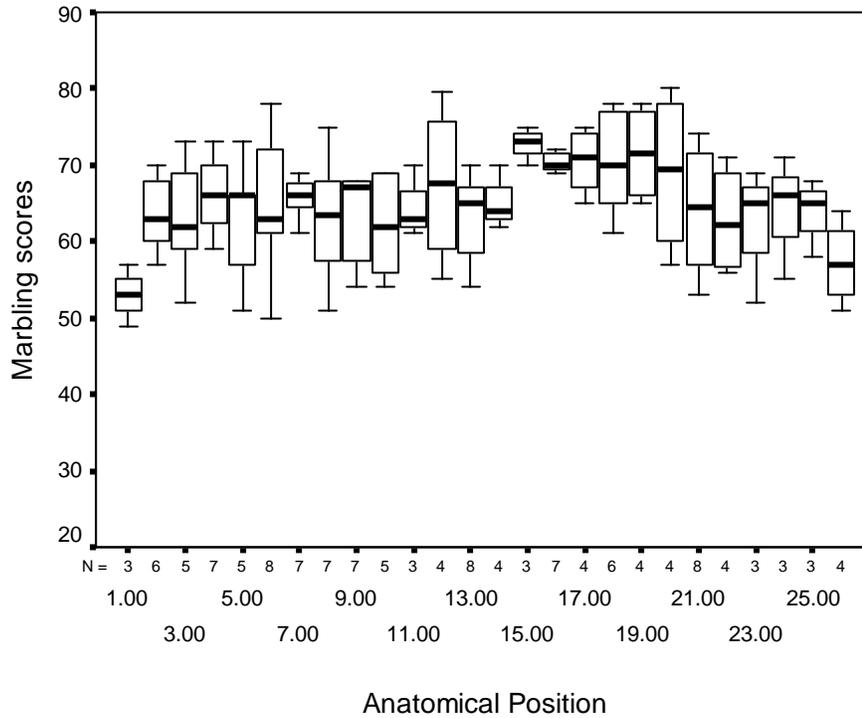
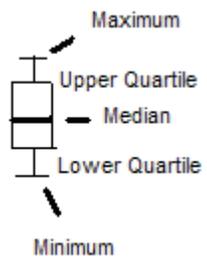


Figure 1. Box plots of visual marbling scores among anatomical positions for USDA Prime *longissimus dorsi* muscles.

N = number of total slices within each anatomical position

Marbling scores: 30 = Slight⁰⁰, 40 = Small⁰⁰, 50 = Modest⁰⁰, 60 = Moderate⁰⁰, 70 = Slightly Abundant.

Anatomical position 1 = most anterior....26 = most posterior



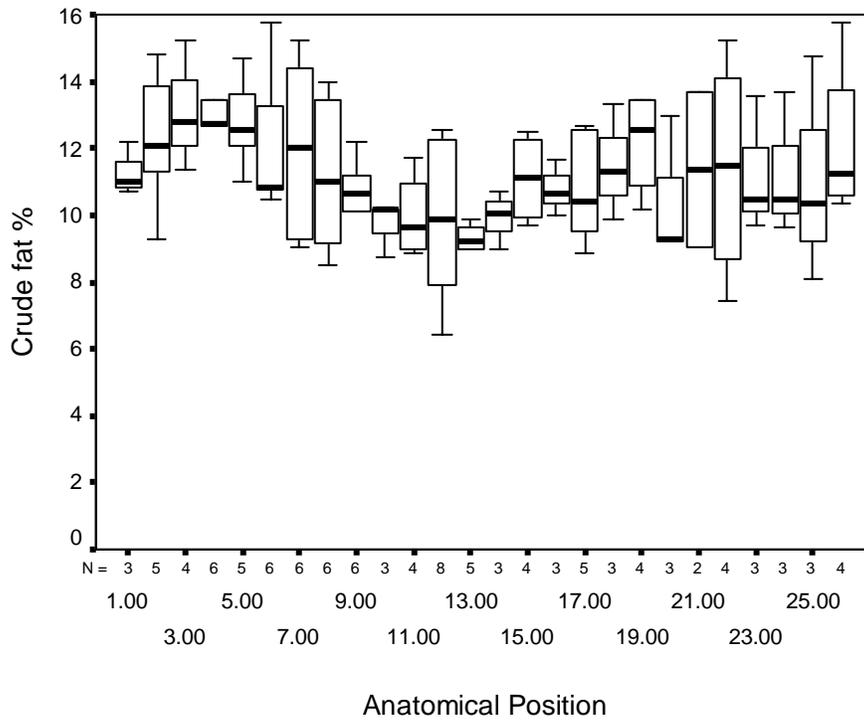
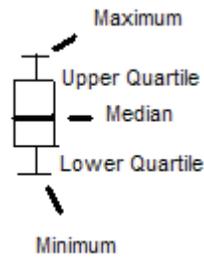


Figure 2. Box plots of crude fat percentage among anatomical positions for USDA Prime *longissimus dorsi* muscles.

N = number of total slices within each anatomical position
Anatomical position 1 = most anterior....26 = most posterior



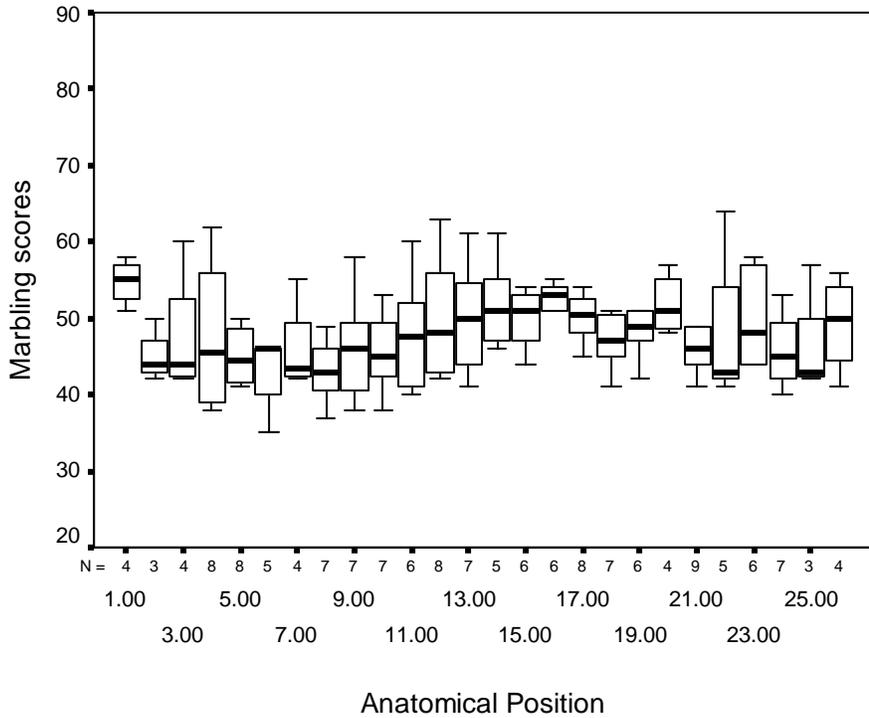
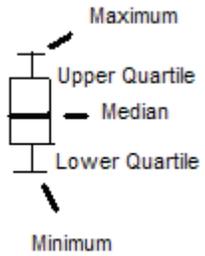


Figure 3. Box plots of visual marbling scores among anatomical positions for USDA Premium Choice *longissimus dorsi* muscles.

N = number of total slices within each anatomical position

Marbling scores: 30 = Slight⁰⁰, 40 = Small⁰⁰, 50 = Modest⁰⁰, 60 = Moderate⁰⁰, 70 = SlightlyAbundant.

Anatomical position 1 = most anterior....26 = most posterior



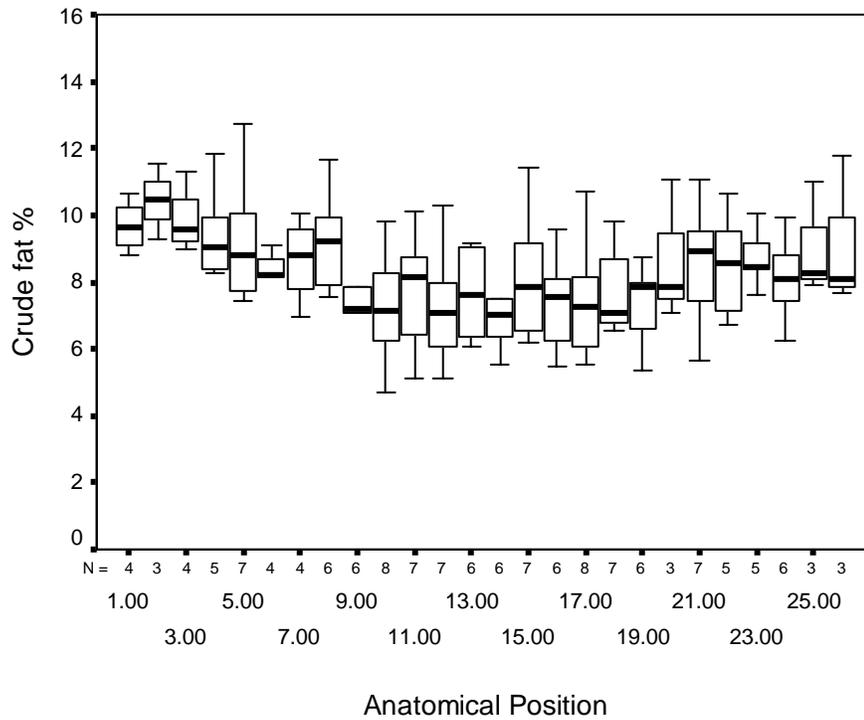
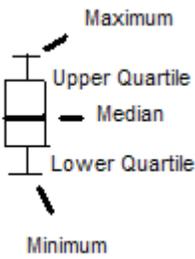


Figure 4. Box plots of crude fat percentage among anatomical positions for USDA Premium Choice *longissimus dorsi* muscles.

**N = number of total slices within each anatomical position
 Anatomical position 1 = most anterior....26 = most posterior**



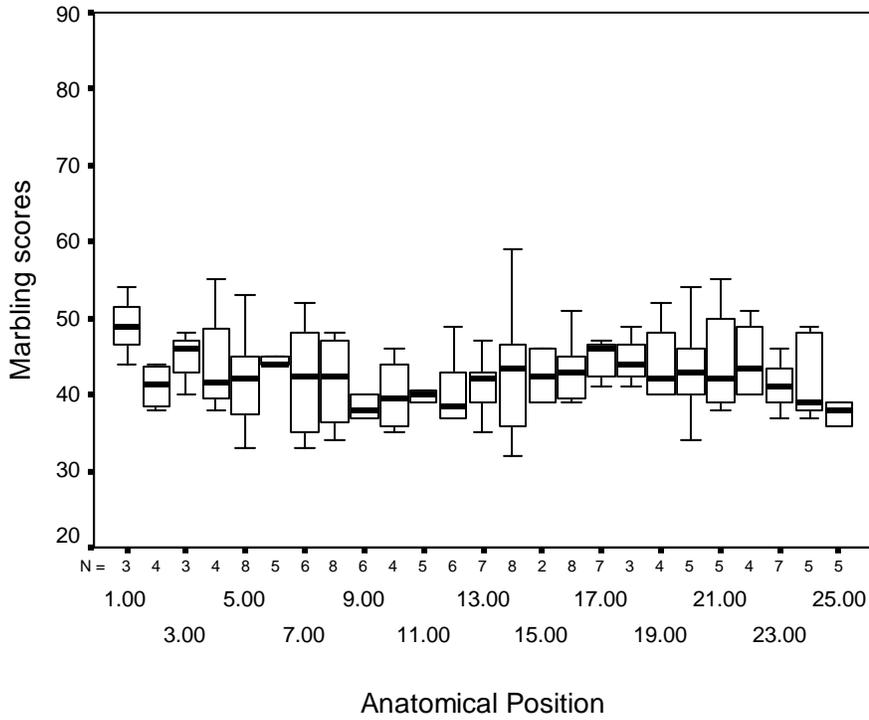
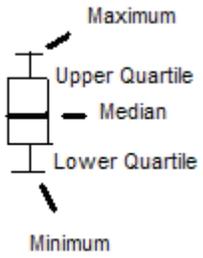


Figure 5. Box plots of visual marbling scores among anatomical positions for USDA Low Choice *longissimus dorsi* muscles.

N = number of total slices within each anatomical position
Marbling scores: 30 = Slight⁰⁰, 40 = Small⁰⁰, 50 = Modest⁰⁰, 60 = Moderate, 70 = Slightly Abundant.
Anatomical position 1 = most anterior....25 = most posterior



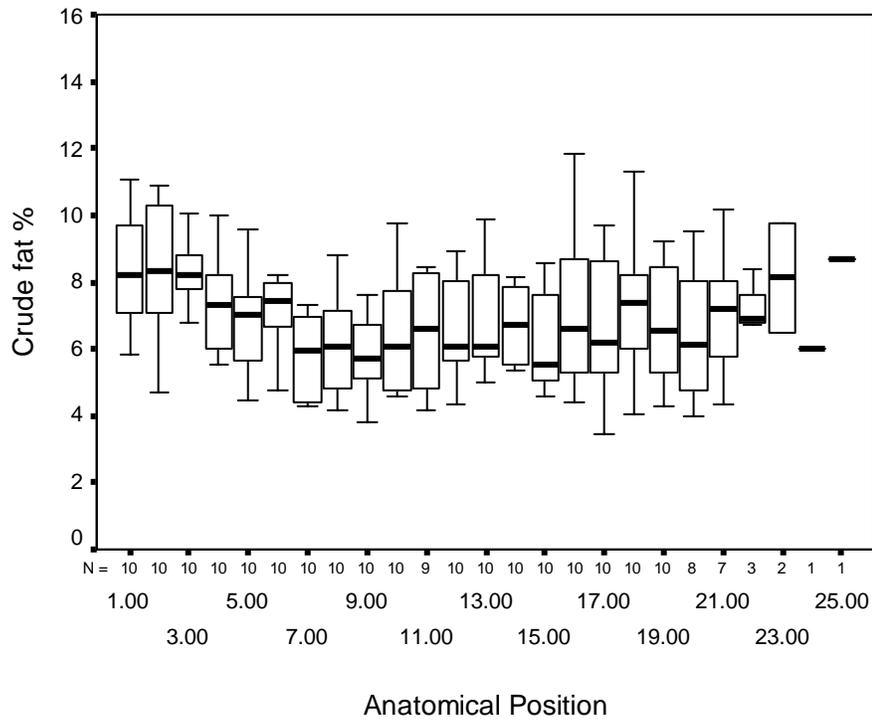
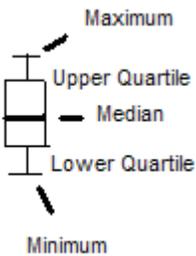


Figure 6. Box plots of crude fat percentage among anatomical positions for USDA Low Choice *longissimus dorsi* muscles.

**N = number of total slices within each anatomical position
 Anatomical position 1 = most anterior....25 = most posterior**



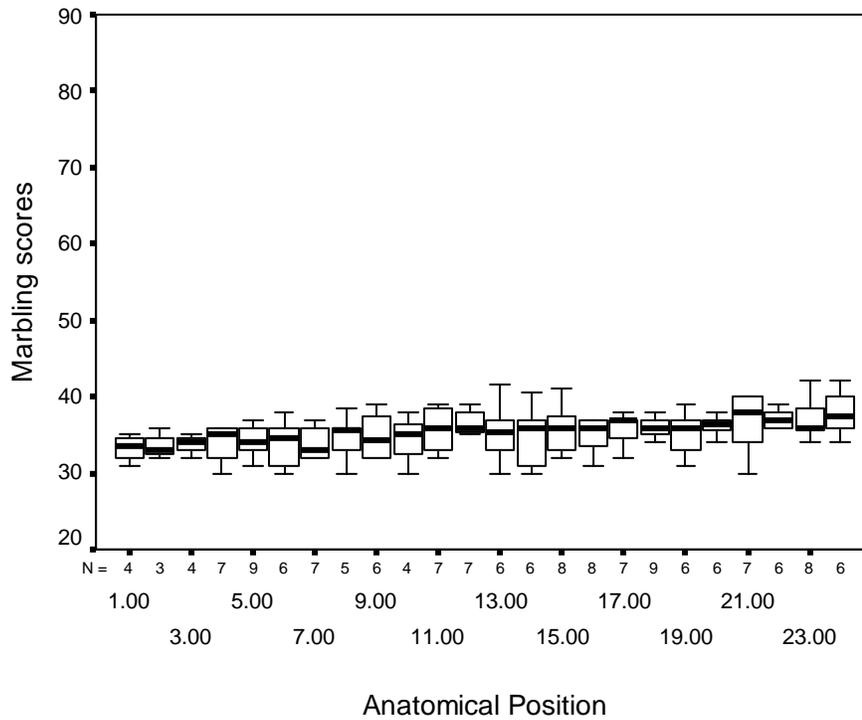
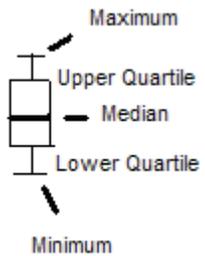


Figure 7. Box plots of visual marbling scores among anatomical positions for USDA Select *longissimus dorsi* muscles.

N = number of total slices within each anatomical position
Marbling scores: 30 = Slight⁰⁰, 40 = Small⁰⁰, 50 = Modest⁰⁰, 60 = Moderate⁰⁰, 70 = Slightly Abundant.
Anatomical position 1 = most anterior....24 = most posterior



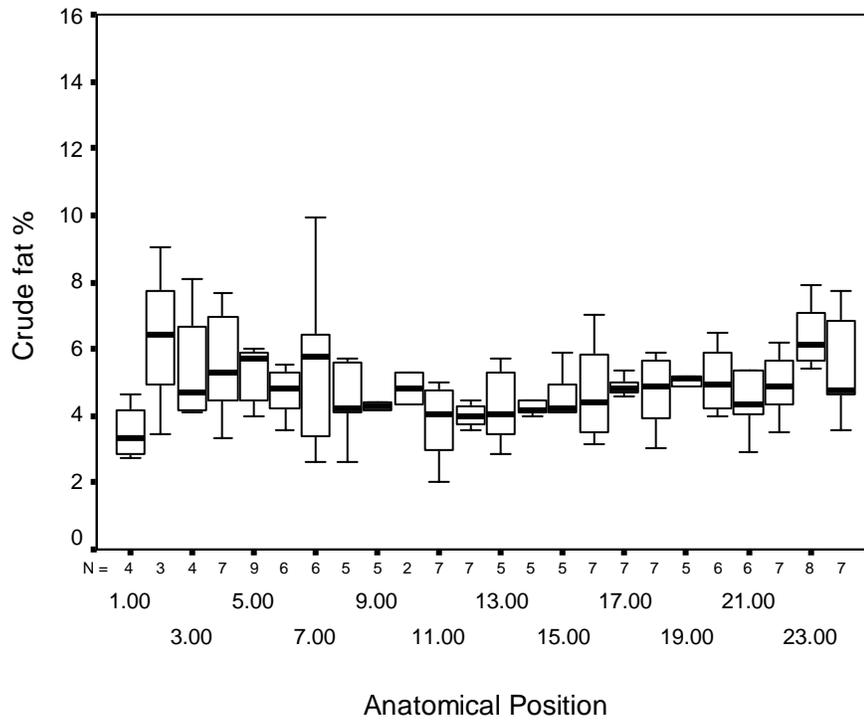
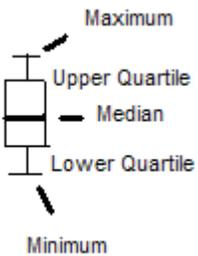


Figure 3.14. Box plots of crude fat percentage among anatomical positions for USDA Select *longissimus dorsi* muscles.

N = number of total slices within each anatomical position
Anatomical position 1 = most anterior....24 = most posterior



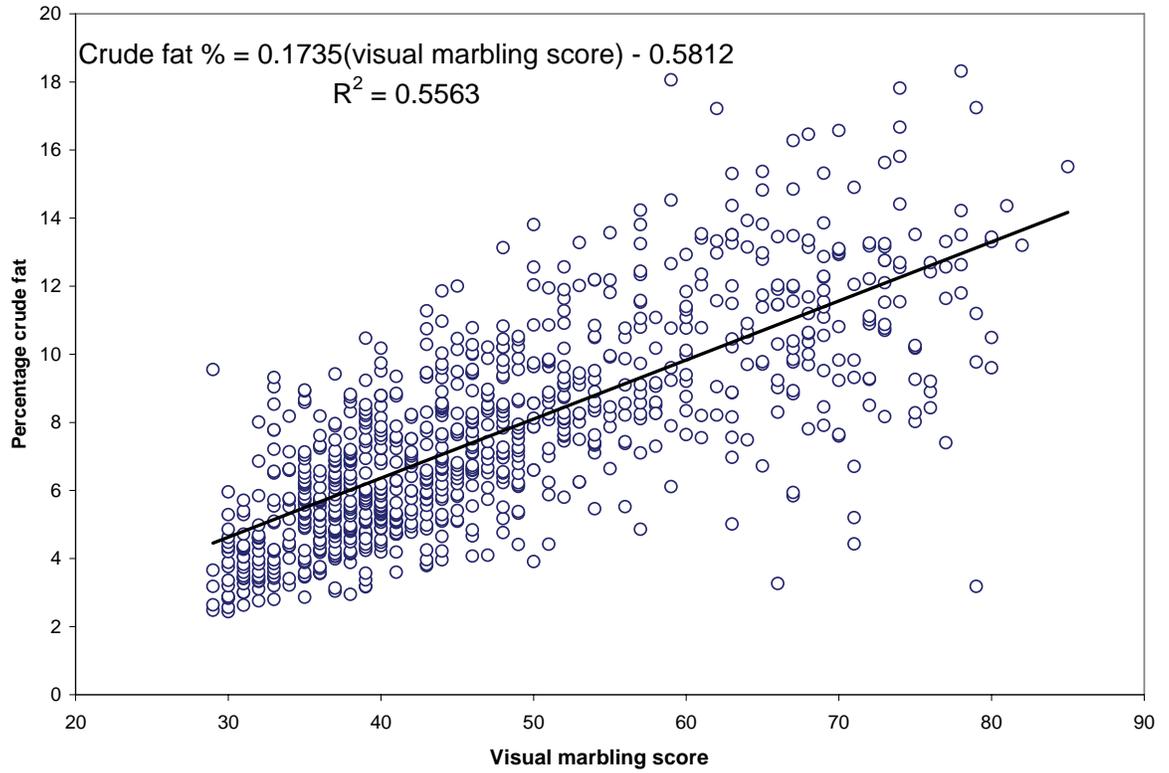


Figure 9. The relationship between visual marbling scores and crude fat percentage of beef *longissimus dorsi* muscles.

Marbling scores: 30 = Slight⁰⁰, 40 = Small⁰⁰, 50 = Modest⁰⁰, 60 = Moderate⁰⁰, 70 = Slightly Abundant, 80 = Moderately Abundant

**THE EFFECTS OF CURING AND DRYING ON THE MUSCLE FIBER,
SARCOMERE AND TENDERNESS OF REHYDRATED
AND COOKED CHEVON**

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Key Words: muscle fiber, sarcomere, dried chevon, rehydrated

Introduction

Any process, be it physical or chemical applied to meat cause changes in the ultrastructural characteristics of the meat tissues. These changes affect meat characteristics, which subsequently influence water-binding capacity, protein solubility and tenderness of meat. In processed meat products, the total effect depends on the kind and degree of muscle structure damage and on the method this damage was inflicted on the structure. Tenderness of the final meat product is influenced by several factors with which the meat has been exposed to. For dehydrated meat, the degree of tenderness is affected by the behavior or response of the contractile and connective tissues to the processing method. The importance of their relative contribution depends on circumstances such as the degree of contraction of the myofibrils, the type of muscle, amount and type of connective tissue and the cooking temperatures (Lawrie, 1998; Purchas, 1989).

To determine the degree of muscle fiber shrinkage and destruction and its corresponding effect on the tenderness of rehydrated chevon (goat meat) products, fiber diameter and sarcomere length of fresh, dried and rehydrated meat samples were determined. Tenderness was evaluated objectively by measuring the shear force required to cut the cooked meat pieces.

Objectives

The general objective was to evaluate the degree of muscle fiber shrinkage and destruction in dried and rehydrated chevon. Specifically,

1. To measure the muscle fiber diameter and sarcomere length of dried-cured and rehydrated chevon.
2. To determine the rehydration potential of dried-cured chevon.
3. To measure the tenderness of dried-cured, rehydrated and cooked chevon thru the shear force values

Methodology

Meat preparation. The *longgisimus dorsi* muscles from six male goats of mixed breeds with slaughter weights ranging from 18–28 kg were used for this research. Meat pieces were sliced into approximately 5-10 mm thickness across the grain, mixed properly and divided into three lots corresponding to the two curing treatments: %14 salt solution, 14% salt solution with nitrite and the control (fresh meat). The cured meat pieces were either sundried or smoked/oven-dried to reduce moisture to 15-20%.

Fiber diameter. Pieces of fresh dried and rehydrated muscle samples taken from the *longgisimus dorsi* were placed in 20% HNO₃ solution. After 24 hours the muscle samples were transferred to test tubes of distilled water and shaken vigorously. Then samples were transferred to saturated solution of aluminum potassium sulfate, and several muscle fibers were placed in slides and measured in a compound microscope fitted with ocular micrometer. Twenty fibers from each treatment were randomly selected for fiber diameter measurement. Each fiber was measured in three points: on the two ends and in the middle section of the fiber. The values were averaged to get the diameter of one fiber. Measurement of the fiber length was not possible due to curling and fragmentation of almost all of the dried and rehydrated samples.

Sarcomere length. Small pieces of fresh, dried and rehydrated samples were likewise taken from the *longgisimus dorsi* of the same animals. The muscle pieces were teased while soaked in 3% glutaraldehyde as fixative solution. Several muscle fibers were separated and transferred to airtight plastic test tubes containing 3% glutaraldehyde and submitted to the National Institute of Molecular Biology and Biotechnology (Biotech) Electron Microscopy Service Laboratory for processing, viewing and measurement in the transmission electron microscope (TEM, Hitachi H-300 model). Suitable images were subsequently photographed and printed.

Rehydration. Sample meat pieces from the different dried products were rehydrated by soaking in warm water (50°C) for 2 hrs. The rehydrated meat pieces were then weighed and the rehydration yield computed following Ibarra et. al., (1988).

Tenderness. Rehydrated samples were cooked into a local recipe called *caldereta*. Tenderness of the cooked meat samples was evaluated using the Warner Bratzler Shear Machine. Thin strips of cooked samples were layered and rolled and fitted in a ½ inch diameter metal bore. Three readings per meat sample were taken.

Statistical analysis. Data on the effects of curing and drying on the rehydration and tenderness characteristics were analyzed using Analysis of Variance (ANOVA) under 2x2 factorial in Randomized Complete Block Design (RCBD) with the animals as blocks, curing solution as factor A and drying method as factor B. On the other hand, simple ANOVA in RCBD was done to determine the effect of treatment on the fiber diameter. Comparison of means was done using Duncan's Multiple Range Test (DMRT).

Results And Discussion

Muscle Fiber Diameter

Table 1 presents the mean muscle fiber diameter of cured-dried (CD) and cured-dried-rehydrated (CDR) chevon. For unhydrated CD meat samples, fiber diameter of

sundried samples (T_2 and T_4) were comparable with the control (T_5) regardless of the curing brine used ($P > 0.05$). On the other hand, samples, which were smoke-oven dried, (T_1 and T_3) have significantly smaller fiber diameter ($P < 0.05$). Smoke-oven dried meat pieces were subjected to increasing temperature of up to 80°C during drying which denatured the sarcoplasmic and myofibrillar proteins and part of the connective tissues, causing the decrease in muscle fiber diameter. Hamm (1966) established that the coagulation of myofibrillar proteins begins between 30°C and 40°C and is almost complete at 55°C . At 62°C , most of the sarcoplasmic proteins are denatured (Bendall, 1964 as cited by Hamm, 1966) and at 80°C they all become insoluble (Hashimoto and Yasui, 1957 as cited by Hamm, 1966). This then results in the stiffening of the structure of the muscle fibers. Shrinkage of collagen at 60°C also contributed to the subsequent shrinking of the muscles.

On the other hand, sundried meat samples were exposed to below 50°C drying temperature. A large part of the globular muscle protein is not yet coagulated at temperature below 50°C and therefore minimized the structural changes that occurred in the cells.

Table 1 also shows the mean fiber diameter of rehydrated meat pieces. There were no significant variations in the diameter among the treated samples ($P > 0.05$). T_2 and T_3 were the most comparable with the control ($P > 0.05$). There was an observed increase in the diameter of the smoke-oven dried samples over their dried counterparts. Surprisingly however, fiber diameter of sundried samples, decreased and yielded measurements smaller than the dried samples despite the higher percent rehydration. One possible explanation is the non-uniform swelling of muscle fiber in rehydrated sundried samples. The study of Knight, et al (1989), demonstrated that the non-uniform swelling of muscle fiber is due to the destruction of cell membrane and a sheath of endomysial connective tissue surrounding the fiber. Non-uniform breakdown of these layers, he added, could be a cause of variable behavior of muscle fiber. Wilding et al (1986) as cited by Knight et al (1989) showed that greater swelling occur at the broken endomysium and he further showed that fibers lacking endomysium on prolonged immersion in 0.25 M KI shrink after swelling whereas smaller swelling fibers with intact endomysium is maintained.

In this experiment, sundried meat samples underwent less heat denaturation, thus, there was less stiffening of the endomysium which made them softer over those that were smoke-oven dried. With vigorous shaking in distilled water to separate muscle fibers, more cells were damaged. Observations also showed that there were more fragmented and damaged fibers in these samples. Some fibers may have retained their endomysial sheath while others were stripped either partially or fully creating breaks and becoming vulnerable to excessive but unstable swelling. On the other hand, smoke-oven dried muscle bundles were more difficult to separate into individual fibers possibly due to more stiffening of the fiber structure. However, individual fibers in this treatment were observed to have more uniform measurements along its entire length probably due to a more intact and stable endomysial sheath acting as restraint.

The 2-hour rehydration time and higher temperature could have been more than enough to cause excessive swelling and subsequent collapse of muscles with damaged endomysium resulting to smaller fiber measurements.

Sarcomere Length

Longitudinal cuts of muscle fibers from fresh, dried and rehydrated samples were prepared for viewing in the transmission electron microscope (TEM) to determine the length of the sarcomeres. Figure 1 shows the micrographs of the sarcomeres of fresh goat meat showing distinct A-bands (), I-bands (), and Z – lines (). These sarcomeres have an average length of 1.16 μm (n=20). In salt-cured samples (Figure 2), there is obvious contraction of contractile structures. Z-lines are no longer recognizable while A-bands are much closer to each other. There was crimping of the sarcomeres. When these treatment samples were rehydrated (Figures 3 and 4) structures are not distinguishable anymore. Black aggregate substances were prominent which are probably denatured proteins separated/broken from each other during hydration. These observations show that upon drying, the A and I filaments are meshed together irreversibly. According to Purchas (1989), the myosin of the A filament is the most heat sensitive. He described further that adjacent A filaments begin to coalesce from about 55°C and continues up to 60-70°C. At the latter temperature I filaments of actin begin to disintegrate. At 70 – 80°C these filaments lose their identity. All that remains according to the same author is a coagulum of actomyosin in which very fine “through-running” filaments can be perceived. This, he suggested are the T-filaments which survive even after 4 hours of 100°C. These T filaments could be the remaining thin and straight running structures along the length of the fibers as in Figure 4 (arrow) and embedded in a coagulum derived from the wreckage of the filaments (Purchas, 1989).

Rehydration

Percent rehydration (Table 1) shows no significant differences among treatments. Drying and curing did not affect significantly the rehydration capacity of the processed meat. Slight variations could be attributed to the differences in temperature with which the samples were exposed during processing. The higher the temperature the more proteins are denatured that would result to lowered capability of meat tissues to reabsorb water. Percent rehydration in this study was negatively correlated with shear value at - 0.21. The relationship, however, is statistically not significant.

Tenderness

Table 1 likewise, contains the tenderness evaluation of treated cooked chevon as measured by the shear value (SV). Shear value measures the shear force required to cut right through the sample and follows that it increases with toughness of meat (Lawrie, 1998). The control (T₅) is shown to have significantly lower shear value than all the dehydrated samples. As has been shown in Figure 1, sarcomeres of T₅ are intact and relaxed. Smulders et al (1990) showed the relationship between sarcomere length and tenderness in their study on beef tenderness and sarcomere length. They found out that longer sarcomeres were clearly associated with greater tenderness. The average sarcomere length of the fresh goat meat in this study is 1.16 μm , shorter than those of the mature sheep (2.3 μm)(Ockerman, 1980). All treated samples have similar shear value (P > 0.05) but are significantly tougher than the control (P < 0.05).

Although collagen content and solubility were not measured in this study, its effect could be predicted based on its established response to heat. Research by Mutungi et al (1995) showed increased strength of perimysium with cooking temperature until 50°C. However at higher temperature and prolonged cooking, collagen is transformed into water-soluble gelatin, which is responsible for the softening and disintegration of tissues. When meat samples in this study were subjected to prolonged cooking, connective tissues were gelatinized which contributed to the relatively tender cooked meat product (maximum 2.5 kgF). Devine et al (1990) as cited by Swan et al (1998) claims that meat is considered acceptably tender if the mean shear force value is less than 8.0 kgF.

Conclusions

When unhydrated, muscle fiber diameter of sundried samples, regardless of the curing brine used, were comparable with the fiber diameter of the fresh chevon. On the other hand, those smoke-oven dried have significantly smaller fiber diameter. When rehydrated, all cured-dried samples had significantly smaller fiber diameter than the fresh samples.

The sarcomere lengths of the treated samples were not measured due to contraction and crimping of the contractile structures. The Z-lines and other structures were no longer recognizable and A-bands were much closer to each other. Rehydrated samples showed indistinguishable, disorganized and swollen structures. This shows that upon drying, A and I filaments are meshed together irreversibly and rehydration did not restore the structures.

Percent rehydration were similar among the treated samples.

The cured-dried chevon had similar tenderness values. However, they were all significantly tougher than the control (fresh). The shear force values nonetheless, are still within the tender range since maximum value considered to be acceptably tender is 8.0 kgF (Swan et. al. 1998).

Drying and curing, to some degree, damaged the contractile structures of the muscle cell, diminishing the cell's ability to reabsorb water during rehydration and consequently reducing the tenderness of the product.

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Tables and Figures

Table 1. Fiber diameter, percent rehydration and shear value of rehydrated cooked chevon.

Treatment	Fiber Diameter, μm		% Rehydration	Shear Value (KgF/cm ²)
	Dried	Rehydrated		
T1	2.82 b	3.48 b	19.15	2.0 a
T2	3.98 a	3.65 b	24.42	2.1 a
T3	3.29 b	3.71 b	20.61	2.5 a
T4	3.94 a	3.38 b	26.00	2.0 a
T5	4.16 a	4.16 a		1.0 b

¹Means in the same column without or have a common letter in their superscript do not differ ($P>0.05$).

²T1 = Nitrite-cured/Smoke-oven dried; T2 = Nitrite-cured/Sun dried; T3 = Brine-cured/Smoke-oven dried; T4 = Brine-cured/Sun dried; T5 = Fresh

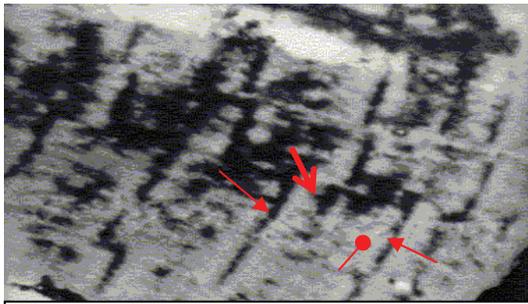


Figure 1. Electron micrograph of a longitudinal section fresh chevon muscle fiber showing contractile structures: Z-line (—►); A-bands (→) and I-bands (—●); x 15,000. Bar = 1 μ m

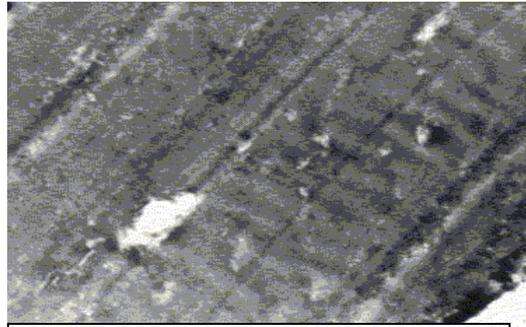


Figure 2. Electron micrograph of salt-cured/sundried chevon: contraction of sarcomeres ; x 15,000. Bar = 1 μ m

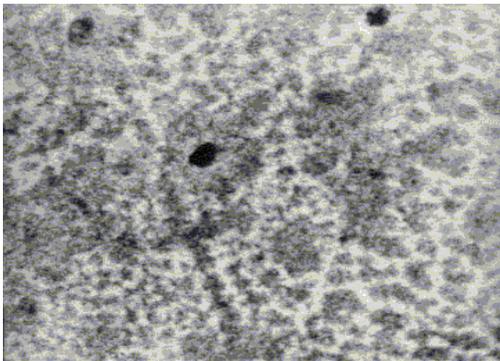


Figure 3. Micrographs of rehydrated chevon (salt-cured/ smoke-oven dried) showing disorganized and disfigured ultrastructures; x10, 000. Bar = 1 μ m

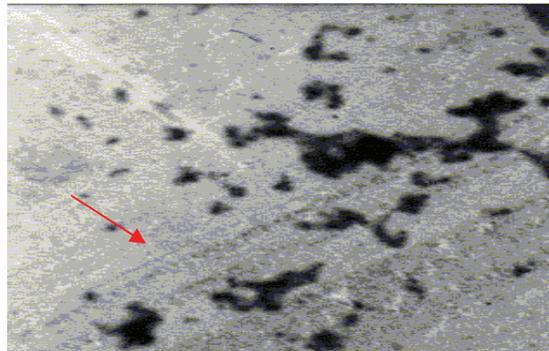


Figure 4. Longitudinal micrographs of rehydrated chevon (salt-cured/sundried): structures are no longer distinguishable, regions are devoid of contractile materials and organelles, black aggregate substances are probably denatured protein and organelles. Arrow points to possible T-filaments, x5, 000. Bar = 1 μ m.

PRESENCE OF GLYCOPROTEINS IN CHICKEN MYOFIBRILLAR FRACTIONS

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Key Words: Chicken myofibrils, glycoprotein, myofibrillar protein

Introduction

Protein glycosylation is an effective method for improving the functional properties of food proteins. Non-enzymatic glycosylation is a complex series of reactions between amino groups of proteins in living systems. Recently, Saeki et al. (1997) and Sato et al. (2000) noted that fish and shellfish myosin in myofibrils became water-soluble in a physiological condition. Syrový and Hodný (1993) revealed that in adult rat skeletal muscle heavy chains of myosin, actin, and tropomyosins were glycosylated *in vitro*. There are not yet known glycoprotein or glycosylated myofibrillar proteins in skeletal muscle of domestic animals.

Objectives

The aim of this work is to investigate the presence of the glycoprotein in chicken myofibrillar fractions.

Methodology

Preparation of myofibril fractions: Myofibrils were prepared from the fresh chicken pectoral muscles as described by Tatsumi et al. (1993), suspended in a basal solution containing 0.1 M KCl, 5 mM EDTA, 1 mM DTT, 1 mM NaN₃ and 10 mM Tris-maleate buffer, pH 7.0, and spun down for 10 min at 1000 × g_{max} to eliminate mitochondria, fragmented sarcoplasmic reticula and cell membranes. The washing of myofibrils was continued 5 times. The resulting sediment was collected, and dialyzed against the distilled water, four times, for at least 6 hours each. The dialysate was concentrated using the collodion bags and then lyophilized (Mf fraction).

Deglycosylation from Mf fraction: Glycoproteins (50 µg) were hydrolyzed in 2.5 M trifluoroacetic acid (TFA) at 100°C for 6 h in order to liberate neutral and amino sugars. TFA was removed from the hydrolysate by rotary evaporation. The hydrolysate was lyophilized and dissolved in water. This solution was passed through an anion-exchange column (AG 1-X8 Resin hydrogen form, Bio-Rad Laboratories, USA), and the elution was done with distilled water. The eluted fraction was then passed through a cation-exchange column (AG 50W-X8 Resin hydrogen form, Bio-Rad Laboratories, USA), was

eluted with distilled water, the fraction was collected, and freeze-drying. The ovalbumin (OA) was used as a positive control.

SDS-PAGE and Glycoprotein blots: Myofibril fractions were suspended with a solution containing 5 mM EDTA and 5 mM Tris-HCl buffer, pH 8.0, and solubilized in a solution containing 1 % SDS, 1% 2-mercaptoethanol, 10% glycerol, 5 mM EDTA and 5 mM Tris-HCl buffer, pH 8.0, then heated at 100°C for 2 min. Samples were subjected to SDS-PAGE by the method of Laemmli (1970) using 7.5 to 17% polyacrylamide and 1.75 to 3.3% glycerol gradient gels (Ahn et al., 2003), and 15% polyacrylamide gels. In the method of Weed (1976), the myofibril samples containing 10 mM Tris-Bicine (pH 8.3), 1% SDS, 2% 2-mercaptoethanol, 10% glycerol, and bromphenol blue tracking dye, were heated at 100°C for 5 min and then applied to 7.5% polyacrylamide gels. For the electrophoretic transfer of proteins to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, USA), myofibrillar proteins separated by SDS-PAGE were electrophoretically transferred on to PVDF membranes by the method of Towbin et al. (1979), and were visualized by methods reported previously (Kondo et al., 1991) using a kit (GP Sensor, Seikagaku Corp., Japan).

Protein and Sugar determination: Concentrations of proteins were measured by the biuret reaction (Gornall et al., 1949) standardized by the micro-Kjeldahl process. Sugar concentration was measured by the phenol-sulfuric acid method (Dubois et al., 1956).

Results & Discussion

To confirm the glycoprotein in Mf fraction, we would try to visualize the glycoprotein using the Periodic-Acid Schiff based reactions between acid-Schiff base with biotin hydrazide and, streptavidin–HRP on the PVDF membrane. Figure 1 shows the existence of sugar chain on the dot-blot. The protein concentrations in OA and Mf fractions were 15 µg and 45 µg, two images were the protein staining (Fig.1A) and the sugar staining (Fig.1B). Two kinds of images showed the protein staining (Fig.1A) and the sugar staining (Fig.1B). Figure 1B indicates the positive, so the myofibrillar protein may conjugate the sugar chain, thus, may be glycoprotein. Table .1 showed the sugar concentration in Mf fraction. Neutral and acid sugar fraction prepared from Mf, and the total sugar content in Mf was 2.52 ± 0.37 µg/mg freeze-dry weight, 3.47 ± 0.52 µg/mg myofibrillar protein. OA derived fraction, positive control, have 31.43 ± 3.22 µg/mg freeze-dry weight, 34.93 ± 3.57 µg/mg protein.

To estimate the glycoprotein in Mf, we carried out the protein staining and the sugar staining in 7.5-17% polyacrylamide gradient gels (Fig.2), 7.5% gel (Fig.3), and 15% gels (Fig.4). Figure 2 showed several positive bands, two bands were high molecular weight more than myosin heavy chain, and four bands were low molecular weight less than α -actinin. In other buffered electrophoresis system (Fig.3), there were two positive bands around myosin heavy chain, and one lower molecular weight-band than α -actinin. In Fig.4, we estimated one lower molecular weight-band than α -actinin. Compared with the immunostaining image using anti- α -actinin antibody, there was not a band in same position. It is assumed that this band is the 85 kDa band. We suggested that there were several glycoproteins among the myofibrillar proteins.

Conclusions

We prepared myofibril fraction from chicken pectoral muscle. This fraction showed the positive spot against sugar staining and this sugar concentration had 3.47 $\mu\text{g}/\text{mg}$ myofibrillar protein. Two types of the buffered system SDS-PAGE, there were several positive bands against sugar staining. Among the different concentration gels, there were common positive bands containing 85-kDa band. We suggested that there were glycoproteins in myofibril fraction.

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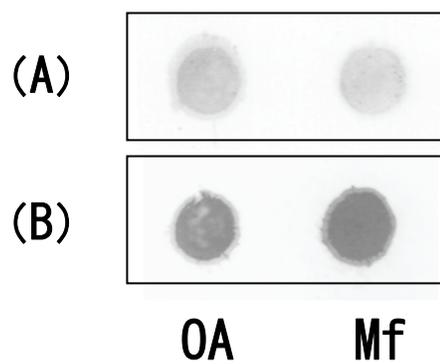


Figure 1 Sugar and protein staining images
Sugar staining; (B) CBB staining OA, ovalbumin; Mf,
myofibril fraction

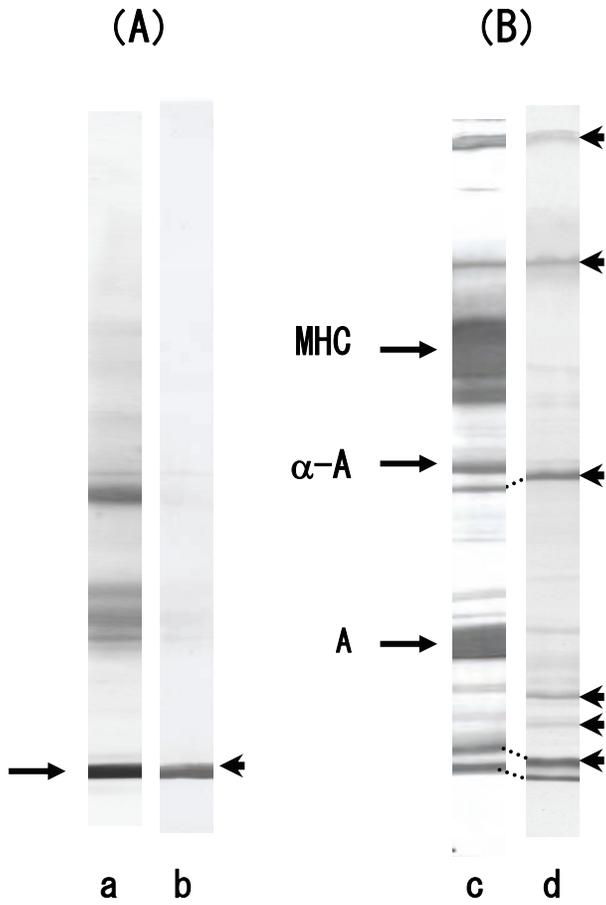


Figure 2 7.5-17% SDS-PAGE image
 CBB staining (a,c); Sugar staining (b,d) A,
 ovalbumin; B, myofibril fraction

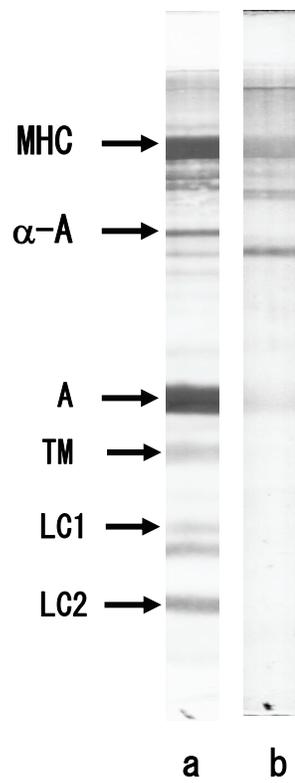


Figure 3 7.5% SDS-PAGE image
CBB staining (a); Sugar staining (b)

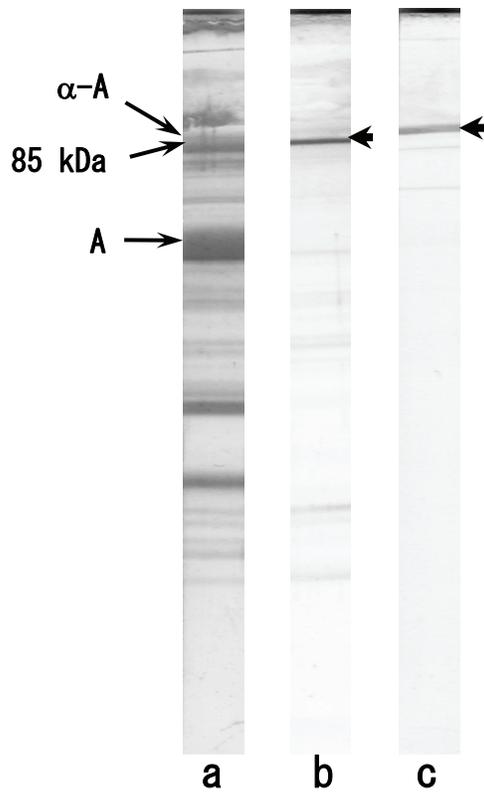


Figure 4 15% SDS-PAGE image
CBB staining (a); Sugar staining (b) ;Anti- α -
actinin antibody staining (c)

IDENTIFICATION OF GENETIC COMPONENTS OF β -RYR ASSOCIATED WITH PSE CHARACTERISTICS IN TURKEYS

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Key Words: PSE, ryanodine receptor, channel activity, single nucleotide polymorphism, ryanodine binding assay

Introduction

The modern turkey industry has been successful in producing fast-growing birds with large breast muscle due to intensive genetic selection. However, these improvements in growth rate and size of birds have led to an increased incidence of pale, soft and exudative (PSE) meat (Toelle et al., 1991; Pietrzak et al., 1997).

Since the term PSE was first coined to describe a meat quality problem in pork (Wismer-Pederson, 1959), there have been extensive studies on the mechanism of PSE meat development. The development of PSE pork, in part, results from an inherited skeletal muscle disorder in swine known as porcine stress syndrome (PSS). A single point mutation, Arg615Cys, has been identified in the skeletal muscle calcium release channel (ryanodine receptor 1, RyR1) which is responsible for PSS (Fujii et al., 1991) and, in turn, is associated with the development of PSE meat. As there are many striking similarities in the development of PSE meat between turkey and pork (Sosnicki et al. (1996) and Pietrzak et al. (1997)), it has been suggested that there is a genetic defect in turkey skeletal muscle RyR which alters the channel activity and causes the development of PSE turkey (Owens et al., 2000).

The ryanodine binding assay has been utilized as a method to measure the channel activity of pig RyR (Mickelson et al., 1986, 1988). Our laboratory has optimized the ryanodine binding assay to assess RyR channel activity in turkey skeletal muscle (Zhang, 2000). Sarcoplasmic reticulum (SR) vesicles from genetically improved turkeys showed a two-fold greater ryanodine affinity compared to the unimproved turkeys ($K_d = 8.4$ vs. 16.0 nM, respectively). However, another line of genetically improved turkeys showed similar calcium channel properties to those of random-bred turkeys ($K_d = 19.1$ vs. 16.0 nM, respectively; Zhang, 2000). This suggests that there is a diversity of the calcium channel activity among different turkey populations.

Despite similarities in development of PSE pork and turkey, we cannot assume that the mutation associated with development of PSE pork would also be present in turkey. More than 20 mutations in RyR1 have been associated with malignant hyperthermia, the human analog of PSS (Jurkat-Rott et al, 2000). Moreover, a key difference between turkey and pig skeletal muscle is that there are two ryanodine receptor isoforms (α and β) expressed in avian skeletal muscle, while there is only one major isoform (RyR1) in

mammalian skeletal muscle (Airey et al., 1993). Therefore, there could be alteration(s) in either or both isoforms which could contribute to the PSE turkey meat problem.

Although both RyR isoforms are expressed in equal abundance in turkey skeletal muscle, the observed ryanodine binding is solely the result of β -RyR activity, since α -RyR activity is almost completely suppressed under the assay conditions (Murayama and Ogawa, 2001). Therefore, we hypothesize that the ryanodine binding assay can be used as a phenotype reflecting the β -RyR genotype.

Objectives

The overall goal of this project is to test the hypothesis that mutations in β -RyR of turkey skeletal muscle are associated with the development of PSE turkey meat.

Methodology

Turkey breast muscle sampling and meat quality evaluation

Genetically unimproved random-bred control (RBC1) turkeys (n=40) were obtained from Dr. K. Nestor (Ohio Agricultural Research and Development Center, Wooster OH). Growth selected turkeys (n=40) were a gift of Hybrid Turkeys, Inc (Kitchener, ON). Turkeys were slaughtered according to standard industry practices. The pectoralis major muscle from one side was collected within 5 min of death, and was immediately sectioned, snap frozen in liquid nitrogen and stored at -80°C for total RNA, genomic DNA extraction and SR vesicle preparation. The remaining pectoralis muscle was used to evaluate meat quality indicators, including $\text{pH}_{15\text{min}}$ and color (L^*) at 24-h post mortem.

Isolation of SR vesicles and [^3H]ryanodine binding

Crude sarcoplasmic reticulum vesicles were purified as previously described (Zhang, 2000). Briefly, fifty μg of CSR protein was incubated in buffer containing 0.1 M NaCl, 0.5 M sucrose, 10 mM MOPS (pH 7.2) and a CaCl_2 -EGTA-nitrilotriacetic acid buffer to give a $[\text{Ca}^{2+}]_{\text{free}}$ of 10 μM . [^3H] ryanodine (2-70 nM as final concentration) was added to each tube. Triplicate samples of each [^3H] ryanodine concentration were incubated for 6 h at 37°C , and filtered through Whatman GF/B filters. Filters were washed three times with 5 mL of ice-cold wash buffer (10 mM MOPS, 0.1 M NaCl, 50 μM CaCl_2 , pH 7.0), and the amount of [^3H] ryanodine retained on the filter was determined by scintillation counting. Non-specific binding of ryanodine was determined by adding unlabeled ryanodine at 1000 times the [^3H] ryanodine in the incubation buffer. Specific ryanodine binding was determined by subtracting the non-specific binding from the total binding. The K_d (ryanodine binding affinity) and B_{max} (maximum binding capacity) values for ryanodine binding were analyzed by Scatchard analysis.

Screening for polymorphism(s) in turkey β -RyR

Ryanodine binding and meat quality data from 77 turkeys were segregated based on their K_d values and meat quality traits as illustrated in Table 1. On this basis, sixteen muscle samples were selected for β -RyR polymorphism screening.

Total RNA from turkey skeletal muscle was extracted using *Trizol* reagent (Invitrogen; Carlsbad, CA). The target cDNA sequence was amplified through RT-PCR using Access RT-PCR system (Promega; Madison, WI) and the PCR products were sequenced directly. The sequencing was done in both directions using the MSU-DOE DNA sequencing facility. The entire coding region of β -RyR was screened.

Statistical Analysis

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 2000) was used to analyze data.

Results & Discussion

Measurement of calcium channel activity

β -RyR channel activity was measured by ryanodine binding assay. Dissociation constants (K_d) for ryanodine binding for 77 birds were distributed over a broad range (20~74 nM) and did not show any significant correlation with other meat quality traits. Genetically improved turkeys and random-bred turkeys did not show significant difference in K_d from each other ($p>0.05$). Because there was no significant correlation between K_d and meat quality indices, the data from the 77 turkeys were combined for further analyses. Muscles from ten birds were selected based on the functional properties as typical PSE (+) meat and PSE (-) meat: pH < 5.8 and $L^* > 52.0$ and pH > 5.8 and $L^* < 50.0$, respectively (Table 2). Channel activity of PSE (+) turkeys was significantly higher than that of PSE (-) turkeys (Table 3).

Screening of the coding region of β -RyR gene

As a result of polymorphism screening of cDNA of β -RyR, 10 single nucleotide polymorphisms (SNPs) were identified (Table 4). All of them were synonymous and therefore the primary structure of the protein was not changed. None of these SNPs or haplotypes was correlated with meat quality indices or K_d .

The cDNA screening revealed that the primary structure of β -RyR protein was conserved for the 16 birds, but their channel activity data were distributed over a broad range. It is possible that post-translational modification of the protein played a role in the large variation in channel activity among turkeys with the same amino acid sequence.

Conclusions

Based on channel activity differences in turkey muscle β -RyR, 16 birds were selected for screening for polymorphisms in the β -RyR cDNA. Ten SNPs were identified but none of them was correlated with meat quality traits or channel activity. Although β -RyR is very well-conserved, the channel activity of turkeys was distributed over a broad range; the variation may result from the post-translational modification. It has been reported that there are two different genomic alleles for α -RyR and several transcript variants (Chiang et al., 2004). Therefore we suggest that α -RyR channel activity is highly adaptable to environmental stress while the coding region of β -RyR is highly conserved.

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Tables and Figures

	High K _d	Low K _d
Better meat quality (high pH, low L*)	N=4 (K_d 57.60 nM) (pH 6.21, L* 51.23)	N=5 (K_d 29.53 nM) (pH 6.24, L* 51.50)
Poorer meat quality (low pH, high L*)	N=3 (K_d 57.24 nM) (pH 5.79, L*53.12)	N=4 (K_d 26.65 nM) (pH 5.77, L*55.43)

Table 1. Sixteen turkeys selected for screening of β -RyR cDNA.

PSE (-)	pH	L*	K _d	PSE (+)	pH	L*	K _d
29-11	6.63	47.42	38.64	31-33	5.8	55.13	28.65
31-13	6.42	48.53	30.03	31-28	5.79	56.58	27.1
29-15	6.31	47.45	44.05	31-25	5.76	55.07	21.01
29-21	6.28	46.68	37.04	31-36	5.75	52.03	24.57
29-37	6.01	49.72	51.28	31-19	5.74	52.24	30.86
29-03	5.96	47.18	41.15	31-32	5.73	54.95	29.85
31-26	5.9	49.56	26.46	31-22	5.73	53.69	41.67
31-03	5.9	45.01	39.22	31-20	5.72	53.45	30.67
29-40	5.86	49.72	59.52	29-08	5.71	56.25	39.22
29-34	5.86	49.08	45.05	31-12	5.64	54.31	44.25

Table 2. Selected turkey samples for channel activity comparison

Group	LSMean of K _d (nM)
PSE (-) (N=10)	31.785
PSE (+) (N=10)	41.244

P<0.05

Table 3. Mean K_d of turkeys in PSE and non-PSE groups

SNP ID	Base	Amino acid
2352	A/G	Val
9111	G/A	Ser
9681	A/G	Lys
9762	C/T	Leu
10434	C/T	His
11094	C/T	Asn
11097	G/A	Ala
12159	C/T	Ser
12675	T/C	Asn
14076	A/G	Lys

Table 4. Ten SNPs identified in the entire coding region of β -RyR.

ALTERNATIVE SPLICING IN TURKEY SKELETAL MUSCLE RYANODINE RECEPTOR α RYR

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Key Words: PSE, ryanodine receptor, alternatively spliced transcript variant

Introduction

The term “pale, soft, exudative (PSE)” is a descriptor for a meat quality defect, primarily pork and turkey, that has an abnormal light color, often flaccid consistency, poor water-holding capacity, and substantially reduced cook yield (De Smet et al., 1996). PSE meat is the result of denaturation of myofibrillar proteins caused by the combination of high carcass temperature and low muscle pH during the early stage of conversion of muscle to meat. The combination of high body temperature and acidic muscle pH in the meat animal mainly is attributed to a disorder of skeletal muscle Ca^{2+} regulation which leads to hypermetabolism and accelerated glycogenolysis during muscle contraction.

A dysfunctional homotetrameric Ca^{2+} release channel protein in skeletal muscle known as ryanodine receptor 1 (RYR1) is believed to contribute to abnormal Ca^{2+} regulation. A point mutation, Arg⁶¹⁴Cys, identified in RYR1 has been correlated with the development of PSE pork (Fujii et al., 1991). In addition, more than 20 missense mutations of human RYR1 have been identified (Jurkat-Rott et al., 2000). The overall physiological effect of the mutations in RYR1 is an elevation of resting muscle Ca^{2+} levels as a result of increased Ca^{2+} permeabilities of the mutant channels (MacLenna, 2000). Although a strong correlation has been shown between the mutated RYR1 and the development of PSE pork, little is known about the molecular mechanism of PSE turkey meat development.

The similarities of postmortem biochemical changes between PSE turkey and PSE pork including increased rates of postmortem pH decline, ATP depletion, and glycolysis (Pietrzak et al., 1997) suggest that the occurrence of PSE turkey is likely caused by a dysfunctional RYR in turkey skeletal muscle. However, the mechanisms of Ca^{2+} regulation in avian skeletal muscle are more complex than in mammalian species. These include a co-expression of two RYR isoforms (α RYR and β RYR; Ottini et al., 1996) and expression of different alternatively spliced transcript variants (ASTVs) of α RYR in turkey skeletal muscle (Chiang et al., 2004). Turkey skeletal muscle α RYR and β RYR have been cloned and sequenced in our laboratory. During cloning, at least eight ASTVs of α RYR have been identified. This paper describes our current research of turkey skeletal muscle α RYR and discusses the significance of the research in identifying the molecular mechanism regulating turkey meat quality.

Objectives

To determine the primary structures of ASTVs of turkey skeletal muscle α R_{YR} and to correlate the changes of the primary structure of α R_{YR} with differences in turkey meat quality.

Methodology

Cloning of turkey skeletal muscle α R_{YR} and β R_{YR} cDNAs

Total RNA was extracted from turkey pectoralis major muscle using Trizol reagent (Invitrogen). First strand cDNA was synthesized as follows: a 50 μ l reverse transcription reaction contained 10 μ g of RNA, 0.1 mM of first-strand primer, 9% glycerol, 1 \times first-strand buffer, 10 mM DTT, 0.5 mM dNTP mix, 30% saturated trehalose, and 600 U of SuperScript II RNase H⁻ reverse transcriptase (Invitrogen). First strand cDNA synthesis was followed the method of Carninci and Hayashizaki (1999) with modifications.

PCR amplification of the resulting first-strand cDNA was conducted using Advantage cDNA polymerase (BD Biosciences). For highly GC-rich regions, amplification was carried out using the Extended Long Template PCR system (Roche). Depending on the melting temperature of the primers, the annealing temperature used during amplification was set in the range of 58 $^{\circ}$ -65 $^{\circ}$ C. The PCR reaction was started with an initial denaturation step at 94 $^{\circ}$ C for 1 min, followed by 35 cycles consisting of 94 $^{\circ}$ C for 10 sec., 58 $^{\circ}$ to 65 $^{\circ}$ C for 15 sec., and 70 $^{\circ}$ C for 1-2 min. Reaction was terminated by a final extension at 70 $^{\circ}$ C for 10 min. Degenerate PCR primers used in the reverse transcription or PCR reaction were designed based on the conserved regions of the R_{YR} sequences among different species. Other turkey R_{YR} specific primers were designed from the sequence of the established turkey R_{YR} cDNA sequence. PCR products were cloned and nucleotide sequences were determined from both strands. In each PCR reaction, all the amplified PCR fragments were cloned and sequenced and these fragments were used to identify ASTVs.

Analysis of cDNA sequences

Nucleotide sequences data from individual clones were assembled into a full-length contig based on overlapping sequences between the adjacent clones. Comparisons of sequence identity among turkey R_{YR}s and published R_{YR} sequences were performed using the UW-GCG program. The splice junction of each ASTV was identified by comparing the sequence of the spliced transcript variant and the full length cDNA sequence.

Results & Discussion

Alternatively Spliced Transcript Variants (ASTVs) of Turkey Skeletal Muscle α R_{YR}

The complete full length cDNA sequences for α R_{YR} and β R_{YR} from turkey skeletal muscle have been successfully cloned and sequenced in our laboratory. There are 15,150 bps in the open reading frame of the α R_{YR} cDNA sequence which encode a protein of 5,050 amino acid residues and a deduced molecular mass of 565,120 Da. There are 14,604 bps in the open reading frame of the β R_{YR} cDNA sequence which encode a protein of 4,868 amino acid residues and a deduced molecular mass of 552,778 Da. The α R_{YR} and β R_{YR} isoforms share 66% amino acid sequence identity. Major sequence variations in the primary structure of the two isoforms were identified in three divergent regions (DRs).

Analysis of full length cDNA sequences of α R_{YR} and β R_{YR} revealed at least eight ASTVs of α R_{YR}, but no ASTV was identified in β R_{YR}. The eight ASTVs of α R_{YR} are clustered in three different regions of α R_{YR} (Fig 1). Since each of these regions is characterized by a high frequency of alternative splicing, we refer to these regions as alternative splicing regions 1-3 (ASRs 1-3). Interestingly, the locations of ASR2 and ASR3 coincide with DR2 and DR1 of α R_{YR}, respectively. ASR1 encompasses two ASTVs (AS-81 and AS-193; Chiang et al., 2004). ASR2 encompasses five ASTVs, AS-1043, AS-401, AS-454, AS-370 and AS-268. With the exception of AS-401, which represents the removal of exon 28, the other four ASTVs occur at aberrant alternative splice junctions. Unlike the conventional 5'/3' splice junctions identified by the GT/AG consensus sequences, the alternative splice junctions of AS-1043, AS-454, AS-370 and AS-268 are characterized by polycytosine (poly-C) tracts for both the 5' and 3' splice junctions. We were unable to determine the exact cytosine residue for the splicing junction of these ASTVs because the sequences at the 5' and 3' junctions were indistinguishable (Fig. 2). The splicing observed within the poly-C tract is novel and does not match to any of the published nonconforming splicing sites (Senapathy et al., 1990; Jackson, 1991). ASR3 resides in the C-terminal region of α R_{YR}. One transcript variant, AS-816 was identified and this transcript variant generates a deletion of 272 amino acids encoded by exon 91 of α R_{YR}.

The observation that ASR2 and ASR3 are contained within DR2 and DR1, respectively, of turkey skeletal muscle α R_{YR} suggests that sequence variation in DR2 and DR1 can arise via nucleotide substitution and by alternative splicing.

Functional effects of alternative splicing on α R_{YR}

The biological determinants of the alternative splice sites in the ASRs of α R_{YR} are not clear. Developmental stage and environmental stresses such as temperature changes can regulate the alternative splice pattern of pre-mRNA (Futatsugi et al., 1995; Takechi et al., 1994). As we analyzed the expression of ASTVs in the ASR1 of α R_{YR}, we found that birds treated with different levels of heat stress expressed different patterns of full length, AS-81 and AS-193 transcript variants (Fig 3). This suggests that the expression

pattern of the full length and the ASTVs of α R_{YR} is a dynamic process which may result in differential channel activity.

Although it is commonly thought that R_{YR} exists as a homotetrameric channel under a normal physiological condition, heterotetrameric channels containing a mixture of R_{YR}3 spliced variant and wild type subunits have been reported (Jiang et al., 2003). The heterotetrameric R_{YR}3 channel shows altered channel activity. All eight α R_{YR} ASTVs, except AS-81 and AS-816, would cause frame-shifts which introduce premature stop codons in the open reading frame. Thus, these transcript variants would not encode a complete functional channel protein. AS-81 and AS-816 result in continuous open reading frames and may encode subunits with deletions of 27 and 272 amino acid residues, respectively. The α R_{YR} subunits encoded by AS-81 and AS-816 could combine with subunits encoded by the same ASTV to form a homotetrameric channel. Alternatively, subunits encoded by AS-81 or AS-816 could combine with subunits encoded by different ASTVs and/or wild type subunits to form a heterotetrameric channel. Channels formed by subunits which are encoded by AS-81 or AS-816 are expected to show a different channel function than the wild type. This is based on the structural and functional analysis of mammalian R_{YR} showing that the ASRs of turkey α R_{YR} are localized in the functional domains of α R_{YR}. For example, the 27-amino-acid-residue deletion generated by AS-81 in ASR1 resides within the cytoplasmic clamp domain which has been proposed to serve as part of the protein-protein contact site of R_{YR}1 with dihydropyridine receptor (DHPR; Wu et al., 1997; Baker et al., 2002). Deletion in the ASR1 of α R_{YR} could alter the interaction between α R_{YR} and DHPR and the lack of interaction between these two proteins which, in turn, could modulate Ca²⁺ release via both α R_{YR} and β R_{YR} (O'Brien et al., 1995).

ASR3 resides in part of the transmembrane domain of α R_{YR} (Krogh et al., 2001) where the Ca²⁺ sensor of R_{YR} has been located (Du et al., 2000). A deletion of 272-amino-acid-residue generated by AS-816 in ASR3 is therefore expected to affect the kinetics of Ca²⁺ diffusion through α R_{YR}.

It is logical to expect the regulation of Ca²⁺ fluxes in turkey muscle will be altered as the α R_{YR} channel formed by different subunits encoded by ASTVs rather than the wild type. The changes of the Ca²⁺ fluxes can affect postmortem metabolism and glycogenolysis rate of muscle, and that influence the meat quality.

Conclusions

1. Eight ASTVs of α R_{YR} were identified and they are clustered in three ASRs of primary structure of α R_{YR}. ASR2 and ASR3 overlap with DR2 and DR1 of α R_{YR}.
2. The expression of AS-81 and AS-193, which carry deletions in the ASR1 of α R_{YR}, was regulated by heat stress.
3. The expression of ASTVs of α R_{YR} is expected to affect α R_{YR} channel activity and which will, in turn, influence turkey meat quality.

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Tables and Figures

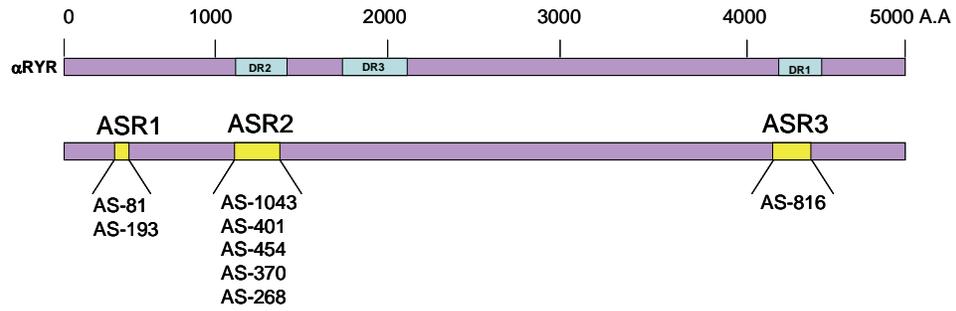


Figure 1. Alternative splicing regions of turkey α RyR. Eight alternatively spliced transcript variants of turkey skeletal muscle α RyR isoform are clustered in three alternative splicing regions 1-3 (ASR1-3). ASR2 and ASR3 coincide with the DR2 and DR1 of α RyR.

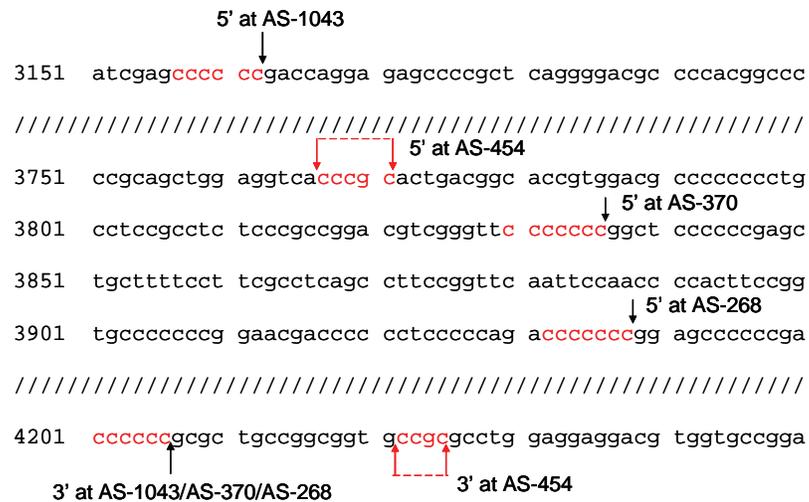


Figure 2. Alternative splicing of turkey α RyR within polycytosine tracts in the ASR2 region. The putative 5' and 3' alternative splice junctions of each transcript variant are indicated by arrows. The two possible splice sites at 5' and 3' junctions of AS-454 are indicated by the arrows connected by a dotted line. The slashed lines are added to replace the omitted nucleotides.

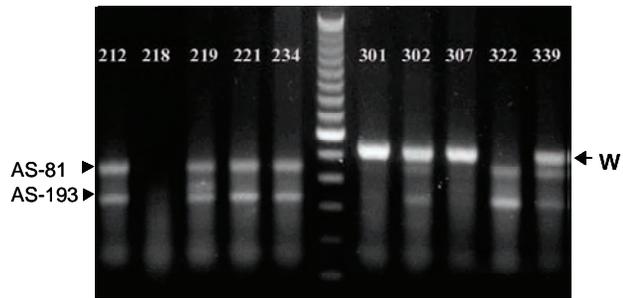


Figure 3. Expression of wild type (W), AS-81 and AS-193 transcript variants under two different heat stress levels. The 100-bp DNA marker is in the center of the gel image. Five individual birds on the left side of the marker are heat stressed two days less than the birds on the right side of the marker. The identification number of each bird is given at the top of the gel.

FREQUENCY OF PORCINE STRESS SYNDROME MUTATION IN COMMERCIAL POPULATIONS IN MEXICO

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Key Words: Porcine stress syndrome, Malignant hyperthermia, Halothane sensitivity, Gene prevalence.

Introduction

Porcine stress syndrome (PSS) is a hereditary predisposition manifested in affected individuals as sudden death induced by stressful situations. This syndrome may be triggered while handling or transporting susceptible animals, during mating, delivery, or exposure to extreme environmental temperatures. After sacrifice, PSS-susceptible pigs develop a muscle degeneration known as pale, soft, exudative muscle (PSE). Furthermore, PSS and PSE susceptible pigs show extreme susceptibility to certain drugs such as succinylcholine or volatile anesthetics such as halothane. Exposure to this compound causes a syndrome known as malignant hyperthermia (MH) (Mitchell and Heffron, 1982).

It is currently known that the same genetic defect is responsible for PSS, PSE and MH in pigs, which is a mutation involving a substitution of cytosine (C) for thymine (T) at the nucleotide 1843 in the ryanodine receptor gene (RYR), which codifies for a calcium release channel at the sarcoplasmic reticulum. This mutation has been identified in practically all commercial pig breeds (Fujii et al., 1991; Otsu, Khanna, Archibald, MacLennan, 1991; Harbitz, Kristensen, Bosnes, Kran & Davies, 1992).

The PSS caused annual losses estimated at 230 to 320 million dollars per year to the American pork industry. It had been estimated that approximately 30% of South African and German Landrace pigs were susceptible to MH (Mitchell et al., 1982). Similarly, gene frequency for MH was 0.42, 0.34 and 0.22 in Swedish Landrace, Yorkshire and Duroc breeds respectively (Gahne and Juneja, 1985).

The epidemiology of porcine stress syndrome is currently unknown in Mexico. Furthermore, the economic impact of this condition on the national pork industry and the need to systematically control the disease, have not been assessed.

Objectives

The objective of the present study was to determine the frequency of the RYR mutant allele in Mexican commercial porcine populations.

Methodology

2.1. Samples

A total of 464 blood samples were drawn from hybrid commercial pigs at the moment they were sacrificed at municipal slaughterhouses of Ecatepec in the State of Mexico (n=158), Pachuca (n=271) and Tizayuca (n=35) in the State of Hidalgo. Samples were selected randomly during several visits to each slaughterhouse over a 3 months period.

2.2. Mutation characterization

DNA was extracted from the blood samples using the sodium chloride exclusion method (Miller, Dykes, Polesky, 1988). A DNA segment containing the porcine ryanodine gene was amplified by the polymerase chain reaction (PCR). Amplified products were immobilized in a nylon membrane and hybridized with radioactively labeled oligonucleotides specific for the normal and mutant alleles (Fujii et al., 1991).

The amplification reactions were done in a final volume of 50 μ l containing 50 mM KCl; 10 mM Tris-HCl, pH 8.3 10; 1 μ g/ml gelatin; 1.5 mM MgCl₂; 200 μ M of each triphosphate deoxynucleotide (dATP, dCTP, dGTP, dTTP); 100 μ g genomic porcine DNA; 50 pmols of each primer: RYR1 (5'-CTCCAGTTTGCCACAGGTCCTACC-3') and RYR2 (5'-CATTACACGGAGTGGAGTCTCTGAG'-3') (Fujii et al., 1991) and 2.5 units Taq-DNA polymerase (Biogenica, México). Amplification conditions were as follows: an initial denaturing cycle at 94^{0}°C for 3 min; 30 subsequent cycles consisting of denaturation at 94^{0}°C, 30 s; annealing at 62^{0}°C, 30 s; and extension at 72^{0}°C, 30 s; and a final extension cycle at 72^{0}°C for 3 min. The amplification products were assessed on 3% agarose gels stained with ethidium bromide and visualized under UV light according to conventional methods (Sambrook, Fritsch, Maniatis, 1989). Afterwards, 30 μ l of 0.6M NaOH was added to 20 μ l of the amplification product. After 10 minutes incubation, 50 μ l of cold 2M ammonium acetate was added, and 40 μ l of this mixture was applied on two nylon membrane replicas (Magna, MSI, INC. Westboro, MA. USA) and UV crosslinked. After that the membranes were hybridized either with the mutant specific oligonucleotide 15T (5'-GGCCGTGTGCTCCAA), or with the normal allele specific oligonucleotide 17C (5'-TGGCCGTGCGCTCCAAC) at 55^{0}°C. Both oligonucleotides were labeled with polynucleotide kinase and gamma-P^{32}ATP following previously described protocols (Sambrook et al., 1989). Hybridized membranes were washed twice with 6X SSC, 0.1% SDS at 55^{0}°C (for the 15C probe) or at 65^{0}°C (for the 17C probe), and later exposed to X-ray film (Kodak X-Omat) for 1-2 days at -70^{0}°C. Each membrane contained the amplified products of previously typified animals (CC, CT and TT) in duplicate as internal hybridization controls. Genotyping was performed by comparing the hybridization signals of each replica (Figure 1).

2.3. Genetic and statistical analysis

Allelic frequencies were estimated directly by allele counting. In addition, observed heterozygosity (Ho) and expected heterozygosity (Hs) were used to estimate the fixation

index $F_{is} = 1 - H_o/H_s$, (Nei, 1987), which indicates the excess or deficiency of homozygotes in a population. In order to test whether this value differs from zero, we used the chi-square test with one degree of freedom $\chi^2 = nF_{is}^2$; where n equals the number of individuals in a population.

Results & Discussion

Although several methods have been used to predict the quality of pork (Cheah, Cheah, Lahucky, Mojto, Kovac, 1993), molecular biology techniques are very promising. The identification of the RYR mutant and normal alleles by allele-specific oligonucleotide hybridization used here was appropriate to assess large number of samples, and was a reliable and sensitive method, in spite of the disadvantages implicit in using radioactive isotopes. We also tested the use of restriction enzymes to detect the mutation (O'Brien, Shen, Cory, Zhang, 1993), however this assay was unsuccessful due to the recurrent low activity of the commercial restriction enzyme available.

The prevalence of the mutation responsible for the porcine stress syndrome, namely the total number of animals carrying at least one mutant allele (mutant homozygotes plus heterozygotes), was 40.3% in commercial Mexican pigs (Table 1). Studying over 10,000 pigs, O'Brien et al. (1993) reported that the prevalence of the mutant allele was 16%. The prevalence of the mutation in Mexican commercial populations was more than 2.5 fold higher. In the same study, O'Brien et al. (1993) found a high prevalence of the mutant allele in commercial breeds, presumably breeding lines: 97% in Pietran, 35% in Landrace, 19% in Large White, 19% Yorkshire, 15% Duroc, and 14% in Hampshire breeds.

In Mexico most of the commercial pig populations are obtained by crossing hybrid dams (Hampshire-Yorkshire or Landrace-Yorkshire) with purebred sires, mainly Duroc or Hampshire. Although, also are used hybrid sires with crosses of Hampshire, Yorkshire or Duroc breeds (CONAPOR, 1998). Because, it is known the association of the mutant T allele to greater muscularity and lean carcasses (Leach, Ellis, Sutton, McKeith, Wilson, 1996), this mutation has been introduced in breeding pig lines in order to have heterozygotes in the final commercial pig. However, this breeding tactics imply the rational use of typed animals. The high prevalence of the T allele in our data indicates that the T allele is being intensely introduced into the Mexican porcine populations. This probably is the result of using breeding animals with high prevalence of the T allele. Unfortunately, there is no current available data of the frequency of RYR genotypes in Mexican breeding pig populations and it seems the pig industry is not performing surveillance and control of this gene.

Table 2 shows the allelic frequencies for the RYR gene, the observed heterozygosity (H_o), the expected heterozygosity (H_s) and the fixation index (F_{is}) estimated in the slaughterhouse porcine population. The gene frequency for the T allele was 0.214. Remarkably, the population was not in genetic equilibrium ($\chi^2 = 6.62$, $P < 0.05$). This disequilibrium was mainly due to an excess of heterozygous genotypes in this population, as measured by negative F_{is} values (-0.119), and was apparently due to a reduced number of the frequency of expected mutant homozygotes (2.6% vs 4.6%) (see Table 1).

Our results suggest that there is a strong selection pressure against the T allele in commercial animals reaching the slaughterhouses. This would explain why the actual genotype frequency of TT (2.4%) was lower than expected (4.6%) In fact, the contribution of TT individuals to the chi-square value was almost 4 units of the total estimated chi-square value of 6.6. This means that almost half of these animals die due to porcine stress syndrome. Because our samples came from different farms and locations, we were expecting to find the existence of isolated reproductive populations (Wahlund effect; Nei, 1987) with an homozygosis excess. However, we rather found a homozygous deficiency. This means that the entire pig populations is behaving as a mendelian population, and the selection process is affecting negatively the TT genotypes.

Considering that around 13.5 million pigs per year are sacrificed in Mexico (Gallardo-Nieto, Villamar, Barrera, Ruiz, 2004), and if the 95% confidence interval for the T allele frequency is between 0.1617 and 0.2663; then, it would be expected that annually around 351,514 and 953,399 pigs would be homozygotes TT. Moreover, and if we regard an homozygote mortality estimated of 43.58%, then we may expect that between 153,005 and 414,992 pigs could die in some point prior to the sacrifice due to the porcine stress syndrome. Of course, the later the loss within the productive process, the greater the economic harm. However, considering that the investment cost for each pig that reaches the slaughterhouse is about \$100.00 dollars, the economic losses caused by porcine stress syndrome can be estimated at 15.3 to 41.5 million dollars per year. Obviously, these figures may be even higher if we consider the losses caused by the PSE.

The samples that we studied were obtained from municipal slaughterhouses who process pigs from at least 17 different Mexican states. Although this is a preliminary study, it offers the first estimation of the prevalence of the RYR T 1843 C mutation in commercial porcine populations of Mexico. It will be important further studies in larger samples and in breeding populations.

Conclusions

Considering the high incidence found in Mexican commercial pig populations, and its association with PSS and PSE, the decision to maintain or eliminate the RYR mutant allele is a fundamental issue to be raised in the Mexican porcine industry. It seems that in most countries the current recommendation is the systematic elimination of the T allele from pig populations. If Mexican porcine industry follows the same trend, then it will have to be establish genetic services to identify carrier animals, as well genetic controls for the imported and locally commercialized breeding animals. This eventually will develop better surveillance and quality control systems of the porcine germoplasm, leading to few economic losses for the local porcine industry.

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Tables

Table 1. Observed and expected absolute and relative genotypic frequencies of the ryanodine receptor alleles in Mexican commercial pigs.

	CC		CT		TT		Total
	N	%	N	%	N	%	
Observed	277	59.7	175	37.7	12	2.6	464
Expected	286.75	61.8	155.90	33.6	18.5	4.6	464

N= Number of individuals registered with each genotype.

%= percentage of individuals identified for each genotype.

Table 2. Allelic frequencies for the ryanodine receptor gene, observed heterozygosity (Ho), expected heterozygosity (Hs) and fixation index (Fis) estimated in the porcine population.

C	T	s.e.	Ho	Hs	Fis	χ^2
0.786	0.214	0.0267	0.377	0.337	-0.119	6.62*

C= normal allele. T= mutant allele. s.e.= Standard error. χ^2 = Chi-square values.

*=P<0.05. ç

COMPARISON OF HYDROXYNONENAL-INDUCED REDOX INSTABILITY OF PORCINE AND BOVINE MYOGLOBINS

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Key Words: Myoglobin; Lipid oxidation; HNE

Introduction

Lipid oxidation generates a variety of secondary oxidation products, for example – aldehydes, which attack other biological molecules in the cellular systems. These secondary products are responsible for off-flavors and off-odors associated with rancidity in muscle foods. The aldehyde products, when compared to free radicals, are more stable and readily diffuse into the cellular environment where they can exert toxicological effects (Esterbauer et al., 1991). Several aldehydes are formed as a result of meat lipid oxidation, and these can enhance oxidation of oxymyoglobin (OxyMb) (Chan et al., 1997). 4-Hydroxynonenal (HNE), an α , β -unsaturated aldehyde formed by oxidation of ω -6 unsaturated fatty acids (Schneider et al., 2001), is reactive towards proteins (Sakai et al., 1995) and has substantial toxicological and biological activity (Esterbauer et al., 1991). HNE has been detected in meat and was suggested to be used as a reliable marker to assess the quality of muscle foods containing higher amount of polyunsaturated fatty acids (Sakai et al., 1995). The concentration of HNE in beef and pork was reported to be approximately 0.15 mM (Sakai et al., 1995). HNE accelerated equine OxyMb oxidation in vitro, and covalent modification of equine Mb by HNE was demonstrated by Faustman et al. (1999).

Lipid oxidation and subsequent discoloration of meat can be minimized by use of antioxidants in animal feeds. Specifically, vitamin E (α -tocopherol) protects highly oxidizable polyunsaturated fatty acids from peroxidation by reactive oxygen species and free radicals (Burtiss & Diplock, 1988). Supranutritional supplementation of α -tocopherol in the finishing diet of cattle increased color and lipid stability of beef obtained from these animals (Faustman et al., 1989). However, in vitamin E supplemented pork even though lipid oxidation was reduced significantly, a color stabilizing effect was not readily observable (Lanari et al., 1995; Cannon et al., 1996; Jensen et al., 1997; Houben et al., 1998). Interestingly, porcine muscle generally has a greater proportion of unsaturated fatty acids than beef and it would be reasonable to hypothesize that pork lipids would undergo oxidation more readily and produce more secondary products than beef. In turn, this would be expected to result in decreased OxyMb (color) stability in pork, which would be prevented by α -tocopherol. However, findings that pork did not demonstrate improved Mb redox stability from α -tocopherol supplementation led us to consider the

possibility that pork Mb differs from beef Mb relative to its susceptibility to lipid oxidation.

Objectives

The present study was carried out to compare HNE-induced redox instability in porcine and bovine Mb at storage temperatures and pH values typical of meat.

Methodology

Oxymyoglobin Preparation: Myoglobin was isolated from fresh cardiac muscle obtained from a local slaughterhouse, and OxyMb was prepared as outlined by Faustman and Phillips (2001). Briefly, a tissue-free supernatant was obtained from homogenized cardiac muscle, filtered through cheesecloth and subjected to ammonium sulfate fractionation. Following dialysis to remove ammonium sulfate, gel permeation chromatography was used to further purify Mb. To ensure 100% starting OxyMb the protein was treated by dithionite-mediated reduction and residual dithionite was removed by dialysis (3 x 40 volumes) against 50 mM sodium citrate (pH 5.6) buffer.

Reaction with HNE: OxyMb was incubated with HNE (Porcine OxyMb 0.075 mM + HNE 0.5 mM; Bovine OxyMb 0.15 mM + HNE 1.0 mM) at 4°C and pH 5.6. For each species, the concentrations of OxyMb were adjusted to simulate those in beef and pork muscles. However the molar ratio of Mb: HNE was maintained at 1:7 for both species. Controls consisted of OxyMb plus a volume of ethanol equivalent to that used to deliver the aldehyde to treatment mixtures. Samples (0.5 mL) were removed from the reaction assays at 0, 24 and 48 h; passed through a PD-10 desalting column to remove unreacted HNE and frozen at -80 °C for subsequent analysis.

Matrix Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS) of Mb: HNE adducts: Native and HNE treated myoglobins were analyzed by MALDI-TOF MS to detect changes in the total mass resulting from HNE adduction. Briefly, 1µL sample was mixed with 1µL freshly prepared 1% solution of sinapinic acid in acetonitrile: 0.1% aqueous trifluoroacetic acid (60:40 vol / vol); 0.5µL mixture was spotted on the MALDI target plate, spread uniformly and allowed to dry. The spots were treated with 1µL 0.1% aqueous trifluoroacetic acid to remove buffer salts, prior to MALDI-TOF MS analysis. Protein molecular ions were analyzed in a linear, positive ion mode using a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA) set at an acceleration voltage of 25 KV. Each spot was analyzed a minimum of 3 times, accumulating spectra composed of 250 laser shots per analysis. The resulting spectra were analyzed by Data Explorer (Applied Biosystems, Foster City, CA), noise-smoothed, baseline-corrected and mass-calibrated using an external standard of equine apomyoglobin.

Results & Discussion

MALDI-TOF analysis of the HNE adducted myoglobins revealed that prior to incubation the primary peaks present were equivalent to the mass of porcine Mb (16954 Da) and bovine Mb (16940). However, following 48 h incubation with HNE, a relatively small peak of mass 17112 appeared in addition to the native porcine Mb (Fig. 1). This corresponded to a mono-adduct of HNE with porcine Mb and was 158 Daltons greater than the mass of porcine Mb, a mass shift equivalent to the molecular weight of HNE. In bovine samples, a mono-adduct peak (17097) was prominent after 24 h incubation and a di-adduct peak (17254) apparent at 24 h, was prominent by 48 h incubation. The unadducted Mb, mono-adduct and di-adduct were separated by a mass shift of 157 Daltons and these results indicated that HNE adducts were formed by Michael addition (Fig. 2). If the adduct had resulted from Schiff base formation, a molecular mass addition of 140 Da would have been expected as one mole of water is lost per mole of HNE: Mb in the reaction. After 48 h incubation with HNE, porcine Mb formed only mono-adducts and the abundance of mono-adduct was considerably less than unadducted Mb (Fig. 1). Whereas in bovine Mb, mono- and di-adducts were present after 48 h incubation with HNE, the intensity of the mono-adduct peak was similar to that of unadducted Mb indicating its similar relative abundance (Fig. 2). These results are similar to those observed by Phillips et al. (2001) who identified the formation of up to three covalently bound HNE molecules per bovine Mb molecule following incubation at pH 7.4 and 37°C.

Conclusions

The results of the present study suggested that bovine Mb is more susceptible to nucleophilic attack by HNE and subsequent adduction than porcine Mb at typical meat storage conditions. This could explain the relatively greater apparent stability of pork color in the presence of lipid oxidation when compared to beef. Work is underway to further characterize the specific sites of adduction on porcine myoglobin.

Figures

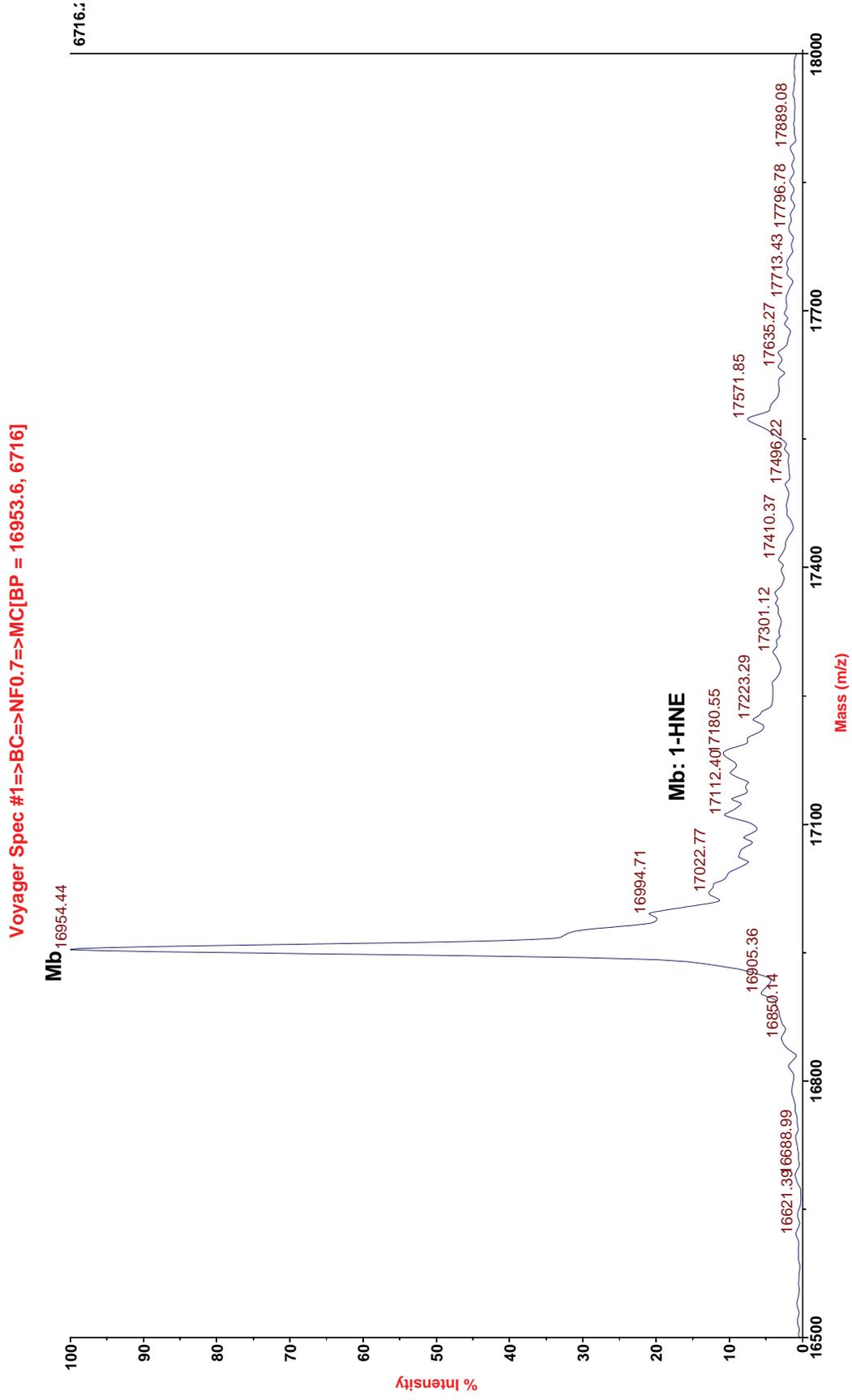


Fig. 1. MALDI-TOF MS spectra of porcine oxyhemoglobin (0.075 mM) following reaction with HNE (0.5 mM) at pH 5.6 and 4°C for 48 h.

Voyager Spec #1=>BC=>NF0.7=>MC[BP = 16938.1, 58156]

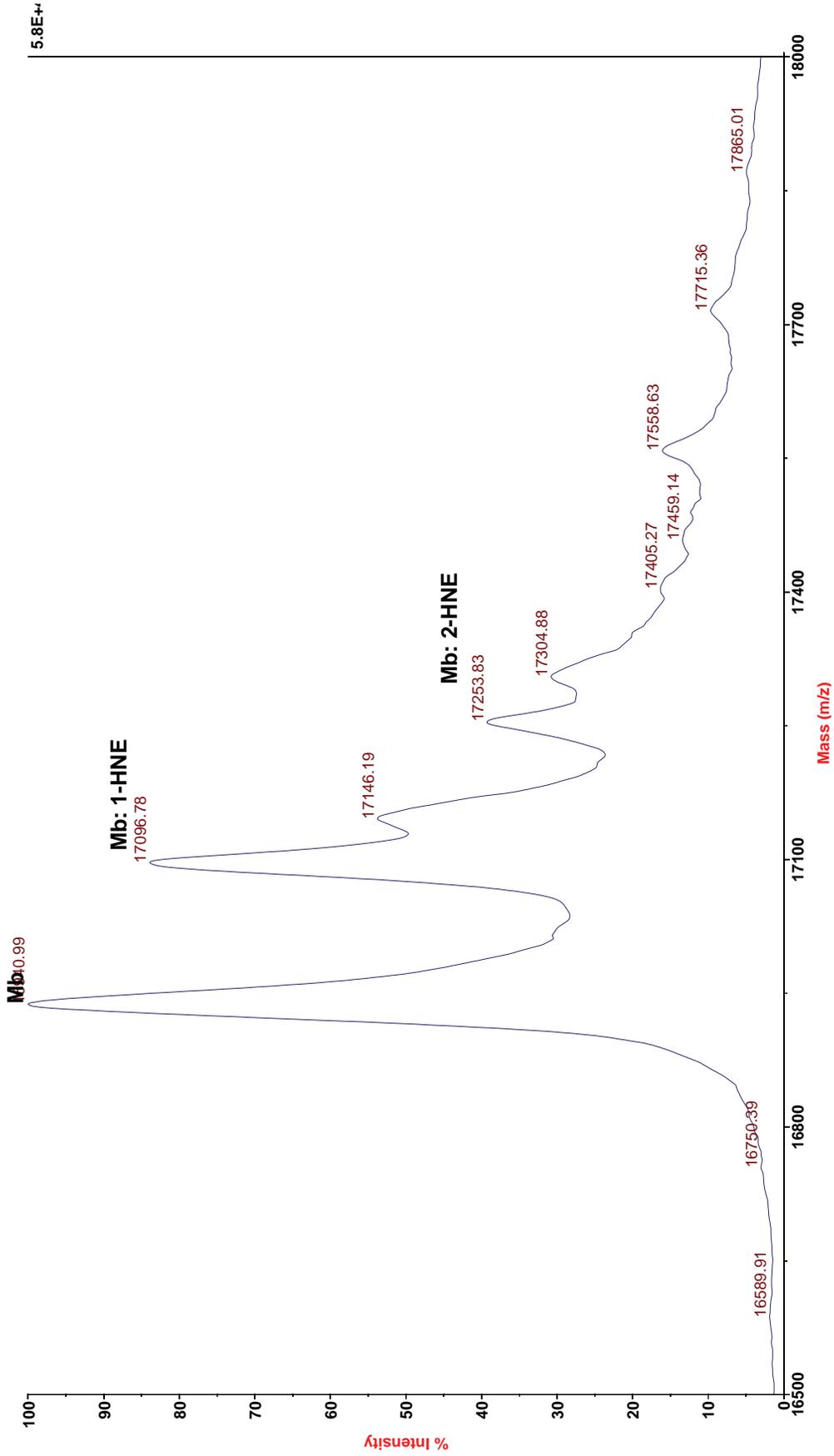


Fig. 2. MALDI-TOF MS spectra of bovine oxymyoglobin (0.15 mM) following reaction with HNE (1.0 mM) at pH 5.6 and 4°C for 48 h.

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PROTEIN EXPRESSION PROFILING OF SKELETAL MUSCLE THROUGH 2-DIMENSIONAL GEL ELECTROPHORESIS AND PROTEIN IDENTIFICATION BY MASS SPECTROMETRY

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Key Words: proteomics, 2-dimensional gel electrophoresis, skeletal muscle, MALDI-TOF, mass spectrometry

Introduction

Many biological treatments, such as nutrient restriction, application of growth hormones and knockout a specific gene, induce phenotype changes which involve the expression alternation of a large number of genes. Therefore, holistic methodologies which can monitor the expression of a large quantity of genes are needed. Currently, cDNA microarray is the main method used for this purpose in animal research (Reverter et al., 2003). One problem associated with gene expression array is that it is not directly related to proteins, due to the widely existed translation control and the modification of newly synthesized protein. And proteins are the final determinant of phenotype. Therefore, a gap exists for cDNA microarray to assess mRNA expression and phenotype, which necessitates a new method that can directly monitor the expression level of a large number of proteins (Pavlickova et al., 2004). This can be achieved by using protein expression profiling through employing 2-dimensional gel electrophoresis and mass spectrometry for protein identification (Pandey and Mann, 2000). Basically, tissue homogenates are subjected to 2-dimensional gel electrophoresis to separate proteins according to their pIs and molecular weights, which then is visualized and the density of spots is analyzed in computer softwares for the differential expression. Protein spots with differentiated expression among treatments can be identified by peptide fingerprint analysis and sequence tag (Sanchez et al., 2001). Further, by searching protein databases and genebanks, the expression control and function of many proteins can be obtained and thus facilitate discovery of mechanism associated with specific biological changes (Choudhary et al., 2001; Sanchez et al., 2001). Therefore, protein expression profiling is a potentially very useful method for muscle growth and nutrition studies.

Objectives

The objective of this study was developed a method for the profiling of protein expression in the fetal skeletal muscle using 2-D electrophoresis and mass spectrometry.

Methodology

Animals and skeletal muscle sample

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. On day 20 of gestation, ten ewes of mixed breed were assigned to either a diet meeting the requirement for maintenance for an early pregnant ewe (NRC requirements) (NRC, 1985), or a diet provide 50% of NRC requirements. The detailed description of the procedures has been published formerly (Vonnahme et al., 2003; Zhu et al., 2004). Ewes were euthanized on Day 80 of gestation and portion of fetal *longissimus dorsi* was immediately sampled, snap-frozen in liquid nitrogen and then stored at -80 °C until analyzed.

Two-dimensional electrophoresis

Muscle (0.1 g) was homogenized in a polytron homogenizer (7-mm dia. generator) with 400 µl of ice-cold buffer containing 137 mM NaCl, 50 mM HEPES, 1 mM MgCl₂, 1 mM CaCl₂, 1% NP-40, 10% glycerol, 2 mM PMSF, 10 mM sodium pyrophosphate, 2.5 mM EDTA, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 2 mM Na₃VO₄, 100 mM NaF, pH 7.4 (Zhu et al., 2004).

Muscle homogenate (40 µl) was transferred to 600 µl lysis buffer containing 8 M urea, 4% CHAPS and 20mM Tris HCl, pH7.4. The mixture was shaken at 4°Ñ for 1 h and centrifuged at 12,000 x g for 10min at 4°Ñ. The supernatant containing the soluble protein was collected and used to rehydrate commercially available immobilized-pHgradient (IPG) strips (pH 3-10, 1× 17 cm, Bio-Rad laboratories, Hercules, CA). Rehydration of strips was conducted under manufacture instruction and was followed by isoelectric focusing with a maximal voltage of 8,000V to reach 100,000 voltage-hr. Then the strip is equilibrated in solution 1 (6M urea, 2% SDS, 30% glycerol, 50 mM Tris-HCl pH 8.8, 1% DTT) and solution 2 (6M urea, 2% SDS, 30% glycerol, 50 mM Tris-HCl pH8.8, 2% iodoacetamide with 0.01% bromphenol blue) for 15 min respectively. The strip was loaded onto a pre-cast 5%-20%, 200 × 200 mm, gradient gels for second dimension separation. To fix the strip on top of gradient gel, low melt agarose solution (0.188M TrisHCl pH 8.8, 0.1% SDS, 9% glycerol, 1% agarose, 0.01% bromophenol blue) was heated to 65°Ñ and then pipetted onto the IPG strip, followed by second dimension SDS electrophoresis. Electrophoresis was conducted in electrophoresis solution (25mM Tris Base, 192mM glycine, 1g/LSDS) at 5°Ñ, with 10 W/gel. After the front dye line arrived at the end of gel, the gels were removed and fixed (40% ethanol, 10% acetic acid) before being stained with Coomassie blue. After staining, gels were scanned by an Image Scanner II (Amershan Biosciences, Piscapaway, NJ)(Arthur et al., 2002).

Quantitative analysis of protein expression and in-gel trypsin digestion

Image Master (Amershan Biosciences) software was used for matching and quantitative analysis of the protein spots on the gels according to manufacture instruction. Proteins with differential expression (P<0.05) were selected for identification by a

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF). Briefly, protein spots with differential expression were excised, cut into 1 mm³ pieces, and washed two more times with 50% acetonitrile, 50 mM ammonium bicarbonate for 15 min with gentle shaking or until the Coomassie dye has been completely removed. Gel pieces were dehydrated in 100% acetonitrile for 5 min and incubated in a solution containing 50 mM ammonium bicarbonate and 10 mM dithiothreitol for 30 min at 56 °C, and then incubated in another solution containing 50 mM ammonium bicarbonate and 55 mM iodoacetamide (freshly made) for 30 min in the dark. The gel pieces were washed with acetonitrile, air dried, and rehydrated with 20µg/ml trypsin (sequencing grade, Promega, Madison, WI) in 50 mM ammonium bicarbonate. Then, rehydrated gel pieces with trypsin were incubated at 37 °C overnight. Following centrifugation at 12,000 x g for 5 min, supernatant (containing tryptic peptides) was transferred to sterile centrifuge tube. An aliquot of extraction solution (25-50µl, composing 60% acetonitrile and 1%TFA) was added to gel pieces and agitated gently by vortexing at lowest setting for 10 min. Repeated the above step once and supernatants were pooled together. Then, supernatants were dried under vacuum and 5 µl of resuspension solution (50%acetonitrile, 0.1%TFA) was added to each tube to resolve peptides (Arthur et al., 2002).

Mass Spectrometry and protein identification

Peptides were mixed with an equal volume of 10 mg/ml α -cyano-4-hydroxycinnamic acid in 65% acetonitrile/0.3% trifluoroacetic acid and applied to the steel plate for MALDI-TOF peptide fingerprinting analysis. The instrument (Voyager DE-STR, Applied Biosystems, Foster City, CA) was set at a positive ion reflector mode, and the laser strength and voltage were optimized to obtain the highest signal:noise ratio.

Protein identification was done by searching against MSDB and NCBI protein database through the Mascot Peptide Mass Fingerprint software which is available free for academic use at website: <http://www.matrixscience.com/>. The parameters for searching were as following: the fixed carbamidomethyl modification was chosen; allow up to 1 missed cleavage; and peptide tolerance was set at 1.0 Dalton (Kwon et al., 2003; Vitorino et al., 2004).

Statistical analyses

The density of spots was analyzed using GLM (General Linear Model of Statistical Analysis System, SAS, 2000). The differences in the mean values were compared by the Tukey's multiple comparison, and mean values and standard errors of means (SEM) were reported.

Results & Discussion

Two-dimensional gel electrophoresis provides a powerful tool for the analysis of protein expression in large scale. The 2-dimensional gel electrophoresis pictures of fetal skeletal muscle from control and nutrient restriction ewes are shown in Figure 1. A large number of proteins were clearly separated. Proteins spots on those pictures were automatically identified by using the Image Master software. Around 1,200 spots were

identified for each gel. Those identified spots were auto-matched by the software and the density of each spot was quantified. Then, the quantitative data of all protein spots were subjected to statistical analysis by SAS. Altogether, there are 36 spots with differential expression. Of them, 14 spots were excised for MALDI-TOF analysis. Proteins in those spots were hydrolyzed by trypsin and peptides were eluted. Peptides were subjected to MALDI-TOF analysis. About 200 to 500 peptide peaks were identified in MALDI-TOF spectra, which was used for Peptide Mass Fingerprint by online submission using Mascot software (Arthur et al., 2002; Chamrad et al., 2004; Vitorino et al., 2004). The proteins of eleven spots were tentatively identified (Table 2), which needs to be further confirmed by sequence tag using MS/MS tandem mass spectrometry or immunoblotting (Vitorino et al., 2004).

Unlike cDNA array, one problem associated with protein profiling is the difficulty of protein identification (Kwon et al., 2003). Due to its cheap and relatively fast measurements, Peptide Mass Fingerprint is the most frequently used method. However, the confidence for the identification is usually low and thus further confirmation is frequently needed (Vitorino et al., 2004). The MS/MS tandem mass spectrometry can be used to sequence a short stretch of peptide, which is called sequence tag and can be used to blast against protein database to find matched proteins with high confidence. However, it is relatively expensive. Both MALDI-TOF and MS/MS tandem mass spectrometry are available in proteomic centers of most major universities. It is a good choice to send protein spots to these centers for identification.

Another problem associated with protein profiling is its sensitivity. Due to the presence of large quantity of proteins in tissue, lots of proteins can not be detected by using tissue homogenate. To solve this problem, interested proteins need to be enriched first. For example, if the interested proteins are located inside nuclei, those proteins can be enriched by separating nuclei and then used for 2-dimensional gel electrophoresis (Escobar et al., 2005). In this way, the interference from the large quantities of proteins in cytoplasm can be removed. Since silver staining can improve the sensitivity by around 50 times compared to Coomassie blue staining, using silver staining can improve detection sensitivity. However, from our experience, silver staining usually has a higher background compared to Coomassie blue staining and the linearity of spot density to protein quantity is not good, plus a much complicated procedure involved in silver staining, thus we recommend Coomassie blue staining instead of silver staining.

Conclusions

In skeletal muscle, proteins with differential expression can be identified by using 2-dimensional gel electrophoresis plus mass spectrometry, which provides a potentially very powerful tool for skeletal growth, meat quality and nutrition studies.

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Tables and Figures

Table 1. Tentative identification of selected protein spots by MALDI-TOF

Protein name	pI	MW (kD)	Con*	NR*	SEM	P
Parvalbumin alpha	4.82	12	4.96	4.36	0.18	0.048
Cytochrome C oxidase	5.72	14	0.18	0.10	0.03	0.050
Superoxide dismutase	7.13	21	2.92	3.62	0.26	0.093
Apolipoprotein	5.23	22	0.56	0.28	0.08	0.043
Glutathione peroxidase	6.12	25	0.24	0.13	0.02	0.006
Enolase	5.91	47	1.18	0.85	0.13	0.100
Pyruvate kinase isozyme	6.81	53	3.63	1.04	0.50	0.007
Myosin-binding protein H	5.98	64	0.38	0.30	0.02	0.021
Albumin	6.07	76	0.52	0.19	0.08	0.024
Heat shock protein	5.25	89	0.14	0.08	0.02	0.084
Elongation factor 2	6.79	94	3.48	1.57	0.49	0.025

*Values were calculated based on the spot density and was arbitrary.

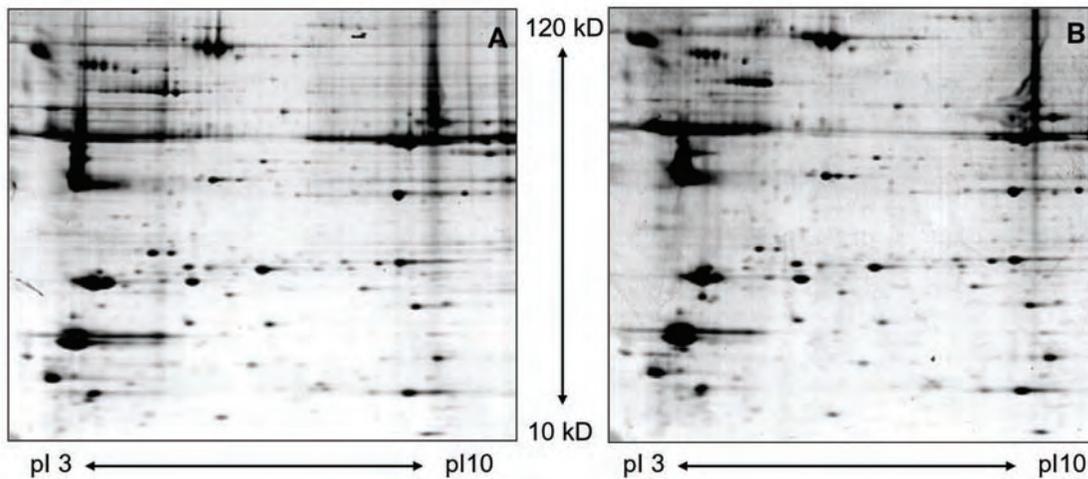


Figure 1. 2-Dimensional gel electrophoresis pictures of fetal skeletal muscle. Panel A: fetal skeletal muscle from Con ewes; Panel B: fetal skeletal muscle from NR ewes.

PURIFICATION OF PHOSPHOLIPASE A₂ FROM PORCINE SKELETAL MUSCLE

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Key Words: porcine skeletal muscle, phospholipase A₂, purification

Introduction

An intense lipolysis was observed during the processing of dry-cured ham. There was a close relationship between the decrease of phospholipids and the accumulation of free fatty acid (Buscaihon, Gandemer & Monin, 1994; Motilva, Toldra, Nieto & Flores, 1994). These observations indicate that phospholipases A plays an important role in lipolysis of dry-cured hams. The main activities are related to basic phospholipases A (Alasnier & Gandemer, 2000). Phospholipase A₂ has been mainly studied on various snake and bee venoms, and also mammalian pancreas and heart, because phospholipase A₂ plays a central role in diverse cellular processes including phospholipids digestion and metabolism, host defense and signal transduction (Kathryn et al., 1998). However, data on phospholipases A in skeletal muscle is limited. Only two studies were devoted to phospholipase A activity in mammalian skeletal muscles, in which phospholipase A activities were compared in the extracted solutions from different metabolic types of muscles (Hernandez, Navarro & Toldra, 1998; Alasnier & Gandemer, 2000). Up to now, pure phospholipase A has not yet been obtained from porcine skeletal muscles.

Objectives

The objective of this study was to purify phospholipase A₂ from porcine skeletal muscle for better understanding the nature of phospholipases A and for further understanding the lipolysis mechanism of the dry-cured ham.

Methodology

Preparation of muscle supernatant

Biceps femoris muscle from pig was homogenized (3×10 s spells at 20,000 rpm with ice cooling) in 4 vols of 0.1M Tris-HCl buffer, pH 8.5, containing 0.1mM PMSF. The homogenate was stirred for 30 min at 4 °C and centrifuged for 20 min at 10,000×g. The supernatant was the source of the crude phospholipase.

Purification of the enzyme

The supernatant (10 ml) was applied to a 2.6×20 cm DEAE-Sephacel column equilibrated previously with 50 mM Tris-HCl buffer, pH 8.5 (buffer I). After washing with 200 ml buffer at a flow rate of 1.5 ml/min, the column was eluted with the same buffer containing 1M NaCl. The fractions containing enzyme activity were collected, concentrated and dialyzed for 12h against 20 mM Tris-HCl buffer, pH 8.0 (buffer II). The dialyzed enzyme was applied to a Blue -sepharose 6 F.F. column (1.0×8 cm) equilibrated previously with buffer II. After washing with the same buffer at a flow rate of 0.5 ml/min, the column was eluted with the same buffer containing 1M NaCl. The fractions with enzyme activity were pooled, concentrated and dialyzed against 20 mM sodium phosphate buffer (pH 6.8) (buffer III). The resulting sample was applied onto a Heparin-sepharose CL-6B column (1.0×8 cm) equilibrated previously with buffer III. The unadsorbed material was washed out with 20 ml buffer III at a flow rate of 0.5 ml/min, and the adsorbed material was eluted with 20 ml buffer III containing 150 mM NaCl, then, eluted with 1 M NaCl and 3 mg/ml heparin in buffer III.

Assay of phospholipase A₂ activity

A radiochemical assay was used to monitor phospholipase A₂ activity in fractions from chromatography (Lumb et al., 1990; Alasnier et al., 2000). The substrate L- α -1-palmitoyl-2-[1-¹⁴C] arachidonyl sn-glycerophatidylcholine (Perkin Elmer Life Sciences, specific activity 1.89 GBq mmol⁻¹) together with unlabelled L- α -1-palmitoyl-2-arachidonyl sn-glycerophatidylcholine (Sigma Chemical Co.) was solubilised in toluene:ethanol (1:1, v/v). The concentration of substrate was adjusted to 250 μ mol/l phosphatidylcholine and 300 MBq l⁻¹. For the majority of studies 200 μ l enzyme was incubated with 5 μ l substrate at 40 °C in a shaking water bath for 20 min. The reaction was terminated by the addition of 2 ml fatty acid extraction mixture (toluene: chloroform: methanol 1.0: 0.5: 1.2 v: v: v), followed by 40 μ l of 1.0 mol/l NaOH to ensure alkaline conditions. After vigorous vortexing the samples were centrifuged (1000×g for 10 min) to form the aqueous and organic phases. 200 μ l upper aqueous phase was extracted to a scintillation vial containing 4 ml liquid scintillation cocktail (optiphase 'supermix', Perkin Elmer Life Sciences), radioactivity was counted in a liquid scintillator (LS 6000 IC, Beckman). Each sample was measured in triplicate. All results were corrected for the blank. Phospholipase A₂ activity was expressed as nmol fatty acids released per hour per mg protein (nmol h⁻¹ mg⁻¹ protein).

Results & Discussion

On a DEAE-Sephacel column, two active peaks were eluted from the adsorbed and unadsorbed fractions, respectively (Fig.1). These observations suggest that there are at least two types of phospholipase A₂ in porcine skeletal muscle. The second active protein peak was pooled and further purified on Blue-sepharose 6 F.F. and Heparin-sepharose CL-6 B affinity chromatography. The elution profiles are shown in Fig 2 and 3. The fractions with phospholipase A₂ activity on Heparin-sepharose CL-6 B affinity chromatography were pooled and an aliquot subjected to SDS-PAGE. A single protein band was observed (Fig. 4) and the molecular mass of the enzyme was estimated to be 66

kDa under conditions with β -mercaptoethanol. The results of purification of phospholipase A₂ from porcine skeletal muscle are summarized in Table 1. The enzyme was finally purified about 226-fold with 7.6% of recovery.

The purification of phospholipase A₂ in the first active peak of DEAE- Sephacel column and the characterization of properties of the purified enzyme are ongoing.

Conclusions

A phospholipase A₂ from porcine skeletal muscle was purified to electrophoretic homogeneity.

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Tables and Figures

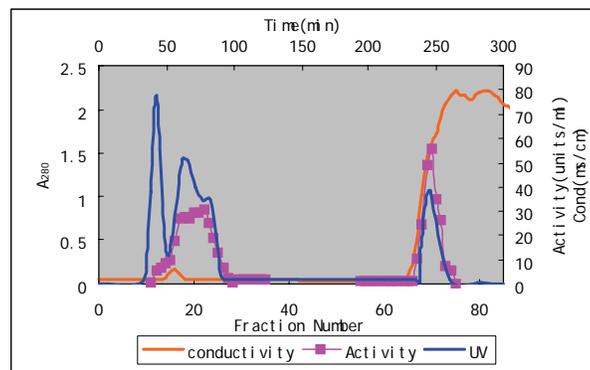


Fig.1 DEAE-Sephacel chromatography of the muscle supernatant

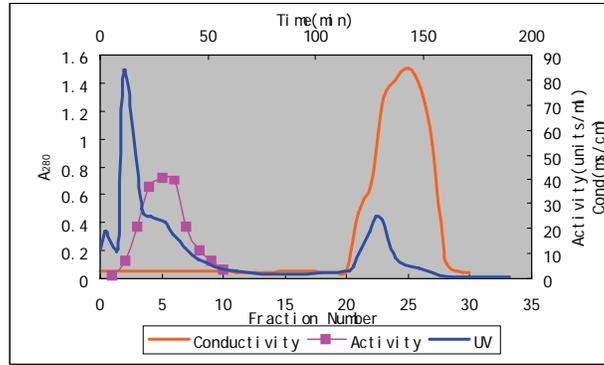


Fig.2 Blue-sepharose 6 F. F. chromatography of the active fractions obtained from DEAE-Sephacel chromatography (the second active peak in Fig.1)

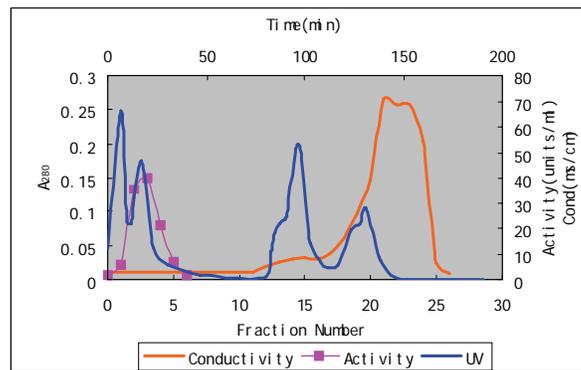


Fig.3 Heparin-sepharose CL-6 B chromatography of the active fractions obtained from Blue-sepharose 6 F. F. chromatography

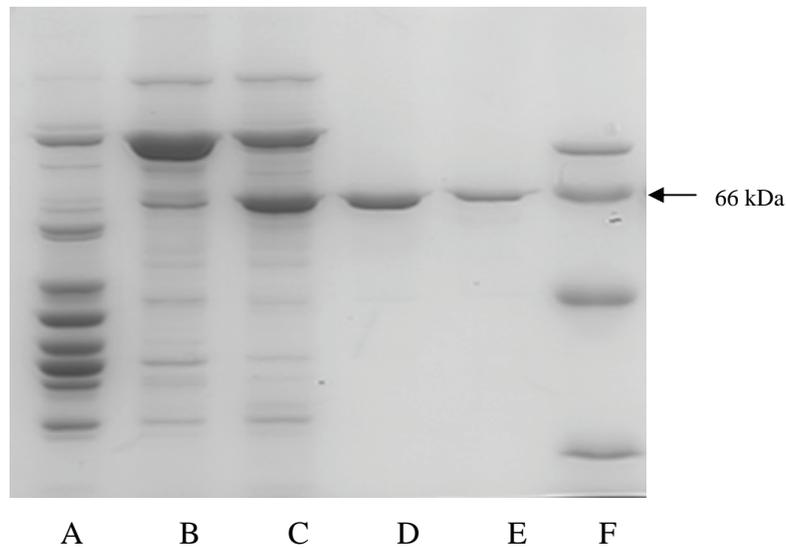


Fig.4 Profiles of SDS PAGE: (A) muscle supernatant; (B) the active peak 2 on DEAE-Sephacel chromatography; (C) the active fraction of Blue-sepharose 6 F.F. chromatography; (D-E) the active fraction of Heparin-sepharose CL-6 B chromatography, the purified phospholipase A₂; (F) low MW standard. The concentration of acrylamide was 12%.

Table 1. Summary of the purification of phospholipase A₂ from porcine skeletal muscle

procedure	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude extract	3550	29110	8.2	1	100
DEAE-Sephacel	410	16605	40.5	4.94	57.0
Blue-sepharose 6 F.F	30	10836	361.2	44.05	37.2
Heparin-sepharose CL-6 B	1.2	2223.84	1853.2	226.00	7.64

One unit of enzyme activity was defined as the amount of enzyme releasing 1nmol fatty acids per hour per mg protein.

COULD THE RELATIVELY LOW ACTIVITY OF GLYCOGEN DEBRANCHING ENZYME ATTENUATE A SUDDEN PH FALL?

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Key Words: glycogen debranching enzyme, phosphorylase, oxidative and glycolytic muscles, pig, cattle

Introduction

The rate and extent of pH decrease in muscles *post mortem* are the most important factors determining meat quality (1, 2). The pH decrease is a result of efforts to maintain the ATP level constant by the anaerobic breakdown of glycogen to lactate. Normally the ATP turnover rate and thus *post mortem* pH decrease are faster in porcine than in bovine muscles and in fast twitch and glycolytic (FG) than in slow twitch and oxidative (SO) (3). Furthermore, the time of onset of anaerobic glycolysis depends on the amounts of myoglobin-bound oxygen in muscles (4), which is generally higher in bovine than in porcine muscles (3).

After slaughter, pH decreases to the ultimate values of around 5.4-6.3, depending on the metabolic and contractile type of a muscle (1, 5), if the glycogen content in muscles before slaughter is not a limiting factor (6). However, it has been showed that some glycogen always remains in bovine muscles after the ultimate pH had been attained (7). Several factors, including glycogen debranching enzyme (GDE), have been suggested to stop *post mortem* glycolysis.

GDE together with glycogen phosphorylase (PHOS) are responsible of the complete degradation of glycogen (9). Glycogen molecules consist of branched glucose chains (10). The GDE breaks down the branching points of glycogen (so-called limit dextrin state), enabling the further action of PHOS (9). The activity of PHOS has been studied quite extensively in meat production animals. Less attention, however, has been paid to GDE despite suggestions of its role as a rate limiting factor in *post mortem* glycogenolysis (8, 11). Hence, the GDE may have an influence on formation of PSE meat, on tenderness and on shelf-life of meat.

Objectives

The aim was to study the activity of GDE and the relationship between the activities of PHOS and GDE in porcine and in bovine muscles, which differ in rate of contraction (slow and fast) as well as in aerobic capacity (oxidative and glycolytic).

Methodology

Muscle samples (fast twitch and glycolytic (FG): *longissimus dorsi*; slow twitch and oxidative (SO): *masseter*) from 27 pigs and from 19 bovines were obtained from a commercial abattoir about 35 min after stunning. The samples were cut into small pieces and frozen and stored in liquid nitrogen.

The activity of GDE was determined using the method of Nelson et al. (12) with minor modifications (8). The method is based on monitoring the shift in the absorbance due to conversion of limit dextrin to glycogen by GDE. The GDE activity measurements were made in triplicate and the activity was calculated from the slope of the linear portion of the absorbance curve. The reaction times used were 1, 1.5, 2.5 min for porcine *longissimus dorsi* muscle, 1, 2.5, 4 min for porcine *masseter* muscle and 1, 3, 6 min for bovine muscles. In addition, the absorption spectra between 375 nm and 800 nm of the method blank and the reaction mixtures were obtained and compared to each other to ensure the conversion of limit dextrin to glycogen (results not shown).

Lactate content, glycogen content as total glucose content, and the activity of phosphorylase were measured as described Kylä-Puhju et al. (8). Glycolytic potential was calculated according to Monin et al. (17): glycolytic potential (mmol LA equiv./kg) = $2([\text{glycogen}] + [\text{glucose}] + [\text{glucose-6-phosphate}]) + [\text{lactate}]$.

The pH values were measured from meat extracts (1 g muscle + 10 ml 5 mM Na-Iodoacetic acid solution) using a Knick Portamess 752 pH-meter equipped with a Mettler-Toledo Inlab 427 electrode. Extracts were made from muscles which were frozen immediately after sampling (pH₃₅), and from samples which were frozen 24 h (porcine muscles) or 48 h (bovine muscles) *post mortem*. Two latter ones were designated the ultimate pH (pH_u). To perform the measurement, the frozen samples were homogenised in icy Na-Iodoacetic acid and all pH values were measured at room temperature.

Data analysis was conducted using SPSS 10.0 for Windows (13). The GLM procedure was applied when calculating the estimated marginal means for variables of porcine muscles. When the error variances were unequal, Dunnet's T3 test was used, otherwise Tukey's HSD test. Tukey's test was applied also when comparing porcine and bovine muscles. The differences between bovine muscles were tested using independent samples t-test. Pearson correlations were calculated between parameters of a given muscle.

Results & Discussion

The results of the present study indicated that porcine muscles have a higher capacity to degrade glycogen than bovine muscles. In particular the activity of GDE was higher in porcine muscles than in bovine muscles (Table 1). The activities of GDE and PHOS were higher in porcine than in bovine *longissimus dorsi* muscle. In porcine *masseter* muscle the activity of GDE was higher and there was also a tendency ($p=0.0588$) of higher activity of PHOS than in bovine *masseter* muscle. The activity of glycolytic enzymes together with the aerobic capacity and the rate of ATP turnover have an effect on the rate of *post mortem* glycolysis, which is frequently higher in porcine than in bovine muscles (3). In the present study, the lower rate of anaerobic glycolysis in the bovine *longissimus*

dorsi muscle shortly after slaughter was seen as a lower lactate content and higher pH at the sampling compared to the corresponding porcine muscle.

The activities of GDE and PHOS increased with the fast twitch and glycolytic character of a muscle of a given animal (Table 1). Tsutou et al. (14) obtained similar results with rabbit slow and fast muscles. Both in pigs and in cattle, the activity of GDE was about two times higher in FG *longissimus dorsi* muscles than in SO *masseter* muscles. However, there was even greater increase in the activity of PHOS, and thus the PHOS/GDE ratio was higher in FG muscles than in SO muscles. Ylä-Ajos et al. (15) obtained similar results with chicken FG breast muscle and SO leg muscle, however, in chicken the ratios were much higher than that found in the present study.

The lower PHOS/GDE ratios in SO muscles compared to FG muscles may indicate that glycogen degrading enzymes were more in balance in the SO muscles. Consequently, in SO muscles the degradation of glycogen may proceed without a delay caused by the low activity of GDE. Alternatively, the proportionally low activity of GDE in relation to the activity of PHOS in FG muscles may be a protective mechanism against a sudden pH decrease, as suggested for chicken muscles (15). FG muscles are capable of short-term strenuous contractile activity, but they fatigue quite easily (19). Thus, in strenuous physical stress, the high PHOS/GDE ratio in FG muscles enables a short burst of glycolysis, which leads to fast increase in H^+ content. The high buffering capacity of FG muscles (18, 20) protects these muscles against a sudden pH decrease, but the proportionally low activity of GDE compared to the activity of PHOS may be needed to further restrain the glycogenolysis. Since GDE and PHOS activities were not measured in same units the ratio does not quantify the real difference between the activities of these enzymes within a muscle. The ratios between muscles, however, are comparable.

The fast glycolysis in FG muscles was enabled by the high glycolytic potential (Table 1) in association with the high activity of glycogen degrading enzymes, allowing a rapid conversion of glycogen into lactate. However, rapid glycolysis is not essential for SO muscles. In these muscles aerobic energy production is preferred, because of better blood supply, higher myoglobin content, more mitochondria and a more active pathway from glycogen through the Krebs cycle to CO_2 and H_2O than in FG (3, 17, 18). Several papers have reported that enzymes in the pathway from glycogen to lactate are more active in FG than in SO muscles (17, 18, 19), however, until now the activity of GDE in the muscles of meat production animals has not been extensively studied.

The activity of GDE may play a role in controlling the rate of *post mortem* pH decrease. Although the activity of GDE did not correlate with the pH_{35} or with the pH_u for either porcine or bovine muscles, a negative relationship between the activity of GDE and pH_u was seen when the results for individual muscles of a given species were combined (Figure 1 a, b). The ultimate pH remained high in SO muscles where also the activity of GDE was low. However, one should bear in mind that there were differences also in glycogen content and in the activity of PHOS between individual muscles of a given animal and it is well known that particularly glycogen content has an influence on muscle pH_u . Although the activity of GDE may not have a critical role in normal *post mortem* reaction sequence, its role may be significant e.g. in a situation where muscle glycogen is low at slaughter and the carcass is chilled rapidly. When the glycogen content is low, muscles soon end up in a situation where glycogen limit dextrin has to be broken down by GDE to maintain the ATP level. At that moment the rate and continuation of the

pH decrease is determined by the activity of GDE rather than phosphorylase. However, the decrease in the activity of GDE is substantial when muscle temperature is lowered by rapid chilling (8). Furthermore, low activity of GDE may reduce the incidence of PSE meat by restraining fast glycolysis in porcine muscles, thus giving time to temperature decrease before reaching the critical pH.

Conclusions

In porcine muscles the activities of glycogen degrading enzymes, GDE and PHOS, are higher than in bovine muscles, thus providing a chance for rapid pH decrease in porcine muscles. The activities of both GDE and PHOS increase with the increasing fast twitch and glycolytic character of a muscle of a given animal, however, the increase in the activity of PHOS is more considerable. The relatively low activity of the GDE may restrict the rate of glycolysis in fast twitch muscles, and thus act as a protective mechanism towards a sudden pH decrease in living muscle.

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Tables and Figures

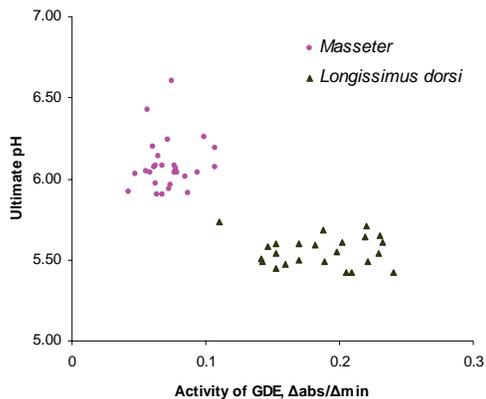


Figure 1a. The ultimate pH in relation to the activity of GDE in porcine muscles

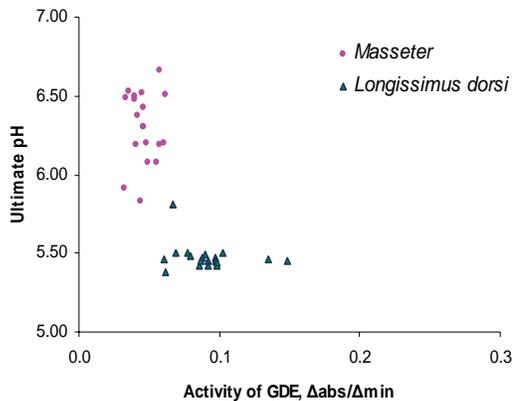


Figure 1b. The ultimate pH in relation to the activity of GDE in bovine muscles

Table 1. The activity of GDE ($\Delta\text{abs}/\Delta\text{min}$), the activity of PHOS (U/g muscle), the ratio between PHOS and GDE activities, pH_{35} and pH_u values in porcine and bovine muscles⁴

	Porcine muscles		S.E. ¹	p-val. ²	Bovine muscles		S.E. ¹	p-val. ²
	<i>longissimus dorsi</i> (n=27)	<i>masseter</i> (n=27)			<i>longissimus dorsi</i> (n=19)	<i>masseter</i> (n=19)		
GDE	a0.187	c0.073	0.006	<0.001	b0.091	d0.046	0.004	<0.001
PHOS ³	a12.6	3.2	0.3	<0.001	b8.1	1.3	0.3	<0.001
PHOS/GDE ³	a70	46	4	<0.001	b89	29	6	<0.001
Glycogen	a66.7	c51.4	3.2	0.022	b105.3	d31.5	3.3	<0.001
Lactate	a49.5	19.3	2.0	<0.001	b28.4	18.5	1.3	<0.001
GP	a182.9	c122.1	5.7	<0.001	b239.0	d81.5	6.3	<0.001
pH_{35}	a6.71	6.81	0.03	0.073	b6.97	6.79	0.03	<0.001
pH_u	5.56	c6.08	0.03	<0.001	5.47	d6.30	0.04	<0.001

¹S.E. = standard error of the mean, ²p-value for a difference between muscles of a given animal, ³for porcine muscles n=27, for bovine muscles n=12, ⁴Different letters within rows indicate significant differences (P<0.05) between porcine and bovine *longissimus dorsi* muscles (a, b) or between *masseter* muscles (c, d)

GLYCOGEN PHOSPHORYLASE DRIVES RAPID EARLY POSTMORTEM METABOLISM IN PORCINE MUSCLE

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Key Words: halothane, Rendement Napole, postmortem metabolism, meat quality

Introduction

Development of pork quality attributes is largely governed by the rate and extent of postmortem pH decline (Briskey, 1964, Sellier and Monin, 1994). Postmortem pH decline is largely dependent on the energy status of muscle at or around slaughter.

Two major genes, halothane (HAL) and Rendement Napole (RN), alter pH decline by causing a defect in Ca²⁺ regulation and glycogen storage, respectively. Halothane positive pigs (nn) exhibit increased sarcoplasmic Ca²⁺ concentration (Cheah and Cheah, 1976, Mickelson, et al., 1989), which sustains muscle contraction, increases ATP utilization and results in accelerated postmortem glycolysis. RN mutant pigs (RN-RN-), however, possess an increased capacity for extended pH decline and low ultimate pH, which is attributed to higher muscle glycogen levels (Monin and Sellier, 1985). These genes generate inferior pork quality through distinctly different mechanisms of post mortem metabolism, yet their ability to modulate metabolism when present together is not known.

Differences in the inherent properties of rate limiting enzymes would be expected to influence the rate and extent of anaerobic metabolism. Yet, enzyme capacity does not explain abnormal glycolysis (Allison, et al., 2003, Schwagele, et al., 1996). Modulators of rate limiting enzymes alter enzyme activity based on energy charge of the cell, and this affects flux through glycolysis and contributes to aberrant postmortem metabolism. Thus, inherent variation in metabolism may control glycolytic flux. Kastenschmidt et al. (1968) and Hammelman et al. (2003) showed disproportionate activity of glycogen phosphorylase in the first hour postmortem compared to subsequent time points in muscles undergoing normal rates of glycolysis, whereas phosphofructokinase appeared to be a more critical glycolytic control point in muscles undergoing a rapid rate of postmortem glycolysis.

Objectives

Objective: Utilize the HAL and RN genetic mutations to examine the effects of accelerated postmortem pH decline and elevated muscle glycogen, separately and in

combination, on the rate limiting reactions of glycolysis and their relationship to pork quality attributes.

Hypothesis: Different enzymes become rate limiting at different times during postmortem metabolism, and this contributes to pH decline and pork quality development.

Methodology

A heterozygous (Nn/RN-rn⁺) population was used to generate animals possessing all possible genotypes. HAL and RN genotype was determined using the polymerase chain reaction and restriction fragment length polymorphism technique outlined by O'Brien et al. (1993) and modified procedure of Meadus and MacInnis (2000), respectively. Animals homozygous for both genotypes (NN/rn⁺ rn⁺, n = 12; NN/RN-RN⁻, n=17, nn/rn⁺ rn⁺, n = 11; nn/RN-RN⁻, n = 16) were slaughtered and exsanguination was considered time = 0.

Longissimus muscle pH values were recorded adjacent to the last rib at 0, 15, and 45 min postmortem. Muscle samples for metabolite analysis were taken from the lumbar region of the *longissimus* at 0, 30, 60, 120 min and 24 h (1440 min). At 24 h postmortem, subjective color, marbling (NPPC, 2000) and firmness (NPPC, 1991) of the *longissimus* muscle were evaluated at the 10th rib, and ultimate pH was determined on the cut muscle surface. Objective color measurements (L*, a*, b*) were determined on one 2.54 cm chop using a Hunter Colorimeter, and water holding capacity was determined on an adjacent 2.54 cm chop using the drip loss method (Rasmussen and Stouffer, 1996). Muscle glucose, glucose 6-phosphate, glycogen and lactate concentrations were determined using enzyme analytical methods (Bergmeyer, 1974) modified to a 96-well configuration (Hammelman, et al., 2003). These metabolites were used to calculate glycolytic potential (GP) using the formula outlined by Monin and Sellier (1985): GP = 2(glucose + glucose 6-phosphate + glycogen) + (lactate). ATP and phosphocreatine levels were determined by a standard sequential enzymatic assay (Passonneau and Lowry, 1993). Data was analyzed using the PROC MIXED procedure of SAS, with the REPEATED statement for variables measured at more than one time point. Significance is represented at P < 0.05.

Results & Discussion

The higher drip loss and lower subjective firmness scores of RN mutants compared to RN normal genotype suggests the RN⁻ allele decreases water holding capacity. RN mutants also had lower marbling scores than the wild type. HAL mutants possessed inferior meat quality compared with the normal genotype, as evidenced by increased reflectance (L*), yellowness (b*) and drip loss values, as well as lower subjective color, firmness, and marbling scores. There were no HAL×RN genotype interactions for quality traits.

During postmortem metabolism, ATP must be generated in order to keep muscle in the relaxed state. Energy levels are first buffered by using phosphocreatine (PCr) to phosphorylate ADP to ATP. The HAL normal genotype had higher PCr concentrations

than mutant pigs at both time points. HAL normal animals maintained higher ATP concentrations than HAL mutants during the first 30 min postmortem.

The RN- allele increased GP in the *longissimus* muscle. RN mutants had higher glycogen concentrations compared to the wild type. The mutant HAL gene increased glycogen breakdown and lactate concentrations at 0, 30, 60 and 120 min postmortem (Figure 1). The rapid utilization of ATP and PCr, followed by increased rates of glycogen breakdown and lactate accumulation early postmortem, denotes the faster rate of glycolysis classically observed in HAL mutant animals. At 45 min, HAL mutants, regardless of RN genotype, possessed similar pH that was lower than HAL normal genotypes. These data imply that the higher glycogen content does not contribute to more rapid degradation of glycogen, greater lactate accumulation or lower pH at 45 min.

Glycogen debranching enzyme breaks the α -1,6 linkages of glycogen and releases free glucose, which accumulate in postmortem muscle. Glucose concentrations were increased by both mutant HAL and mutant RN genotypes (Figure 2). HAL mutants had elevated glucose concentrations at 30, 60 and 120 min and 24 h, supporting that glycogen debranching enzyme does not block rapid glycolysis and pH decline when the temperature is high. In contrast, RN mutant pigs had greater glucose concentrations at 120 min and 24 h.

Glycogen phosphorylase cleaves the outer chains of the glycogen molecules, generating glucose 1-phosphate which is isomerized to glucose 6-phosphate (G6P). HAL and RN genotype combination influenced G6P concentration over time (Figure 3). G6P concentrations in HAL mutants decreased during the first 30 min postmortem and remained at a similar level thereafter. The high rate of lactate formation in HAL mutants indicates high glycolytic flux during the first hour postmortem. Curiously, G6P concentrations in the HAL/RN mutant tended to increase in the first 30 min, and then decrease through 60 min. Despite the large difference in G6P between HAL mutant and HAL/RN mutant pigs at 30 min, the rate of lactate accumulation was similar. Thus, glycogen phosphorylase and debranching enzyme activity are capable of aggressive glycogenolysis to supply adequate G6P for the reactions of glycolysis. This suggests phosphofructokinase may be rate limiting during rapid early postmortem metabolism in HAL/RN mutants. Conversely, the high ATP levels and decreased energy demand of normal and RN mutant genotypes suggests that the decrease in G6P concentrations from 0 to 60 min is likely due to decreased glycogen phosphorylase activity.

Alternatively, during normal rates of glycolysis, greater glycogen degradation after 60 min corresponds with increased G6P accumulation. The accumulation of G6P after 60 min supports that glycogen phosphorylase activity was sufficient to meet demand, and that either phosphorylase activity was increased or phosphofructokinase activity was reduced. Acidic conditions and declining ATP concentrations after 60 min may compromise phosphofructokinase activity in muscle undergoing normal rates of glycolysis and prevent it from sustaining the glycolytic flux provided by phosphorylase.

Conclusions

Together, the HAL and RN genes maximize activation of glycogen phosphorylase by enhancing sarcoplasmic Ca^{2+} concentration and glycogen availability, and depleting ATP. The difference in G6P concentrations between HAL and HAL/RN mutants despite

high glycolytic flux indicates that phosphofructokinase may be rate limiting during aggressive early postmortem glycogenolysis. In contrast, decreased G6P concentrations in the first 60 min postmortem in muscle undergoing normal rates of glycolysis is likely due to high ATP levels and decreased energy demand, which would limit glycogen phosphorylase activity. The accumulation of glucose and glucose 6-phosphate in muscles with normal rates of glycolysis suggests phosphofructokinase is rate limiting to glycolysis after one hour postmortem. Therefore, different enzymes may be rate limiting at different times postmortem depending on the rate of metabolism. A greater understanding of glycolytic flux through phosphofructokinase is critical for minimizing adverse pork quality development.

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Tables and Figures

Figure 1. LS means of postmortem *longissimus* muscle lactate concentrations in halothane and RN genotypes.

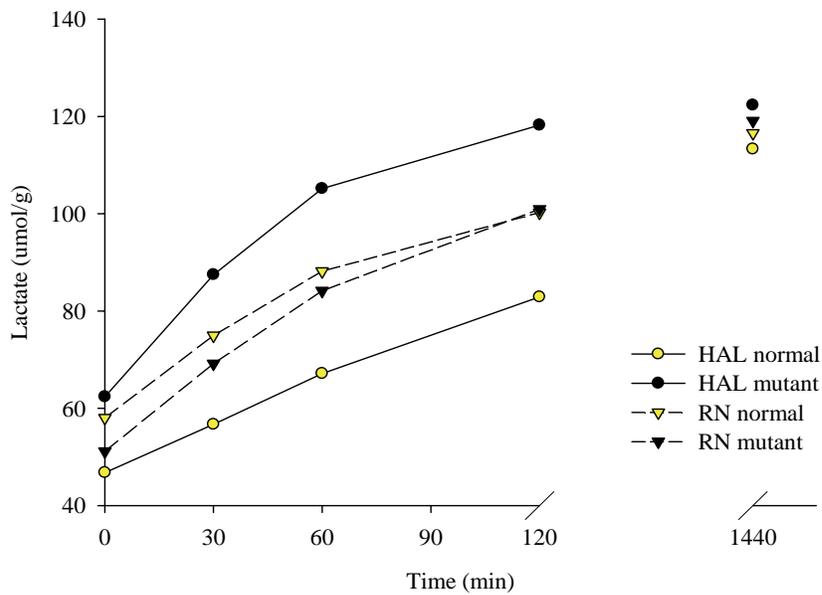


Figure 2. LS means of postmortem *longissimus* muscle glucose concentrations in halothane and RN genotypes.

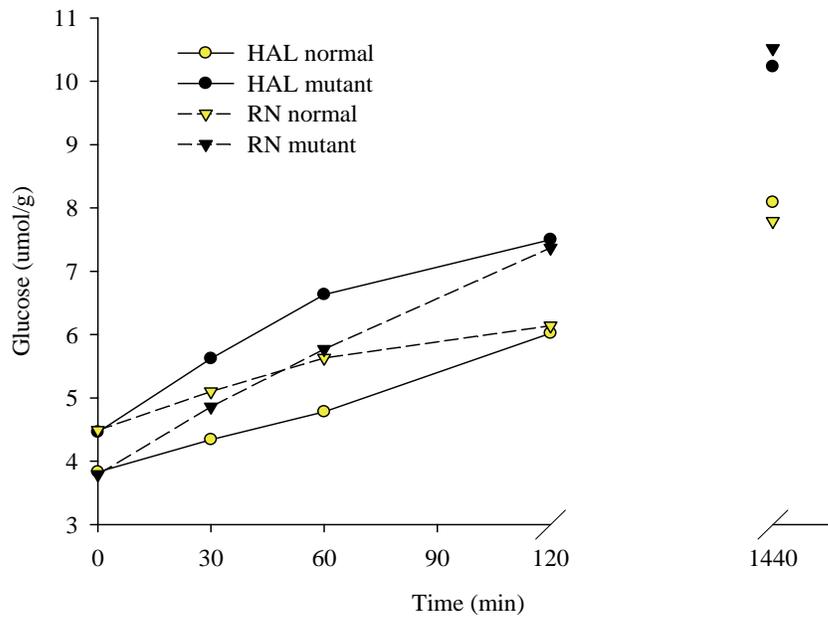
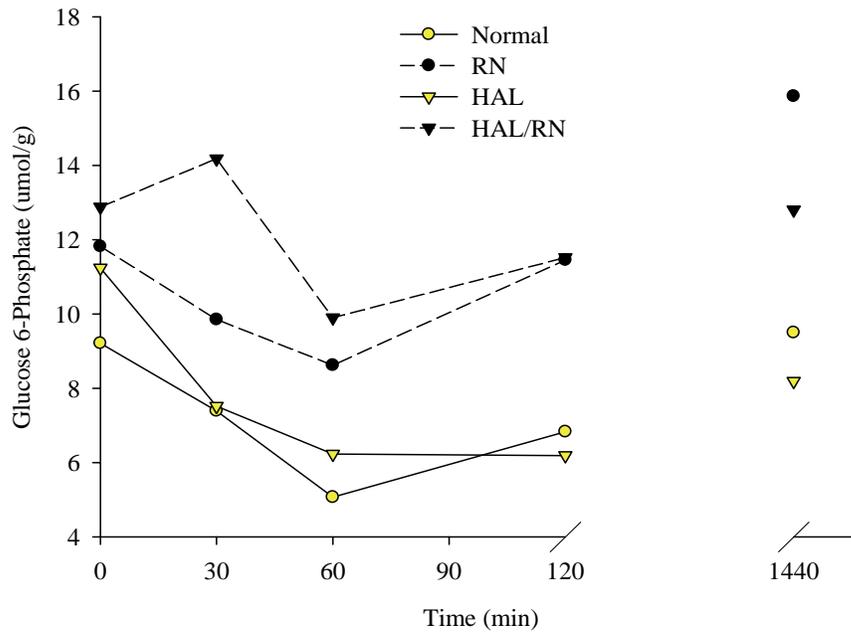


Figure 3. LS means of postmortem *longissimus* muscle glucose 6-phosphate concentrations in halothane and RN genotypes.



**PROTEOME ANALYSIS OF THE SARCOPLASMIC FRACTION OF PIG
SEMIMEMBRANOSUS MUSCLE: CORRELATION WITH MEAT L* VALUE**

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Key Words: proteomics, pig, semimembranosus, L*value

Introduction

Despite considerable achievements in the field of genetic improvement of pigs, the meat processing industry still suffers from a large variability in the quality of raw material. One obvious reason is that meat quality results not only from the animal genetic equipment but to the response of genes to external factors such as breeding and slaughter conditions, post-slaughter treatments etc. Thus, the traditional genetic approach, based on monitoring the expression of a limited number of candidate genes, does not completely take into account the complexity and multiplicity of interwoven biochemical mechanisms. Post-genomics tools (transcriptomics, proteomics) appear suitable to describe relationships between various metabolic pathways, thereby assisting in proposing mechanisms of meat quality development. For that particular purpose, as reported by Hamelin et al. (2004), it is considered appropriate to study specifically sarcoplasmic proteins, as they contain the majority of enzymes and regulators.

Proteomics has been successfully applied to describe *post mortem* modifications of pig muscle proteins (Lametsch et al., 2002; Morzel et al., 2004) or to finely characterize PSE zones in *semimembranosus* muscle (Laville et al., 2005). Other studies have investigated the correlation between proteins and fragments abundance with meat quality attributes such as texture (Lametsch et al., 2003) or L* value and drip loss (Hwang, 2004). Monitoring L* value is of particular interest for meat destined to ham production since it is a relatively simple measure, indicative for example of defective PSE meat (Mueller and Domel, 2000). However, measure can be performed only after carcass preparation. Earlier predictors, probably of a molecular nature, would constitute an improvement to the phenotypic measurement.

Objectives

The overall objective of the present work is of a double nature: contributing to the identification of biochemical mechanisms responsible for meat color characteristics and searching for early predictors of technological quality of pig meat destined to ham production. For that purpose, a differential proteome analysis is performed on the

sarcoplasmic proteins of *semimembranosus* muscles, characterized by high or low L* values measured at 36 hours *post mortem*.

Methodology

Animals, sampling and L value measurement*

In order to generate important genetic variability in meat quality traits, the studied population was composed of 1200 pigs originated from F2 crossing between Pietrain and a synthetic line (Large White x Duroc x Hampshire). Pigs were slaughtered at a liveweight of 110 kg. 30 min after slaughter, a 5g sample was taken from the dorso-superficial region of *semimembranosus*, thereby avoiding the inner part of the muscle that may be affected by PSE zones (Laville et al., 2005). L* value was measured with a Minolta CR 300 chromameter 36h *post mortem*, on the internal surface of muscle near the bone.

Sarcoplasmic protein extraction and electrophoresis

Muscle was homogenized in 40mM Tris (pH 7), 2mM EDTA and a protease inhibitors cocktail (SIGMA) at 4°C, at the ratio of 1:4 (w/v). The homogenate was centrifuged at 4°C for 10mn at 10000g. Supernatant, referred to as the sarcoplasmic extract, was stored at -80°C. Protein concentration was determined by the Bradford assay (Bio-Rad). 900µg of sarcoplasmic proteins were included in a buffer containing 7M Urea, 2M Thiourea, 2% (w/v) CHAPS 0.4% (v/v) carrier ampholyte and bromophenol blue. Samples were loaded onto immobilised pH-gradient strips (pH 5-8, 17cm Bio-Rad) and isoelectric focusing was performed using a Protean IEF cell system (Bio-Rad). Gels were passively rehydrated for 16h. Rapid voltage ramping was subsequently applied to reach a total of 85 kVh. In the second dimension, proteins were resolved on 12% SDS-PAGE gels using Protean II XL system (Bio-Rad). Gels were Coomassie Blue stained. Three gels were produced per sample.

Image analysis and statistical treatment of data

Gels were visualised and analysed using the 2DE image analysis software PDQuest (Bio-Rad). Detected and matched spots were statistically analysed using SAS software. A spot was considered significant when it was associated to $p < 0.05$ in ANOVA and/or pair-wise Student test. Clustering of significant spots was also performed using xlstat software.

Protein identification by mass spectrometry

Spots of interest were excised and the proteins were subjected to in-gel tryptic digestion and peptide extraction using Montage In-Gel Digest96 ZP kit (Millipore). Peptides were analysed using a MALDI-TOF mass spectrometer Voyager DE-Pro model (Perseptive BioSystems). Proteins were identified from their peptide mass fingerprinting using the MASCOT software (<http://www.matrixscience.com>). The initial search

parameters allowed a single trypsin missed cleavage, carbamidomethylation of cysteine, partial oxidation of methionine and a m/z error of 25 ppm.

Results & Discussion

Among 1200 animals, 2 groups of 12 animals (chosen by pair within 12 families) were selected based on extreme L^* value of meat. Average L^* values were 61.3 and 43.2, in the groups later referred to as ‘light’ and ‘dark’ respectively.

Figure 1 shows an example of a 2-D gel of sarcoplasmic proteins. Proteins of interest, discussed below, are circled. Out of 290 matched spots, 25 spots showed a significant differential abundance between the two groups. Among those, 17 are discussed in the present manuscript. Table 1 provides identification of spots, and information related to the validity of search results. At the exception of spots 4319, 6108, 6211 and 5214, which are potential fragments, position of all spots in the gel corresponded well with the provided theoretical MW, suggesting that 13 spots are entire proteins. Figure 2 is a cluster representation of the 17 identified proteins and their relative abundance in the two groups. The two main clusters naturally separate proteins over-represented (shadowed) and under-represented in dark samples.

All proteins over-represented in the light group are cytosolic proteins involved in glycogenolysis and glycolysis (enolase and enolase fragment, glycerol-3P dehydrogenase, glycogen phosphorylase fragment) or the associated cytosolic energy metabolism enzyme creatine kinase, in the form of fragments. These results corroborate previous findings that glycolytic muscle metabolism and proportion of type IIb glycolytic fibers enhance meat lightness in pig (Larzul et al., 1997). Regarding creatine kinase fragments, their higher abundance probably also indicates a higher proteolysis, that can be induced *intra vitam*, for example by exercise (Niikawa et al., 2002) and that is observed *post mortem* as we have described recently in PSE zones (Laville et al., 2005).

Proteins over-represented in the dark group can be divided into 4 groups: mitochondrial proteins involved in the respiratory chain (ATPase beta subunit, succinate dehydrogenase, NADH dehydrogenase), hemoglobin, chaperone proteins (HSP 27, alpha-crystallin) and antiquitin. Mitochondrial proteins clearly indicate a more pronounced oxidative metabolism, which is consistent with the inverse finding in the light group. Regarding hemoglobin, it is always present in muscle as remains of blood (Warriss, 1971). Since the pool of haem pigments is correlated with color of pig meat (Warriss et al., 1990), it comes as no surprise that hemoglobin quantity is higher in dark samples. Its higher abundance is most likely linked with the greater vascularity found in oxidative type muscles (Ruusunen and Puolanne, 2004) or with a higher hemoglobin content in the blood itself as observed in veal carcasses (Klont et al., 1999). Interestingly, the two chaperone proteins (HSP 27 and alpha-crystallin) were grouped in the same cluster, suggesting a co-regulation in the studied muscles. Their general function, including in skeletal muscle, is to offer a protection against protein denaturation. Kim et al. (2004) observed the higher abundance of one form of HSP 27 in white rather than red pig muscle. However, HSP 27 exist in many forms (Scheler et al., 1999), and the pool of immunologically detected HSP 27 have been reported to be over-expressed in oxidative fibers and in response to an increased demand in oxidative metabolism (Neufer and Benjamin, 1996). Since excessive protein denaturation is known to induce discoloration,

for example in fast pH fall PSE meat (Sayre and Briskey, 1963), the higher presence of chaperone proteins may contribute somehow to the darker color in this group. Finally, antiquitin is a protein implicated in cellular turgor pressure (Tang et al., 2002). Further investigation would be necessary to confirm that it plays a role in color development, but again, a reduced quantity would be consistent with fluid linkage and protein denaturation, as observed in excessively pale PSE meat (Monin, 1995).

Conclusions

The differentially expressed sarcoplasmic proteins in *semimembranosus* muscle confirm that increased meat lightness is associated with a more glycolytic / less oxidative metabolism. Concomitant changes in chaperone proteins and in antiquitin are observed. It would be interesting to determine whether such proteins can have an effect, even limited in the *post mortem* period, that could contribute to the installation of final color development. Further investigation is also necessary to determine whether any of the described proteins can be used as early predictor of technological quality of meat.

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Tables and Figures

Table 1. Identification of 17 differentially represented proteins (SC: Mascot score, MP: number of matched peptides, C: percentage of sequence coverage)

Spot	Identity	Accession number	SC	MP	C	Theoretical Mr/pI
0405	beta-subunit ATP synthase	gi 104	115	9	38	38.6/4.9
1712	NADH dehydrogenase	gi 57110953	206	23	34	79.6/5.8
4106	HSP 27	gi 55926209	75	7	35	22.9/6.2
4304	glycerol-3-phosphate dehydrogenase	gi 2149959	91	10	48	39.9/5.0
4319	enolase (fragment)	gi 109215	163	18	40	46.8/8.1
4404	enolase	gi 4503571	60	8	19	47.1/7.0
4513	antiquitin	gi 25108887	111	13	25	55.3/6.2
5214	glycogen phosphorylase (fragment)	gi 55636467	76	12	13	97.1/6.7
5314	enolase	gi 57086343	132	18	33	55.4/8.5
5614	succinate dehydrogenase	gi 284648	114	13	24	72.8/7.2
6108	creatine kinase (fragment)	gi 54111517	62	8	18	43.0/6.6
6211	creatine kinase (fragment)	gi 54111517	74	7	19	43.0/6.6
7005	hemoglobin chain D	gi 5542425	198	13	91	16.0/6.7
7008	hemoglobin alpha chain	gi 70237	90	6	58	15.0/8.7
7009	hemoglobin chain D	gi 5542425	190	11	77	16.0/6.7
7010	alpha-crystallin	gi 7441290	147	15	56	20.0/6.7
8003	hemoglobin chain D	gi 5542425	175	13	91	16.0/6.7

Figure 1. 2-D electrophoretic separation of sarcoplasmic proteins of *semimembranosus* pig muscle. Identified proteins differentially represented between the two groups are circled and numbered.

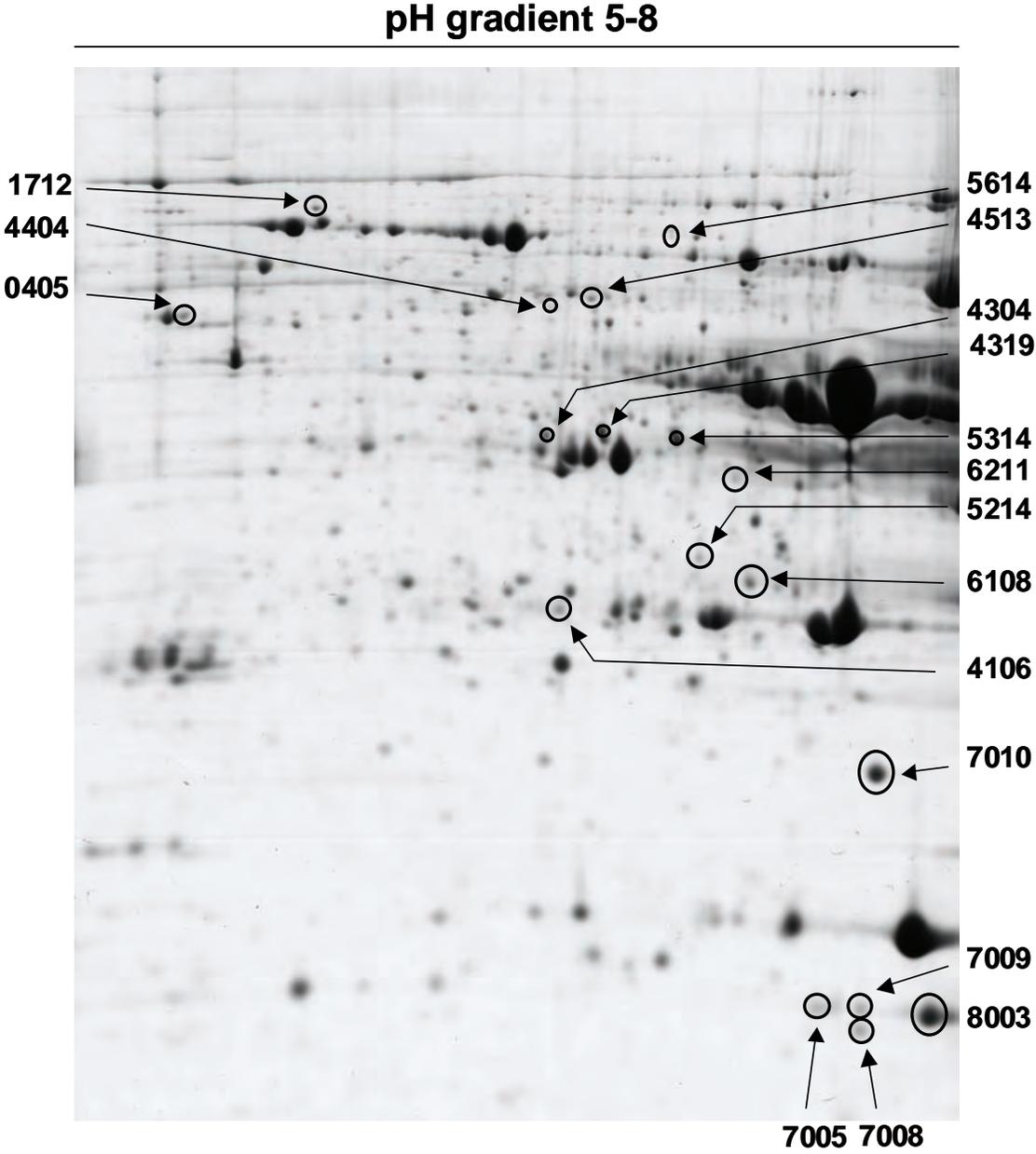
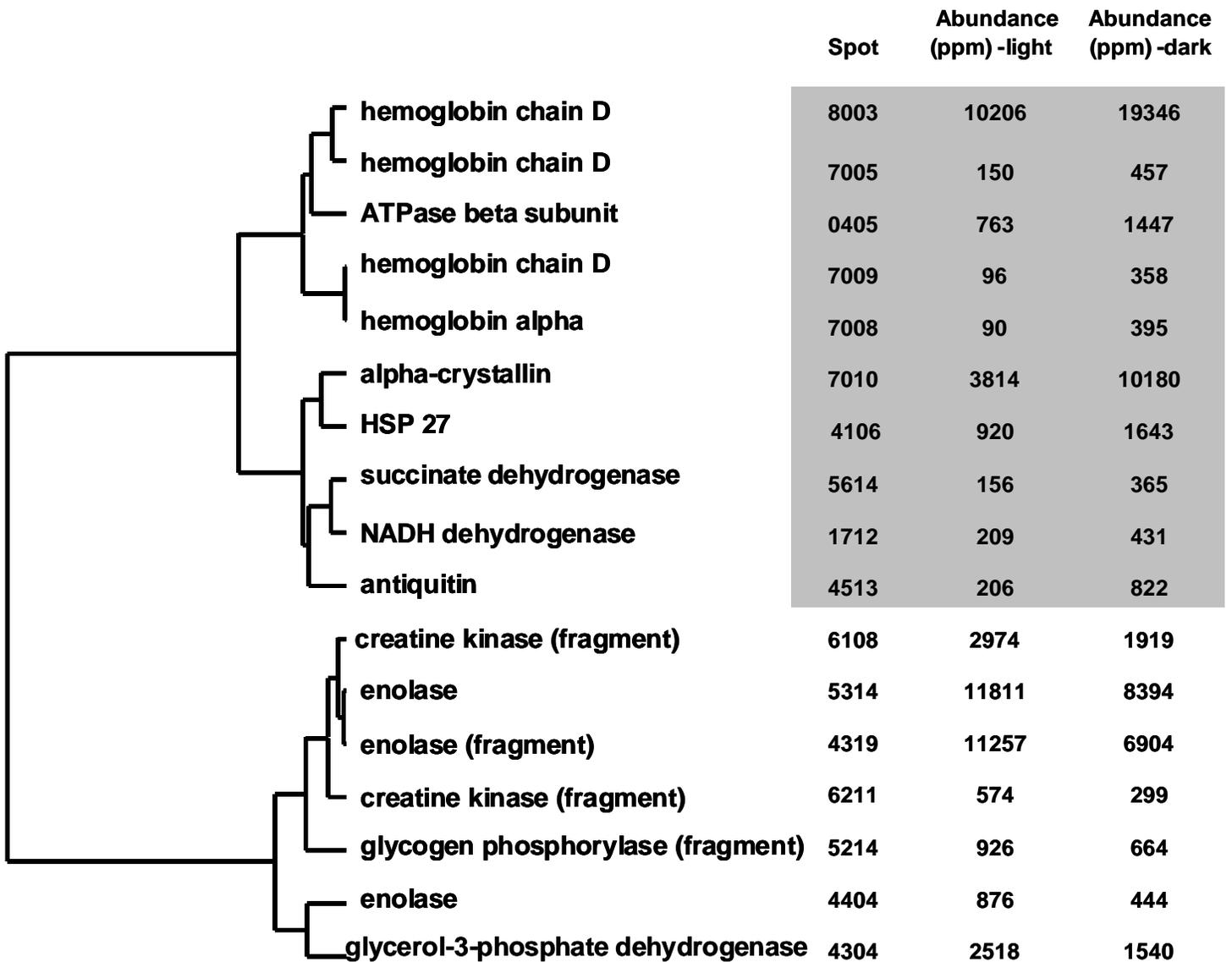


Figure 2. Cluster representation of identified proteins of interest and relative abundance (ppm) in the groups with high (“light”) and low (“dark”) L* value.



A STUDY OF MUSCLE CELL MORPHOLOGY DURING *RIGOR MORTIS* MEASURED BY IMAGE ANALYSIS

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Key Words: rigor mortis, image analysis, osmotic pressure, water transport

Introduction

After death, blood circulation in animals ceases, but the catabolic processes of the muscle cells (myofibers) continue as long as energy is available. Firstly, remaining oxygen is used up, followed by ATP-dependent anaerobic metabolism. This leads to accumulation of lactic acid and lowering of pH. When pH-level reaches a certain level, it interferes with the synthesis of ATP, eventually stopping it completely (Robb, 2001). The muscle cells enters *rigor mortis* when ATP level reaches a minimum (Currie & Wolfe, 1979). In rigor almost all of the myosin heads form cross-bridges to actin but in an abnormal, fixed and resistant way (Bendall, 1951; Marsh, 1953). This actomyosin complexes are often used to explain the rigidity or stiffness characterizing *rigor mortis* (Partmann, 1963). However, it is also agreed that rigor/stiffness is resolved without any changes of these bridges (Toyohara & Shimizu, 1988; Ando *et al.*, 1991). Clearly, other factors must be introduced to fully explain rigor-stiffness.

Several studies show osmotic- and extracellular changes in postmortem muscle (Heffron & Hegarty, 1974; Winger & Pope, 1980-81; Oplatka, 1994; Veiseth *et al.*, 2004). This can be a potentially important factor contributing to rigor/stiffness (Slinde *et al.*, 2003; Balevik *et al.*, 2004). Slinde *et al.* (2003) suggests that rigor/stiffness is a result of high osmotic pressure, due to increased number of molecules within the cells. This causes water movement from inter- to intracellular space, and as a consequence the cells size and shape changes.

Objectives

The purpose of the present study was firstly to use image analysis to detect changes in the morphological shape of muscle cells at different stages postmortem. Secondly; to discuss any findings in relation to the theory presented by Slinde *et al.* (2003), and possibly strengthen the evidence of intramuscular water transport into the cells during rigor. Fish muscle from Atlantic salmon (*Salmo salar* L.) was selected as model, as the swimming muscle (M. lateralis) is very homogenous with regard to fibre- and connective tissue composition (Hultin, 1984).

Methodology

Histology: Muscle pieces, 0.5x0.5x0.5 cm³ were cut from slaughtered Atlantic salmon, 1 (pre-rigor), 22 (in-rigor) and 72 (post-rigor) hours postmortem. The rigor/stiffness was observed by a finger technique as described by (Botta, 1994). Cross sectioned samples were stained with PAS (Periodic Acid Schiff) and examined by a light microscope (Leica DMR, Leica mikroskopie and Systeme GmbH, Wetzlar, Germany) x 10, and a digital camera (Olympus DP10, Olympus optical CO, GmbH, Hamburg, Germany). Pictures were downloaded to software Image ProPlus v. 4.0 (Media Cybernetics L.P., Silverspring, Maryland, U.S.A).

Image analysis of muscle cells: The colouring of the muscle-slides resulted in intensity images without any colour information. In general, the muscle cell borders and the neighbouring interior (in comparison) had a dark and a light pink colour respectively. In consequence the image analysis was performed on only the green colour-layer of the RGB-digital-image. Variation in both the light function and colour staining over individual slides were suppressed by background correction, also called flat fielding (Seul et al., 2000). The cell borders were segmented using a threshold value of $T = 100$. Skeletonizing was then used to achieve a one pixel thick representation of the cell borders (Gonzalez & Woods, 1992; Borgefors & Nyström, 1997). This initial automatic segmentation was followed by a manual segmentation, where a skilled researcher sanctioned which cells had been segmented correctly. Alternatively cell borders were added and/or corrected when necessary. The segmented cells were stored as circular lists representing the n polygon vertices of the cell border; $(i_0, j_0), \dots, (i_k, j_k), \dots, (i_n, j_n)$; $(i_0, j_0) = (i_n, j_n)$. Standard descriptive variables for each cell, as area, compactness and rectangularity, were calculated from these list representations (Sonka et al., 1998).

(1)

$$\begin{aligned}
 \text{area} &= \frac{1}{2} \left| \sum_{k=1}^{n-1} (i_k j_{k+1} + i_{k+1} j_k) \right| \\
 \text{compactness} &= \frac{(\text{borderlength})^2}{\text{area}} \\
 \text{rectangularity} &= \frac{\text{area}}{\text{areaboundingrectangle}}
 \end{aligned}$$

The bounding rectangle is the rectangle of minimum area that bounds the cell region.

(2)

$$\alpha(i, j) = i \cos \theta + j \sin \theta \quad \beta(i, j) = -i \sin \theta + j \cos \theta$$

This rectangle was found as the minimum and maximum of all boundary points (i_k, j_k) and directions θ (0-90°). Compactness = 1 for perfect circles. Rectangularity has values in the interval (0,1], with 1 representing a perfectly rectangular region. A fourth measurement, especially designed for this problem domain, was concavity.

(3)

$$\text{concavity} = \frac{\text{convexarea} - \text{area}}{\text{area}}$$

The *convexarea* is the area of the smallest convex region enveloping the respective cell region (Figure 1). The convex region can be computed efficiently from the circular list representation (see above) (Sklansky, 1982; Orłowski, 1985; Shin & Woo, 1986). If one imagines a region with an elastic border, the *concavity*-variable (3) measures how 'inflated' the region is, i.e. how outstretched the region border is. *Concavity* has possible values in the interval $[0, \infty)$. A cell without any windings or concavities will have *concavity* = 0.

Statistics: The statistical procedures were performed using the SAS software package v.8.0.2. (SAS Institute Inc., Cary, North Carolina, USA). Least square means were calculated for each stage of rigor. T-tests were used to detect significant differences between LS-means. Effects of fish were also included in the model to avoid individual differences dominating the results. All *p*-values below 0.05 were considered significant. *P*-values above 0.20 were considered not significant (denoted n.s).

Results & Discussion

The shape of the muscle cells cut in- and post-rigor was significantly different to the shape of the muscle cells cut pre-rigor (Table 1). The area of the cells clearly increased from pre- to in- and post-rigor. Slinde *et al.* (2003) suggests that after death, glycogen is converted to glucose and further metabolised to lactate. In addition, other catabolic reactions such as proteolysis, lipolysis and enzymatic reactions also contribute to increased number of molecules within the cells. This increases the osmotic pressure ($\pi = cRT$) and generates a water transport into the cells (Figure 1). And as a result the cell area expands. The pre-rigor cells had an, in comparison to in- and post rigor cells, more wavy and concave border (Figure 2). Following Slinde's theory this is due to low osmotic pressure and relative modest water transport into the cells pre-rigor. The low pressure inside the cells is not able to expand the cell borders fully. In contrast, during rigor, high osmotic pressure causes water transport into the cells expanding the cell borders. The shape of in- and post-rigor cells is more inflated compared to pre-rigor cells (Table 1, Figure 2). The cells became more compact (lower compactness values) and less concave (lower concavity values) during rigor. As a cell expands the concavities are filled out (Figure 1). A possible explanation for the increase in hardness according to Slinde *et al.* (2003) is that the cells now follow the surrounding connective tissue more closely. The release of hardness/stiffness post-rigor may therefore be explained by rupture of the muscle cell membranes as the muscle deteriorates postmortem.

It is important to be aware of that cutting, freezing and chemical treatment may have different effects on muscle samples pre-, in- and post-rigor. For instance, the PAS-staining of the muscle samples resulted in a pink colour pre-rigor, while the in- and post muscle samples became purple (Figure 2). This is probably due to the lowering of pH during rigor. Sampling techniques have been done systematically in all state of rigor. Because of this, artefacts may have been converted to "white noise". The result presented still support the theory by Slinde *et al.* (2003).

Conclusions

The image analysis successfully detected a change in cell-shape from concave to more inflated during rigor. This supports the theory suggested by Slinde *et al.* (2003) that there is an influx of water into the cells. It is however important to remember that rigor is a complex process. The above observed changes in muscle cell morphology may therefore only be on piece of the puzzle explaining muscle stiffness during *rigor mortis*.

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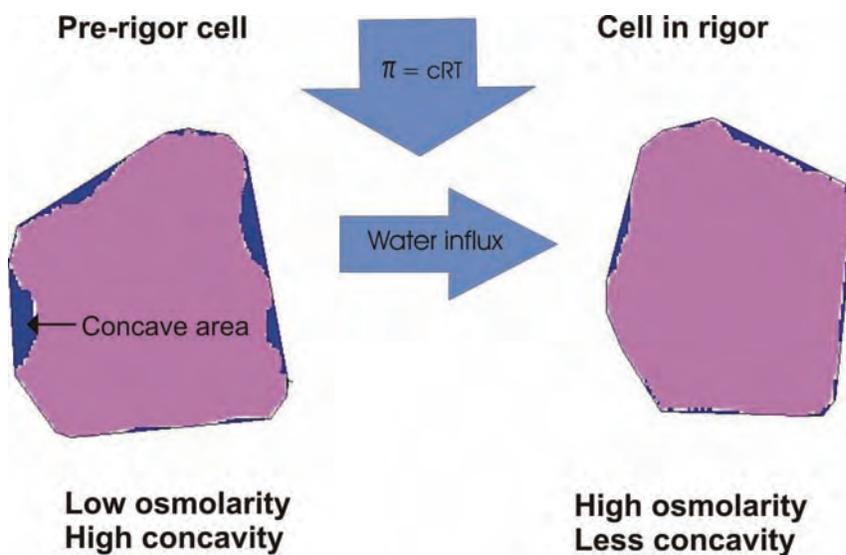
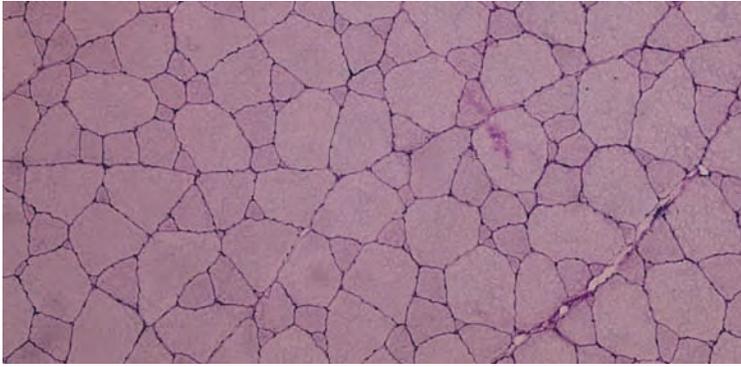
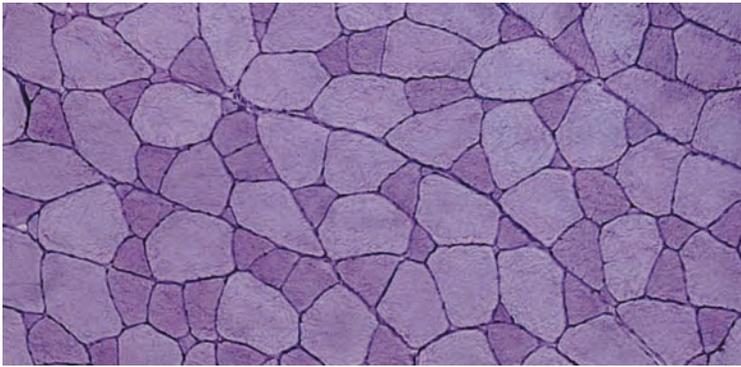


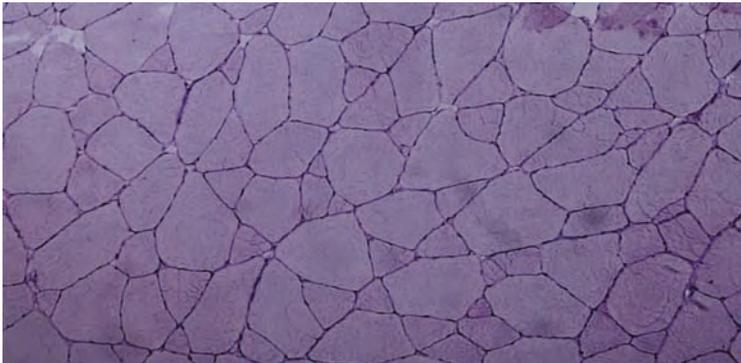
Figure 1. The convex area to a region is the least convex region containing the region. As the cell enters rigor, the osmotic pressure increases within the cell, $\pi = cRT$, and give rise to water transport into the cell. The cell expands and decreases its concavity.



A. Pre-rigor



B. In rigor



C. Post-rigor

Figure 2. Muscle cells in cross sections from white epaxial muscle of Atlantic salmon and stained with PAS. Picture A, B and C show muscle cells from pre-, in rigor and post-rigor state, taken 1, 22 and 72 hours after death respectively.

Variable	Rigor-status	Pre-rigor	In-rigor	Post-rigor
Area (μm^2)	Pre-rigor	12399 \pm 395	14581 \pm 452	14041 \pm 447
	In-rigor	$p < 0.001$	$p < 0.001$	$p = 0.006$
	Post-rigor	$p = 0.006$	n.s	n.s
Compactness	Pre-rigor	19.552 \pm 0.088	18.567 \pm 0.100	18.455 \pm 0.099
	In-rigor	$p < 0.001$	$p < 0.001$	$p < 0.001$
	Post-rigor	$p < 0.001$	n.s	n.s
Rectangularity	Pre-rigor	0.694 \pm 0.003	0.709 \pm 0.003	0.709 \pm 0.003
	In-rigor	$p < 0.001$	$p < 0.001$	$p < 0.001$
	Post-rigor	$p < 0.001$	n.s	n.s
Concavity	Pre-rigor	0.066 \pm 0.002	0.051 \pm 0.002	0.046 \pm 0.002
	In-rigor	$p < 0.001$	$p < 0.001$	$p < 0.001$
	Post-rigor	$p < 0.001$	$p < 0.084$	$p < 0.084$

Table 1. Statistical analysis of morphological variables of n = 1404 muscle cells in different state postmortem Least square means \pm Standard Error for each rigor-status (in-, pre- and post-rigor) and p -values for differences of the least square means (t-test).

POSTMORTEM STABILITY PROFILE OF BOVINE MUSCLE RNA

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Key Words: RNA, Stability, Bovine

Introduction

The production of consistently tender, well-flavoured and juicy meat products remains elusive in the meat production sector, with the complex *postmortem* tenderisation process still not fully understood. Meat quality may be affected by differential expression of muscle genes and their protein products in response to factors such as nutrition, handling, environment or growth rate. The new scientific orientation towards transcriptome and proteome analysis has opened up new possibilities for discovering molecular predictors (genes, proteins or molecular polymorphisms) that control muscle growth and meat quality traits (Hocquette *et al.*, 2001). The identification of such genes expressed in skeletal muscle tissue is a necessary step toward a better understanding of the genetic basis of meat production (Davoli *et al.*, 1999) and may have a revolutionary impact on understanding the biology of meat quality. However to carry out such analysis, high quality undegraded RNA is necessary. The half-lives of mRNAs may vary from a few minutes to more than 24 hours and changes in mRNA stability are ultimately reflected in the amount of protein produced, the stability of mRNA has emerged as a key step in the regulation of eukaryotic gene expression (Tourrière *et al.*, 2002). As there can be a considerable time lag in commercial abattoirs between time of slaughter and muscle excision online, it is important to determine whether RNA stability in muscle tissue, particularly in *M. longissimus dorsi* (striploin), is influenced by time *postmortem*.

Postmortem stability in fibrous tissue, such as muscle, has not been extensively studied, and is of particular interest in respect of analyzing gene expression in meat-producing animals in relation to meat quality.

Objectives

The objective of this research was to determine the RNA integrity and quantity from bovine muscle tissue, collected *postmortem* in an abattoir environment. Total RNA stability was measured over a 14-day *postmortem* period, by assessing cellular RNA integrity using electrophoresis, the Agilent Bioanalyzer system, RT-PCR and real time RT-PCR.

Methodology

Heifers were slaughtered under controlled conditions at the abattoir facility at the National Food Centre, Dublin, Ireland. Samples were excised from the *longissimus dorsi* (LD) muscle at intervals of 1 hour to 14 days for the first animal (14 days is the normal length of the *postmortem* ageing process) and 0.5 hour to 2 days for the second animal (thereby focusing on the early *postmortem* period). Samples were stored in RNeasy Lysis Buffer or flash frozen in liquid nitrogen and stored at -20°C or -80°C as appropriate. RNeasy Lysis Buffer (Qiagen, Crawley, UK) is a tissue storage reagent that rapidly permeates tissue to stabilise and protect cellular RNA without jeopardising the quality or quantity of RNA (Reisz-Porszasz *et al.*, 2003). Total RNA was isolated from 100mg of frozen tissue by (1) homogenisation in TRIzol reagent and precipitation with chloroform and isopropanol or (2) with the commercial Qiagen RNeasy mini-kit. All samples were DNaseI treated to remove contaminating genomic DNA.

The Agilent Bioanalyzer (Agilent Technologies, Germany) was used to calculate the integrity and concentration of total RNA, as it offers the advantage of assessing RNA integrity in addition to making a quantitative measurement and is regarded as a quality control standard for all routine gene expression experiments, i.e. microarray and realtime PCR analysis. Samples with rRNA ratios (28S/18S) of 1 or greater and values of greater than 13 for '% of Total Area of the 28S peak' are considered to be of sufficient quality for downstream gene expression analysis.

Randomly primed reverse transcription was carried out for first strand cDNA synthesis. PCR was performed using primers designed specifically for the housekeeping genes glyceraldehyde-3 phosphate dehydrogenase (GAPDH) (Accession number U85042) and beta-actin (Accession number AF191490). RT-PCR products were analysed on agarose gels using electrophoresis. Realtime RT-PCR analysis was carried out for DNA detection using SYBR Green 1 (Roche Diagnostics, Ltd., UK).

Results & Discussion

The *postmortem* stability profile of bovine muscle is of particular interest as regards gene expression analysis since *postmortem* tissues have traditionally been regarded as unsuitable sources of RNA for gene expression studies due to the rapid degradation of RNA.

Using gel electrophoresis, 28S and 18S rRNA bands were detectable up to 8 days *postmortem*, with visible degradation being observed as the *postmortem* interval increased up to 14 days (Figure 1).

The Agilent Bioanalyzer was used for a more accurate and reproducible assessment of total RNA integrity and concentration. Samples analysed initially with the Bioanalyzer showed that total RNA integrity decreases dramatically from 2 day to 14 days *postmortem* so further analysis focussed on the 0.5 hour to 2 day *postmortem* interval. Meat samples stored in RNeasy Lysis Buffer from which RNA was isolated using TRIzol reagent or the commercially available kit gave high quality total RNA up to 2 days *postmortem* (Figure 2). Good rRNA ratios (>1) and values for '% of Total Area of 28S peak' (>13) were obtained for these samples. These extracts are of sufficient quality for gene expression analysis using total RNA. Total RNA isolation from meat samples flash frozen in liquid

nitrogen gave a lower RNA integrity than those stored in RNAlater (Figure 2). Poly(A) RNA (mRNA) makes up between 1-5% of total cellular RNA and is most frequently used for detection and quantitation of extremely rare mRNAs, and while high quality total RNA indicates good overall RNA stability, mRNA integrity will have to be assessed for generating an accurate gene expression profile.

RT-PCR transcripts of varying intensity were evident up to 10 days *postmortem* for GAPDH and 9 days *postmortem* for beta-actin (Figure 3). Band intensity decreased between day 2 and day 3 *postmortem*. Despite the overall stability of RNA specific for GAPDH and beta-actin, individual RNAs specific for other genes may undergo more rapid decay and is currently being investigated. Realtime RT-PCR analysis, which is a more sensitive technique to determine transcript levels, showed that GAPDH transcripts were evident up to 6 hours *postmortem* after which RNA integrity and transcripts begin to decrease (Figure 4).

Conclusions

Determining RNA integrity and quantity is an essential step in generating an accurate gene expression profile. Storing muscle samples in RNAlater, stabilised and protected RNA samples for extraction of high quality total RNA. The Agilent Bioanalyzer is a fast and reliable method of assessing RNA integrity in addition to making a quantitative measurement. High quality total RNA, suitable for gene expression analysis, was isolated from beef up to 2 days *postmortem* demonstrating that a time delay between slaughter and sample collection should not adversely affect cellular RNA quality.

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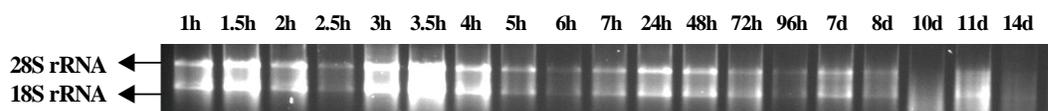


Figure 1. Total RNA samples isolated over a *postmortem* ageing period of 1 hour to 14 days and analysed using gel electrophoresis. RNA degradation was observed as the *postmortem* interval increased up to 14 days.

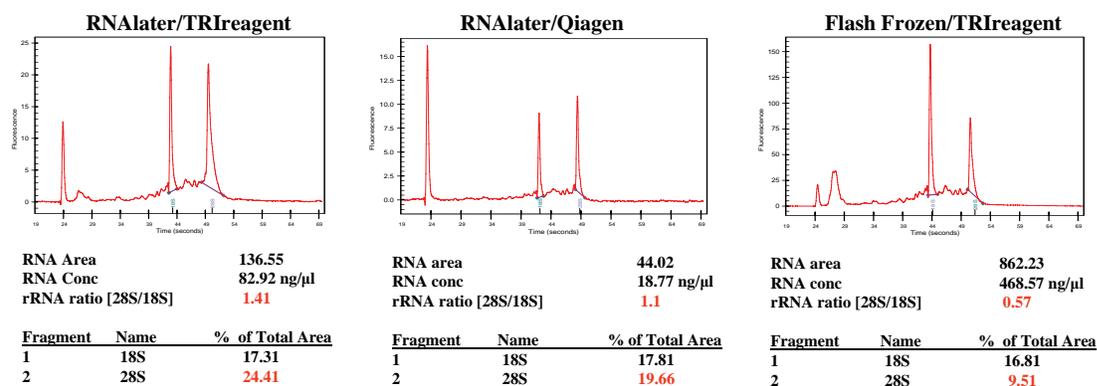


Figure 2. Examples of Bioanalyzer results of RNA samples stored in RNAlater or flash frozen in liquid nitrogen and total RNA isolation using TRIreagent or Qiagen RNeasy mini-kit. Samples with rRNA ratios [28S/18S] of >1 and ‘% of Total Area of 28S peak’ values of > 13 are considered to be indicative of high quality RNA.

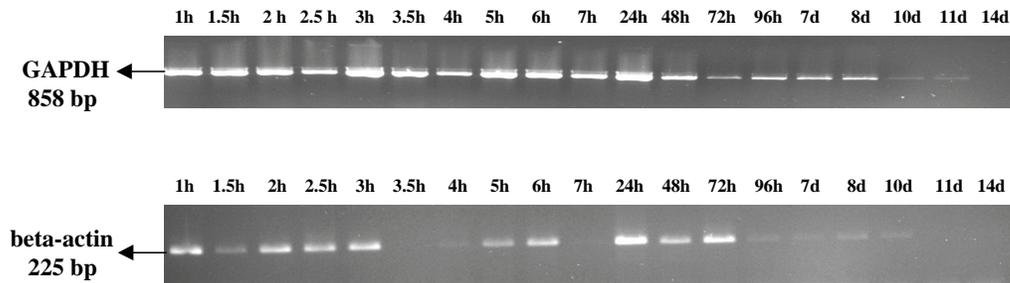


Figure 3. RT-PCR was carried out on RNA samples (1h – 14d) using GAPDH (858 bp) and β -actin (225 bp) specific primers and PCR products were analysed using agarose gel electrophoresis. Band intensity decreased as the *postmortem* interval increased up to 14 days.

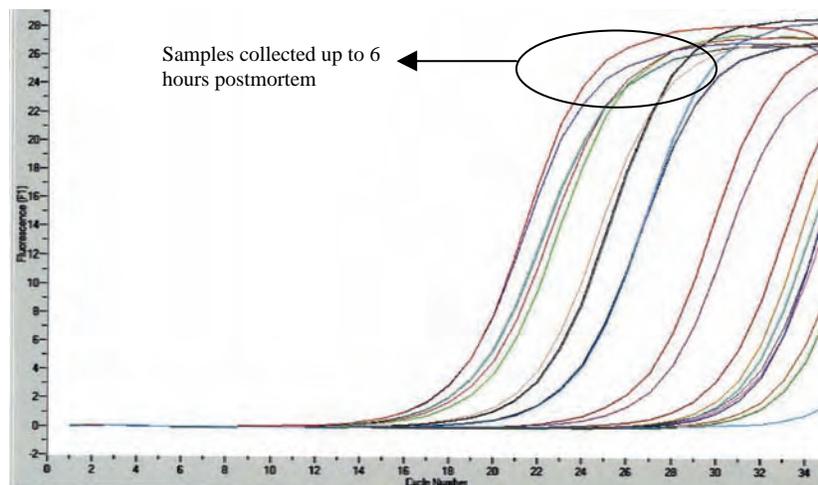


Figure 4. Realtime RT-PCR analysis was carried out using GAPDH specific primers on RNA samples (1h – 14d). PCR products were detected using SYBR Green 1. Transcript integrity decreased as the *postmortem* interval increased up to 14 days.

CLONING AND EXPRESSION OF DNA FRAGMENT ENCODING PARATROPOMYOSIN BINDING CONNECTIN/TITIN DOMAINS AT THE A-I JUNCTION OF A SARCOMERE

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Key Words: chicken myofibrils, connectin/titin domain, A-I junction, paratropomyosin, postmortem ageing

Introduction

A myofibrillar protein, paratropomyosin weakens the rigor linkages between actin and myosin, and contributes to meat tenderization. Paratropomyosin was found at the A-I junction of sarcomeres in living muscle and in muscle immediately postmortem, and translocated from its original position to thin filaments by an increase of calcium ion concentration to 0.1 mM during postmortem storage of muscles (Hattori and Takahashi, 1988). We have shown that in chicken breast muscle paratropomyosin bound to both β -connectin/titin 2 and the 43-kDa fragment, the proteolytic product of β -connectin/titin 2, in examining binding of paratropomyosin at the A-I junction region, and determined the N-terminal sequence of the 43-kDa fragment to identify more precise paratropomyosin binding site in the intact molecule (Fei, *et al.*, 1999; Yamanoue *et al.*, 2003). The sequence is similar to a part of I51 domain of human cardiac connectin/titin domain structure presented by Labeit and Kolmerer (1995), which is located at the A-I junction region of a sarcomere. So, it is important to clarify whether the domains constituting the 43-kDa fragment are also located at the A-I junction of chicken sarcomere or not.

In the present study, we cloned DNA fragment encoding the 43-kDa fragment and produced recombinant domains constituting the fragment in *E. coli*, and then raising the antibody against a recombinant domain for indirect immunofluorescence microscopy.

Objectives

The objective of this study was to clarify the location of the 43-kDa fragment in chicken sarcomere in order to explain the details of paratropomyosin localization at the A-I junction.

Methodology

Cloning and sequencing of connectin/titin DNA fragment encoding the 43 kDa fragment. Degenerate PCR was performed to amplify connectin/titin DNA fragment

encoding the 43-kDa fragment using chicken cDNA library as a template. Oligonucleotide primers for PCR were synthesized and amplification conditions were 30 cycles of the reaction of 1 min at 94°C, 1 min at 50°C, and 1 min at 70°C after the initial denaturation for 5 min at 94°C, followed by a final 10 min incubation at 72°C. 2nd (nested) PCR product was ligated to pGEM-T-vector (Promega), and the vector was introduced into *E. coli* DH5 α strain. Plasmid DNA was purified from selected colonies using the alkaline sodium dodecyl sulfate extraction procedure and the sequence of the plasmid DNA was analyzed by ABI 310 Genetic Analyser (Applied Biosystems).

Expression and purification of recombinant connectin/titin domains. For construction of expression vectors, DNA fragments encoding connectin/titin domains were amplified by PCR using a set of specific primers based on cDNA of 43-kDa fragment and cloned into the *Nde* I - *Bam*H I site of the expression vector, pET- 22b (Novagen). After the expression vectors were introduced into the *E. coli* BL21 (DE3) strain, production of the recombinant proteins were induced by the addition of IPTG. Transformed cells were harvested and sonicated. The cell lysates were centrifuged and the supernatants were applied on a Sephadex G-75 gel filtration. Fractions containing recombinant protein were collected and added solid ammonium sulfate to 1.4 M, then applied on a Butyl-TOYOPEARL 650M or an Ether-TOYOPEARL 650M column equilibrated with 1.4 M ammonium sulfate. Purified recombinant proteins were passed through the column or eluted with a linear gradient of 1.4 to 0 M ammonium sulfate, appearing as single band on SDS-PAGE (Laemmli, 1970) gels.

Indirect immunofluorescence microscopy. Antiserum against purified recombinant domain was raised in a rabbit. A drop of myofibrillar suspension was mounted on a slide and covered with a cover glass. Myofibrils were treated with diluted antiserum against recombinant domain, and then washed thoroughly with a solution containing 75 m NaCl and 10 m sodium phosphate buffer, pH 7.2. The fluorescein isothiocyanate (FITC)-labeled anti-rabbit immunoglobulin G (IgG) were reacted with the myofibrils and free IgG were washed out thoroughly. The specimen was observed under a fluorescence microscope and photographed.

Results & Discussion

The sketch shown in Fig. 1 describes the location of the 43-kDa fragment based on human cardiac titin/connectin domain structure. In the agarose gel electrophoresis patterns of the first and the second (nested) PCR products, the bands of about 400 bp, 900 bp and 700 bp corresponding to A, B, and C regions appeared respectively by the second PCR (Fig. 1). After TA cloning of the PCR products and transformation of *E. coli*, the sequences of plasmid DNAs were analyzed. As the result, nucleotide sequence of 1849 bp from A to C region was determined and DNA fragment of 1158 bp seemed to encode the 43-kDa fragment (data not shown). When the sequence was compared with that of human cardiac connectin/titin, 79.6% similarity was shown. The deduced amino acid sequence of the 43-kDa fragment showed 87.6% similarity to the correspondence part of human cardiac connectin/titin sequence. The 43-kDa fragment was composed of four fibronectin type-3 domains and one immunoglobulin domain as similar as the domain structure of human cardiac connectin/titin.

DNA fragments encoding each domain of 43-kDa fragment were expressed in *E. coli* and recombinant domains were purified from cell lysates by the method described in **Methodology**. Figure 2 shows SDS-PAGE pattern of a chicken recombinant domain, CK-I53 domain, based on human cardiac connectin/titin domain structure. The recombinant domain appeared in *E. coli* after addition of IPTG and was purified by hydrophobic interaction chromatography. The antibody against recombinant CK-I53 domain was raised in a rabbit for indirect immunofluorescence microscopy. When the anti-serum was reacted with chicken myofibrils, the fluorescence was recognized at the A-I junction as a result detected by the FITC labeled second antibody (Fig. 3), indicating that CK-I53 domain is located at the junction of a sarcomere.

Conclusions

Chicken DNA fragment encoding the 43-kDa fragment from β -connectin/titin 2 was cloned and expressed in *E. coli*. Antiserum against a recombinant domain constituting the 43-kDa fragment reacted at the A-I junction of a sarcomere. Thus, it was confirmed that the 43-kDa fragment is located at the junction where paratropomyosin is localized in living muscle and in muscle immediately postmortem.

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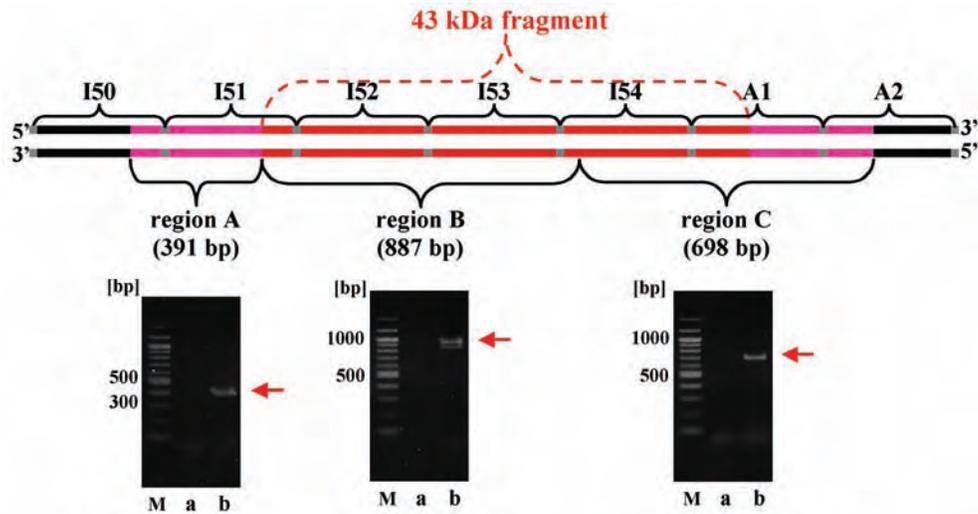


Fig. 1. PCR amplification of DNA fragments encoding chicken connectin/titin 43-kDa fragment.

Lane M, marker; a, 1st PCR product; b, 2nd PCR product.

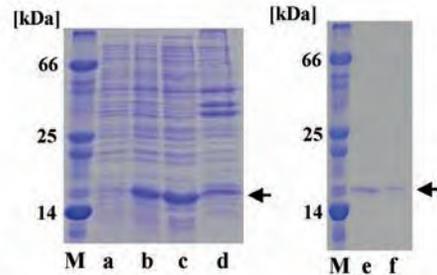


Fig. 2. SDS-PAGE patterns of expression and purification of recombinant CK-I53 domain.

Lane M, marker; a, without IPTG; b, with IPTG; c, supernatant from cell lysate; d, precipitate from cell lysate; e, Sephadex G-75 fraction; f, Butyl-TOYOPEARL 650M fraction. Arrows indicate recombinant CK-I53 domain.

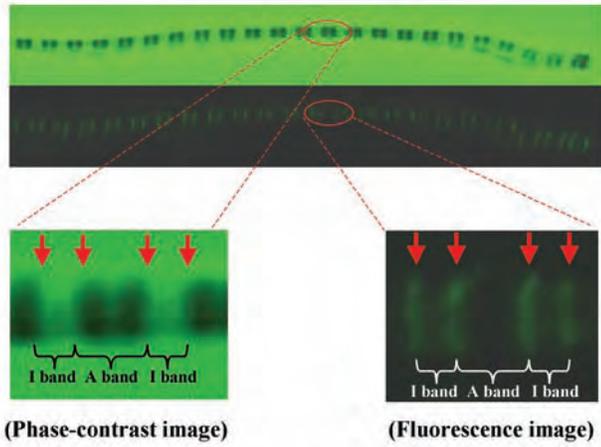


Fig. 3 Localization of connectin/titin I53 domain in chicken breast myofibrils. Arrows indicate the A-I junction.

OPTIMIZATION OF THE PURIFICATION PROCESS OF ACTOMYOSIN AND G-ACTIN FROM POST-RIGOR PORCINE SKELETAL MUSCLE

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Key Words: myofibrillar proteins, myosin, actin, purification

Introduction

In processed meat products the majority of used meats are in post-rigor state and the separation of the constituents of muscle is necessary for various physiological and biochemical studies. Therefore, the isolation of myofibrillar proteins from post-rigor muscles is important to evaluate the functional properties of post-rigor proteins at the molecular level. Also, in order to study their effect on the interaction with other molecules such as volatile components presents in meat products (Gianelli et al, 2003), it is necessary to obtain high yields of myofibrillar proteins using simplified purification processes. In this sense, there are many extraction procedures for isolation of myofibrillar proteins from muscle (Dudziak and Foegeding, 1988; Pardee and Spudich, 1982) which are very tedious including many extractions processes (Syrový, 1984). Therefore, it is important to obtain a simplified isolation process for both proteins actomyosin and actin with a purity enough to be used in studies of molecular interactions with volatile compounds.

Objectives

The objective of this investigation was to optimize the isolation of actomyosin and actin from post-rigor porcine muscle based on differential solubility, gel filtration chromatography and extraction times in order to use them for further studies on molecular interactions of myofibrillar proteins with volatile compounds.

Methodology

The methodology used for the purification of proteins (Figure 1) consists on the extraction of pork meat with a buffer containing 20 mM EDTA for three times to eliminate the sarcoplasmic proteins. The extraction of myofibrillar proteins is made with Hasselbach- Shneider solution (0.6 M KCl, 10 mM $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$, 1 mM MgCl_2 20 mM EGTA 0.1 M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ pH= 6.4) (Dudziak and Foegeding, 1988). The pellet obtained is used to prepare the acetone powder by successive three extractions with acetone for 20 min and further filtration. The acetone powder is used for the extraction of G-actin by using buffer A (2 mM tris Cl 0.2mM ATP 0.5 mM 2-mercaptoethanol 0.2mM CaCl_2 0.005% sodium azide pH=8.0 at 25 °C) with different extraction times.

SDS-PAGE electrophoresis. The purity of the isolated fractions was monitored by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 10 % gels and staining with Coomassie blue R-250 (Laemmli, 1970). The protein concentration in the fractions was determined according to the method of the biochinconic acid (Smith et al., 1985) using bovine serum albumin as standard.

Results & Discussion

The actomyosin was purified from post rigor muscle using differential solubility processes and gel filtration chromatography (Figure 1). The successive steps of the isolation process were followed by SDS-PAGE electrophoresis as it is shown in figure 2 and the protein concentration is shown in table 1. The first steps extracted the majority of the sarcoplasmic proteins and afterwards, the myofibrillar proteins were extracted using a high ionic strength solution (Dudziak and Foegeding, 1988). The fraction containing the actomyosin protein (M4p) was purified using a gel filtration column in order to obtain a purified actomyosin fraction (M8, Figure 3). The yield obtained for this purification process was 0.14 mg of actomyosin per g of meat. This process is shorter than the proposed by Dudziak and Foegeding (1988) although with a similar yield. However, we did not be able to separate actomyosin from myosin in the chromatography step.

In the same process, the second major myofibrillar protein (actin) was isolated. In order to purify the protein G-actin, the pellet obtained after the extraction with the high ionic strength solution (M3p) was used to acquire the acetone powder instead of using the extractions proposed by Pardee and Spudich (1982). Furthermore, the acetone powder was used for the isolation of actin by successive extractions with buffer A (Figure 1) instead of using depolymerization and repolymerization steps as indicated Pardee and Spudich (1982). A filtration step was added to eliminate the presence of myosin contamination in fraction A4S obtaining a final fraction (A4Sw) highly concentrated in G-actin. The successive steps of the isolation process of G-actin were followed by SDS-PAGE electrophoresis as it is shown in figure 4 and the protein concentration is shown in table 1. The yield of the process was 18.6 mg of G-actin per 1 g of acetone powder or 2.5 mg per g of meat.

Conclusions

Actomyosin and G-actin were isolated from post-rigor porcine skeletal muscle reducing the extraction times in both proteins purification processes. Both proteins were obtained with a recovery of 0.014 g and 0.25 g per 100 g of meat, respectively. However, during the actomyosin purification process it was not possible to separate actin from myosin although an enriched fraction of G-actin was obtained on the further purification process.

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Tables and Figures

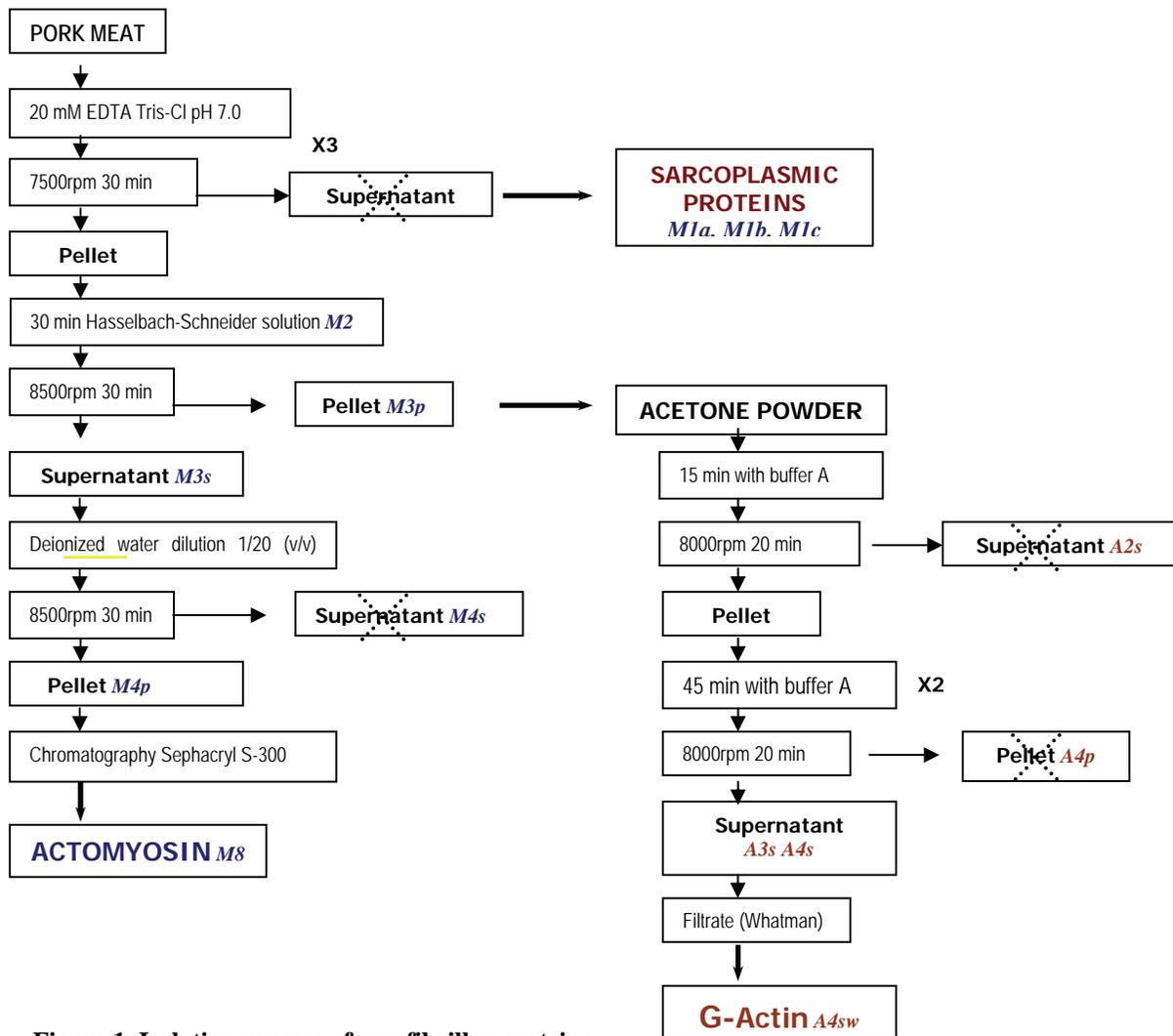


Figure 1. Isolation process of myofibrillar proteins

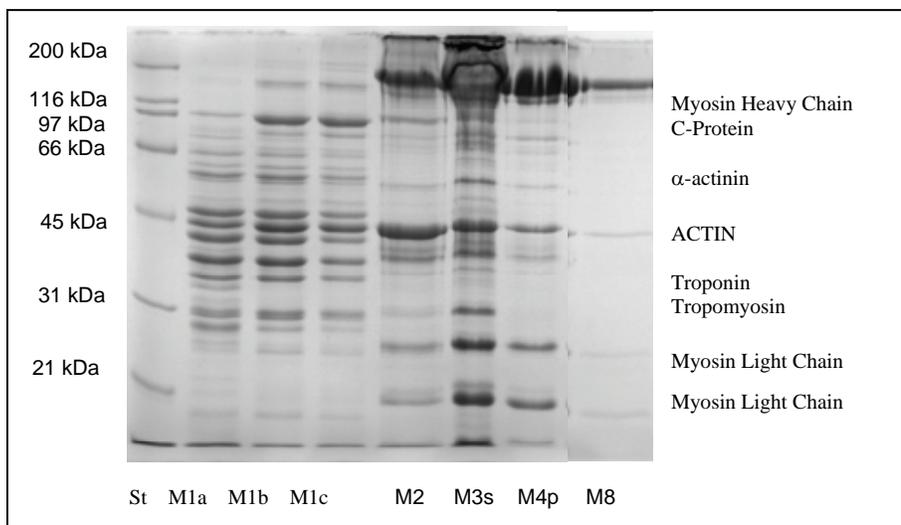


Figure 2. Ten percent SDS-PAGE gel of the actomyosin purification process.

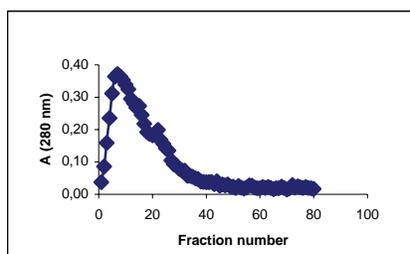


Figure 3. Gel filtration chromatography (Sephacryl S-300) of M4P fraction.

Table 1. Protein concentration (mg/ml) in the isolation steps.

Protein Isolation Step					
<i>Sarcoplasmic proteins</i>					
M1a	M1b	M1c			
18.40	4.70	2.80			
<i>Actomyosin purification</i>					
M2	M3S	M4p	M8		
7.30	6.50	7.10	0.31		
<i>Actin purification</i>					
A2s	A2sw	A3s	A3sw	A4s	A4sw
0.84	0.86	0.45	0.42	0.86	0.86

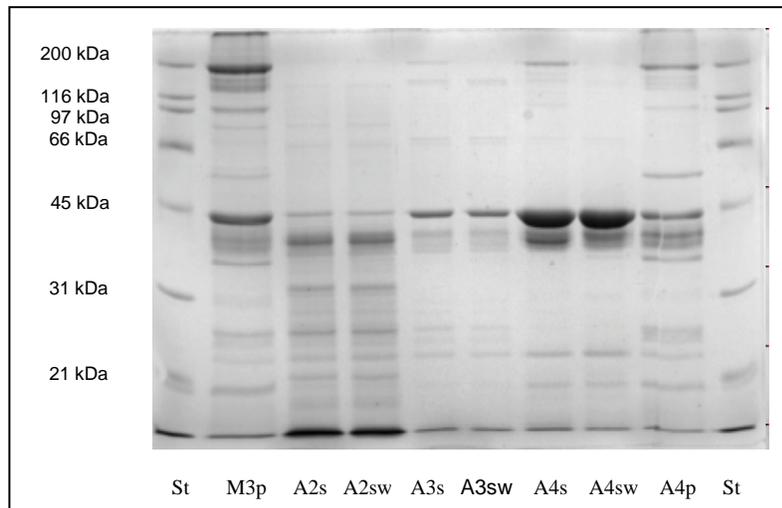


Figure 4. Ten percent SDS-PAGE gel of the G-actin purification process.

**ACTION MECHANISM OF ANGIOTENSIN I-CONVERTING ENZYME
INHIBITORY PEPTIDES OBTAINED FROM HYDROLYZATE OF CHICKEN
BREAST MUSCLE**

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Key Words: Angiotensin I-converting enzyme inhibitor; spontaneously hypertensive rats; hypotensive activity; chicken breast muscle extract; peptide

Introduction

Hypertension has raised the risks of stroke, cerebral infarction. Blood pressure is controlled by various regulatory factors in the body and angiotensin I-converting enzyme (ACE) is one such factor. In the previous paper, we reported that administration of hydrothermal extract of chicken breast muscle prevented rises in blood pressure in spontaneously hypertensive rats (SHRs) by 50mmHg compared with the case in which saline was administered(1). We discovered an ACE-inhibitory peptide (Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe; P4 peptide) in the extract digested by gastric proteases (pepsin, trypsin–chymotrypsin, and small intestinal enzymes). The P4 peptide was as active as a lactotripeptide and a sardine peptide, which have been reported to be ACE-inhibitory peptides.

Objectives

The P4 peptide is a promising ingredient for functional foods, and its characteristics should be analyzed in detail. We synthesized a peptide that has this amino acid sequence, examined whether the synthetic peptide suppressed rises in blood pressure, and investigated the inhibition mechanisms and active regions of the peptide.

Methodology

Measurement of Blood Pressure and Heartbeat in SHRs

Eight-week-old male SHRs were fed a commercial diet and water for 2 weeks *ad libitum* in an environment-controlled room (23°C, 55% humidity), and then either saline or a peptide (30mg/kg weight) dissolved in saline was administered through a tail vein. Their tail systolic blood pressure and heartbeat were determined by tail-cuff method.

Student's *t*-test was used to analyze whether there were significant differences among the data.

Assaying of Inhibitory Activity toward Angiotensin I-converting Enzyme (ACE).

The following assay components, in a final volume of 0.25ml, were incubated at 37°C for 30min: 100mM sodium borate buffer (pH8.3), 5mM Hip-HL, 500mM NaCl, 20 mU of rabbit lung ACE, and peptide. The enzyme reaction was stopped by the addition of a 1N HCl solution. The rate of hydrolysis of Hip-HL was determined by measuring the absorbance of the released hippuric acid at 228nm. The ACE inhibitor concentration required to inhibit 50% of the ACE activity under the conditions described above was expressed as IC₅₀.

Analysis of the N-terminal Amino Acid Sequences of Peptides

The N-terminal amino acid sequences of the isolated hypotensive peptides were determined with a protein sequencer G1005A (Hewlett Packard Co. Wilmington, DE).

Determination of ACE Inhibition Manner

Six mM of the peptide was incubated with 10 mU of ACE at 37 °C for 3 h. After the incubation, the mixture was heated to 100 °C for 10 min. The supernatant from centrifugation (10,000g, 10 min) was applied on an ODS column and the chromatographs before and after incubation with ACE were compared. The peaks were recovered, and the molecular weights were determined by electrospray-ionization mass spectrometry.

Results & Discussion

Suppression of Rises in Blood Pressure by the P4 Peptide

Changes in blood pressure when the P4 peptide was injected through a vein are shown in **Figure 1**. Rises in blood pressure were not suppressed in the group to which saline was administered. The blood pressure of the group to which the P4 peptide was administered was significantly lower than that of the control group, and this effect continued for over 30 minutes. In the previous paper, we showed that oral administration of chicken breast muscle thermal extract reduced blood pressure by up to 50 mm Hg in 3 hours. The fact that intravenous administration of the P4 peptide caused drops in blood pressure immediately after the administration suggests that the hypotensive peptide is produced within the body by digestive enzymes and reduces blood pressure.

Manner of Inhibition by the P4 Peptide

The inhibitory mechanisms by the P4 peptide was analyzed in detail by calculating the inhibitory constant (K_i). The inhibitory activity was measured by changing the concentration of the substrate (Hip-HL) at a constant concentration of the P4 peptide. The results were plotted (Dixon plot) to determine the mode of inhibition and the K_i . Because the plots obtained by changing the substrate concentration intersect with the X-axis, the inhibition of ACE by the peptide was found to be noncompetitive inhibition. From the

value at which the line intersects with the X axis, the K_i value was calculated to be 11.5 μM . This value is lower than Hip-HL (2.3 mM) and angiotensin I (70 μM). This indicates that the affinity of the P4 peptide to ACE is higher than those of these ACE substrates. We then investigated whether the P4 peptide is decomposed when the peptide is incubated with ACE, by using HPLC. Incubation with ACE resulted in two additional peaks besides the peak for the original peptide. The molecular weight corresponding to peak 2 was 804, which is the same as that of a peptide lacking the Gly-Phe at the C-terminus of the P4 peptide. The peptide solution after incubation with ACE was heated, and the ACE inhibition activity was investigated again. The IC_{50} of the heated solution was 52.5 μM (the IC_{50} before heating was 46 μM). The area of the HPLC plot showed that about 90% of the P4 peptide had been decomposed.

Length of Peptide Chain and ACE Inhibition

Regions in the P4 peptide involved in the expression of ACE inhibition were investigated by preparing synthetic peptides removed amino acids from the C- and N-termini of the P4 peptide and measuring their ACE inhibition (**Table 1**). A peptide consisting of three residues at the C-terminus showed an inhibitory activity 1/20th of that of the original peptide ($\text{IC}_{50} = 443 \mu\text{M}$). On the other hand, Hyp-Gly-Leu-Hyp-Gly-Phe and Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe were as active as the original P4 peptide, suggesting that Hyp-Gly-Leu at the fourth to sixth positions of the C-terminus, is responsible for the affinity with the enzyme. Removal of the C-terminus produced marked drops in activity, and the drop was especially large when Phe was removed from the C-terminus. All the ACE-inhibitory peptides that have already been reported, such as Ile-Pro-Pro (hydrolysates of casein) (2), Val-His-Leu-Pro-Pro (hydrolysates of corn proteins) (3), and Phe-Gln-Pro, Ile-Tyr, Asp-Tyr-Gly-Leu-Tyr-Pro and Ile-Lys-Pro-Leu-Asn-Tyr (hydrolysates of sardine proteins) (4), have an aromatic amino acid at their C-termini, such as Pro, Phe, or Tyr.

Conclusions

Intravenous administration of synthetic P4 peptide resulted in significant drops in the blood pressures of SHR. This peptide showed noncompetitive manner, and the affinity site was suggested Hyp-Gly-Leu in its sequences.

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Tables and Figures

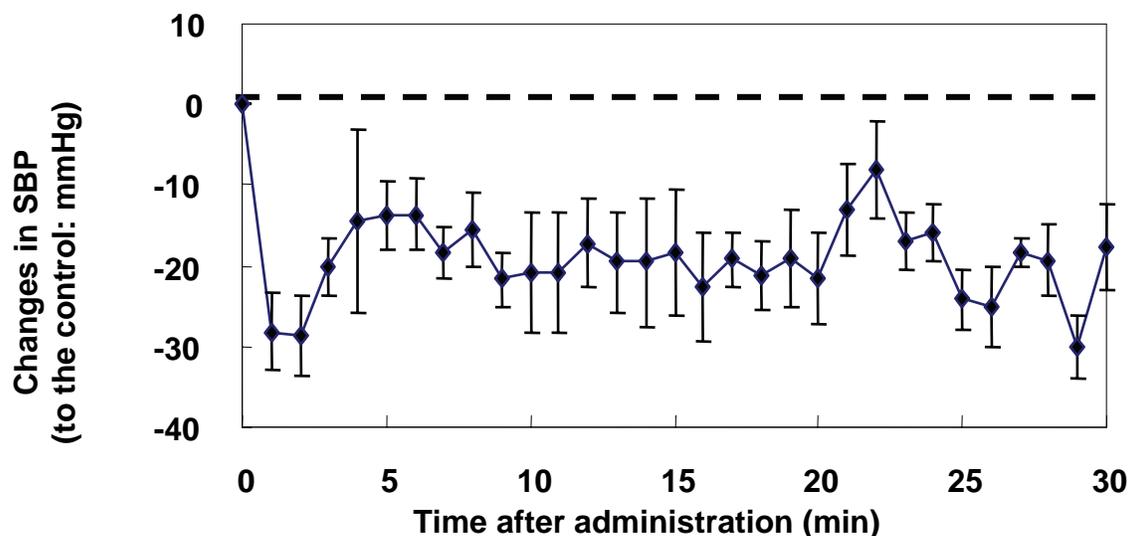


Figure 1. Changes in blood pressure on intravenous administration of the P4 peptide; ■, Synthetic P4; ---, saline (control). The blood pressure of 9-week-old SHR was measured by the tail-cuff method after intravenous administration of the P4 peptide (30mg/kg of rat weight). Average blood pressure values in five SHR are shown. Each bar shows the standard error. The values at whole time for 30min except for at 4 and 22min were significantly lower than those of the control ($p > 0.05$).

Table 1. Identification of the Inhibition Activity Site in the P4 Peptide

Synthetic peptide		IC ₅₀ (μ M)
P4	Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe	26
No.1	Hyp-Gly-Phe	433
No.2	Hyp-Gly-Leu-Hyp-Gly-Phe	10
No.3	Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe	19
No.4	Phe-Hyp-Gly	171
No.5	Phe-Hyp-Gly-Thr-Hyp-Gly	406
No.6	Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly	25000

MITOCHONDRIA DEPENDENT REDUCTION OF METMYOGLOBIN IN VITRO

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Key Words: Metmyoglobin; mitochondria; reduction; NADH; NADPH

Introduction

Oxidation of ferrous myoglobin (Mb) to ferric metmyoglobin (MetMb) is responsible for fresh meat discoloration. Reduction of MetMb can delay this process and prolong desired color.

MetMb has been reported to be reduced enzymatically (DT-diaphorase, MetMb reductase) and non-enzymatically by NADH or NADPH (1-5). The MetMb reductase includes NADH-cytochrome b₅ oxidoreductase and mitochondrial outer membrane cytochrome b (OM cytochrome b); they are located in the mitochondrial outer membrane (6). Mitochondria appear to have the potential to play an important role in MetMb reduction; however, no direct reduction of MetMb by mitochondria has been demonstrated.

NADH is required for non-enzymatic and enzymatic reduction of MetMb and can be produced in cytosolic and mitochondrial fractions. Mitochondrial NADH is generated from the Krebs cycle, or by reversal of electron transport (7-13). Electrons from succinate or cytochrome c can be transferred to NAD⁺ in the reverse direction of the electron transfer chain (ETC), and the energy required for this process can be supplied by endogenous or added ATP (10;11). If mitochondria are subjected to anaerobic conditions, or the ETC is inhibited at complexes III or IV, equivalents from succinate are first transferred to ubiquinone, and from there, instead of moving down the cytochrome region of the chain as in forward electron transfer, they are transferred to the NADH dehydrogenase region. Subsequently, the reduced dehydrogenase flavoprotein can reduce NAD⁺ which is then available for NADH-linked reductions. This mechanism has been reported in the reduction of acetoacetate and glutamate synthesis (14-16). Similarly, Giddings (17) proposed the concept of MetMb reduction by NADH derived from reversal of electron transport in the early 1970s, but its occurrence was not demonstrated.

Collectively, mitochondria have the potential to mediate MetMb reduction by (1) providing appropriate enzyme systems, and (2) generating NADH.

Objectives

The objective of this study was to characterize potential bases for mitochondria-dependent reduction of MetMb.

Methodology

Materials and Chemicals

Beef hearts were obtained locally within 1 h of exsanguination, placed on ice and transported to the laboratory. All chemicals were reagent grade.

MetMb Preparation

Commercial equine heart Mb was dissolved in 50 mM Tris-HCl buffer (pH 7.4), and the MetMb concentration was determined to be 96% (18).

Bovine Mitochondria Isolation

Mitochondria were isolated from bovine cardiac muscle (2 h postmortem) according to Smith (19) with minor modification. Briefly, one hundred g of ground cardiac muscle was washed with 250 mM sucrose twice, and suspended in 200 ml mitochondrial isolation buffer (250 mM sucrose, 10 mM Tris-HCl, 1 mM EGTA, pH 7.4). The suspension was stirred slowly and hydrolyzed with Nagarse protease (protease/tissue = 0.5 mg/g) for 20 min; the pH was maintained between 7.0 and 7.4. After proteolytic digestion, the suspension was diluted to one liter with mitochondrial isolation buffer and subjected to two homogenization processes. The first of these was accomplished in a KONTES DUALL grinder (Vineland, NJ, USA) with three passes, and was followed by one pass with a WHEATON Potter-Elvehjem grinder (Millville, NJ, USA); pestles of these grinders were driven by a heavy-duty drill at 1400 rpm. The homogenate was centrifuged (1,200×g) for 20 min with a Sorvall RC-5B centrifuge (Newtown, CT, USA), and the resulting supernatant was then centrifuged (26,000×g) for 15 min. The pellet was washed twice and suspended in mitochondrial suspension buffer (250 mM sucrose, 10 mM Tris-HCl, pH 7.4) and then used. All steps were performed at 0~4 °C. Mitochondrial protein content was determined by a Bicinchoninic Acid Protein Assay Kit.

Reduction of MetMb

MetMb (2.5 mg/ml) was combined with different reagents in a microfuge open-top tube and incubated at 37 °C. During incubation, samples were removed and centrifuged (15,000×g) with an Eppendorf 5415D centrifuge (Westbury, NY, USA) for 3 min. The resulting supernatant was scanned from 650 to 500 nm with a Shimadzu UV-2101PC spectrophotometer (Kyoto, Japan). The relative proportions of DeoMb, OxyMb, and MetMb were calculated according to Tang et al. (18). Mitochondrial ETC inhibitors, rotenone (0.02 mM) and myxothiazol (0.01 mM), were used in reduction systems;

control experiments did not show any effects of these inhibitors on OxyMb oxidation or MetMb reduction (results not shown).

Statistical Analysis

Results were expressed as mean values of three independent trials. Data were analyzed using the GLM procedure of SAS, and differences among means were detected at the 5% level using Least Square Difference (LSD) with appropriate correction for multiple comparisons (20).

Results & Discussion

Effect of exogenous NADH and NADPH

MetMb was reduced by NADH and NADPH non-enzymatically and the effect was concentration dependent (Figure 1; $P < 0.05$); there was no obvious reduction relative to controls when NADH or NADPH concentrations were 0.2 mM (Figure 1; $P > 0.05$). This is consistent with previous results by Brown and Snyder (1). In the presence of mitochondria, MetMb reduction was observed when NADH was added at 2 and 8 mM (Figure 1, $P < 0.05$), but not at 0.2 mM (Figure 1, $P > 0.05$). The addition of rotenone, an ETC complex I inhibitor, facilitated MetMb reduction in the presence of 0.2 mM NADH and mitochondria, but decreased the extent of reduction in the presence of 2 and 8 mM NADH when compared to controls without rotenone (Figure 1, $P < 0.05$). The increase in MetMb reduction with rotenone addition indicated that mitochondria-dependent reduction was more effective than non-enzymatic reduction at 0.2 mM NAD(P)H. The decreased MetMb reduction with rotenone addition suggested that NADH (2 and 8 mM) was involved in ETC-linked MetMb reduction, as previously reported (21).

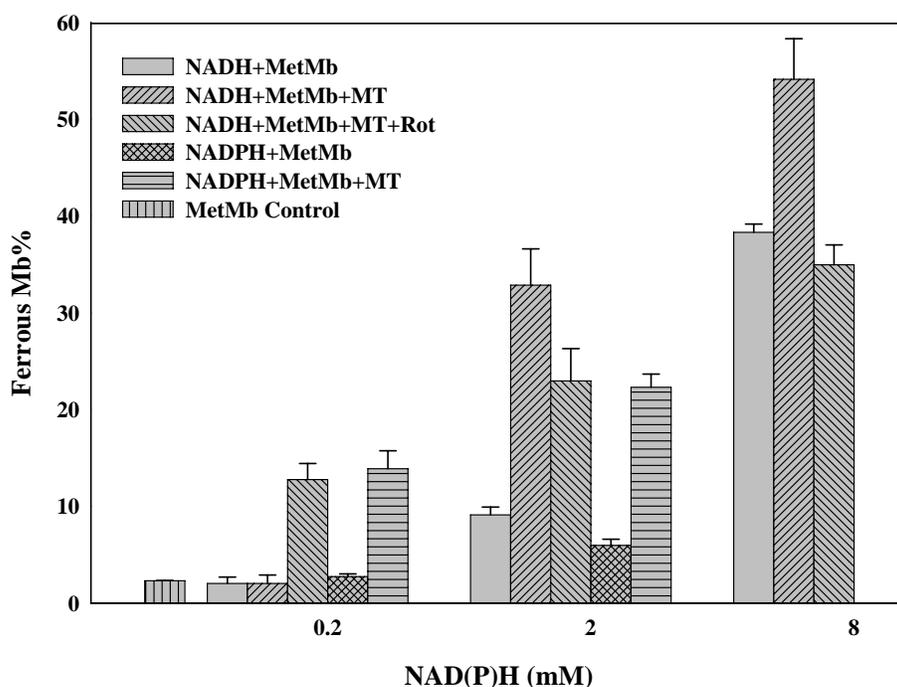


Figure 1. Reduction of metmyoglobin (MetMb, 2.5 mg/ml) by NADH or NADPH in the absence or presence of mitochondria (MT, 1.0 mg MT protein/ml) and rotenone (Rot, 0.02 mM) at 37 °C following 1 h incubation. Controls contained MetMb in buffer only. Each system contained 120 mM KCl, 30 mM Tris-HCl, 5 mM KH₂PO₄ with a final pH 7.2.

NADPH alone or in combination with mitochondria, enhanced MetMb reduction when compared with controls and the reduction was NADPH concentration-dependent (Figure 1, $P < 0.05$). Dicumarol did not inhibit NADPH-dependent MetMb reduction, but rather increased it (Table 1; $P < 0.05$). This suggests that DT-diaphorase did not contribute significantly to mitochondria-dependent MetMb reduction by NADPH. This is not surprising given the reported lower activity of DT-diaphorase in mitochondrial fractions relative to cytosol (22). However, the increased MetMb reduction by dicumarol addition is not readily explained.

Table 1. The Effect of Dicumarol (0.1 mM) on NADPH-dependent Reduction of Metmyoglobin (MetMb) in the Presence of Mitochondria.*

Addition	Control	Control +Dicumarol
Ferrous Mb(%)	12.15a	18.57b

*Controls contained 120 mM KCl, 30 mM Tris-HCl, 5 mM KH₂PO₄, 0.2 mM NADPH, 2.5 mg/ml MetMb, and 1 mg mitochondrial protein/ml with a final pH 7.2; this system was incubated at 37 °C for 1 h. Means with different letters are different ($P < 0.05$).

Effect of the Krebs cycle substrates

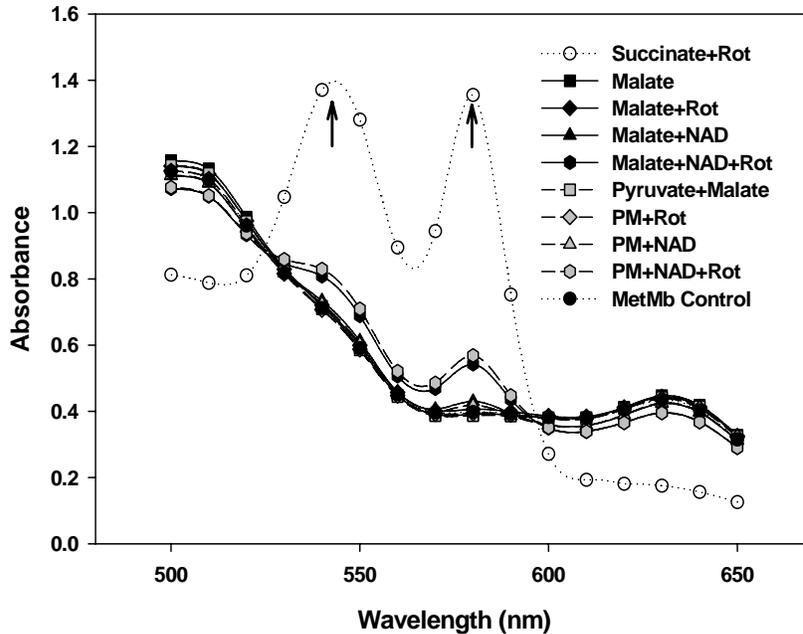


Figure 2. Metmyoglobin (MetMb) reduction by succinate (8 mM), malate (8 mM), and pyruvate (2 mM) plus malate (6 mM) (PM) in the presence of mitochondria (MT) at pH 7.2 and 37 °C for 3 h. Controls contained only MetMb in buffer. Each system contained 120 mM KCl, 30 mM Tris-HCl, 5 mM PH_2PO_4 , 1.0 mg MT protein/ml, and 2.5 mg/ml MetMb. NAD^+ and rotenone (Rot) concentrations were 1.00 mM and 0.02 mM, respectively. Absorbance increases at 544 and 582 nm depicted by arrows indicates MetMb reduction.

Measured increases in absorbance values at 544 nm and 582 nm demonstrated that malate addition led to greater MetMb reduction relative to controls; however, this happened only in combination with NAD^+ and rotenone (Figure 2, $P < 0.05$). Similar results were observed with pyruvate plus malate as substrate (Figure 2, $P < 0.05$). Pyruvate and malate are NADH-linked substrates, and NAD^+ is required for production of NADH by these substrates. The requirement of rotenone for MetMb reduction to be detected in the presence of mitochondria was similar to results with 0.2 mM NADH (Figure 1), and suggested that NADH production was relatively low with NADH-linked substrates. Succinate addition accelerated MetMb reduction relative to controls (with ferrous Mb concentration) by 71.2% following 3 h incubation in the presence of rotenone (Figure 2, $P < 0.05$).

Effect of electron transfer reversal

Table 2. MetMb Reduction by Reversal of Electron Transport in Mitochondria.*

Addition	Control	Control	Control
		+NAD	+NAD+Rot
Ferrous Mb (%)	3.94a	6.22b	4.18a

*Controls contained 0.25 mM sucrose, 50 mM Tris-HCl, 6 mM MgCl₂, 1 mM ATP, 0.01 mM myxothiazol, 8 mM succinate, 2.5 mg/ml MetMb, and 1.0 mg mitochondrial protein/ml with a final pH 7.2. The NAD⁺ and rotenone (Rot) concentrations were 1 and 0.02 mM, respectively. This system was incubated at 37 °C for 3 h. Means with different letters in the same experiment are different ($P < 0.05$).

MetMb reduction by reversed electron transport was proposed by Giddings (17), and Lanier and others (3). In order to reverse electron transport, myxothiazol, a complex III inhibitor, was added to reactions. ATP was used as an energy supply and succinate as an electron donor (10;11). The addition of NAD⁺ increased MetMb reduction relative to controls; this reduction was abolished by the addition of rotenone, a complex I inhibitor (Table 2, $P < 0.05$). This result demonstrated that reversal of electron transport had the potential to reduce MetMb, but it is important to note that the relative effect was not great. Succinate oxidation in the Krebs cycle does not produce NADH, rather, electrons from succinate oxidation are delivered directly to ubiquinone via succinate dehydrogenase. Normally, electrons move to complexes III and IV and ultimately reduce oxygen; however, they can also move in a reverse direction to complex I if oxygen is absent, or if complexes III or IV are inhibited. The reversal of electron transport led to decreased MetMb reduction (Table 2) when compared to forward ETC (6.2% vs. 71.2%) (Figure 2). Thus, our results suggested that forward ETC was more potentially important in mediating MetMb reduction in bovine mitochondria isolated 2 h postmortem.

Summary

Three MetMb reduction pathways mediated by mitochondria were identified: (1) rotenone insensitive and mitochondria-dependent reduction by exogenous NADH; (2) dicumarol insensitive and mitochondria-dependent reduction by exogenous NADPH; and (3) reversal of electron transport from succinate oxidation. NADH produced by oxidation of malate or malate plus pyruvate, or reversal of electron transport from succinate oxidation, also had the potential to reduce MetMb.

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METMYOGLOBIN REDUCTION THROUGH LACTATE-NAD-LDH SYSTEM IN VIVO AND IN VITRO

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Key Words: Lactate, LDH activity, NAD, NADH, MRA, Enhancement, Color stabilization

Introduction

Discoloration of meat surfaces due to brown metmyoglobin (MMb) formation significantly affects consumers' purchase decisions. Hood & Riordan (1973) reported that consumer discrimination against discolored meat was linearly correlated with increases in surface MMb. Renerre & Labas (1987) summarized that even at low levels of MMb, consumers begin to discriminate. Oxidized myoglobin can be converted to deoxymyoglobin (DMb) via metmyoglobin reducing activity (MRA). Then, it can be oxygenated back to oxymyoglobin (OMb). Ledward (1985) suggested that MRA is the most important intrinsic factor controlling the rate of metmyoglobin accumulation in beef. It is now well established that reduction of MMb occurs through both enzymatic and non-enzymatic reducing systems and that NADH is an ultimate reducing substrate for both pathways. However, where NADH comes from has not been established. Watts, Kendrick, Zipser, Hutchins, & Saleh (1966) hypothesized that since postrigor meat contains both lactate and lactate dehydrogenase (LDH), hydrogen may be transferred from lactate to nicotinamide adenine dinucleotide (NAD) by LDH. The reduction of NAD to NADH could be coupled with the reduction of MMb in the presence of intermediate electron carriers such as reductases, quinines, or methylene blue. Mancini, Kim, Hunt, & Lawrence (2004) further reported increased MRA and LDH activity of beef *longissimus* enhanced with 2% lactate. Therefore, we hypothesize that the lactate-NAD-LDH system is partially responsible for both (1) non-enzymatic and enzymatic MMb reduction of postmortem muscle and (2) the increased color life of lactate-enhanced beef. NADH may be replenished via the conversion of lactate to pyruvate by LDH, and the regeneration of NADH can be increased by the addition of substrates such as lactate and NAD.

Objectives

The objectives of this study were to: (1) examine the lactate-NAD-LDH system's ability to reduce MMb in a model system and (2) use an applied model to confirm the influence of lactate enhancement on intrinsic muscle biochemical traits related to color stability such as LDH enzyme activity, NADH contents, and metmyoglobin reducing activity (MRA) in enhanced strip loins.

Methodology

In two experiments, this study investigated the relationship between MMb reduction and the conversion of lactate and NAD to pyruvate with subsequent production of NADH via LDH.

Experiment 1: Lactate+NAD+LDH system in equine MMb model system

Assays of nonenzymatic MMb reduction were carried out at 22-23°C in 10 mm path length cuvettes with 1.0 mL final reaction volume. The standard reaction mixtures at pH 8.0 contained: 0.3 mL of 0.5 mM equine MMb in 30 mM phosphate buffer pH 7.0, 0.1 mL of distilled water, 0.1 mL of 2.0 mM FMN, 0.1 mL of 6.5 mM NAD, 0.1 mL of 200 mM L-lactic acid with Tris 400 mM pH 8.0, 0.1 mL of 0.1 mM methylene blue, 0.1 mL of 50 mM citrate buffer, and 0.1 mL of LDH. The reaction was initiated by adding LDH to the mixture. Absorbance at 580 nm was recorded every 2 sec for 300 seconds. Nonenzymatic reducing activity was calculated as nanomole MMb reduced (equal to nanomole OMB formed) per min during the initial linear phase of the assay, using a difference in molar absorptivity of $12000 \text{ l mol}^{-1}\text{cm}^{-1}$ at 580 nm (the wavelength at which the difference in absorption between MMb and OMB is maximal). Activity is expressed as the mean of triplicate samples. The effect of concentration of NADH, NAD, L-lactic acid and assay pH on the rate of MMb reduction was determined. The final pH of the assay was varied by altering the pH of the citrate and tris buffers. Oxalate or D-lactate (replaced L-lactate) was added to the mixture to investigate their inhibiting effects on LDH in the MMb reducing system.

Experiment 2: NAD+Lactate+LDH system in whole beef loin enhancement

Twelve USDA Select beef strip loins were divided into 4 equal-width sections. One of five treatments was assigned randomly to each loin section using an incomplete block design. Each loin section was enhanced 10% with aqueous solutions consisting of different combinations of lactate (1.5 or 2.5%), phosphate (0.3%), salt, and/or sodium acetate (0.1%). Steaks were packaged in 80% O₂ and 20% CO₂ and stored for 2 or 9 days and then displayed for 5 days at 1°C. Visual and instrumental color were measured on d 2 and 9 to 14 and pH, metmyoglobin reducing activity (Hultquist, 1978), LDH activity (Vassault, 1983; Wahlefeld, 1983) in both directions (Lactate ↔ Pyruvate), and NADH (Klingenberg, 1974; McCormick & Lemuel, 1971) were measured on day 2, 9, and 14 (Fig. 1).

Results & Discussion

Experiment 1: Lactate+NAD+LDH system in equine MMb model system

All necessary constituents for generating NADH were mixed to test the reduction of horse MMb (Table 1).

Table 1. Nonenzymatic reduction of horse MMb with Lactate-LDH system in various mixtures^a at 22°C and pH 8.0

FMN	Methylene Blue	NAD ^b	L-lactic ^c	LDH	Oxalate ^d	D-lactic ^c	Activity (nmole/min)
+	+	+	+	+	-	-	0.69 ± 0.004
-	+	+	+	+	-	-	0.52 ± 0.012
+	-	+	+	+	-	-	0.17 ± 0.003
+	+	-	+	+	-	-	0.02 ± 0.004
+	+	+	-	+	-	-	0.03 ± 0.003
+	+	+	+	-	-	-	0.02 ± 0.000
+	+	+	+	+	+	-	0.40 ± 0.015
+	+	+	-	+	-	+	0.05 ± 0.002

^aSubstances present (+) or absent (-) in mixtures run in triplicate.

^b4.5mM of NAD.

^c200mM of L or D-lactic acid.

^d200mM of oxalate.

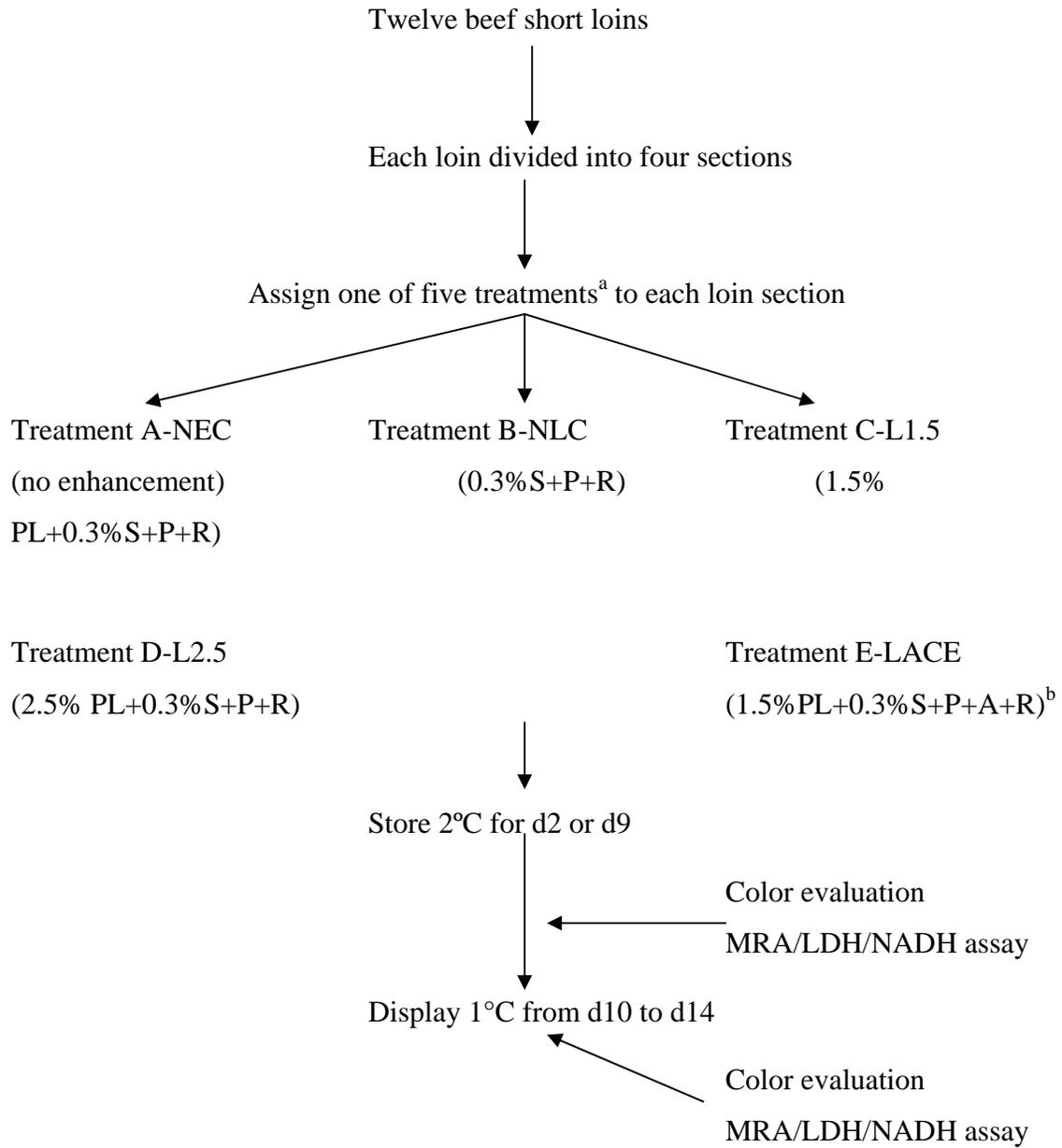


Figure 1. Flow diagram of experimental design.

^aNEC: Non-enhanced control, NLC: No lactate control, L1.5: 1.5% lactate, L2.5: 2.5% lactate, LACE: lactate with acetate.

^bS: Salt, P: Phosphate, PL: Potassium lactate, A: Acetate, R: Rosemary

The nonenzymatic reduction occurred effectively in the lactate-LDH system with NAD. Exclusion of any one necessary constituent (NAD, L-lactic acid, and LDH) reduced or eliminated reduction. Nonenzymatic reduction through the system increased to a level at 4.5 mM NAD in the reaction mixture (Fig. 2).

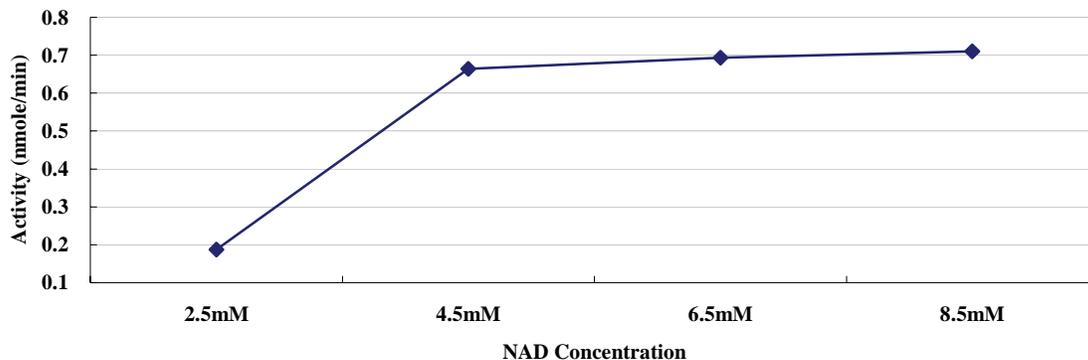


Figure 2. Effects of NAD concentration on horse MMB reduction at 22°C and pH 8.0. All systems contained 200mM of L-lactic acid. Means are the average of 3 determinations. SEM=0.002 to 0.005.

Madhavi & Carpenter (1993) reported that NAD concentrations were directly related to meat color stability because NAD decreased rapidly in post-mortem muscle. They further reported lower NAD concentrations and less MRA in the *psoas major*, a less color stable muscle. Increasing the amount of L-lactic acid also resulted in greater MMB reduction (Fig. 3).

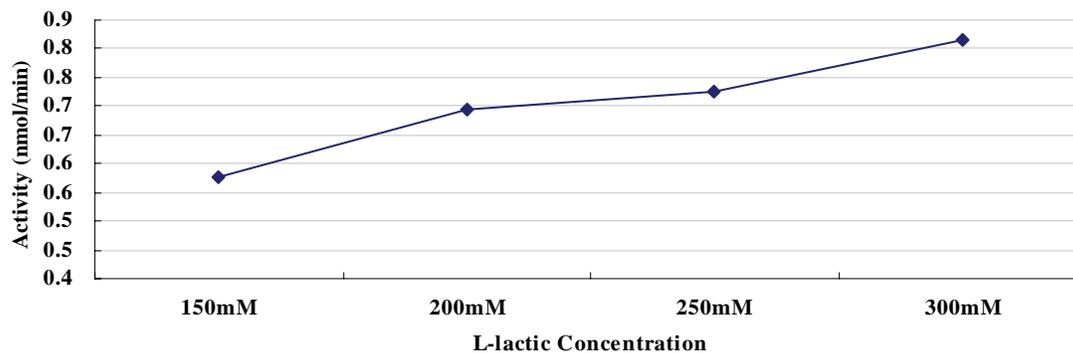


Figure 3. Effects of L-lactic acid concentration on horse MMB reduction at 22°C and pH 8.0. All systems contained 6.5mM of NAD. Means are the average of 3 determinations. SEM=0.003 to 0.005.

Addition of oxalate, a known LDH inhibitor (Wahlefeld, 1983), to the reaction mixture tended to decrease reducing activity (Table 1). Replacing L-lactic acid with D-lactic acid in the assay mixtures reduced MMB reduction (Table 1), probably due to the selective interaction of LDH with L-lactic acid (D-lactic acid is not preferred for metabolism; Hall, 2000). As expected, the reduction reaction through lactate-NAD-LDH system was highly favorable in alkaline conditions (Fig. 4). The equilibrium of the LDH

reaction favors oxidation rather than reduction of NAD due to the acidic condition created by lactic acid (Vassault, 1983). However, although minimal, the nonenzymatic reduction through the lactate-NAD-LDH system still occurred at pH of 5.7, which is near the postmortem muscle pH.

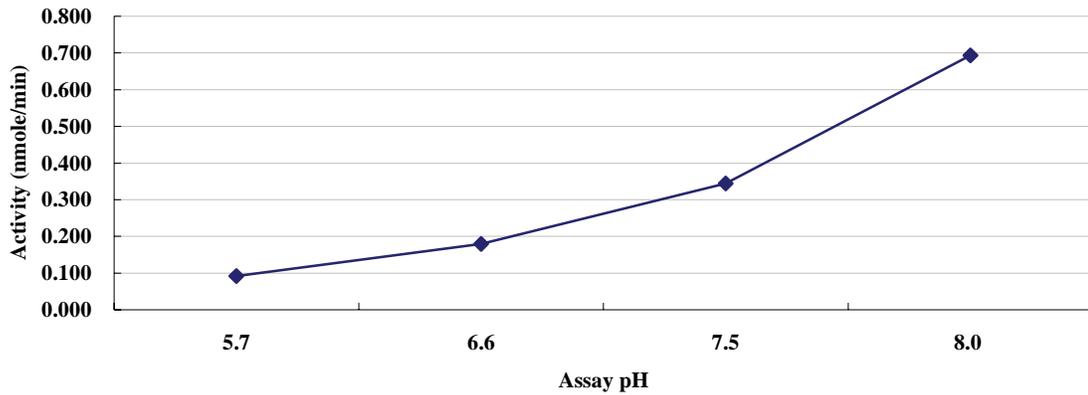


Figure 4. Effects of pH on horse MMb reduction at 22°C.
All systems contained 200mM of L,-lactic acid and 6.5mM of NAD. Means the are average of 3 determinations. SEM=0.0001 to 0.0003.

Experiment 2: NAD+Lactate+LDH system in whole beef loin enhancement

Color, Color Stability and pH

Steaks enhanced with 2.5% lactate had the least visual discoloration, most color stability, and were most red (greatest a^* values) at the end of display (Fig. 5). Non-enhanced controls and enhanced steaks without lactate were most discolored throughout display. Steaks treated with the combinations of lactate and acetate were less dark ($P < 0.05$) than steaks only treated with lactate. All enhanced meat samples had a slightly higher pH than non-enhanced controls at the end of display, likely due to the phosphate and/or lactate (Table 2).

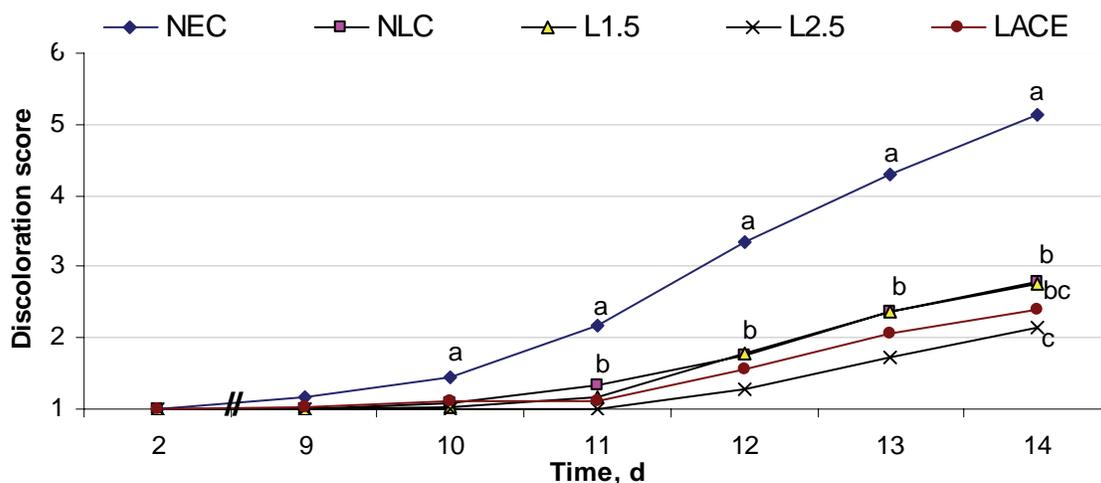


Figure 5. Effects of lactate enhancement on strip loin steak visual color. Discoloration evaluated as % MMb on the surface: 1 = no discoloration (0%), 2 = slight discoloration (1-19%), 3 = small discoloration (20-39%), 4 = modest discoloration (40-59%), 5 = modest discoloration (60-79%), 6 = extensive discoloration (80-99%), 7 = total discoloration (100%). ^{abc}Treatments within a display day with a similar letter do not differ ($P > 0.05$).

LDH Activity, NADH, and MRA

Enhancing steaks with 2.5% lactate significantly increased LDH activity in both directions (Lactate \leftrightarrow Pyruvate) compared to 1.5% lactate, no lactate controls, and non-enhanced controls on d 14 (Table 2). Consequently, increased LDH activity replenished more NADH, which was utilized for MMb reduction.

Table 2. Effects of lactate on pH, metmyoglobin reducing activity (MRA), lactate dehydrogenase (LDH 1 & 2) activity, and NADH content at d 14 of display

Treatment	pH	MRA ^c	LDH1 ^d	LDH2 ^e	NADH ^f
NEC (non-enhanced control)	5.83 ^a	28.2 ^a	145 ^a	161 ^a	1.0 ^a
NLC (no lactate control)	5.94 ^b	30.1 ^{ab}	147 ^a	162 ^a	0.9 ^a
L1.5 (lactate 1.5%)	5.94 ^b	32.7 ^b	151 ^a	164 ^a	1.1 ^{ab}
L2.5 (lactate 2.5%)	5.93 ^b	33.2 ^b	172 ^b	213 ^b	1.4 ^b
LACE (lactate + acetate)	5.90 ^b	31.9 ^b	161 ^{ab}	182 ^a	1.2 ^{ab}

^{ab}Least square means within a column with a similar letter do not differ ($P > 0.05$).

^cMetmyoglobin: nmoles reduced/min/g of muscle.

^{de}Lactate dehydrogenase activity: $\mu\text{mol}/\text{min}/\text{g}$ sample.

^fNADH: $\mu\text{g}/\text{ml}$.

NADH contents for steaks enhanced with 2.5% lactate increased during storage and display and were significantly greater than treatments without lactate on d14 compared to d2. Throughout storage and display, MRA decreased for all steaks. However, steaks

with 2.5% lactate enhancement retained more MRA compared with non-enhanced controls at the end of display. Increased MRA in 2.5% treated steaks was convincing evidence for increased NADH production via enhancement-stimulated LDH activity (Fig. 6).

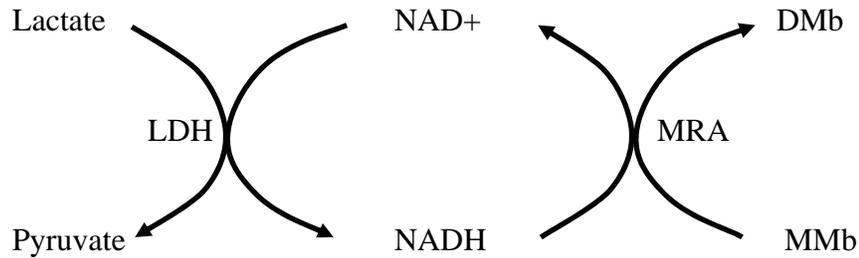


Figure 6. Proposed mechanism for lactate stabilization of meat color.

Conclusions

The results indicate that NADH can be regenerated through the lactate-NAD-LDH system *in vivo* and *in vitro*. Steaks enhanced with lactate had more color stability than steaks without lactate and were considerably more color stable than non-enhanced steaks. Lactate appears to promote color stability by the conversion of lactate to pyruvate via increased activity of lactate dehydrogenase and the concomitant regeneration of NADH. The NADH subsequently reduces metmyoglobin to either oxy- or deoxymyoglobin. The increased color stability due to lactate is accompanied by a darkening of muscle color. However, inclusion of acetate with lactate decreased muscle darkening and slightly improved color stability.

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ANTI-OBESITY EFFECT OF PORK-LIVER PROTEIN HYDROLYSATE IN DIABETES AND OBESITY MODEL RATS

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Key Words: pork-liver protein hydrolysate, obesity, diabetes, OLETF rat, lipogenesis

Introduction

Obesity is defined as the accumulation of excess adipose tissue resulting from various metabolic disorders. It is a strong risk factor of hyperlipidemia, heart disease and type II diabetes mellitus, and it is associated with low capacity of insulin to regulate glucose and lipid metabolisms. Recently, much attention has been focused on several food materials such as soy protein or buckwheat protein in survey of materials to prevent obesity. Liver has been traditionally used as a food ingredient to supply several nutrients. However, limited information is available on the physiological functions of liver extract.

Objectives

The aim of this study was to investigate the efficacy of pork-liver protein hydrolysate (PLH) on body fat accumulation in Otsuka Long-Evans Tokushima Fatty (OLETF) rat that is an animal model of non-insulin-dependent diabetes mellitus and obesity.

Materials & Methods

Preparation of pork-liver protein hydrolysate(PLH)

Pork liver was cut into very small pieces and dissolved in water at 280g/ L. The pork liver solution was hydrolyzed by a proteinase at pH 7.0 and 45 °C for 4 h. A proteinase was added to the protein solution at the 10g/ L. After the enzyme reaction, the enzyme was inactivated by heating at 95 °C for 1 h and then centrifuged at 3,000 x g for 10 min. The supernatant was ultrafiltrated through a membrane which separated molecular weights below 1,000. The permeable solution was spray-dried as a PLH.

Animals and Diets

Male 4 wk old OLETF rats were obtained from Tokushima Research Institute (Otsuka Pharmaceutical Company, Tokushima, Japan) and housed individually. The

animals were fed a standard laboratory chow plus tap water ad libitum until 19 wk old and assigned to experimental groups with the same average body weight. Then, two groups of 7 rats were fed experimental diet with or without PLH, and adapted to the experimental conditions and diets from 20 to 34 wk old. Composition of the experimental diets is shown in Table 1. The rats were individually pair-fed the PLH diet and the casein diet. Food intake and body weight were measured daily. Blood samples were collected from the tail vein at 24, 28 and 32 wk old rat. After the experimental period (34 wk old), rats were killed by decapitation under light anesthesia with diethyl ether. The liver, adipose tissues such as perirenal and epididymal fat and gastrocnemius muscle were excised and immediately weighed. They were then frozen using liquid nitrogen and kept at -80 °C until use.

Analysis of plasma parameters

Plasma concentrations of triglyceride and free fatty acid (FFA) were measured using enzymatic kits obtained from Wako Pure Chemical Industries (Osaka, Japan). Plasma leptin and insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) kit according to the manufacture's directions (Shibayagi, Gumma, Japan and Morinaga, Yokohama, Japan, respectively).

Assays of enzyme activities

Portions of the liver from individual rats were homogenized in an ice-cooled 10 mM Tris-HCl buffer (pH 7.4) containing 0.25M sucrose and 1 mM EDTA. Cytosolic and mitochondrial fractions were prepared as described previously. The supernatant was used for the assay of glucose-6-phosphate dehydrogenase (G6PDH) and fatty acid synthase (FAS). G6PDH and FAS activities were assayed spectrophotometrically as described by Kelly et al. (1, 2). Activity of carnitine palmitoyltransferase I (CPT) in the mitochondrial fractions was measured using L-carnitine, palmitoyl CoA, and 2-nitrobenzonic acid according to the method of Markwell et al. (3). The protein concentration was determined according to the method of Lowry et al. (4), by using bovine serum albumin as a standard.

Statistical analysis

Results were expressed as means \pm SE. Statistical significance of differences between the two groups was evaluated by Student's *t*-test. Results were considered significant at $P < 0.05$.

Results & Discussion

1. Dietary PLH suppressed the increase in body weight

Fig. 1 shows body weight of rats. During the feeding period from 20 to 34 wk old, the rats fed casein and PLH gained 191 g and 136 g, respectively. Supplementation of PLH significantly suppressed the increase in body weight compared with casein diet in 28 to 34 wk old rats. During the experimental period for 14 wks, casein and PLH diet groups consumed 2328 ± 0.1 g casein and 2319 ± 0.1 g PLH, respectively. Thus, PLH diet seems to possess the function to suppress the increase in body weight.

2. Dietary PLH significantly lowered leptin concentration in 24, 28 and 32 wk old rats

Concentrations of plasma biochemical parameters in 24, 28 and 32 wk old rats were shown in Fig. 2. During these periods, plasma concentration of leptin was significantly lower in the PLH group compared to the casein group. Consumption of PLH tended to reduce plasma FFA and triglyceride ($P < 0.10$). There is no significant difference in plasma insulin level between the two groups. These results indicate that the PLH diet influences lipid metabolism. There are few possibilities that PLH diet enhances lipolysis of triglyceride in adipose tissue and leads to lower adipose tissue weight.

3. Dietary PLH lowered abdominal fat pad weight, but did not affect muscle weight

The weight (g) and relative weight (%) of abdominal fat pad in the PLH group was significantly lower than those of the casein group (Table 2). The weight of gastrocnemius was unaffected by the dietary treatment. The ratio of the fat pad and gastrocnemius muscle weights in the PLH group was significantly lower than that in the casein group. It was concluded that dietary PLH lowered abdominal fat pad weight but not other tissues such as muscle.

4. Dietary PLH lowered the activities of hepatic G6PDH and FAS

The activities of hepatic G6PDH and FAS in the OLETF rats were significantly lower in the PLH group compared with the casein group (Table 3). This result indicates that consumption of PLH diet markedly suppressed hepatic activities of lipogenesis enzymes in the liver. The activity of CPT was also significantly lower in the PLH group compared with the casein group (Table 3). However, this slight reduction of hepatic CPT activity does not seem to influence. From these results, the reduction of hepatic lipogenesis by PLH diet seems to bring about anti-obesity of rats and the decrease of plasma markers such as leptin.

Conclusions

We first discovered PLH diet suppress the development of obesity in OLETF rats. The effect of PLH diet on anti-obesity appears to be, at least in part, due to lowered hepatic lipogenesis enzyme activities, especially G6PDH and FAS.

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Tables and Figures

Table 1 Composition of experimental diets

Ingredients	Casein	g/ kg	PLH
Casein ¹	230		66
PLH ²	0		200
Corn starch	270		234
Sucrose	300		300
Cellulose powder	50		50
Soybean oil	100		100
Mineral mixture ³	35		35
Vitamin mixture ³	10		10
DL-Methionine	3		3
Choline bitartrate	2		2

¹ Protein component of casein is 87.0% (w/w) (N X 6.25).

² Protein component of PLH is 77.1% (w/w) (N X 6.25).

³ The mineral mixture and vitamin mixture are prepared according to the AIN-76 mixture.

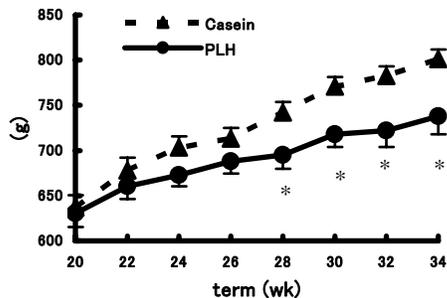


Fig. 1 Effect of dietary PLH on body weight

Table 2 Effect of dietary PLH on the weights of adipose tissue and muscle

	Casein	PLH
Fat pad (perirenal+epididymal)		
wt ^a (g)	114 ± 3	92 ± 5**
relative wt (g/ kg body wt)	139 ± 2	123 ± 3**
Gastrocnemius muscle		
wt ^b (g)	5.5 ± 0.1	5.3 ± 0.2
relative wt (g/ kg body wt)	6.7 ± 0.1	7.1 ± 0.2
Fat/ muscle (a/b)	19.7 ± 0.5	16.4 ± 0.9**

**p<0.01

Table 3 Effect of dietary PLH on the activities of hepatic lipogenesis enzymes

	Casein	PLH
G6P dehydrogenase (nmol/ min mg prtein)	95.0 ± 4.2	18.9 ± 5.2*
Fatty acid synthase (nmol/ min mg prtein)	15.6 ± 0.8	8.8 ± 1.2*
Carnithine palmitoyltransferase (nmol/ min mg prtein)	3.97 ± 0.20	3.26 ± 0.25*

*p<0.05

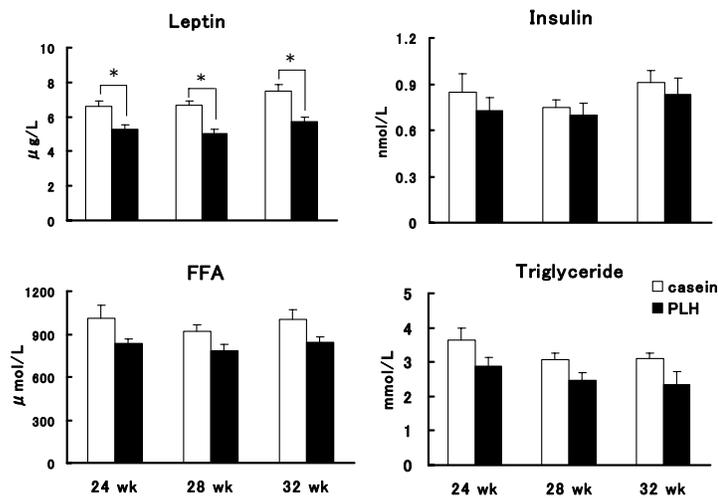


Fig. 2 Effect of dietary PLH on plasma parameters

Production Systems and Meat Quality

**PRACTICAL USE OF AN ECONOMIC INDEX FOR SIMULTANEOUS
GENETIC IMPROVEMENT OF LIVE PERFORMANCE, CARCASS AND
MEAT QUALITY OF THE MODERN PIG**

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Key Words: Pig Genetics, Meat Quality

Introduction

For decades, selection of boars and gilts within lines and selection between lines (crossbreeding) has been the standard procedure for genetically improving traits of economic importance to the pork industry. For producers, production traits such as growth rate, feed conversion, and carcass lean are of great economic importance. Traits of economic importance for packers include carcass lean, and primal, sub-primal, and processing yields. In recent years, as packers have moved from selling “commodity pork” to branded pork products, meat quality has become more economically important (Allen, 1995; Sosnicki et al., 2003). Tenderness, juiciness, flavor, and other organoleptic traits are paramount to the consumer and must also be considered when setting up selection objectives as the ultimate goal for the industry is to produce products that the consumers want at a price they will pay (Miller, 2003; Sosnicki et al., 2003). Although different segments of the industry rely on differing economic criteria, a genetic improvement program must include those traits with the greatest economic importance to the overall pork chain.

Without the ability to directly measure meat quality in live animals, only progeny testing or measuring meat quality in full- or half-siblings has been available to calculate estimated breeding values (EBVs) for meat quality traits. Larzul et al. (1999) demonstrated that glycolytic potential measured *in vivo* was a viable option to predict meat quality, but recent guidelines and standards for animal welfare discourage live animal biopsies. Modern practices that reduce the difficulty of including progeny, full-sibling and half-sibling data allows for traits such as pH to now be more readily incorporated into a genetic improvement program. The wide-ranging effects of ultimate pH on pork quality and the relative ease of collecting the phenotypic data make this an ideal trait for selection purposes. Ultimate pH has been shown to be genetically and phenotypically correlated with many economically important criteria such as color, water holding capacity, and sensory quality (Huff-Lonergan, et al., 2002; 2003). More specifically, a higher ultimate pH is associated with better water holding capacity, translating into lower drip or purge losses during storage, and a higher yield when processing (Eikelenboom et al., 1995). Although continuing to increase pH may have a positive effect on water holding capacity and processing characteristics, flavor of fresh

pork and shelf life may be compromised when ultimate pH exceeds 6.1 (Bidner et al. 2004; Klont et al. 2002). Therefore, genetic companies wishing to improve meat quality must keep the pH level within an upper threshold to both maximize the benefits of darker color and improved WHC while minimizing effects of potential off-flavor and decreased shelf life. Moreover, focusing selection strictly on one area of performance such as meat quality while disregarding potential negative effects on growth or carcass quality could actually create a negative trend for the overall economic performance of the animal. Huff-Lonergan et al. (1998) showed the converse of this to be true where selection emphasis placed strictly on lean growth efficiency while ignoring meat quality traits resulted in lower pH and higher drip loss than in the randomly selected control line.

Until recently, molecular genetics and marker assisted selection (MAS) has provided little contribution to the standard animal breeding program, but this is rapidly changing as more genetic markers and genes are discovered. These techniques allow the animal breeder to more readily exploit the diversity of the current population (Beuzen, et al. 2000). In addition to basic evaluation programs and quantitative selection approaches, PIC has included major genes and markers into the breeding program in order to increase the rate of genetic improvement for traits of economic importance to the pork industry. PIC products sold in North America are guaranteed free of the Hal-1843® mutation and the RN- gene. Additionally, favorable alleles of the PRKAG3 mutation are included in the selection process due to the demonstrated positive effects on glycolytic potential and resultant meat quality traits (Ciobanu, et al., 2001; Fields, et al., 2002; Oliver et al., 2003; Sosnicki et al., 2003). Furthermore, there are several additional meat quality markers in the advanced validation phase that will soon be available for inclusion in the PIC breeding program (Knap, et al., 2002).

When considering a multi-trait, “desired gains” approach to a breeding program it is necessary to verify the approach is both commercially feasible and biologically effective. The purpose of the research presented in this paper is to evaluate the effectiveness and demonstrate that the combination of quantitative selection and marker assisted selection is a viable tool for simultaneous improvement of carcass and meat quality performance in the modern commercial animal.

Objectives

The objective of this paper is to demonstrate the effectiveness of selection of boars based on an economic index to improve growth, feed conversion, carcass leanness, and loin and ham pH of their progeny.

Methodology

Twenty-eight PIC 280M boars of a predominantly Duroc background from a commercial Gene Transfer Center (GTC) were ranked and bi-directionally selected based on their commercial sales index (High genetic merit = High index and Low genetic merit = Low index). Estimated breeding values used to calculate the economic index for each boar came from the PIC Global Best Linear Unbiased Prediction (BLUP) that included the effects of the boar’s PRKAG3 genotype in addition to performance data. The mean

sales index for the “High” indexing boars was 105.1 and 89.4 for the “Low” indexing boars.

Three one-week breeding periods at a commercial farm were set up in March, April, and May 2004. During each breeding period approximately 140 PIC C22 sows were single-sire mated using semen from the selected boars. Prior to cross-fostering, pigs were double-tagged with duplicate numbered colored ear tags. Boar and sow ear tag numbers and farrowing date were recorded for each pig.

Pigs were approximately 10 weeks of age when weighed on-test. Each pig was individually weighed at the start of the growing – finishing period and allotted to one of 50 pens within a room in the finisher by gender and according to whether its sire was a “High” or “Low” sales index boar. Ten pigs were allotted to each pen. Feed consumption was recorded on a pen basis.

All pigs were individually weighed off-test at a target weight of 125 kg and a unique tattoo number was applied to each pig’s shoulder so identification could be maintained at the packing plant. The pigs were harvested at a commercial packing facility that harvests approximately 10,000 pigs per day. Pigs were electrically stunned and placed into a standard holding cooler (~3°C) for 24 hours. A Fat-O-Meat’er (SFK Technologies, Denmark) was used to measure backfat thickness, loin depth, and lean percentage. Hot carcass weight was recorded for all carcasses. For each of the three replicates of pigs produced for the trial, the pigs were harvested on three separate days. Approximately 160 to 170 pigs were harvested on each day.

Loin and ham pH measurements were taken approximately 22 hours post-mortem in the cooler utilizing an MPI “pistol grip” pH meter fitted with a Mettler-Toledo glass tipped probe (Meat Probes, Inc., Topeka, Kansas). Loin pH was measured in the *longissimus dorsi* at approximately the last rib while ham pH measurements were taken in the *semimembranosus* by inserting the probe approximately 2.5 cm caudal of the aitch bone to a depth of approximately 5 cm.

SAS PROC GLM (SAS Institute, Inc., Cary, NC) was used to complete the statistical analyses. Sources of variation accounted for in the analyses for all traits included breeding group, gender of the pig, and the linear regression of the progeny’s phenotype for each trait on its sire’s sales index. For average daily gain and feed conversion, a linear covariate for on-test weight was also included in the statistical model. A linear covariate for age at harvest was included in the statistical model in the analysis of carcass lean percentage. For loin and ham pH, fixed effects for harvest date nested within breeding group was included in the statistical models to account for day-to-day variation. Response graphs were developed showing the changes in each trait as the sales index of the boars increased from 70 to 136 index points (Figure 1).

Results & Discussion

Table 1 presents the estimates of the regression of the progeny’s performance on its sire’s sales index for each trait. For loin pH, the coefficient of .001 indicates that pH will increase .01 units for every 10-point increase in the sales index ($P < .001$). Likewise, ham pH increased .007 units for every 10-point increase in the sales index ($P < .05$). Selection of boars based on the sales index resulted in a .017 improvement in feed conversion and a

.126 % increase in carcass leanness for every 10-point increase in the sales index ($P < .01$ and $P < .001$, respectively).

Figures 1 through 4 graphically represent the incremental change in progeny performance as the sires' sales index increases. All responses are linear in nature. As greater selection intensity is placed on a genetic line (ie. selecting only the top 20% of boars in the herd), improvements can be made more rapidly and the resultant change will have a greater economic impact to the industry.

Conclusions

The research clearly shows that a simultaneous improvement of pig live performance, carcass, and meat quality is both commercially feasible and biologically effective. By using index selection, unfavorable genetic correlations between production, carcass and meat quality traits can be overcome. Ultimately, products that improve profitability for all segments of the pork chain can be brought to the marketplace in a timelier manner.

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Tables and Figures

Table 1. Estimates of the regression of progeny performance on its sire's sales index

Trait	Regression coefficient	Probability
Loin pH	.0010 ± .0003	P < .001
Ham pH	.0007 ± .0003	P < .05
Lean Percentage, %	.0126 ± .004	P < .01
Average Daily Gain, g/day	.078 ± .146	P < .60
Feed Efficiency, feed, kg:gain, kg	-.0017 ± .0005	P < .001

Figure 1.

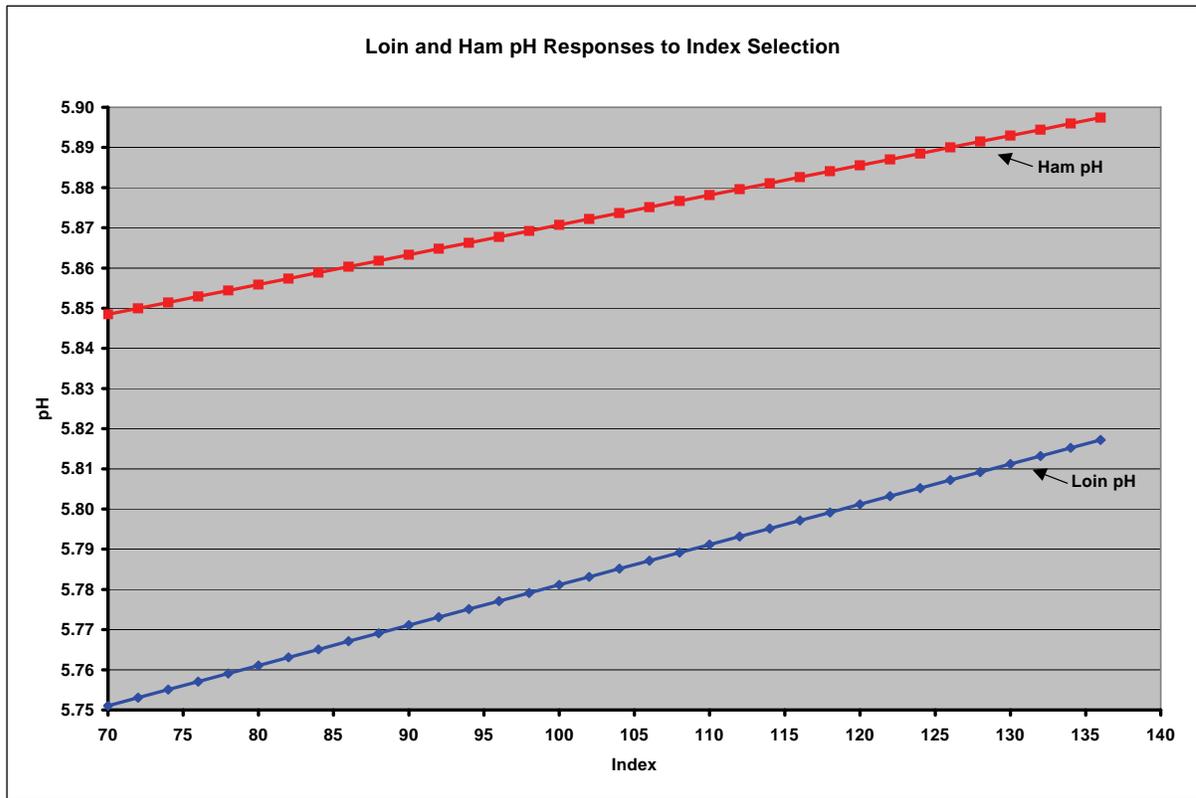


Figure 2.

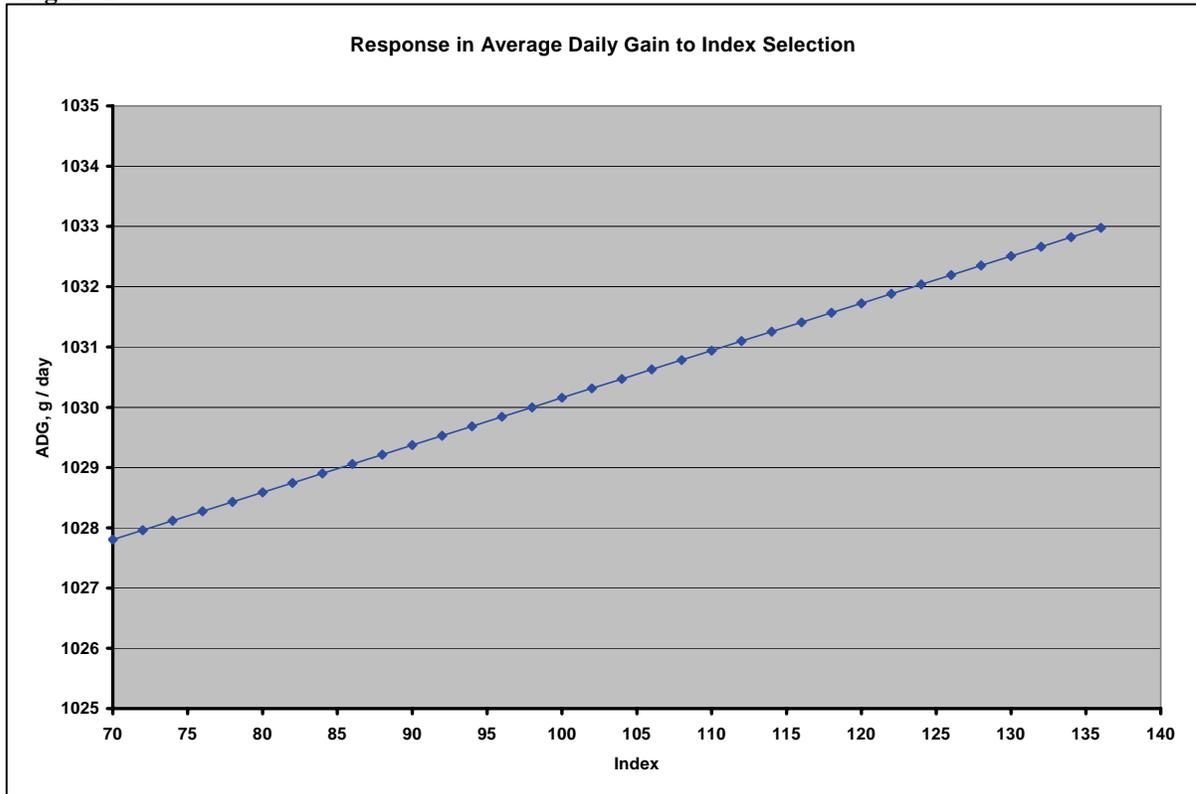


Figure 3.

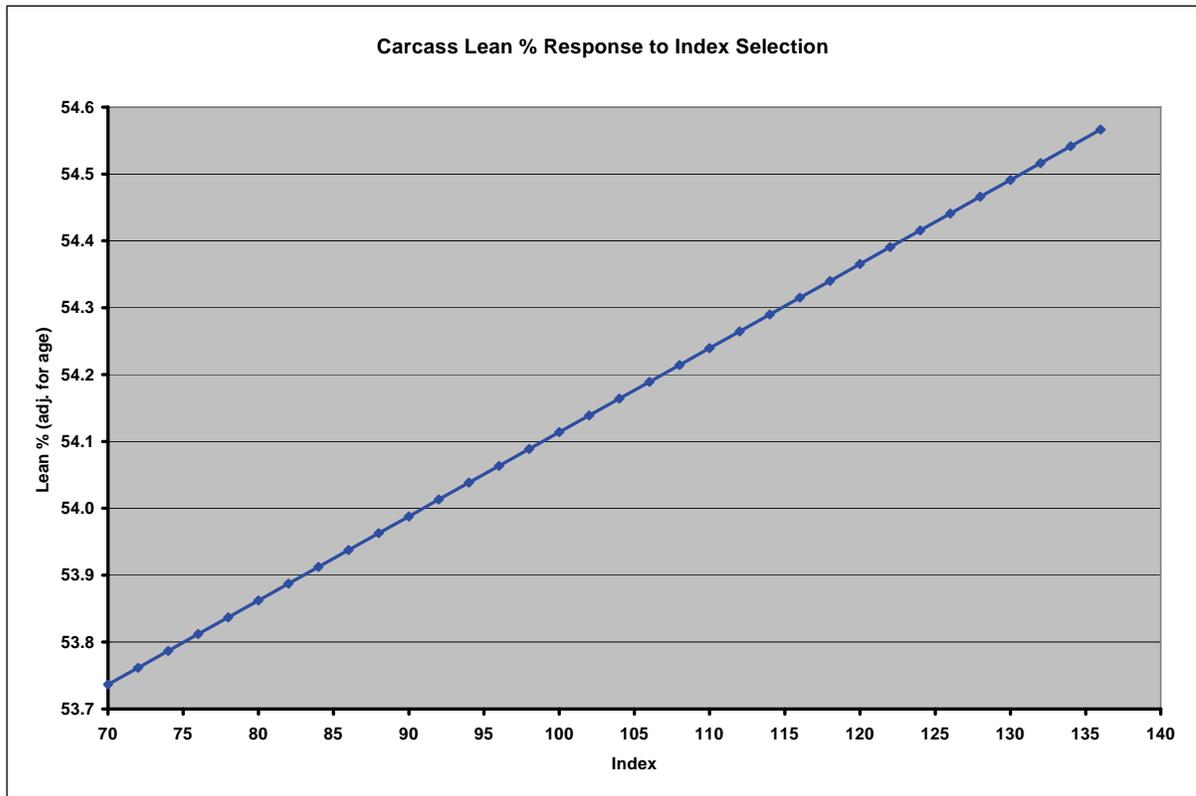
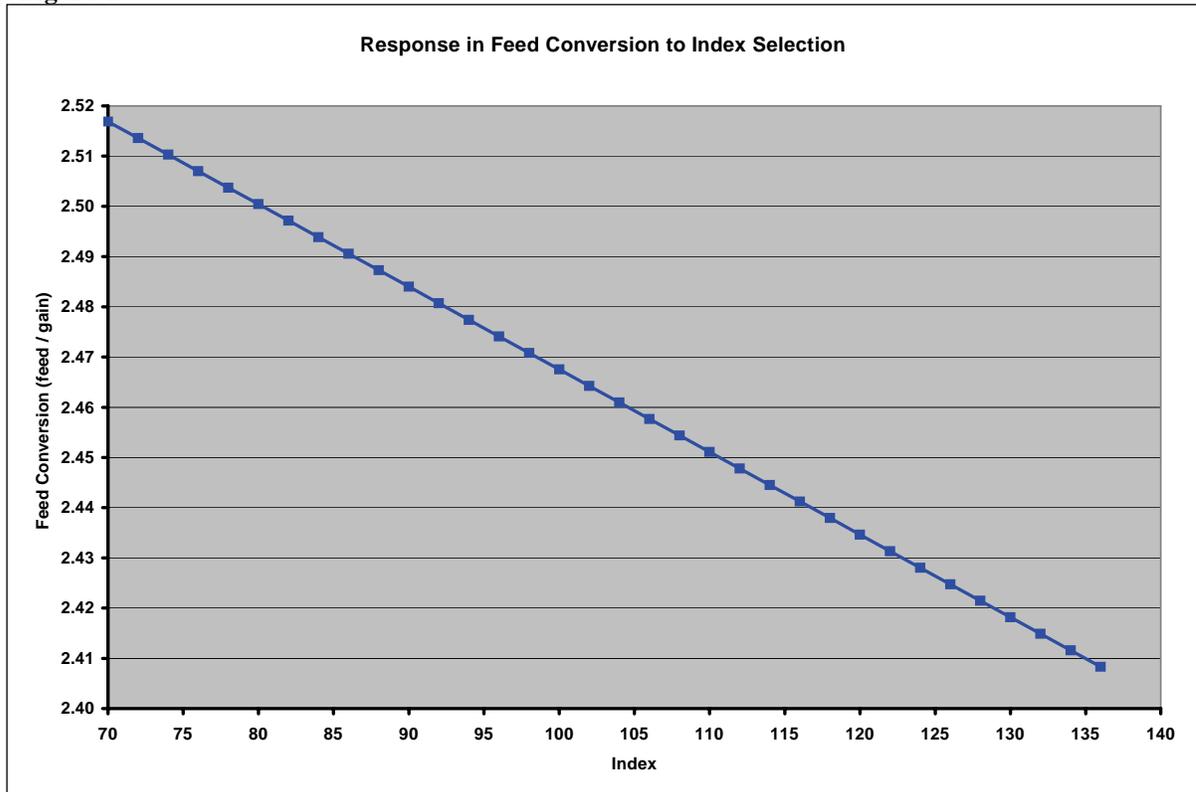


Figure 4.



RELATIONSHIP BETWEEN SIRE EBV'S AND CARCASS AND MEAT QUALITY TRAITS

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Key Words: Lamb, carcass, breeding value

Introduction

As a part of the meat science program of the Australian Sheep Industry CRC (www.sheep.crc.org.au) a resource flock was established to generate progeny to be used in strategic studies on fat and muscle development. One aspect that is under investigation in the multifaceted program is the relationship between the estimated breeding value of sires for specific traits and the performance of their progeny. Hopkins et al. (2004) recently reported that the lightness of the m. *longissimus thoracis* (LL) increased as the estimated breeding value (EBV) for post weaning weight (PWWT) increased and intramuscular fat content in the LL decreased as sire EBV for post weaning muscle depth (PEMD) increased. It was suggested that this latter effect partially explained the finding of a decrease in eating quality in the LL, but this remains to be validated. In the same work Hegarty et al. (2005b) found that selection for increased PEMD decreased carcass fat and increased carcass lean. Whether this effect is maintained as animals grow is unknown.

Objective

To examine the impact of sire EBV's for fat, muscling and growth on carcass composition and meat quality traits in lambs of varying age.

Methodology

Animals

In 2002 a flock of 450 Merino ewes and 120 Border Leicester x Merino (BLM) ewes was created. Using LAMBPLAN (Banks 1994) databases a number of sires were selected based on their EBV's for growth and muscle development. The lamb types generated were as follows; Poll Dorset x Border Leicester x Merino (with a focus on growth in the

sires), Poll Dorset x Merino (with a focus on muscling in the sires), Poll Dorset x Merino (with a focus on growth in the sires), Merino x Merino (with a focus on growth in the sires), Border Leicester x Merino (with a focus on growth in the sires). There were 4 different sires per group. Only progeny sired by Poll Dorset rams and representing 3 of the genotypes will be considered in this paper.

Experimental Design

All lambs were weighed pre-weaning in September 2003 and based on this weight lambs were randomly allocated in a stratified way to one of the 4 slaughter times, balanced for rearing type, gender and sire. The experiment was designed for slaughters across the age range of 4 to 20 months. Data for 4 and 8 month old lambs is presented here these representing unweaned and weaned lambs, respectively.

Slaughter Protocol and Measurements

Lambs to be slaughtered at each age were allocated to 2 slaughter days and 2 slaughter groups within slaughter days based on stratified weight and balanced for sire. All lambs were electrically stunned (head only) and carcasses were trimmed according to the specifications of AUS-MEAT (Anon, 1992). Hot carcass weights were recorded and the GR measured (total tissue depth over the 12th rib, 110 mm from the midline) using a GR knife and the carcasses chilled. The pH was measured in the left-hand portion of the m. *longissimus thoracis et lumborum* (LL) at the caudal end over the lumbar/sacral junction at 24 hours. A section of subcutaneous fat and the m. *gluteus medius* was cut away to expose the LL. pH of the m. *semitendinosus* (ST) was measured at 24 using WPS meters with temperature compensation (TPS, WP-80, PTS Pty Ltd) and a polypropylene spear-type gel electrode (Ionode IJ 44), calibrated at ambient temperature. The left hindleg was removed from the loin section by a cut 30 mm distal to the lumbar/sacral junction and the muscle depth of the rump (m. *gluteus medius*) and of the subcutaneous fat was measured. A section of the LL (~50 grams) from the caudal end was collected for determination of intramuscular fat (IMF) and frozen at -20°C. The percentage of intramuscular fat was determined using a near infrared procedure (NIR) in a Technicon Infralyser 450. NIR readings were calibrated with chemical fat using solvent extraction. The method is further described by Perry *et al.* (2001). The caudal end of the LL was exposed to the air at ambient temperature for 30-40 min and the meat colour measured on the cut surface using a Minolta Chroma meter (Model CR-300) set on the L^* , a^* , b^* system (where L^* measures relative lightness, a^* relative redness and b^* relative yellowness). The chroma meter was operated using Illuminant C and a white tile standard ($Y = 93.1$, $x = 0.3135$, $y = 0.3197$). Three replicate measurements were taken at the same position with special effort to avoid areas of connective tissue or intramuscular fat. A thin (1-2 mm) slice of frozen LL muscle (-20°C) from 1 day aged portions was used for determination of sarcomere length using laser light diffraction as reported by Bouton *et al.* (1973). Muscle samples (2 g) taken from 1 day aged LL were held at -20°C and subsequently used for determination of 'free' calcium concentration. The calcium concentration was determined using a Ca^{2+} electrode (Cole-Parmer, Niles Illinois, USA) as described by Hopkins and Thompson (2001). Subsequent to boning of the left side, the remaining bone was removed by sawing the carcass down the middle of the vertebral

column. The entire right sides were transported chilled by road to Werribee (DPI, Vic) where they were weighed and carcass length measured between the anterior edge of the first rib and the anterior end of the pubic symphysis. The sides were scanned by dual energy X-ray absorptiometry (DXA) using a Hologic QDR 4500A fan beam X-ray bone densitometer (Hologic, Waltham, MA, USA). The half carcasses were positioned flat down on the DXA table with the cut surface down and the percentage of fat, lean and bone estimated. After scanning the entire backstrap (Anon. 1998 product identification number HAM 5100) was removed with overlying subcutaneous fat and the fat depth over the LL at the 12th rib measured (Fat C), as was the depth and length of the LL cross section (EMD and EML respectively). The weight of both the LL and ST was determined.

Statistical Analysis

Traits were analysed using a REML procedure (Genstat 7.1, 2004), which contained the fixed effects of post-weaning eye muscle depth EBV, (PEMD), post-weaning weight EBV (PWWT) and post-weaning weight fat EBV (PFAT), dam breed (BLM or Merino), slaughter group (suckers or weaned), gender (wether or ewe) and significant interactions if appropriate. Non-significant fixed effects were removed accordingly. Sire and slaughter day (1-4) x slaughter time within day (1 or 2) were included as random effects and for carcass measures weight of the carcass was included as a covariate, except for compositional data.

Results & Discussion

In total 198 mixed sex lambs were slaughtered representing 8 different sires. In table 1 the traits, which were significantly related to sire EBV's are shown excluding any significant effects of gender, slaughter age or dam breed. For the following traits there was no relationship to sire EBV's (LL weight, depth, width and cross sectional area, rump fat and muscle depth, carcass length, muscle calcium concentration or sarcomere length). Not surprisingly as the value of the EBV PFAT increased the percentage of carcass lean decreased and carcass fat increased. There was a consistent increase in measures of fat depth as the PFAT EBV increased as would be expected and as verified by the reports of Hall et al. (2002) and Hegarty et al. (2005a). This response indicates that changes in both FatC and GR are reflecting changes in the percentage of fat and lean in the carcass. The increase in intramuscular fat (IMF) percentage with an increase in the PFAT EBV is consistent with the response in carcass fatness indicators. However the fact there was no decrease in IMF as the PEMD EBV increased was contrary to the findings of both Hopkins et al. (2005) and Hegarty et al. (2005) which may be due to the wider age span used in the current study. As both the EBV's for PEMD and PWWT increase the weight of the ST increased and both EBV's are required for this effect to be significant. This indicates that selection for muscling may have a positive effect on hind leg muscle content.

The effect of PEMD EBV on muscle pH was notable indicating that as muscling increases, pH at least at 24 hours after death will also increase in both the LL and ST. However both the EBV for PEMD and PFAT are required for the effect on LL pH ($P = 0.06$), and this effect did not persist when ultimate pH was examined. The response in the

ST is not consistent with muscling leading to increased anaerobic capacity. An increase in the lightness of loin meat with increasing PWWT EBV was also found by Hegarty et al. (2005a), and in this study both the EBV's for PEMD and PWWT were required for there to be a significant relationship with lightness (L^*) and yellowness (b^*).

Conclusions

Results presented here demonstrate that selection emphasis in terminal sires will impact on the carcass and meat quality traits of their crossbred progeny. These effects are not all beneficial (eg higher muscle pH), whereas other effects such as the ability to reduce carcass fat could be considered beneficial. These outcomes demand that the interaction between traits and the absolute effects of sire selection on traits need to be quantified so informed decisions can be made about selection decisions.

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Tables and Figures

Table 1. Effect of Poll Dorset sire EBV's on carcass and meat quality traits

Traits	PEMD	PEMD	PFAT	PFAT	PWWT	PWWT
	Sign.	Coefficient (s.e.)	Sign.	Coefficient (s.e.)	Sign.	Coefficient (s.e.)
Lean %	NS		0.001	-1.87 (0.32)	NS	
Bone %	NS		NS		NS	
Fat %	NS		0.001	1.93 (0.33)	NS	
Intramuscular fat %	NS		0.05	0.36 (0.16)	NS	
ST weight* (g)	0.05	5.51 (2.73)	NS		0.05	1.99 (0.93)
Hot carcass weight (kg)	NS		0.05	0.98 (0.44)	0.05	0.15 (0.07)
GR*	NS		0.001	1.49 (0.45)	NS	
FatC*	NS		0.05	0.61 (0.25)	NS	
LL pH – 24 hour	0.06	0.01 (0.006)	0.05	-0.03 (0.012)	NS	
LL pH - ultimate	NS		NS		NS	
ST pH – 24 hour	0.05	0.04 (0.015)	NS		NS	
<i>L</i>	0.05	0.79 (0.36)	NS		0.05	0.37 (0.12)
<i>a</i> ⁺	0.05	0.32 (0.16)	NS		NS	
<i>b</i> ⁺	0.05	0.20 (0.10)	NS		0.05	0.07 (0.03)

*Adjusted to a carcass weight of 21.1 kg. ⁺Adjusted to a mean pH of 5.67.

**THE EFFECT OF FEEDING CLOVER SILAGE ON POLYUNSATURATED
FATTY ACID AND VITAMIN E CONTENT, SENSORY, COLOUR AND LIPID
OXIDATIVE SHELF LIFE, OF BEEF LOIN STEAKS**

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Key Words: Clover, PUFA, Oxidative and colour stability, Vitamin E

Introduction

Grass and legumes are important constituents of the forage-based feeding systems used for beef in the UK. European Union policy on sustainability of the environment discourages the use of inorganic fertilizers for grass production and encourages the use of legumes. Their inclusion in a sward also produces a higher economic benefit for the livestock sector compared to grass-only systems (Rochon *et al.*, 2004). The inclusion of white and red clovers in swards and silages has been shown to increase dry matter intake and liveweight gain for beef cattle (Yarrow & Penning, 2001). In addition, studies on beef finished on clover pastures/silages showed a beneficial contribution of red clover to higher polyunsaturated fatty acid (PUFA), especially contents of meat n-3 PUFA (Enser *et al.*, 2001a; Scollan *et al.*, 2002). Clover silage produced a lower biohydrogenation and higher flow of PUFA through the rumen (Lee *et al.*, 2003) and this may have been due to higher concentrations of polyphenol oxidase activity in the red clover (Lee *et al.*, 2004)

Green forage and other leafy materials, including good quality hay and silage, are not only good sources of vitamin E but also of other compounds with antioxidant activity such as carotenoids and flavonoids (McDowell *et al.*, 1996; Gatellier *et al.*, 2005). The vitamin E content of fresh herbage is between 5 to 10 times as great as that in some cereals or their by-products, thus meat from grass-fed animals is able to delay lipid oxidation and discoloration thereby maintaining a better shelf-life during retail display compared to meat from standard commercial concentrate-fed beef cattle ((McDowell *et al.*, 1996; Faustman & Wang, 2000)

Whilst an increase in n-3 PUFA can be beneficial in terms of human health, they can put an oxidative stress on meat systems. In a previous trial it was noted that meat from animals that had grazed mixed grass/clover swards were more oxidatively unstable and had lower vitamin E concentrations in the meat than that from animals grazed on a grass-only sward. (Enser *et al.*, 2001; Scollan *et al.*, 2002) A recent study (Al-Mabruk *et al.*, 2004) has shown that milk from cows fed legumes (alfafa, white clover and red clover) had milk with lower oxidative stability compared to that from grass-fed animals and that vitamin E supplementation prevented this increased oxidative deterioration

Objectives

The present study aimed to establish the effect of feeding graded amounts of red clover silage with grass silage on intramuscular fatty acid composition, shelf-life (lipid and colour stability) and eating quality of beef meat and the effect of a vitamin E supplement on these characteristics.

Methodology

Thirty-two (32) Charolais steers with a mean initial live weight of 490 kg (s.e. 6.7 kg) were randomly allocated to one of four treatments, resulting in eight animals per treatment. The animals were fed *ad libitum* on forage and standard commercial concentrates to achieve approximately 0.7 and 0.3 of the dietary dry matter intake respectively. The forages were as follows: GS: 100% grass silage; GCS: grass and red clover silage mix (50:50 DM basis); CS: 100% Red clover silage; CS^{+E}: Red clover silage plus high vitamin E concentrate (500 IU/kg).

The grass silage used was first cut perennial ryegrass (*Lolium perenne*) harvested in May, and the red clover (*Trifolium pratense*) silage was harvested in July of 2003. A biological inoculant (Powerstart, Genus Ltd) was added during collection at the recommended rate to aid the fermentation process. The grass was ensiled in a concrete clamp, covered by plastic and weighted down using tyres and bales and the clover was wilted for a 24 period before ensiling in big bales.

Animals within a treatment were penned together and fed individually using roughage intake control feeders and concentrate feeders. They were accustomed to the diet during a 21-day preliminary period and remained on treatment for 100 days. Animals were transported on the day before slaughter and were kept in lairage overnight with access to water and slaughtered conventionally using captive bolt stunning. Carcasses were held in chill (1-2°C) for 48 h before butchering and sampling.

At 48h post-mortem, samples of m. *longissimus thoracis* at the 11th rib were removed and blast frozen for fatty acid and vitamin E analysis. An additional 180mm section of muscle was conditioned at 1°C for 12 days in vacuum pack. A 100mm section was then frozen at -20°C for sensory analysis. After overnight thawing at 1°C, 20mm thick steaks were cut and grilled to 74°C internal temperature. The meat was assessed by a 10 person trained taste panel using 100mm unstructured line scales (see Vatansever et al., 2000). Four steaks 20mm thick were cut from the remaining sample, packed in modified atmosphere trays (O₂:CO₂, 75:25) and subjected to simulated retail display (700lux lighting for 16h a day, 4°C±1°C). Colour (L*a*b*) was measured on the surface of two steaks at three points, daily with a Minolta Chromameter. The saturation (chroma), $\sqrt{a^{*2} + b^{*2}}$, describing the intensity of the colour was calculated (MacDougall and Rhodes, 1972). The remaining steaks were taken at 7d of display and analysed for lipid oxidation as thiobarbituric acid reacting substances (TBARS) by the methods of Tarladgis et al. (1960).

Lipid was extracted using chloroform/methanol as per Folch *et al.* (1957) and separated into neutral and phospholipid. Fatty acid methyl esters were prepared by alkaline hydrolysis followed by methylation with diazomethane and analysed on a CP Sil 88, 100m x 0.25mm ID column (Chrompack, UK) and individual fatty acids quantified,

as described by Demirel *et al.* (2004). Total fatty acid was taken as the sum of all the phospholipid and neutral lipid fatty acids quantified. The extraction and HPLC separation and quantification of vitamin E was essentially as described by Liu *et al.* (1996) using rac-5, 7-dimethyltocol as internal standard.

Results & Discussion

Carcass, fat and fatty acid composition

Increasing the amount of clover silage in the diet tended to increase the dry matter intake, liveweight gain and carcass conformation of the animals but not significantly so over the 100d feeding period. Total fat content of the muscle (TFA), amount of saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA) tended to increase (non-significantly) with the proportion of clover in the diet (Table 1), whilst the amount of polyunsaturated fatty acids (PUFA) increased significantly ($p < 0.001$) with each increment. This was less clear with the CS+E group, which had less total fat. The PUFA change was due to increase in 18:2n-6 and 18:3n-3 (significant at $p < 0.001$) and long chain PUFA (non-significant). Overall there was a significant decline in the ratio of 18:2n-6:18:3n-3 and a small but significant ($p < 0.01$) increase in the P:S ratio as clover increased. In the neutral lipid fraction (results not shown) there was a similar pattern to that for total lipid, with TFA, SFA and MUFA tending to increase with each increment in clover, whilst PUFA ($p < 0.05$) and 18:3n-3 ($p < 0.01$) increased significantly. There were more changes in the composition of phospholipid fatty acids and as these affect lipid stability, a fuller set of results is shown in Table 2. TFA, SFA and PUFA all increased significantly with red clover addition. The percentage of 18:2n-6 and 18:3n-3 increased significantly with each increment in red clover fed ($p < 0.001$) replacing, in part, the MUFAs, 18:1 *cis*-9 and 20:1 and conjugated linoleic acid (CLA 9-*cis*,11-*trans* C18:2). The fatty acid composition of the muscle of animals fed the CS+E was essentially the same as that from animals without the supplement.

Shelf life

The values for vitamin E concentration, TBARS and colour saturation after 7 days simulated retail display, and selected sensory attributes are shown in Table 3. TBARS increased incrementally with increasing amount of clover in the diet, but the largest and most significant change ($p < 0.001$) was from GCS to CS. This was mirrored by the change in colour saturation with the intensity of redness decreasing with increasing increments of clover in the diet (Figure 1). The amount of unsaturated fatty acids in the meat increased with increasing amounts of clover inclusion in the diet and would have placed a greater oxidative stress upon the system. However, it would appear that these results can be explained, at least in part, by the concentration of vitamin E found in the muscle. As the amount of clover in the silage increased so the concentration of vitamin E in the muscle decreased ($p < 0.001$). Adding a supra-nutritional amount of vitamin E to the 100% red clover diet restored the concentration of muscle vitamin E to that found in meat from grass-fed animals, reduced the TBARS value and increased the colour stability (Figure 1). It has been suggested that optimum stability for meat is obtained when the

muscle concentration of m. *longissimus* is 3-3.5 mg/kg lean muscle (Liu et al., 1996) and this was reached in muscle from GS and CS+ animals.

There was no effect of diet on sensory characteristics, though the CS+E diet produced the toughest meat (which was least fat). There was a trend for the more unstable meat (that from CS) to have slightly more rancid and fishy notes.

Conclusions

Feeding red clover silage as 0.7 of the diet increased the content of the beneficial PUFA in meat, but this was at the expense of both lipid and colour stability. This instability was more easily explainable as being due to a low vitamin E content rather than the increased content of PUFA, since a supra-nutritional supplement of vitamin E in the diet restored the vitamin E concentration and stability of the meat to that seen with other diets.

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Tables and Figures

Table 1. Total fatty acid composition (mg/100g of lean tissue) and ratios in beef loin steak

Muscle	GS	GCS	CS	CS ⁺	sed	sig
Total FA	3081	3639	4001	3074	0.36	NS
SFA	1339	1584	1760	1322	276.9	NS
MUFA ^b	1300	1535	1643	1270	261.7	NS
PUFA	170.7 ^a	206.4 ^b	244.4 ^c	216.8 ^{bc}	13.44	***
18:2n-6	73.2 ^a	92.8 ^b	113.2 ^c	99.3 ^b	6.68	***
18:3n-3	22.5 ^a	34.1 ^b	50.7 ^c	37.5 ^b	3.83	***
P:S ^c	0.07 ^a	0.09 ^{ab}	0.10 ^{bc}	0.12 ^c	0.01	**
18:2n-6:18:3n-3	3.28 ^c	2.73 ^b	2.30 ^a	2.66 ^b	0.15	***

^{a,b,c} means within a row with the same letter do not differ significantly (Fisher's least significant difference procedure, post hoc)

*** $p \leq 0.001$, NS - not significant

GS- grass silage; GCS- mixture (50/50) of grass and red clover silage; CS- red clover silage; CS⁺- red clover silage plus vitamin E supplement (500 IU/kg concentrate)

P:S calculated as (18:2n-6 + 18:3n-3)/(12:0+14:0+16:0+18:0)

SFA calculated as (12:0+14:0+16:0+18:0)

MUFA calculated as (16:1+18:1trans+18:1 cis-9+18:1cis-11+20:1)

PUFA calculated as (18:2n-6+18:3n-3+20:3n-6+20:4n-6+20:5n-3+22:4n-6+22:5n-3+22:6n-3)

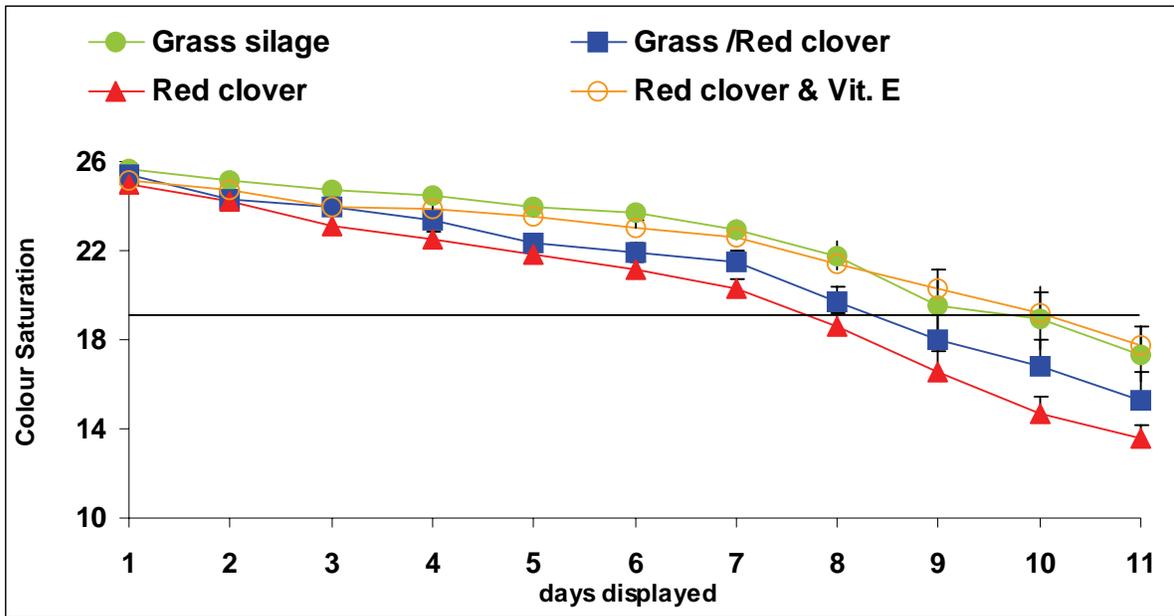
Table 2. Fatty acid composition of the phospholipid fraction of lean tissue
% of fatty acids

	GS	GCS	CS	CS+E	sed	sig
14:0	0.28	0.29	0.39	0.35	0.06	ns
16:0	14.48	14.01	14.97	14.78	0.44	ns
18:0	9.76	10.00	10.09	9.74	0.02	ns
18:1 cis-9	22.55 ^b	20.98 ^{ab}	19.30 ^a	19.02 ^a	1.28	*
18:2n-6	10.54 ^a	12.07 ^b	13.38 ^b	13.57 ^b	0.74	***
18:3n-3	2.58 ^a	3.42 ^b	4.36 ^c	3.98 ^c	0.26	***
CLA	0.21 ^b	0.17 ^a	0.15 ^a	0.15 ^a	0.02	**

Table 3. Meat quality measurements, shelf life and sensory

	GS	GCS	CS	CS+E	sed	sig
TBARS d7	0.64 ^a	1.31 ^b	4.88 ^c	1.13 ^b	0.45	***
Colour saturation d7	22.9 ^a	21.5 ^b	20.3 ^c	22.6 ^{ab}	0.57	***
Vitamin E	3.47 ^a	2.92 ^b	1.80 ^c	3.32 ^a	0.18	***
Toughness	46.5 ^{ab}	41.6 ^a	42.0 ^a	48.2 ^b	2.8	*
Beef flavour	31.7	34.2	34.2	31.0	2.28	ns
Rancid	0.2	0.3	0.6	0.3	0.3	ns
Fishy	0.6	0.2	1.1	0.4	0.5	ns

Figure 1. Effect of diet and days displayed upon colour saturation of beef loin steaks during simulated retail display in MAP.



EFFECT OF PASTURE BOTANICAL DIVERSITY ON THE OXIDATIVE STABILITY OF LAMB MEAT

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Key Words: lipid oxidation, protein oxidation, forage, lamb, antioxidative enzymes

Introduction

Nowadays, increasing attention is paid to the quality of the animal's diet in relation to meat quality. In this context, a lot of studies aimed at increasing the polyunsaturated fatty acid content (PUFA), particularly the n-3PUFA, in animal products. This can be achieved by selecting an appropriate dietary fat source such as forages, fish oil, linseed(oil) (see review e.g. Raes et al., 2004). As PUFA are more prone to oxidation, these dietary strategies could compromise the oxidative stability of the product. Oxidation is a complex process, involving pro- and antioxidants, and can damage lipids as well as proteins and DNA. The effect of antioxidants, of which α -tocopherol(acetate) is the most frequently studied one, has mainly been focused on limiting lipid and pigment oxidation. However, besides tocopherols, plants contain numerous other compounds with antioxidative characteristics, e.g. flavonoids, polyphenols, carotenoids. The effects of this complex of plant antioxidative compounds to prevent oxidation of lipids, pigments and proteins *post mortem* is not well studied yet. In addition, besides the possible contribution from the supply of dietary antioxidants, muscle contains an endogenous antioxidative system of which the enzymes glutathione peroxidase (GSH-Px), catalase (Cat) and superoxide dismutase (SOD) are considered the most important. The effect of diet on the activity of these enzymes has not been investigated thoroughly.

Objectives

The aim of this study was to investigate the effect of botanical diversity in pastures (intensive ryegrass, herbage rich grass and leguminosa rich grass) on the oxidative stability of lamb meat.

Methodology

Experimental set-up

A group of 21 lambs (mean (sd) live weight: 22.3 (3.06) kg) was bought from an organic farm (Berendrecht, Belgium). Prior to the experiment, lambs had been exclusively grazing with their mother. Lambs were divided in three groups (n=7) for grazing on three different pastures from 01/07/2004 till 22/09/2004, i.e. an intensive ryegrass pasture (IR) (Melle, Belgium), a herbage rich pasture (HBG) and a leguminosa rich pasture (LG). The botanical composition of the pastures was determined weekly. The intensive ryegrass consisted mainly of *Lolium perenne*, while the herbage rich grass consisted for 80 to 90% of *Agrostis stolonifera* and *Bromus hordeaceus*. The leguminosa rich pasture contained as predominant plants red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*). After 3 months grazing, animals (mean (sd) live weight: 32.3 (6.53) kg) were slaughtered in a local slaughterhouse (Ronse, Belgium). Carcasses were cooled for 24 h at 2°C. The *longissimus thoracis* was sampled for oxidative measurements (color, lipid and protein stability). Samples for measuring the α -tocopherol content and antioxidative enzymes were vacuum-packed and stored at -18°C.

Meat oxidative stability analyses

Color stability Steaks (2.5 cm thickness) were over-wrapped in a O₂ permeable PVC film and stored at 4°C for 8 days under constant illumination with white fluorescent lights (900 lux). Color and color stability measurements were performed using a HunterLab Miniscan spectrophotometer (D65 light source, 10° standard observer, 45°/0° geometry, 1 in. light surface, white standard). The color coordinates, expressed as CIE L*a*b* values and the reflectance values, to calculate % metmyoglobin (Krzywicki, 1979), were measured daily.

Lipid oxidation was measured as thiobarbituric acid reacting substances (TBARS) at day 4 and 8 of display and is expressed as μ g malonaldehyde (MDA)/g muscle (Tarladgis et al., 1960).

Protein oxidation was measured by following a decrease in the amount of thiol groups and expressed as nmol free SH-groups/mg protein (Batifoulier et al., 2002). Protein oxidation was determined on the same samples used for lipid oxidation measurements.

α -tocopherol content (μ g α -tocopherol/g meat) was determined after saponification and extraction by HPLC on a Supelcosil LC18 column (25mm x 4.6mm x 5 μ m) with UV-detection (λ = 292 nm) (Desai, 1984).

Endogenous antioxidative enzyme activities GSH-Px activity was determined by measuring spectrophotometrically (340 nm) the oxidation of NADPH at 22 °C (DeVore and Greene, 1982). One unit of GSH-Px was defined as one mole NADPH oxidised per min and per g meat. Cat activity was measured as described by Aebi (1983). The reaction (H₂O₂ loss) was monitored by measuring the absorbance at 240 nm at 22°C. One unit (U) of catalase was defined as one mole H₂O₂ decomposed per min and per g meat. Total SOD activity was determined according to the procedure of Marklund & Marklund

(1974) using inhibition of pyrogallol autoxidation by measuring an increase in absorbance at 340 nm at 22°C. One unit was taken as the activity that inhibits the reaction by 50%.

Statistical analysis

Oxidative stability measurements were analysed by one-way analysis of variance using pasture type as fixed factor. Comparison of means was performed using Tukey test ($P < 0.05$) (SPSS for Windows, version 11.0).

Results & Discussion

The intramuscular α -tocopherol content showed a trend towards a higher content in meat from lambs fed intensive ryegrass compared to those fed herbage rich grass or leguminosa (1.72, 1.24 and 1.09 μg α -tocopherol/g muscle for IR, HRG and LG respectively) ($P = 0.091$). No effect of forage type was observed on the color parameters (data not shown) and color stability measurements (Table 1). Also lipid oxidation, measured as TBARS-values, was not influenced by the pasture type (Table 1). Although the TBARS-values increased during time of display, the lipid oxidation was still limited after 8 days (mean (sd) 0.61 (0.30) μg MDA/g muscle). However, a significant effect of the pasture type was observed on protein oxidation, measured by the amount of free thiol groups after 8 days of display (Table 1). The data suggest a more pronounced protein oxidation in the meat from the lambs on the herbage rich pasture. It is not clear why an effect of diet on protein oxidation was observed, while colour and lipid oxidation remained unaffected. Sista et al. (2000) suggested, using a chicken muscle model system, that sulfhydryls were utilized to stabilise primary oxidation products. However, measuring thiol groups is only one method to evaluate protein oxidation.

Oxidation of muscle post mortem can also be limited by the endogenous antioxidative enzymes, which seem to be relatively stable during refrigerated storage (Renerre et al., 1996). The activities of the endogenous antioxidative enzymes, depending on the feeding group, are presented in Table 2. No effect of the pasture type was observed on the SOD activity, while a trend for a higher Cat activity was observed for the intensive ryegrass group compared to the other groups. A significantly higher GSH-Px activity was found for the leguminosa rich pasture group compared to the other groups. This finding is in line with studies on bovine meat showing a strong dependence of the activity of GSH-Px on the finishing mode (i.e. concentrate or grass-fed) (Gatellier et al., 2004). The effect of diet on SOD and Cat seems to be less consistent. An elevation of GSH-Px activity is commonly associated with oxidative stress (Frank and Messano, 1980), which would suggest a higher oxidative stress for the animals on the leguminosa rich pasture. Factors that may be responsible for this dietary effect include differences in the deposition of n-3PUFA (e.g. red clover was shown to limit biohydrogenation of n-3PUFA by Lee et al., 2003), but other antioxidants, minerals and especially Se could also influence the activity of GSH-Px. However, differential effects of diet on protein oxidation and GSH-Px activity were observed, whereas no effect of diet on colour and lipid oxidation was seen.

Conclusions

The oxidative stability of lamb meat was not clearly affected by the type of pasture. Only effects on protein oxidation and activity of GSH-Px were observed.

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Tables and Figures

Table 1. Effect of pasture botanical diversity on color, lipid and protein oxidation in *longissimus thoracis* of lambs (n = 7)

	Intensive Ryegrass	Herbage rich pasture	Leguminosa rich pasture	SEM	P
Color oxidation: % Metmyoglobin					
Day 4	33.4	40.4	38.6	1.63	0.196
Day 8	38.2	41.6	42.9	1.75	0.552
Lipid oxidation: TBARS ($\mu\text{gMDA/g}$ muscle)					
Day 4	0.32	0.41	0.31	0.06	0.359
Day 8	0.68	0.63	0.52	0.12	0.631
Protein oxidation: nmol free SH-groups/mg protein					
Day 4	62.8	62.8	70.4	2.71	0.106
Day 8	61.0 ^a	48.6 ^b	64.4 ^a	1.97	0.000

^{ab} Means within a row with different superscripts are significantly different ($P < 0.05$)

Table 2. Effect of pasture botanical diversity on the activity of endogenous antioxidative enzymes in *longissimus thoracis* of lambs (n = 7)

	Intensive Ryegrass	Herbage rich pasture	Leguminosa rich pasture	SEM	P
GSH-Px	0.09 ^a	0.08 ^a	0.18 ^b	0.07	0.006
Cat	40.8	30.8	30.1	9.96	0.074
SOD	64.4	68.5	72.4	8.99	0.259

^{ab} Means within a row with different superscripts are significantly different ($P < 0.05$)

**REGULATION OF TASTE-ACTIVE COMPONENTS OF MEAT BY DIETARY
BRANCHED-CHAIN AMINO ACIDS – APPLICATION OF BRANCHED-CHAIN
AMINO ACIDS ANTAGONISM**

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Key Words: meat taste, diet, branched-chain amino acids, antagonism, chicken, sensory evaluation

Introduction

Regulation of taste active components of meat is important for improvement of meat quality. It has been generally believed that it was possible to regulate aroma and storage stability by diet, while regulation of meat taste is difficult. In previous study, we indicated that the main taste-active component of meat, free glutamate (Glu) contents, can be controlled by dietary restriction, metabolizable energy and crude protein (1997, 2001, 2004). From these studies, the possibility to regulate taste of meat by diet was suggested. Additionally dietary leucine (Leu) level regulates free Glu contents of meat (2004). The decrease in dietary Leu induced an increase in the free Glu of muscle. From the results of sensory evaluations of meat, the taste in low dietary Leu group was superior to that in the control group. In addition, it was suggested that the glutamate dehydrogenase (GDH) and glutaminase (GA) activities may contribute to the regulation of the free Glu content of muscle by dietary Leu level.

From these results, regulation of dietary Leu level is effective on the progress of meat taste. But it is difficult to formulate the low Leu diet, because most of feed ingredients include Leu in abundance. And so, in this study, the application of branched-chain amino acids (BCAA) antagonism on the progress of meat taste was examined. Leu, valine (Val) and isoleucine (Ile), have very similar structures. BCAA are essential amino acids for animals, and mainly metabolized in the muscle, not in the liver. The antagonism among BCAA reported in chicks (Trevor and Richard, 1978). When animals consume excessive amount of one of the BCAA from diet, the other BCAA contents of plasma and muscle decrease. We considered the possibility that dietary Ile and Val might regulate the Leu level, therefore, free Glu contents of meat were improved.

Objectives

In this study, the effect of dietary Ile and Val levels on meat quality, especially taste, was studied. The meat quality was estimated by free amino acids, ATP metabolites and sensory evaluation. As a result, the Glu level and sensory score were changed by dietary Ile and Val levels. Then, the Glu-related enzyme activities in muscle were measured to elucidate the regulatory mechanism of Glu in the muscle.

Methodology

28 days old female Cobb strain broiler chickens were divided into 3 groups. Leu:Ile:Val (% of NRC requirements (1994)) contents of experimental diet were 100:100:100, 150:100:100 (control) and 150:150:150, respectively. The 3 groups of chickens were allowed free access to each these diet for 10 days. On 10th day, the body weight and feed intake were measured, and blood samples were taken from vein of wing. On 11th day, all chickens were killed, and then breast muscle (*M. Pectoralis superficialis*) was taken for following analyses. Concentrations of free amino acids and ATP metabolites in muscle and plasma were measured by HPLC. Sensory evaluation was carried out with trained 18 panelists using paired difference test and Scheffe's paired comparison test. For investigation of the mechanism of Glu regulation, the muscle GDH, glutaminase (GA) and glutamine synthetase (GS) activities were measured by enzymatic methods (Bergmeyer, 1978).

Results & Discussion

From growth performance, there were no difference in the weight gain, feed intake and feed efficiency. Therefore we considered that these BCAA levels didn't affect growth performance.

The plasma amino acid analysis showed that free Ile and Val contents of 100:100:100 and 150:150:150 groups increased compared to these of the control ($P<0.05$). By contraries, free Leu contents of 100:100:100 and 150:150:150 groups decreased compared with control ($P<0.05$). Free Glu contents of plasma have no difference among all experimental groups.

From the results of free amino acid contents of muscle, it was indicated that all BCAA contents had same tend of plasma. On the other hand, free Glu contents of muscle was significantly changed by dietary BCAA levels. Free Glu of 100:100:100 was increased in comparison with control, the tendency was similar to previous study (2004). Additionally free Glu contents of the 150:150:150 diet significantly increased by 34% compared to that of the control ($P<0.05$). Therefore, free Glu of muscle increase with increasing of dietary Ile and Val levels. While, there were no differences were shown in the 5'-inosinic acid contents of muscle in all groups.

In sensory evaluation, 16 of 18 panelists answered that the difference of meat taste between 150:150:150 and control by paired difference test ($P<0.01$). Additionally, the 150:150:150 group was estimated high score in overall preference, taste intensity, umami taste and chicken like taste in Scheffe's paired comparison test. Especially there was significant difference in taste intensity ($P<0.05$). On the other hand, there were no differences in aroma. These results suggested that the taste of meat in 150:150:150 group was superior to that in the control group.

The GA activities of 100:100:100 and 150:150:150 groups demonstrated an upward tend in comparison to control. The GDH and GS activities had not changed by dietary BCAA levels. From these results, we considered that the GA activities might contribute to the regulation of the free Glu content of muscle by dietary BCAA level.

Conclusions

The effect of dietary BCAA levels on the meat quality, especially free Glu contents and taste of meat was studied. We estimated free amino acids, ATP metabolites and sensory evaluation of meat. From these results, it was suggested that the dietary Ile and Val levels affected the free Glu contents in muscle. The increase in dietary Ile and Val induced an increase in the free Glu of muscle. In sensory evaluation of meat, the taste improved by high dietary Ile and Val levels. We conclude that dietary Ile and Val levels regulates the free Glu content in muscle, and high dietary Ile and Val level can improve taste of meat.

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Tables and Figures

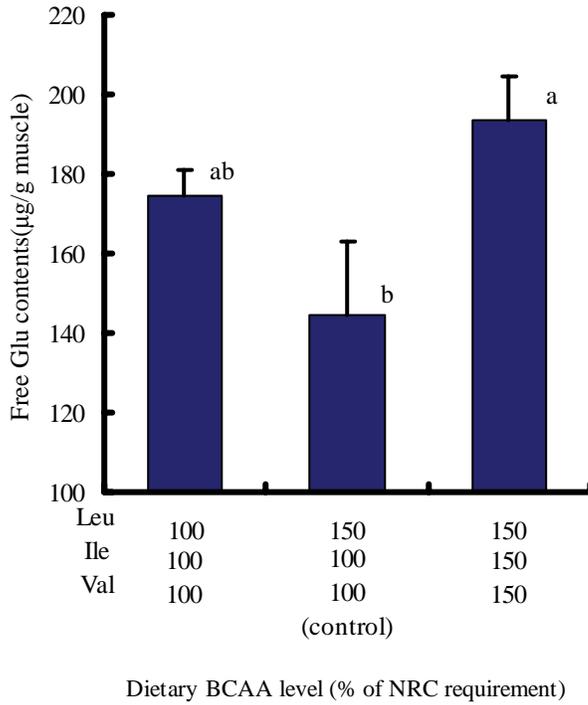


FIGURE 1. Effect of dietary Leu levels on free Glu contents in chick muscle. Values are means \pm SEM (n = 6). Bars with different superscripts^{a-b} are significantly different, P < 0.05.

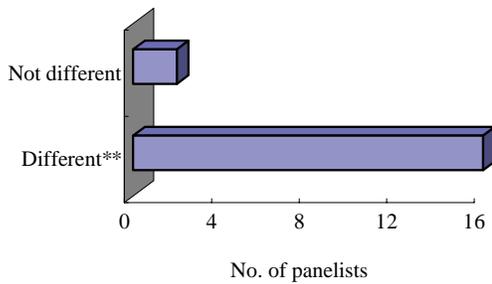


FIGURE 2 The result of paired difference test of 150:150:150 group versus control. Panelists were 18. **: P < 0.01.

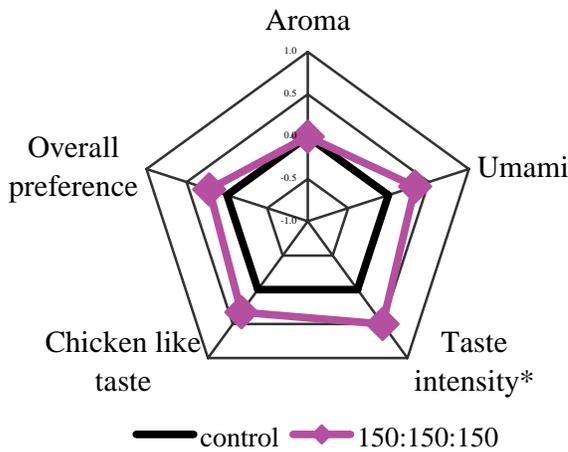


FIGURE 3 The result of Scheffe's paired comparison test of 150:150:150 group versus control. Panelists were 18. *: P < 0.05.

WET DISTILLERS GRAINS PLUS SOLUBLES DOES NOT INCREASE LIVER-LIKE OFF-FLAVORS IN COOKED BEEF

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Key Words: Distillers Grains, Livery, Knuckles, Flavor

Introduction

Feed and supplementation costs account for 40-70% of total production costs in the beef industry (Funston, 1998). One viable option to lower production costs while still producing a high yielding, desirable product is to utilize distillers grains or distillers grains plus solubles. Distillers grains, the by-products of dry milling processes, offer a low cost and effective protein and energy source that is currently used in feedlot rations (Stock, Lewis, Klopfenstein, and Milton 2000). Wet distillers grains have been shown to increase daily gain and gain/feed when compared to dry-rolled corn and may help control subacute acidosis (Stock et al., 2000).

Results from the Beef Customer Satisfaction Study demonstrate the importance of flavor in relation to consumer acceptance (Lorenzen et al., 1999; Neely et al., 1999; Savell et al., 1999). Recently, purveyors, retailers, and consumers have reported a liverlike off-flavor in beef cuts. Miller (2001) noted that cuts cooked to higher degree of doneness, cuts with higher levels of myoglobin, and cuts with greater degrees of lipid oxidation typically express a liver-like off-flavor. More specifically, Yancey (2002) identified thirteen compounds that were higher in samples with liver-like off-flavor when compared to samples without liver-like flavors. Of these byproducts, six were aldehydes formed from the oxidation of oleic and linoleic acid.

Distillers grains supplementation increases unsaturated fat content of the diet which can subsequently escape rumen biohydrogenation and become incorporated into the phospholipid fraction of muscle tissue (Koger et al., 2004), thus increasing the possibility of lipid oxidation and subsequent off-flavors.

Objectives

Our objectives were to determine if feeding wet distillers grains plus solubles (WDGS) increases liver-like off-flavors in beef, and to determine the sensory attributes of cattle finished with WDGS.

Methodology

Cattle from this study were a subset of the cattle described by Vander Pol, Erickson, Klopfenstein, and Greenquist (2005). Briefly, two hundred eighty-eight crossbred yearling steers were randomly assigned to a dietary treatment containing 0, 10, 20, 30,

40, or 50% (DM basis) WDGS, where WDGS replaced a high-moisture/dry-rolled corn mixture (1:1 DM basis). Steers were implanted on d 28 with Revalor-S®, fed for 125 d and then harvested at a commercial processing facility. At harvest, university personnel randomly selected 15 Choice and 15 Select carcasses from each treatment group. Carcass data (hot carcass weight, fat thickness, and ribeye area) were collected by university personnel while USDA marbling score and yield grade were determined by a USDA grader. Following grading, the knuckles (IMPS #167) were removed from the carcasses, vacuum-packaged, and shipped to the Loeffel Meat Laboratory at the University of Nebraska.

Following the 7 d aging period at 1°C, the *M. rectus femoris* (knuckle centers) were isolated and cut into 2.54 cm steaks, freezer wrapped, and frozen (-16°C) until sensory analysis was conducted. Steaks were allowed to thaw in a cooler at 1°C for 1 d prior to cooking for sensory evaluation.

Sensory Evaluation

Steaks were cooked to an internal temperature of 70°C on an electric broiler (FSR200, Farberware Inc., Porspect, IL). Internal temperature was monitored with a digital thermometer (Omega Engineering, model 450-ATT, Stamford, CT) with a type T thermocouple (Omega Engineering, Stamford, CT). Once the internal temperature reached 35°C, the steak was turned once until the final temperature was reached. The steak was then cut into 1.27 cm x 1.27 cm x 2.54 cm cubes and served warm to the panelists, approximately 5 minutes post cooking.

Panelists for this study were selected and trained according to the guidelines and procedures outlined by Meilgaard, Civille, and Carr (1991). In order to prevent bias, panelists were seated in individual booths equipped with red fluorescent lights and partitioned to reduce collaboration between panelists and eliminate visual differences (Meilgaard et al., 1991). Each panelist was served distilled water and unsalted, saltine crackers and given three minutes between samples to cleanse their palates. Six samples, identified using three-digit codes, were served on each day. Eight-point descriptive attribute scales (Muscle fiber tenderness: 1=extremely tough, 8=extremely tender; Connective tissue: 1=abundant, 8=none; Juiciness: 1=extremely dry, 8=extremely juicy; Off-flavor intensity: 1=extreme off-flavor, 8=no off-flavor) were used. Panelists were trained to identify the specific off-flavors (liver-like, metallic, sour, charred, oxidized, rancid, or other) contributing to the off-flavor score for the steak.

Statistical Analysis

Data were analyzed as a randomized complete block design by analysis of variance (ANOVA) using the MIXED procedure of SAS (Version 9.1, Cary, N.C, 2002) with a predetermined significance level of $P \leq 0.05$. Carcass served as the experimental unit and was considered a random effect. Main effects of treatment, grade, and their two-way interaction were included in the model. Since the treatment x grade interaction was not significant for any attribute, least square means were not reported. The Kenward-Roger option was used to determine denominator degrees of freedom. When significance was indicated by ANOVA, means separations were performed using the LSMEANS and PDIFF function of SAS.

Results & Discussion

Carcass Data

A complete analysis of performance data from cattle sampled for this study has been reported by Vander Pol et al. (2005). Carcass data are presented here to characterize the meat source utilized in this trial. Results are consistent among the two analyses. Treatment had an effect on hot carcass weight and USDA yield grade ($P=0.0001$ and 0.036 , respectively). Cattle finished on the 0%, 10%, and 50% diets had similar hot carcass weights, which were lighter than those from cattle fed 20%, 30%, and 40% diets (Table 1). Carcasses that were finished with any level of WDGS had higher USDA yield grades, which was also reported by Koger et al. (2004). Adjusted fat thickness, ribeye area, and USDA marbling score were not ($P=0.37$, 0.08 , and 0.31 , respectively) different in the present study. Koger et al. (2004) reported that cattle finished with distillers grains had greater adjusted fat thickness than cattle finished with the control diet. Distillers grains have higher fat content than corn, which may contribute to higher yield grades.

Table 1. Least squares means for main effects for hot carcass weight, adjusted fat thickness, yield grade, and marbling score.

	Hot	Adjusted		USDA	USDA
	Carcass	Fat	Ribeye	Yield	Marbling
Effect	Weight,kg	Thickness,cm	Area,cm ²	Grade	Score ^a
Treatment ^b	0.0001 ^c	0.37 ^c	0.08 ^c	0.036 ^c	0.31 ^c
0	356.3 ^d	1.12	82.47	2.4 ^d	503
10	366.5 ^{de}	1.31	82.09	2.7 ^e	521
20	371.4 ^{ef}	1.28	81.92	2.7 ^e	494
30	378.0 ^f	1.23	81.65	2.7 ^e	508
40	381.4 ^f	1.20	77.81	2.9 ^e	504
50	361.1 ^{de}	1.24	78.81	2.7 ^e	503
SEM ^g	3.92	0.01	1.38	0.11	8.10
Quality Grade	0.72 ^c	0.24 ^c	0.95 ^c	0.10 ^c	0.0001 ^c
Choice	368.92	1.26	80.76	2.76	564
Select	367.78	1.20	80.82	2.61	465
SEM ^g	2.26	0.04	0.80	0.06	4.68

^a400= Slight^{oo} and 500= Small^{oo}.

^bTreatments: Percentage of wet distillers grains plus solubles included in diet.

^c P -value from analysis of variance tables.

^{de}Mean values within a column and followed by the same letter are not significantly different ($P>0.05$).

^gStandard error of the mean.

Sensory Analysis

Treatment had no effect on the sensory attributes muscle fiber tenderness, connective tissue amount, juiciness, and off-flavor intensity (Table 2). Gill, Roeber, and DiCostanzo

(2004) also reported that finishing cattle with distillers grains had no effect on taste panel tenderness, flavor, or juiciness.

USDA Choice steaks were more tender, had less amounts of detectable connective tissue, were juicier, and had a greater off-flavor intensity when compared to Select steaks. Our findings are consistent to those of Smith et al. (1987), who reported that Choice carcasses were more tender and more juicy than USDA Good steaks. Although off-flavor intensity was not recorded, Smith et al. (1987) reported that flavor and overall palatability scores for USDA Choice steaks were greater when compared to USDA Good steaks.

Treatment did not significantly influence off-flavor intensity (Table 3), although the frequency of liver-like off-flavor notes was approaching significance ($P=0.07$). The liver-like off-flavor occurred most frequently in the 0% and 10% WDGS diets (14.44 and 19.63, respectively) while steaks from animals fed the 30% or 50% WDGS diets had the lowest incidence of liver-like off-flavor (7.41 and 8.52, respectively). Liver-like and metallic off-flavors were more frequent in Select carcasses ($P=0.02$ and $P=0.0002$, respectively). Although oxidative rancidity was not measured in our study, we hypothesize that the increase in off-flavor intensity, liver-like, and metallic off-flavors is due to lipid oxidation. Vipond, Lewis, Horgan, and Noble (1994) and Koger et al. (2004) indicated that animals finished with distillers grains had significantly higher amounts of unsaturated fatty acids in muscle tissue. These unsaturated fatty acids can catalyze lipid oxidation. Miller (2001) reported that as lipid oxidation progresses, off-flavors such as metallic and liver-like increase. Findings in our study indicate that a greater percentage of panelists detected the liver-like off-flavor (15.19 vs. 9.51) and the metallic off-flavor (39.26 vs. 26.17) in USDA Choice steaks when compared to USDA Select steaks. All other off-flavor notes were not significant in terms of quality grade

Table 2. Least squares means for main effects for muscle fiber tenderness, connective tissue amount, juiciness, and off-flavor intensity.

	Muscle Fiber	Connective Tissue		Off- Flavor
Effect	Tenderness ^a	Amount ^b	Juiciness ^c	Intensity ^d
Treatment ^e	0.37 ^f	0.72 ^f	0.46 ^f	0.47 ^f
0	5.80	4.86	5.18	5.72
10	5.62	4.73	5.04	5.49
20	5.82	4.91	5.24	5.69
30	5.51	4.65	4.90	5.74
40	5.53	4.67	4.96	5.54
50	5.60	4.73	5.05	5.73
SEM ^g	0.13	0.14	0.13	0.11
Quality Grade	0.0001 ^f	0.0001 ^f	0.0009 ^f	0.0020 ^f
Choice	5.90 ⁱ	5.01 ⁱ	5.24 ⁱ	5.51 ^h
Select	5.39 ^h	4.51 ^h	4.87 ^h	5.80 ⁱ
SEM ^g	0.07	0.08	0.08	0.07

^aMuscle Fiber Tenderness: 1= Extremely Tough; 8= Extremely Tender.

^bConnective Tissue Amount: 1= Abundant Amount; 8=No Connective Tissue.

^cJuiciness: 1= Extremely Dry; 8= Extremely Juicy.

^dOff-Flavor Intensity: 1=Extreme Off-Flavor; 8= No Off-Flavor.

^eTreatments: Percentage of wet distillers grains plus solubles included in diet.

^f*P*-value from analysis of variance tables.

^gStandard error of the mean.

^{hi}Mean values within a column and followed by the same letter are not significantly different ($P>0.05$).

Table 3. Least squares means for main effects for liver-like, metallic, sour, oxidized, rancid, and other off-flavors.

Effect	Liver-Like ^a	Metallic ^a	Sour ^a	Charred ^a	Oxidized ^a	Rancid ^a	Other ^a
Treatment ^b	0.07 ^c	0.73 ^c	0.82 ^c	0.37 ^c	0.21 ^c	0.75 ^c	0.10 ^c
0	14.44	34.07	48.89	7.41	10.37	12.22	2.96
10	19.63	27.41	50.37	8.52	11.85	8.52	0.74
20	11.85	31.85	50.74	5.56	18.52	11.11	3.33
30	7.41	31.85	55.19	4.44	11.48	10.74	3.33
40	12.22	34.81	49.63	8.89	16.67	11.11	2.59
50	8.52	36.30	50.37	5.56	10.37 ^h	11.36	4.82
SEM ^d	0.03	0.04	0.03	0.02	0.03	0.02	0.01
QGE ^e	0.02 ^c	0.0002 ^c	0.65 ^c	0.14 ^c	0.30 ^c	0.24 ^c	0.12 ^c
Choice	15.19 ^g	39.26 ^g	51.48	7.78	11.98	11.36	3.58
Select	9.51 ^f	26.17 ^f	50.25	5.68	14.44	9.38	2.35
SEM ^d	0.02	0.02	0.02	0.01	0.02	0.01	0.01

^aOff-flavors are expressed as a percentage of panelists that identified the off-flavor.

^bTreatments: Percentage of wet distillers grains plus solubles included in diet.

^c*P*-value from analysis of variance tables.

^dStandard error of the mean.

^eQuality grade.

^{fg}Mean values within a column and followed by the same letter are not significantly different ($P>0.05$).

Conclusions

Results from this study indicate that finishing cattle with wet distillers grains plus solubles does not cause the liver-like off-flavor or any other off-flavors in the *M. rectus femoris*. Wet distillers grains plus solubles offers cattle feeders cost effective means to finish cattle with minimal effects on meat palatability.

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EFFECTS OF FEEDING PERILLA SEEDS TO PIGS ON FATTY ACID COMPOSITIONS AND PALATABILITY OF PORK

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Key Words: egoma, perilla, pork, alfa-linolenic, palatability

Introduction

Perilla known as *shiso*/Japanese basil is commonly used in the Japanese cuisine as mostly a garnish. A type of perilla called *egoma* has a long history of cultivation for extracting oil. *Egoma* seeds contain 35-40% of oil and extracted *egoma* oil has an unique fatty acid profile in which alfa-linolenic acid is contained as high as 60%. Alfa-linolenic acid is one of n-3 fatty acids and many nutritionists and researchers feel that compared with n-6 fatty acids n-3 fatty acids are under consumed. The Japanese health authority is suggesting a recommended n-6:n-3 ratio as close as 4. Well informed and health orientated consumers have started to opt for n-3 fatty acids rich food ingredients and this would be even true for animal products. Such value added products are increasingly advantageous. If a certain resource of n-3 fatty acids is available, a profile of fatty acids of the pork can be altered so that it contains increased n-3 fatty acids by adding n-3 fatty acids to feed but its efficacy should be assessed and the resource of the fatty acids should be carefully chosen so that it does not affect or intervene production cost, productivity and health of the animals and also palatability of the products.

Objectives

The present study is aimed to produce pork with enhanced n-3 fatty acids by supplementing conventional concentrate with ground *egoma* seeds. Two levels of *egoma* supplementation were set for comparison of its efficacy. Assessment of palatability of the pork was also made by analysing free amino acids profiles and sensory tests by a panel.

Methodology

Experimental design

Fifteen barrows (Landrace x Large white x Duroc mean live weight 30kg) were allocated to three groups to receive diets which were made of conventional concentrate supplemented with *egoma* seeds at 3% level (ES3), 1% level (ES1) and concentrate only as a control diet. Soybean meal was also added to the ES1 and control diets at 4 and 6% levels, respectively, so that lipid contents were similar among the diets. The basal concentrate was premixed and commercially available but had different constituents for

growth and fattening. The basal diet was changed when the pigs reached weight approximately 70kg. Biopsy was performed to take subcutaneous fat tissues at the buttocks every three weeks to monitor changes in fatty acids composition. The pigs were slaughtered when a mean live weight reached approximately 105kg for the whole group and performances of meat production were recorded for each pig. *M. longissimus dorsi* (loin) were separated from the carcasses between the 12th and 13th ribs and were stored at -30 °C for chemical analyses.

Fatty acid assays

Approximately 10g of each sample were homogenized in 50ml of physiological saline and lipids were extracted by a method described by Folch *et al.*. The extracted lipids were weighed as total lipids and were then dissolved with 10ml of chloroform and an aliquot sample was taken into a test tube with a screw cap and the solvent was removed by blowing with N₂ gas. The intermuscular, inner and outer subcutaneous fat tissues of the loins were separated and 0.1g of the aliquot samples was put into test tubes. Methanol containing 0.5N sodium hydroxide was added to the tubes for saponification then boron trifluoride methanol solution was added to form methyl esters for gas chromatography. A Hitachi G-3000 gas chromatography apparatus was employed to analyse fatty acids. The apparatus was equipped with a flame ionisation detector (FID) and fitted with a 0.3mm x 30m glass column packed with DB-WAX. Temperatures for the FID and the column oven were constant and set for 300 and 210 °C, respectively. Nitrogen carrier gas was used at a rate of 30ml/min.

Free amino acid and peptide assays

The loins were thawed under refrigeration and aged for up to four days. Samples were taken on the 2nd and 4th day of aging. Aliquot samples, approximately 5g, were homogenized with 75% ethanol and the filtered extracts were made up to a 100ml volume. The extracts were added with 1ml of 2% sulfosalicylic acid, supernatants were injected to an amino acid analyser (Shimazu, LC-10).

Sensory evaluation

Slice samples were taken from the loin on the 2nd and 4th day of aging under refrigeration. The samples were fried with a small amount of cooking oil on a hot plate. Palatability for flavour, umami, texture, oiliness and total palatability were scored from 1 for poor to 5 for excellent by a panel consisting of 5 volunteers.

Results & Discussion

Meat production performances

A summary of meat production performances is shown in Table 1. Mean dressing percentage of ES3 group was significantly ($P < 0.01$) greater than the control group (74.5% vs 63.3%) although means of carcass weight were all similar among the groups

ranging between 71.9 and 74.4kg. The mean dressing percentage of the ES1 was between the two groups without any statistical significance. There were no significant differences in final live weight and back fat thickness among the groups.

Lipid contents and fatty acid compositions in diets

Total lipid contents in each diet and fatty acid compositions are shown in Table 2. All the diets were prepared to be containing similar amounts of lipids but they varied within a tolerable range. The fattening diets supplemented with *egoma* at 3 and 1% levels contained 13.2 and 7.1% of alfa-linolenic acid, respectively compared with 2.7% of the control diet. Contents of other major fatty acids such as myristic, palmitic, palmitoleic, stearic acids were all similar among the diets. Ratios of n-6:n-3 fatty acids in ES3, ES1 and the control diet for fattening were 2.98, 6.28 and 17.3, respectively.

Changes in fatty acid composition in fat tissues during fattening

Changes in fatty acid composition in fat tissues taken by biopsy are shown in Table 3. Levels of alfa-linolenic acid in fat tissues of ES3 and ES1 pigs increased with advancement of growth and fattening while that of the control groups was not greatly changed. Levels of linoleic acid stayed similar levels throughout the period. Statistical significances were obtained among the groups throughout the period for level of alfa-linolenic acid and ratio of n-6:n-3 fatty acids.

Lipid contents and fatty acid compositions in loin

Levels of total lipids were 3.47%, 3.33% and 3.19% for ES3, ES1 and the control groups, respectively. No statistical difference was found among the group. Table 4 shows fatty acid compositions of total lipids, intermuscular, inner and outer subcutaneous fat tissues of the loin. Levels of alfa-linolenic acid in total lipids were 1.34%, 0.73% and 0.39% and linoleic acid were 7.61%, 8.08% and 9.28% for ES3, ES1 and the control groups, respectively ($P < 0.01$). There was no significant difference in other polyenic fatty acids. Consequently, ratios of n-6:n-3 fatty acids were 3.28, 5.37 and 8.34 for ES3, ES1 and the control groups ($P < 0.01$). Similar tendencies were found for intermuscular, inner and outer subcutaneous fat tissues.

Changes in free amino acids and peptides

Generally, free amino acids and peptides, which are major contributing factors to improve taste and flavour of meat, increase with the process of aging. This is believed to be caused by endo- and exopeptidase activities on fragments of protein and free peptides produced during tenderisation. Changes in free amino acids and peptides in the loin are shown in Table 5. Levels of glutamic acid in the control group increased most during aging but no statistical significance was obtained. Glycine and alanine are related to sweetness and these amino acids interact with glutamic acid to enhance umami taste. Levels of glycine of the control group ($83.5\mu\text{mol}/100\text{g}$) were slightly higher than ES3 ($73.2\mu\text{mol}/100\text{g}$) and ES1 ($76.8\mu\text{mol}/100\text{g}$) on the 2nd day of aging whereas respective alanine levels were $71.2\mu\text{mol}/100\text{g}$, $86.3\mu\text{mol}/100\text{g}$ and $78.3\mu\text{mol}/100\text{g}$. Amino acids such as phenylalanine, tyrosine, leucine, isoleucine, valine are considered to contribute to bitterness. These amino acids in this study were considerably low compared with the

levels of alanine and glycine except for leucine. Total levels of free amino acids increased on the 4th day of aging. Dipeptides tended to increase in ES3 and ES1 groups.

Sensory evaluation

Sensory evaluations of the pork from each group are summarised in Table 6. The panel tended to show greater palatability for the pork of the control group than that for the ES groups although any statistical significance was not obtained. Palatability increased for all the groups from the 2nd to 4th day of aging.

Conclusions

Supplementing with *egoma* seeds to pigs successfully produced n-3 fatty acids enhanced pork with improved dressing percentages. Pork produced from the pigs supplemented at a 3% level achieved an almost ideal n-6:n-3 fatty acids ratio. Although statistical significance was not observed and the results were not conclusive, palatability might be affected by supplementing with *egoma* seeds.

Tables and Figures

Table 1. Meat production performances of pigs supplemented with *egoma* seeds

	Live weight (kg)	Carcass weight (kg)	Dressing (%)	Back fat thickness(cm)
ES3	98.4	73.4	74.50 ^a	1.6
ES1	106.5	74.4	69.78 ^a	1.9
Control	113.0	71.9	63.55 ^b	1.8

Values with different superscripts are statistically significant (P<0.01)

Table 2. Total lipid contents and fatty acid compositions of diets (%)

	Total lipids	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	n6:n3
Basal diet growth									
ES3	6.4	0.67	16.4	2.33	6.39	35.5	32.7	6.06	5.39
ES1	5.8	0.73	16.2	2.02	6.20	35.1	35.7	4.11	8.67
Control	5.9	0.70	16.5	2.17	6.41	36.6	35.8	1.83	19.58
Basal diet fattening									
ES3	4.8	0.38	14.1	0.59	3.83	28.7	39.2	13.15	2.98
ES1	4.0	0.38	14.4	0.59	3.69	29.3	44.6	7.10	6.28
Control	4.1	0.39	15.1	0.58	3.88	31.3	46.1	2.67	17.28

Table 3. Changes in fatty acid composition in fat tissues taken by biopsy (%)

	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	n6:n3	
3 rd week									
ES3	1.18	21.7	4.07	11.2	45.0	14.9	2.03 ^a	7.3 ^a	
ES1	1.30	21.9	3.88	12.0	45.2	14.7	1.09 ^b	13.5 ^b	
Control	0.13	20.0	3.78	11.0	45.8	17.3	0.97 ^c	17.9 ^c	
6 th week									
ES3	1.14	21.5	2.98	11.9	43.9	15.0	3.67 ^a	4.1 ^a	
ES1	1.18	21.4	3.27	12.2	44.1	15.5	2.42 ^b	6.4 ^b	
Control	1.32	21.6	3.56	11.9	45.7	14.8	1.13 ^c	13.1 ^c	
9 th week									
ES3	1.25	21.7	2.85	12.9	42.8	14.1	4.43 ^a	3.2 ^a	
ES1	1.20	23.7	2.47	14.6	43.7	12.2	1.80 ^b	6.8 ^b	
Control	1.22	21.9	3.26	12.5	45.3	14.9	0.97 ^c	15.3 ^c	

Values with different superscripts are statistically significant (P<0.01)

Table 4. Total lipid contents of loin and fatty acid compositions of total lipids, intermuscular, inner and outer subcutaneous fat tissues (%)

	Content	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	n6:n3
Total lipids									
ES3	3.47	1.38	24.7	4.30	11.9	48.8	7.61 ^a	1.34 ^a	5.7 ^a
ES1	3.33	1.20	24.5	3.89	13.8	47.8	8.08 ^b	0.73 ^b	11.1 ^b
Control	3.19	1.18	23.6	4.18	12.5	48.9	9.28 ^c	0.39 ^c	23.8 ^c
Intermuscular									
ES3		1.30	24.5	1.28	16.0	38.9	13.6	4.43 ^a	3.06 ^a
ES1		1.24	25.4	1.21	17.7	38.8	13.6	2.06 ^b	6.62 ^b
Control		1.22	24.5	1.31	16.6	40.8	14.5	1.00 ^c	14.53 ^c
Inner subcutaneous									
ES3		1.44	24.0	1.20	15.9	39.9	13.3	4.31 ^a	3.08 ^a
ES1		1.30	24.8	1.08	17.6	39.1	14.1	2.06 ^b	6.84 ^b
Control		1.28	23.8	1.22	16.2	40.8	15.8	1.01 ^b	15.65 ^c
Outer subcutaneous									
ES3		1.20	23.6	1.48	15.4	40.1	13.7	4.50 ^a	3.05 ^a
ES1		1.27	24.0	1.21	17.1	39.6	14.7	2.16 ^b	6.79 ^b
Control		1.15	22.5	1.38	14.1	42.3	17.3	1.22 ^c	14.15 ^c

Values with different superscripts are statistically significant (P<0.01)

Table 5. Changes in free amino acids and peptides in the loin with process of aging ($\mu\text{mol}/100\text{g}$)

	Glutamic acid	Glycine	Alanine	Valine	Iso-leucine	Leucine	Phenyl-alanine	Taurine	Carnosine	Anserine
2 nd day										
ES3	11.9	73.2	86.3	4.0	tr	36.6	15.8	178.7	1198.3	128.4
ES1	14.0	76.8	78.3	4.8	tr	33.3	16.8	159.5	1175.6	119.0
Control	10.3	83.4	71.2	13.5	8.5	18.8	16.3	205.8	1092.2	104.0
4 th day										
ES3	19.5	77.6	92.8	8.4	tr	26.3	16.7	176.2	1184.8	123.2
ES1	23.6	82.7	67.8	13.2	4.7	41.7	19.3	199.2	1173.4	113.2
Control	21.6	86.3	86.3	11.2	10.9	58.3	20.1	208.1	1126.9	110.7

Table 6. Mean scores of sensory evaluation for palatability of loin from the pigs supplemented with egoma

	Flavour	Umami	Texture	Oiliness	Total palatability
2 nd day					
ES3	3.0	3.0	3.2	2.9	3.1
ES1	3.2	3.3	3.0	3.1	3.1
Control	3.6	3.6	3.6	3.2	3.6
4 th day					
ES3	3.3	3.1	3.2	3.0	3.1
ES1	3.0	3.3	3.4	3.2	3.4
Control	3.8	3.5	3.3	3.6	3.6

EFFECTS OF A DIETARY CHITOSAN-ALGINATE-FE(II) COMPLEX ON MEAT QUALITY PIG LONGISSIMUS MUSCLE DURING AGEING

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Key Words: Pork Quality, Chitosan-Alginate-Fe(II) Complex, Fatty Acids, Ageing

Introduction

Chitosan is a cationic polysaccharide made from alkaline N-deacetylation of chitin. The component has multifunctional properties, including antibacterial (Jia et al., 2002; Xie et al., 2001; Sudarshane et al., 1992) fungicidal (Allan and Hadwiger, 1979), and antioxidant functions (Xie et al., 2001; Jeon et al., 2003). On the other hand, early studies showed that alginate, the polymer of β -D-mannuronate and its C-epimer form of α -L-guluronate inhibited the production of NO and H₂O₂ (Mo et al., 2003), and consequently exhibited antioxidant and antitumor effects by improving metabolic activity (Michio and Terukazu, 1992).

Given the previous studies, it was reasonable to assume that if chitosan and/or alginate are deposited in muscle tissue, lipid oxidation and microorganism growth could be significantly retarded. Chun et al. (2003) demonstrated a possible use of chitosan-alginate complex in the pig industry by showing that more than 90% of piglets with diarrhea benefited from oral feeding of the complex. By applying an isotope tracing technique, we previously showed that approximately 0.43% of isotope-conjugated chitosan-alginate-Fe(II) complex (CAFC) was deposited in pig muscle tissue (Korean Department of Agriculture and Forestry, 2002). However, its effect on meat quality has not yet been evaluated

Objectives

The objective of this study was to investigate the effects of dietary CAFC supplementation on carcass and meat qualities of pig m. longissimus during chiller ageing.

Methodology

Animals, experimental design and treatment: A total of 122 LYD (Landrace \times Yorkshire \times Duroc) pigs were sampled from an industrial population. Seventy-four pigs (32 gilts and 42 barrows) were administered to a 3 mL of dietary supplementation of CAFC, diluted in water, per day from 25 to 70 days of age, while the remaining 48 pigs (20 gilts and 28 barrows) were fed with the same commercial diet without the

supplementation. After the experimental period, an ordinary feeding regime was applied for all pigs until approximately 110 kg of slaughter weight was reached. All pigs were slaughtered after being stunned by an electronic stunner (230 volts for 2.5 s) at an industrial abattoir, and placed at a 1°C chiller until the following day. CAFC was manufactured by polymerizing two volumes of chitosan (MW 2,000-3,000, Tahoan Chemicals, Korea) and one volume of alginate (MW 1,500-2,000, EcoBio Inc, Korea) with 3% of Fe₂SO₄ at 90°C for 2 h (Korean Department of Agriculture and Forestry, 2002).

Sampling and measurement of objective meat quality: The day following slaughter, carcass grade was evaluated by carcass graders from the Korean Animal Products Grading Service (APGS, 2001). To evaluate the effect of CAFC supplementation on stability in objective meat quality during chiller ageing, 20 barrows (10 of each treatment) were randomly sampled, and longissimus muscles (from the 7th thoracic vertebrae to the last lumbar vertebrae) were taken from the left sides. The muscle samples were cut into 6 portions of ca. 150 g, vacuum packed, and randomly assigned to six ageing treatments (3, 7, 12, 16, 20, and 25 days post-mortem).

Results & Discussion

As seen in Table 1, the treatment had no significant effect on pH, meat color and WHC during ageing. pH at 24 h post-mortem has a direct effect on meat color and WHC through its effects on protein denaturation and the surface reflectance of muscle fiber (Bertram et al., 2004). The studies implied that the objective meat qualities of meat color and WHC were largely related to post-mortem glycolytic. Given the fact, the current result of the similar pHs and objective meat qualities between the treatments indicated that the dietary supplementation did not influence post-mortem glycolytic rate and likely amount of energy resources at the time of slaughter.

TBARS values as indicators of lipid oxidation have been used by numerous research groups (e.g., Jeon et al., 2003). The most significant result of the current study was that the dietary supplementation significantly retarded lipid oxidation, as assessed by TBARS. As seen in Fig. 1, meat from CAFC-fed pigs had significantly ($p < 0.05$) lower TBARS values from 20 days of storage at 1°C, but at the practical storage temperature of 4°C, the beneficial effect would be detectable before that ageing time. The current study did not determine absorption and deposition rate of CAFC in longissimus muscle. However, we previously traced isotope-conjugated CAFC for pig, and found that a large portion of the feed additive was accumulated in the digestive organs, but also 0.43% of the fed-dose was detected in muscle tissue (KDAF, 2002). Given the apparent effect of both chitosan and alginate on antioxidant activity (Peng et al., 1998), it can be assumable that the current results suggest that the deposited CAFC maintained their functions to some extent during chiller ageing.

Another noticeable result was the significant effect of CAFC supplementation on VBN formation during ageing (Fig. 1). VBN is a measurement of the nitrogen component of protein degradation, but also includes metabolite products such as AMP (Takasaka, 1975). Significantly lower levels of VBN after 12 days of storage for the CAFC-fed pigs suggests a significant reduction in protein degradation for that group. Taken that negligible effect of the treatment on pH and temperature, significantly retarded formation

of VBN for the CAFC-supplied pigs was unlikely related to the previously proposed mechanisms, and suggested that other beneficial characteristic was involved. The current study did not determine the initial bacterial loads and their changes during ageing time. However, previous studies have demonstrated that chitosan and alginate had antibacterial functions (Jia et al., 2002), and that TBARS and VBN values were significantly increased when meat were contaminated by microorganisms (Chae et al., 2004). It is possible that antibacterial activity of the supplement alone could have caused reduction in the formation of TBARS and VBN.

Conclusions

The current study showed that dietary supplementation of CAFC slowed down the formations of TBARS and VBN during chiller ageing.

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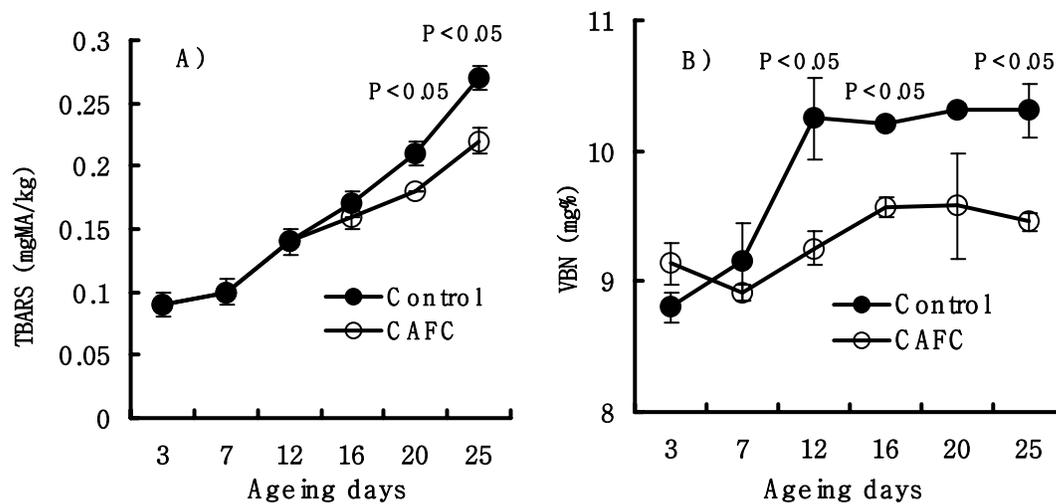


Fig. 1. Effects of dietary supplementation of chitosan-alginate-Fe(II) complex (CAFC) on changes in (A) thiobarbituric acid reactive substances (TBARS) and (B) volatile basic nitrogen (VBN) in *m. longissimus dorsi* during chiller ageing. Bar: standard deviation.

Table 1. The effects of dietary supplementation of chitosan-alginate-Fe(II) complex (CAFC) on changes in pH, objective meat color, water-holding capacity (WHC) and cooking loss (Cook loss) in *m. longissimus dorsi* during chiller ageing

		Day of ageing ^ψ					
		3	7	12	16	20	25
pH	Control	5.6±0.06	5.6±0.00	5.7±0.00	5.6±0.03	5.7±0.00	5.7±0.03
	CAFC	5.5±0.03	5.7±0.03	5.8±0.07	5.6±0.07	5.6±0.03	5.6±0.03
CIE L*	Control	54.4±0.32	56.5±2.21	57.4±2.10	56.8±1.7	56.8±0.64	57.5±0.82
	CAFC	54.6±0.85	53.1±2.43	55.6±1.81	57.4±1.6	57.7±1.06	55.2±0.63
CIE a*	Control	7.5±0.22	7.9±0.53	7.4±0.18	7.8±0.16	7.9±0.30	8.9±0.68
	CAFC	7.4±0.87	8.4±0.67	8.0±0.76	8.4±0.72	8.5±0.64	9.7±0.66
WHC (%)	Control	54.5±1.23	53.4±1.24	56.3±1.35	58.9±0.67	53.4±0.53	51.8±1.81
	CAFC	52.9±1.26	55.9±1.34	55.6±1.36	59.8±0.56	53.6±0.93	54.9±0.35
Cook loss (%)	Control	32.5±1.05	33.7±1.14	33.7±0.86	33.3±0.54	32.0±1.14	32.7±1.68
	CAFC	32.9±0.77	32.7±1.15	33.3±1.12	32.0±0.31	32.7±1.09	32.1±1.29

^ψ There was no significant effect of the feed supplementation on all meat quality traits within each ageing time (p>0.05).

CARCASS CHARACTERISTICS OF CREOLE GOAT OF GUADELOUPE (FWI) AS A FUNCTION OF FEEDING MANAGEMENT

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Key Words: Creole goat, feeding level, live weight, carcass yield, carcass composition, conformation

Introduction

In Guadeloupe goats are mainly raised under the suckling system for meat production and grazing is the most common mode of production. Due to its high production level (Alexandre *et al.*, 1999) and good adaptation to tropical conditions, the Creole goat could be a valuable genetic resource for the region. For flocks reared under intensive grazing systems, the reproductive performances are high and the pre-weaning growth of kids varies from 60 to 95 g/d (Alexandre *et al.*, 1999). Although it is a meat breed, very few studies deal with the assessment of carcass characteristics (Alexandre 1987). The carcass weight, varying from 6 to 10 kg among breeders, does not meet the needs of the formal modern sub-network. So there is a need to increase the growth during fattening and the carcass performances while ensuring the promotion of the local breed. However, tropical forage, even when exploited intensively, is of average food value (Humphreys, 1991) and is a major limiting factor in animal production. High levels of productivity partly depend on the supplementary intake of more energy-rich foods. Therefore, experiments are going on to test the effects of the growth potential and the feeding mode upon meat production. The aims of this paper is to give the first trends in this area.

Objectives

Creole meat type goats, a small-sized breed (28 kg LW) were used in two concurrent experiments from weaning to slaughter. Entire male kids were weaned at 87 ± 5 days. In the first experiment animals were reared on rotationally grazed tropical pasture (P) every 28 days (9.05 MJ ME and 123 g CP per kg DM) at an average stocking rate of 1200 kg LW/ha and received no supplementation. In the second experiment, the animals were supplemented (S) and reared in collective pens on a slatted floor. The diet was composed of the same stand of tropical pasture as mentioned above. In addition, they were offered commercial pellet (10.4 MJ ME and 180 g CP per kg DM), composed of maize (32.5%), wheat issues (40.0%), soya bean meal (15.0%) sugar cane molasse (6.0%) and minerals (6.5%). In both experiments, two groups of kids were determined (Table 1) according to their pre-weaning ADG level low (L; 69 ± 10 g/day) and high (H; 91 ± 14 g/day). Thus treatments were defined as LP vs. HP and LS vs. HS. Regular drenchings were carried out, in order to control gastro-intestinal parasitism, monthly for kids from birth to

weaning and every two months for weaned kids and goats. External parasites were controlled every two weeks for young and adults (spraying of acaricides).

Kids of P experiment (12 in each group) were slaughtered at the end of 8 months of growth whereas those of S experiment were slaughtered as soon as the mean live weight of the group reached 19 to 20 kg (14 and 18 in LS vs. HS group, respectively). Prior to slaughter, each goat was weighed. Length of fast (24 hrs) prior to slaughter was standardized for all goats in order to minimize variation in fill. Weights of all items (head, feet, pelt, lungs, liver, heart, and viscera) were taken during the slaughter process. The weight of the gastro-intestinal tract and all of its contents were recorded prior to and after cleaning. The cleaned GI-tract was separated and weighed as large and small intestines and mesenteric and intestinal fat. Hot carcass weight and chilled carcass weight (24 hr. post slaughter) and cut weight (leg, shoulder, neck, loin, breast, ...) were obtained according to procedures outlined by Colomer-Rocher *et al.* (1987). Kidney and pelvic fat was removed and weighed on the chilled carcass before cutting. Empty body weight (EBW) was calculated by subtracting values of gut content from slaughter weight. Two dressing percentages were calculated, true carcass yield calculated as cold (chilled) carcass weight related to EBW and commercial carcass yield expressed as proportion of hot carcass weight to slaughter weight (SW). Carcass was graded according to conformation, colour of the meat and fat cover score (Colomer-Rocher *et al.*, 1987).

Results & Discussion

The weights at weaning, and at slaughter (Table 1) of HP and LP kids were 10.6 vs. 8.4 ($P < 0.01$) and 18.7 vs. 18.4 ($P > 0.05$), respectively. Same values for HS and LS kids were 9.1 vs. 7.6 ($P < 0.01$) and 19.4 vs. 20.8 ($P > 0.05$), respectively. The HS kids spent significantly less time in the feedlot stall than the LS ones (107 vs. 178 days ; $P < 0.01$).

The hot carcass weight (Table 2) was similar for P kids (6.5 kg) while it was significantly lower ($P < 0.05$) for HS vs. LS group: 8.2 vs. 9.5 kg. At the same time the white full organs represented 38% vs. 31% of the carcass weight ($P < 0.05$) for LP vs. HP kids; same values for HS and LS kids were 27% vs. 23 % ($P < 0.05$). These proportions are linked to the feeding mode of the animals. In the case of P kids, the diet is 100% grazed tropical forages known for its high level of structural elements (Humphreys 1991) leading to a high gut content. The true carcass yield reached 52% for P kids and 5 points more for S kids (no significant difference occurred between groups of L vs. H kids). Commercial yields of S goats were higher than those of Black Bengal goats reared in comparative intensive conditions (42 %; Moniruzzaman *et al.*, 2002) but slaughtered at a lighter live weight (10 to 12 kg). Those of P kids were lower than those of Criollo kids reared in commercial farms of South Chile (46 %, Gallo *et al.*, 1996) although the slaughter weight was similar (19 kg LW).

Whatever the pre-weaning ADG level or the feeding mode during fattening, the proportion of cuts was similar, among them 30% long leg, 20% shoulder, 12% neck and 12 % breast. This is in concordance with the conclusion of Sheridan *et al.* (2003) for Boer goats reporting that diet did not affect the weight of commercial cuts as a proportion of carcass weight.

The meat colour was mainly pale for P kids while it was mainly pink for S kids (Table 3). Fat cover score and fat weights did not differ significantly between L and H

kids within P or S experiment (Table 3). However these variables seem to be lower for pasture fed kids against supplemented kids: 8 and 3 points less for mesenteric and kidney fat, respectively. This might be due to the lower energetic value of the P diet vs. the S diet. Nevertheless, the subcutaneous fat cover is thin and poorly developed as reported by many researchers that have studied different breeds or management as reviewed by Warmington and Kirton (1990)

Conclusions

The mode of feeding seemed to slightly affect carcass quality and composition while it seemed to have a marked influence upon live and carcass weight as well as upon gut content weight. Marketing practices would depend on the feeding mode. It would be more interesting for breeders, to sell live animals in the case of kids reared at pasture whereas for the others in the form of entire carcasses if a grading system is used. Further studies are required with more animals in order to increase the carcass weight. It is necessary to improve the supplementation strategies (quantity, quality and cost) especially for light weaned kids.

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Tables and Figures

Table 1 Creole kid performances during fattening period in two experiments: at pasture (P) or supplemented (S) according to preweaning ADG, low level (L) and high level (H)

Kid group		Weaning weight (kg)	Slaughter weight (kg)	Age at slaughter (d)	Fattening period (d)
Pasture	LP	8.4 ^a ± 0.7	18.0 ± 2.1	349.5 ± 3.4	249.0 ± 3.1
	HP	10.6 ± 1.1	18.7 ± 2.1	348.3 ± 3.9	249.0 ± 3.1
Supplement	LS	7.6 ^a ± 0.7	20.8 ± 2.2	293.4 ^a ± 41.6	177.8 ^a ± 41.0
	HS	9.1 ^b ± 0.5	19.4 ± 1.3	217.9 ^b ± 13.4	106.7 ^b ± 9.1

^{a,b)} data within same column with different superscripts differ significantly, P<0.05

Table 2 Carcass weights and yields of Creole kids in two experiments : reared at pasture (P) or supplemented (S) according to preweaning ADG, low level (L) and high level (H)

Kid group		GI ¹ and gut fill (% EBW)	Hot carcass (kg)	True ² carcass yield (% EBW)	Commercial ³ carcass yield (% LW)
Pasture	LP	31 ^a [29-38]	6.5 ± 0.9	51 [41-56]	40 [37-44]
	HP	38 ^b [32-50]	6.5 ± 1.0	53 [47-71]	38 [37-42]
Supplement	LS	23 ^a [18-26]	9.5 ^a ± 1.2	58 [57-61]	50 [48-54]
	HS	27 ^b [24-30]	8.1 ^b ± 0.5	56 [57-60]	47 [43-51]

¹) GI : gastrointestinal tract weight plus gut fill weight related to empty body weight (EBW); ²) calculated as cold carcass weight related to empty body weight (see in text); ³) calculated as hot carcass weight related to live weight at slaughter; ^{a,b}) data within same column with different superscripts differ significantly , P<0.05; [x-y]: variations

Table 3 Carcass quality of Creole kids in two experiments : fed at pasture (P) or supplemented (S) according to preweaning ADG, low level (L) and high level (H)

Kid group		Meat Colour ¹	Fat cover score ²	Mesenteric fat (g)	Kidney fat (g)
Pasture	LP	1.7 ± 0.4	2.2 ± 0.6	9 ± 5	7 ± 3
	HP	1.7 ± 0.5	1.7 ± 0.6	7 ± 4	7 ± 2
Supplement	LS	2.0 ± 0.5	3.1 ± 0.5	30 ^a ± 19	13 ± 7
	HS	2.5 ± 0.6	2.8 ± 0.4	20 ^b ± 7	12 ± 4

¹) colour of meat, classification based on Colomer-Rocher et al. 1987 (1= pale, 2 = pink and 3 =red); ²) classification based on Colomer -Rocher et al. 1987 (scale from 1 to 5); ^{a,b}) data within same column with different superscripts differ significantly , P<0.05

EFFECT OF KIDS FEEDING WITH LINSEED CAKE: PRODUCTIVE PERFORMANCES AND SOME MEAT QUALITY TRAITS

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Key Words: Kids, linseed cake, meat quality, fatty acids

Introduction

The cultivation of the linseed, particularly suitable in the Southern Italy areas, can be revalued thanks to a high content in linolenic acid (actually about 58%) considered the precursor of the essential ω 3 fatty acids. For this reason, the nutritionists particularly recommend the use of this oil to satisfy the human nutritional requirements. Being an oil with such a high level of unsaturation, therefore susceptible to rapid oxidation, the cold-extraction is considered a good kind of production, since it ensures lipid stability. The linseed cake, residual by-product from this extraction system, is an animal feed particularly appetizing and very rich of refreshing quality proteins, as well as mainly provided of high quality lipids. Then it can be employed in animal feeding as an alternative protein source to the GMO (genetically modified organism) soy, really cultivated in the world in a quantity of around 63%, so guaranteeing an GMO free meat product for the consumer as well as good health and performance of the animal.

Previous studies on the use of linseed meal performed on growing cattle report results comparable to the ones of the soybean meal on the liveweight gains and on the carcasses composition (Berge *et al.*, 1993) or even superior in the inclusion of fat in the carcasses (Dumont *et al.*, 1997) when the protein level furnished from linseed meal increases. Omega-3 fatty acids are in part transferable to the ruminants fat (Scollan *et al.*, 2001; Raes *et al.*, 2002; Raes *et al.*, 2004; Demirel *et al.*, 2004) and develop an action in the prevention of the vascular diseases, showing an anti-thrombogenic and anti-atherogenic effect (de Lorgeril *et al.*, 2001; Demirel *et al.*, 2004; Ragni *et al.*, 2004), as an effect of prevention in the colon cancer (Roynette *et al.*, 2004). Many authors, besides, have used the linseed meal in the feeding for ruminants, especially cattle, since positive responses are obtained on the digestibility (Khorasani *et al.*, 1994; Dixon *et al.*, 2003a; Dixon *et al.*, 2003b), on the performances (Dixon *et al.*, 2003a; *et al.*, 2003b; Raes *et al.*, 2004), on the meat quality (Berge *et al.*, 1993; Raes *et al.*, 2002; Scollan *et al.*, 2001; Raes *et al.*, 2004) and on the fatty acid content (Wood *et al.*, 2004). There are few information about the use of the linseed cake for the kids.

Objectives

The present study aimed to investigate productive performance and some of the most important meat quality traits such as colour and tenderness, the chemical composition and fatty acid profile in kids fed on a linseed cake in comparison with a traditional diet based on soybean meal.

Methodology

In order to evaluate the effect of linseed cake as a feed for kids on some quantitative characteristics, meat colour and tenderness, chemical and fatty acid composition, 16 male Garganica kids, weaned at 40 ± 3 days of age, homogeneous for age and body weight, were used. After a week of adaptation to the pellet feeding, the kids were divided into two groups of 8 subjects each, and fed *ad libitum* for 6 weeks on either a concentrate pelleted diet containing soybean meal s.e. (Control group) or on a diet containing 20% linseed cake (LC group). Diets were planned so as to contain approximately the same amount of protein and crude fibre. Each animal was placed in a single box with respect to the animal welfare.

During the trial, the daily feed intake of each subject was detected, while the liveweight was monitored weekly in order to establish the daily weight gain and the feed conversion index.

The pH values were measured on the *Longissimus lumborum* (*Ll*) and *Semimembranosus* (*Sm*) muscles at slaughtering (pH_1) and after the carcasses were refrigerated for 24 hours at 4°C (pH_2). From each right half carcass the Lumbar region and Pelvic limb were separated and then dissected into their tissue composition: lean, fat and bone. The colorimetric indexes were assessed on the *Ll* and *Sm* muscles, once isolated from the respective cuts, using the Hunter Lab system (Colourmeter Miniscan XE, D65). From each muscle, half an inch of diameter samples were taken and subjected to the shear force according to the Warner Bratzler Shear device system by an Instron 5544 instrument. Besides, to determine cooking loss percentage, from each *Ll* muscle, meat samples homogeneous for dimensions (about 5 cm thick) were obtained, weighed before and after cooking in an electric ventilated oven at 165°C , until the internal temperature of 75°C was reached in the core of the meat sample (ASPA, 1996), recorded by a thermocouple (Hanna Instruments). Then from cooked *Ll*, 1×1 cm section pieces were subjected to the cutting force. Moreover, chemical analysis and fatty acid profile were performed only on raw meat from *Ll* muscle. Lipids were extracted according to the 2:1 chloroform-methanol method described by Folch *et al.* (1957), whereas the acidic profile was assessed using a Chromopack CP 9000 gas chromatograph. Then the thrombogenicity index was also calculated (Ulbricht and Southgate, 1991). The data were analysed for the variance using the procedure GLM of SAS (1999). Means were compared by the “t” test of Student.

Results & Discussion

From the results reported in Table 1, any significant difference between the two diets didn't emerge, although the kids of the Control group presented final live weights (20.97

vs 19.87 kg) and weight gains (0.152 vs 0.140 kg/d) slightly higher. Moreover, the kids fed on linseed cake evidenced a lower and significant ($P<0.01$) feed intake (0.642 vs 0.765 kg/d) and a better transformation of the feed, since its feed conversion index value was 4.71 against 5.15 kg/kg of the Control group subjects. The net cold dressing percentage (after 24 h at 4°C) was not influenced by the linseed cake integration in the diet (52.18 vs 51.10%).

The pH values of the *Sm* and *Ll* muscles (Table 2) at slaughtering didn't show differences between the two diets, while when measured on the m. *Longissimus lumborum*, after the carcasses refrigeration for 24 h at 4°C, they resulted significantly lower ($P<0.05$) in the LC group (5.75) than in the Control one (6.01).

On the dissecting the Pelvic limb (Table 3), no relevant difference was found in the lean and fat fractions, while the bone incidence resulted higher ($P<0.05$) in the LC (30.70%) than in the Control (28.75%). As regards the Lumbar region, the use of linseed cake would have not determined any difference in the bone and fat percentages, though both higher, and a lower incidence of lean ($P<0.05$). The cooking loss percentage (Table 4) resulted higher ($P<0.01$) in the m. *Ll* meats of the LC group in comparison with the Control one (17.98 vs 13.30%). As for the colour, differences didn't appear on the m. *Ll* in agreement with the considered parameters, while the m. *Sm* meats from the subjects fed on linseed cake resulted redder and yellower.

With regards to the shear force, differences were observed only on the m. *Ll* raw meats in the LC diet, even if they disappeared after cooking, whereas they were never noticed on the raw m. *Sm*. The employment of the linseed cake influenced the meat chemical composition by providing a product with reduced moisture content ($P<0.01$) as well as richer in fat in comparison with the control group (Table 5). As regards the fatty acid profile, the lipids of the meat from the kids fed on the linseed by-product showed a higher percentage of C14:0 ($P<0.01$) and a lower presence of C16:0 ($P<0.05$) and of C18:0 ($P<0.01$) than the control ones (Table 6). The data concerning the fatty acids as a whole allowed us to affirm that the linseed cake positively influenced the fatty acid profile of the meat, improving the unsaturated fraction (61.65 vs 55.90%; $P<0.01$), and particularly the monounsaturated one (53.15 vs 47.67%; $P<0.01$). The use of the linseed cake in the diet affected the PUFA classes amount, result interesting under a dietetic point of view: in fact, it was observed a lower content ($P<0.05$) of $\omega 6$ fatty acids (6.52 vs 7.15%) and a higher one ($P<0.01$) of $\omega 3$ fatty acids (1.97 vs 1.07%) in comparison with the control.

Meat from the kids fed on LC showed a better ($P<0.01$) thrombogenicity index (0.95 vs 1.34) and a $\omega 6/\omega 3$ ratio (3.39 vs 7.09) than the control one, with positive effects on human health (Galli, 1999), since the $\omega 6/\omega 3$ ratio value recommended by the Human Nutrition Society is equal to 4 (Carnovale and Marletta, 1997).

Conclusions

Based on the results of this test, it may be deduced that the integration of the linseed cake allowed to get weight gains and final weights comparable to the ones of kids traditionally fed on soybean meal. In general, the latter provided some meat physical characteristics better than the linseed by-product. As regards the dietetic properties of the meat, the linseed cake produced a positive effect on the fatty acid profile, improving the

thrombogenic index and the ω -3 fatty acids content, with good consequences on human health.

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Tables and Figures

Table 1 - Productive performances

Samples (n.)	Control	LC	SED DF = 14
	8	8	
Initial weights (kg)	13.50	13.05	1.706
Final weights (kg)	20.97	19.87	1.992
Weight gain (kg/d)	0.152	0.140	0.026
Feed intake (kg/d)	0.765A	0.642B	0.078
FCI (kg/kg)	5.15	4.71	0.935
Net cold dressing percentage (%)	51.10	52.18	1.387

A, B: P<0.01

Table 2 - Measurements of pH on *Ll* and *Sm* muscles

	Diet		SED DF = 14
	Control	LC	
<i>Ll</i> : pH ₁	6.61	6.65	0.149
pH ₂	6.01a	5.75b	0.219
<i>Sm</i> : pH ₁	6.34	6.37	0.132
pH ₂	5.88	5.77	0.301

a, b: P<0.05

Table 3 - Dissecting data

	Diet		SED
	Control	LC	
	% on Pelvic limb weight		DF = 14
Pelvic limb weight (kg)	1.27	1.23	0.164
Lean	63.97	61.97	2.202
Fat	7.27	7.32	1.431
Bone	28.75b	30.70a	1.503
	% on Lumbar region weight		
Lumbar region weight (kg)	0.31	0.30	0.054
Lean	49.04a	44.36b	4.343
Fat	19.83	22.90	3.533
Bone	31.12	32.73	2.176

a, b: P<0.05

Table 4 - Meat quality traits

	Diet		SED
	Control	LC	
	<i>Longissimus lumborum</i>		DF = 14
Cooking loss (%)	13.30B	17.98A	1.938
L	43.36	42.33	2.077
a	7.59	7.61	0.696
b	8.44	8.76	0.799
Shear force - raw (kg/cm ²)	7.07B	8.49A	0.639
Shear force -cooked (kg/cm ²)	3.62	3.57	0.589
	<i>Semimembranosus</i>		
L	42.65	41.44	2.760
a	7.73B	9.94A	1.303
b	7.40b	9.13a	1.346
Shear force - raw (kg/cm ²)	6.40	5.30	1.363

A, B: P<0.01; a, b: P<0.05

Table 5 - Chemical composition of *Longissimus lumborum* muscle (% on raw meat)

	Diet		SED
	Control	LC	
Samples (n.)	8	8	DF = 14
Moisture	75.44A	74.53B	0.291
Protein	18.97	19.12	0.361
Fat	3.29B	4.37A	0.279
Ash	1.08	1.02	0.081
Undetermined	1.21	0.96	0.300

A, B: P<0.01

Table 6 - Fatty acid profile (%) and T.I. in *Longissimus lumborum* muscle

	Diet		SED DF = 14
	Control	LC	
Samples (n.)	8	8	
C _{12:0}	0.17	0.25	0.073
C _{14:0}	2.30B	3.15A	0.419
C _{16:0}	21.55a	20.10b	1.058
C _{18:0}	16.95A	10.72B	0.384
Saturated	44.10A	38.35B	1.126
Monounsaturated	47.67B	53.15A	1.348
Polyunsaturated	8.22	8.50	0.635
Unsaturated	55.90B	61.65A	0.126
ω6	7.15a	6.52b	0.555
ω3	1.07B	1.97A	0.291
ω6/ω3	7.09A	3.39B	1.664
Thrombogenicity index	1.34A	0.95B	0.056

A, B: P<0.01; a, b: P<0.05

**EFFECTS OF SEASONAL ENVIRONMENT, ON-FARM HANDLING,
TRANSPORT STOCKING DENSITY, AND TIME IN LAIRAGE ON CORE
BODY TEMPERATURE AND PORK LEAN QUALITY
OF MARKET WEIGHT PIGS**

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Key Words: Pork Quality, Metabolism, Ibutton, Welfare

Introduction

The U.S. pork industry has become increasingly concerned with pork lean quality and its possible effects on both domestic and foreign pork demand (NPB, 2003). Muscle metabolism ante-mortem and early post-mortem has a direct impact upon ultimate pork quality endpoints (Briskey, 1964). Pre-harvest stress has a direct association with muscle metabolism (Gregory, 1998). Evaluating ante-mortem body temperature along with lean quality parameters would help to answer more questions in reference to pre-harvest stress as it associates with non-conforming lean quality.

Core body temperature serves as an indirect indicator of metabolic rate (Webb, 1995). During pre-harvest stress, pigs are often near their upper threshold of thermal tolerance (Lambooj and van Putten, 1993). The temperatures within the digestive tract of live animals should produce values representative of core body temperature

Numerous research initiatives have been conducted to quantify the relationship between ante-mortem stress, animal welfare, and lean quality of market hogs. However, no known study has utilized a non-invasive, objective assessment of transport stress on a large-scale as an indicator of animal well-being and lean quality parameters.

Objectives

The present series of studies were conducted to evaluate two seasonal environments (temperate and cold stress), two on-farm handling intensities (normal and passive), two transport stocking densities (tight and loose) and two lairage lengths (45-min and 3-h) on digestive tract temperature and pork lean quality.

Methodology

Sensor Ingestion

Market hogs at an average live weight of 125 kg were harvested at two representative environmental situations: temperate (TMP) 15-25°C, < 50% RH (n = 111) and cold stress (CS) < 0°C (n = 113). Approximately 16-h prior to harvest, all test animals were withheld from conventional feeding. Approximately 1-h later, all animals were individually snared by a conventional hog snare, then ear tagged for treatment identification and tattooed for carcass identification. At the same time, a computer-activated temperature logging device marketed as an Ibutton (Dallas Semiconductor Corp. Dallas, TX www.ibutton.com) was placed within a conventional small balling gun, placed within the animal's mouth, and the plunger was depressed. Following Ibutton ingestion, animals were allowed to rest prior to transport to the abattoir. The Ibuttons were calibrated to log temperature at 10min intervals.

Handling During Loading

Loading for transport initiated at 0400-h. Half of all test animals (n = 56) were randomly subjected to passive handling and the other half conventional (n = 56) handling. Each group was loaded on individual trucks with identical trailer dimensions. The passively handled pigs were loaded first with that trailer waiting at the loadout facility for the conventionally handled pigs to be loaded. The passively handled pigs (PAS) were moved at a moderate to slow handling speed, with little to no handler vocalization, and limited utilization of paddles or boards, and absolutely no electric prod usage. The conventionally handled pigs (CON) were subjected to a more rapid-paced handling, extensive vocalization by handlers, and extensive physical manipulation via hog paddles and boards, as well as selective utilization of electric prods. The CON handling treatment is indicative of typical, welfare-conscious on-farm handling. Ear tags were recorded for treatment identification.

Loading Density

As each respective handling group was loaded, a portion of the pigs (approximately half) were allotted to a tight loading density (T) within the trailer (0.4 m² per animal). The other portion of the test group was subjected to a looser, more restful density, (L) allowing all animals to lie down (0.5 m² per animal). Loading density specifications were modified from Lambooj and van Putten (1993). Both trailers were loaded with the same proportions and location within the trailer for each stocking density. In accordance to trailer dimensions, non-test animals (n = 4) were added to each trailer to insure proper stocking densities. At the conclusion of loading, both trailers contained 60 market hogs with 56 from each trailer being test animals. Ear tags were recorded for treatment identification.

Unloading and Lairage

Following a 2.5-h trip to a commercial pork processing facility (Tyson Foods, Columbus Junction, IA), the passively handled animals (the first trailer loaded) were unloaded immediately upon arrival. Half of the test animals within each trailer (n = 56) were randomly allocated to one of two lairage treatments, 45-min (45M) or 3-h (3H). Animals were given free access to water during lairage. Ear tags were recorded for treatment identification. All animals were subjected to handling by the researchers during unloading and by the plant personnel for the walk to the stunning chute. All animals were subjected to humane head-to-heart electrical stunning procedures.

Post-Mortem Measurements

Following humane harvest and evisceration, the eviscera was inspected for retrieval of the Ibuttons. A stud finder with a metal scan setting (Zircon Corp., Campbell, CA) was utilized to expedite object retrieval. Ibuttons (n = 172 of 224) were recovered for downloading of temperature data onto a personal computer after returning to the University of Missouri.

Carcass Composition and Quality Assessment

All carcass parameters were assessed from the right carcass side. At the harvest floor grading station, hot carcass weight was recorded. Additionally, the Carcass Value Technology (CVT) system (Animal Ultrasound Services, Ithaca, NY) was used to evaluate average fat depth and loin muscle depth for the calculation of percent muscle. Intramuscular pH and temperature was assessed approximately 1-h post-mortem within the longissimus muscle (LM) between the 10th and 11th ribs utilizing a portable pH meter (pH Star, SFK Co., Peosta, IA) and temperature probe (Koch Equipment Co., Kansas City, MO). At fabrication, all right side skinned loins (IMPS 410; NAMP, 1997) were collected, weighed, and subsequently shipped to the University of Missouri Meat Laboratory.

Pork Quality Assessment

Further fabrication was initiated at approximately 30-h postmortem. The loin was divided between the 10th and 11th ribs and after allowing a 15-min oxygenation period, ultimate muscle pH and objective lean color analysis (L*, a*, b*) was conducted using a Hunter MiniScan SE with an illuminant setting of D65/10. Following deboning and sirloin removal, one 2.54 cm LM chop was fabricated, originating from the initial 10th/11th rib separation. A 2.54 x 2.54 cm section was further fabricated from the center of the lean tissue of the chop for drip loss analysis. The loin section generated from the loin section posterior to and including the 11th rib was weighed, then vacuum packaged and stored for 7d at 4°C for subsequent assessment of purge loss.

Statistical Analysis

All assessments of lean quality, digestive tract temperature, and carcass data were analyzed using ordinary least squares (PROC GLM, SAS Inst., Inc., Cary, NC). Seasonal

environment (harvest date), on-farm handling intensity, stocking density, and lairage time were utilized as a fixed effect. Ibutton temperatures from individual animals were averaged for three time periods representing loading (0400-h – 0530-h) transport (0531-h – 0800-h) and lairage (0801-h – 1-h following harvest of the last animal). These averages were evaluated as repeated measures within a split plot in time. Mean separation was accomplished using the P-DIFF option of SAS at an Alpha level of 0.05.

Results & Discussion

Carcass Composition

Market hogs from the TMP harvest group had heavier hot carcass weights, and greater loin muscle depths ($P < 0.0001$) than market hogs from the CS harvest group (data not provided). However, market hogs in the TMP group also had greater average fat depth ($P < 0.01$) than animals in the CS group; therefore no difference ($P > 0.05$) was found between harvest groups for calculated percent muscle. This suggests that between animal harvest groups, metabolic rate as it associates with lean mass should be similar.

Pork Quality

Market hogs from the TMP harvest group had higher 1-h pH values ($P < 0.0001$) within the LM and tended to have higher ultimate pH values ($P = 0.07$) than hogs from the CS harvest group (Table 1). Conventionally (CON) handled market hogs loaded at a loose (L) stocking density displayed darker, more desirable L^* values ($P < 0.05$) than PAS handled hogs loaded at the same stocking density (Table 1). This suggests that the time efficiency of CON handling could actually be less stressful than the more time consuming PAS handling of market hogs. Market hogs from the TMP harvest had lower drip loss percentages ($P < 0.0001$) than market hogs from the CS harvest (Table 1). This could be attributed to the higher 1-h pH values reported during TMP weather. This higher pH early postmortem could possibly impede protein denaturation of the musculature, hence improving water holding capacity. Additionally, market hogs loaded at a L stocking density had lower drip loss percentages ($P < 0.05$) than those loaded at a T stocking density (Table 1). This could be attributed to an endocrine stress response increasing the muscle metabolism of the more tightly stocked animals.

Digestive Tract Temperature

Animals from both harvest groups, cold stress and temperate, displayed lower digestive tract temperatures during lairage than during loading and transport ($P < 0.05$), suggesting that the more extended lairage treatment could allow metabolic rate to slow prior to harvest, possibly leading to improved lean quality.

Conclusions

Further analysis and investigation should be conducted to further develop the relationships between on-farm handling intensity, stocking density on the truck, and

lairage time. However, it appears the extra time associated with passively loading market hogs could actually be more stressful than handling them at a conventional rapid pace. Assessment of digestive tract temperature holds promise as an indicator of metabolic rate. Further analysis is needed to assess how digestive tract temperature correlates with lean quality parameters.

Tables and Figures

Table 1. Effect of seasonal environment, on-farm handling intensities, transport stocking density and lairage length on loin muscle quality characteristics

Characteristic	Harvest Group ^a (HG)		Handling Intensity ^b (HI)		Stocking Density ^c (SD)		Lairage Time ^d (LT)		Level of Significance ^e					
	TEM	CS	PAS	CON	L	T	3H	45M	HG	HI	SD	LT	HI X SD	SD X LT
1-h pH	6.24	5.99	6.11	6.13	6.12	6.12	6.10	6.14	***	NS	NS	NS	NS	NS
Ultimate pH			5.77	5.78										
Loose			5.74 ^f	5.81 ^f										
Tight	5.80	5.75	5.79 ^f	5.74 ^f	5.77	5.77	5.75	5.80	x	NS	NS	x	*	NS
Hunter L*			48.07	47.41										
Loose			48.39 ^f	46.81 ^g										
Tight	48.13	47.35	47.74 ^{fg}	48.02 ^{fg}	47.60	47.88	48.26	47.22	x	NS	NS	*	*	NS
Hunter a*	8.20	8.80	8.41	8.60	8.49	8.52	8.56	8.45	**	NS	NS	NS	NS	NS
Hunter b*					14.68	14.83								
3-h					14.97 ^{fg}	15.27 ^f								
45-min	14.70	14.82	14.87	14.63	14.71 ^g	14.27 ^g	15.05	14.47	NS	NS	NS	**	NS	*
Drip, %	3.22	6.74	4.84	4.71	5.10	4.46	4.87	4.70	***	NS	**	NS	NS	NS
Purge, %	2.62	2.27	2.33	2.55	2.60	2.30	2.55	2.35	x	NS	NS	NS	NS	NS

^aHarvest Group: TEM= Temperate, CS= Cold Stress; respectively.

^bHandling Intensity: PAS= Passive, CON= Conventional, respectively.

^cStocking Density: L= Loose, T= Tight, respectively.

^dLairage Time: 3H= 3 hours, 45M= 45 minutes, respectively.

^eLevel of significance: NS= not significant, x= $P < 0.10$, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.0001$, respectively.

^{f, g}Values lacking a common superscript within an interaction differ ($P < 0.05$).

Table 2. Relationships between harvest group and handling intensity with the average Ibutton temperature (°C) within the digestive tract during loading, transport, and lairage

Average Ibutton Temperature(°C) During:			
	Loading	Transport	Lairage
Handling Intensity			
Passive	39.76 ^a	39.47 ^b	39.10 ^c
Normal	39.64 ^a	39.54 ^b	39.21 ^c
Harvest Group			
Cold Stress	39.66 ^a	39.55 ^b	39.41 ^c
Temperate	39.74 ^a	39.45 ^{bc}	38.90 ^d

^{a, b, c, d} Values lacking a common superscript within an interaction differ ($P < 0.05$).

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DEEP BEDDED FINISHING OF PIGS: EFFECTS ON SWINE PERFORMANCE, PORK QUALITY AND ADIPOSE TISSUE COMPOSITION

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Key words: Swine production; hoop structures; pork quality; fatty acid composition

Introduction

An alternative system for finishing pigs utilizes deep-bedded hoop structures. Hoops are large, tent-like shelters with cornstalks or straw for bedding (Honeyman et al., 2001). Items differing between hoops and confinement systems are the use of straw bedding and exposure to the environment. Deep-bedded systems are enrichment strategies that have been shown to stimulate foraging and explorative behavior (O'Connell et al., 2004, De Jong et al., 1998). Finishing in deep-bedded environments has also supported increased spontaneous exercise (Morrison et al., 2003a; Gentry et al., 2002; Beattie et al., 1996). Increased exercise and exploratory behavior may lead to changes in stress susceptibility, influencing performance and ultimate pork quality (Morrison et al., 2003b; Klont et al., 2001). Stress during finishing and before slaughter is known to influence the physiological and biochemical processes in pigs, which will affect the perimortem muscle metabolism and thereby meat quality (Cassens, 1975).

Objectives

Few studies have compared growth characteristics from confinement systems to deep-bedded systems. No research has been conducted comparing pork quality and adipose tissue composition between these two systems. This study was undertaken to compare pigs finished in standard confinement systems to pigs finished in hoop structures and the effects of swine performance, pork quality and adipose tissue composition.

Methodology

Animal Selection: Five groups of 600 pigs were farrowed and reared in intensive confinement conditions at the Iowa State University Swine Nutrition Farm, Ames, IA. At four months of age, gilts were separated from barrows, weighed and allocated into groups stratified by weight. From those weight allocation groups, 100 gilts ranging in weight from 59 – 71 kg were randomly assigned to treatments of hoop (n = 50) and CON (n = 18). Stocking density in each treatment group was 0.70m²/pig. Gilts were fed a two-phase diet ad libitum for a standard period of 45 days. At 45 days, gilts were weighed and

allocated into pre-slaughter groups stratified by weight. One gilt was randomly chosen to represent each weight group to total six pigs per treatment for observations.

Growth and Performance: Beginning weight, 21-day weight and final slaughter weight were obtained for each pig. Average daily gain (ADG, g/day), feed conversion (g:f) were calculated for each pig.

Slaughter and Sampling: After standard slaughter, carcasses were placed in a 0°C cooler and chilled for 24 hours. After 24 hours, two 20 g samples of adipose tissue from the blade end of the loin were obtained for fatty acid analysis and fat firmness measurement. Four 2.54 cm chops were obtained for star probe analysis; the first two chops were assigned an aging period of 24 hours and the second two were assigned an aging period of 120 hours. Three 2.54 cm chops were obtained for objective color and drip loss analysis. Sirloin ends of pork loin were obtained for purge analysis. All samples were vacuum packaged and held until analysis was conducted.

Pork Quality: pH and temperature measurements were taken at 1, 6 and 24 hours postmortem on right side loins by a penetration probe. Carcasses were ribbed between the 10th and 11th ribs and were subjectively analyzed for color and appraised for firmness, wetness and marbling. Objective measurements of tenth and last rib backfat and loin eye area were taken. Fat free lean % (FFL%) was calculated using the NPB percent fat free lean calculation (NPB, 2005). Four 2.54 cm chops from right side loins were stored in a vacuum bag at 4°C for 24 or 120 hours postmortem. After aging, chops were frozen in a -20°C blast freezer until needed for star probe analysis. All procedures were done in accordance to Lonergan and Prusa (2002). Hunter L*, a* and b* values were determined at 1-d postmortem on 2.54 cm thick chops using a Hunter Labscan colorimeter (Hunter Association Laboratories Inc.; Reston, VA). Drip loss was determined using 2.54 cm-thick boneless chops by similar method to Lonergan et al. (2001). Purge Loss was measured on the sirloin and of the loin after 120 hours of storage at 4°C.

Fatty Acid Composition and Total Lipid: Approximately 3 g samples from inner layer only were weighed into a 50 mL test tube for total lipid analysis by the method of Folch et al. (1957). Crude lipid analysis was conducted from the method of 10 ml of folch extract from lipid extraction. Total lipid percentage was determined on a wet weight basis. Fatty acids were methylated using the method of Morrison and Smith (1964) and separated (Jo and Ahn, 2000).

Firmness: Adipose samples were cut into 5 x 3 cm squares and analyzed for firmness using a method modified from Nishioka and Irie (2005). Samples were evaluated using TA-XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY) with a ¼" diameter ball shaped probe. Sample height was noted by the testing machine, and the probe was driven downward at 2mm/sec to a depth of 20% of the sample height. Peak force exerted (kg) and sample height (cm) were recorded for three separate positions on the square.

Results & Discussion

Hoop pigs gained significantly ($P<0.01$) less per day and required more feed for lean growth than CON pigs (Table 1). These results are congruent with Larson et al. (1999). Carcass weights and dressing % did not differ between the two groups. Confinement pigs had lower lean percentages than hoop pigs (55.50 vs 56.87). Significant ($P<0.05$) replication effects were noted in beginning weight, live weight, carcass weight, fat free

lean, and backfat at the 10th rib as well as the last rib. Several other studies have noted lower levels of backfat in pigs finished outdoors or semi-outdoor compared to indoor finished pigs (Gentry et al., 2002; Warriss et al., 1983; Enfält et al., 1997). Group effects and main effect interactions were noted for live weight, carcass weight, backfat (10th & last rib), and % fat free lean (FFL). With no change in feed intake, it is probable that the effect of temperature may play a role in fat deposition and lean gain. Environment did not affect temperature or pH decline (Table 2). There were no differences in loin eye area, color, firmness or wetness of the loin. Confinement pigs had higher levels of marbling in the loin compared to hoop pigs. Environment had no effect on fat firmness. Hoop pigs had lower proportions of C16:0, and higher deposition of C18:1 and C18:2 in the inner layer of adipose tissue (Table 3). These differences led to overall differences in proportions of total saturated (SAT), total monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the adipose tissue. Total SAT was lower (34.06 v 49.80), and total MUFA and PUFA were higher in hoop pigs compared to confinement pigs (48.04 v 35.05 and 17.54 v 15.17 respectively). There was a significant replication effect noted for several individual fatty acids as well as fat firmness. The interactive effect of environment and replication gave insight that individual fatty acids were being deposited at different levels from rep to rep, and also were differing between treatments. The main characteristic differing by replication, irrespective of treatment, was ambient temperature. Temperatures within the confinement ranged from 31° – 9°C, where in hoops temperature ranged from 33° – 2.9°C. Fluctuations in ambient temperature due to alternative production systems have been shown to influence fatty acid composition in pigs (Bee et al., 2004; Lebret et al., 2002). Outdoor pigs have been shown to deposit more unsaturated fats than their confinement counterparts (Hogberg et al., 2004; Bee et al., 2004). Specifically, decreasing temperature affects the fatty acid composition of the back fat leading to higher MUFA and lower SAT and PUFA contents of adipose tissue in pigs finished at cooler temperatures. Pearson correlation coefficients were analyzed for each individual fatty acid against average and low temperatures (Table 4). Individual fatty acids were correlated to temperature, where saturated fatty acids were positively correlated and unsaturated fatty acids were negatively correlated. These differences drove strong correlations between SAT and MUFA with temperature. These data verify that fluctuations in ambient temperature below the thermoneutral zone for pigs accompanied an increased in MUFA and PUFA in the adipose tissue of pigs.

Conclusions

Hoop pigs varied from CON finished pigs, being less fat at the 10th rib with lower amounts of intramuscular fat in the loin. Hoop-finished pigs had significantly higher amounts of MUFA and PUFA and significantly lower amounts of SAT comprising the adipose tissue. Replication effects and treatment by replication interactions caused variations within growth, subsequently affecting fatty acid composition and adipose tissue firmness. The specific role of ambient temperature fluctuation on these attributes needs to be further evaluated.

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Table 1. The effect of finishing environment on swine performance and carcass composition

Variable	Hoop	CON	ENV ¹	SE ²	Rep ³	ENV*Rep ⁴
BW (kg)	73.82	71.40	NS	5.25	**	**
LW (kg)	106.92	110.41	NS	5.64	***	**
CW (kg)	79.15	82.75	**	4.34	***	**
Dressing (%)	74.65	74.90	NS	0.37	NS	NS
10 th rib BF (mm)	13.72	15.24	**	0.03	**	**
LRBF (mm)	19.02	20.32	NS	0.09	**	***
FFL (%)	56.87	55.50	***	0.52	***	***
ADG (kg/day)	0.81	1.07	***	0.09	NS	NS
G:F	0.52	0.42	***	0.09	NS	NS

1 ** P<0.05 *** P<0.01
 2 Standard Error of Treatment
 3 Group 1 - 5
 4 Interaction of replication and treatment

Table 2. The effect of finishing environment on fresh pork quality attributes

Variable	Hoop	CON	ENV ¹	SE ²	Rep ³	ENV*Rep ⁴
Temp – 1 (°C)	36.49	36.82	NS	0.50	NS	NS
Temp – 6 (°C)	9.05	9.77	NS	0.22	NS	NS
Temp – 24 (°C)	1.39	1.32	NS	0.62	NS	NS
pH – 1	6.21	6.18	NS	0.52	NS	NS
pH – 6	5.61	5.62	NS	0.42	NS	NS
pH – 24	5.32	5.40	NS	0.53	NS	NS
LEA (in ²)	6.93	6.96	NS	0.21	**	NS
Color	1.92	2.07	NS	0.12	NS	NS
Marbling	1.42	1.78	***	0.12	NS	NS
Firmness	1.90	1.88	NS	0.06	NS	NS
Wetness	1.83	1.89	NS	0.07	NS	NS
L*	54.48	54.40	NS	0.64	**	NS
a*	8.06	8.26	NS	0.24	**	NS
b*	14.19	14.27	NS	0.35	***	NS
Drip (%)	3.68	4.64	NS	0.92	NS	NS
Purge (%)	2.74	2.28	NS	0.30	NS	NS

1 ** P<0.05 *** P<0.01
 2 Standard Error of Treatment
 3 Group 1 - 5
 4 Interaction of replication and treatment

Table 3. The effects of finishing environment on fatty acid composition and total lipid concentration of adipose tissue¹

Formula	Environment			Significance ⁴		
	Hoop ²	CON ²	SE ³	ENV ⁵	Rep ⁶	ENV*Rep ⁷
C14:0	1.88	2.94	0.55	NS	NS	NS
C16:0	19.16	32	0.50	***	**	**
C16:1, n7	5.5	6	0.51	NS	***	**
C17:0	0.93	0.84	0.11	NS	NS	NS
C17:1, n10	0.73	1.01	0.32	NS	NS	NS
C18:0	11.11	12.28	0.81	NS	***	**
C18:1, n9	39.96	26.52	1.32	***	***	***
C18:1, n7	2.21	1.5	0.42	NS	***	**
C18:2, n6	15.38	13.14	0.81	***	***	**
C18:3, n3	0.84	0.8	0.18	NS	***	**
C20:0	0.67	1.52	0.46	NS	***	**
C20:4, n6	0.63	0.58	0.12	NS	**	**
C20:5, n3	0.46	0.34	0.16	NS	***	**
C22:0	0.31	0.22	0.10	NS	***	**
C22:5, n3	0.18	0.21	0.07	NS	***	**
C22:6, n3	0.05	0.1	0.04	NS	NS	***
Total SAT	34.06	49.8	1.28	***	***	**
Total MUFA	48.4	35.03	1.22	***	***	**
Total PUFA	17.54	15.17	0.85	***	***	***
% Lipid	81.55	83.6	1.68	NS	***	NS

¹ Analysis done on inner layer of backfat tissue. Presented as least squared means.

² Hoop = hoop finished pigs, CON = confinement finished pigs.

³ Standard Error of the treatment mean.

⁴ Significance: **, P<0.05; ***, P<0.01; NS, P>0.05.

⁵ Environmental significance, hoop versus confinement.

⁶ Replication group

⁷ Treatment by replication

⁸ Monounsaturated

⁹ Polyunsaturated

Table 4. Pearson Correlation Coefficients of FA to Average and low temperatures

	C16:0	C16:1	C18:0	C18:1, n9	C18:1, n7	C18:2	Sat	MUFA	PUFA
AVG	0.70 (0.001)	-0.79 (0.001)	0.44 (0.001)	-0.21 (0.01)	-0.28 (0.001)	0.22 (0.01)	0.65 (0.001)	-0.66 (0.001)	-0.18 (0.02)
LOW	0.56 (0.001)	-0.58 (0.001)	0.34 (0.001)	-0.18 (0.02)	-0.27 (0.001)	0.11 (0.15)	0.50 (0.001)	-0.48 (0.001)	-0.07 (0.35)

THE EFFECT OF SPACE ALLOCATION ON SWINE PERFORMANCE, PORK QUALITY AND ADIPOSE TISSUE COMPOSITION

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Key Words: Swine production; pork quality; fatty acid composition

Introduction

Alternatively-managed pigs differ from intensive systems in that pigs have a chance to pursue their natural instincts and have more space to move freely (Honeyman, 1996). This is brought about by variations in housing style, stocking rate, flooring, and bedding type. Variations in stocking rates influence behavior (Schmolke et al., 2004) and swine performance (Hyun, Ellis, Riskowski & Johnson, 1998). Reducing space has been shown to increase observations of abnormal behaviors and levels of aggression (Randolph et al., 1981). Higher incidence of these behaviors could increase stress, thereby impacting perimortem metabolism. Variations in perimortem metabolism will induce changes in the conversion of muscle to meat, leading to differences in ultimate pork quality (Klont et al., 2001). Several studies have reported increased acceptability of pork from pigs finished in systems which allocate more space (Millet et al., 2004; Gentry, McGlone, Miller & Blanton, Jr., 2002a; Beattie et al., 2000). The standard stocking density commonly implemented in confinement systems is 0.72 - 0.90 m²/pig from 70 to 113 kg. (NCR-89, 1993). The optimum space allocation for several alternative environments has yet to be defined.

Objectives

The space requirement of pigs housed in large groups in deep-bedded, semi-outdoor structures has not been adequately evaluated. The following experiment was designed and implemented to determine the degree to which space allocation in a deep bedded system influences swine performance, pork quality and adipose tissue attributes in deep-bedded, semi-outdoor structures.

Methodology

Animal Selection: Five groups of 600 pigs were farrowed and reared in intensive confinement conditions at the Iowa State University Swine Nutrition Farm, Ames, IA. At four months of age, gilts were separated from barrows, weighed and allocated into groups

stratified by weight. From those weight allocation groups, 100 gilts ranging in weight from 59 – 71 kg were randomly assigned to treatments of low ($0.70\text{m}^2/\text{pig}$, $n = 50$) and high ($1.13\text{m}^2/\text{pig}$, $n = 50$) space allocation. Gilts assigned were transported 126 miles to the ISU Western Research Farm, Castana, IA. The alternative method employed in the current study was the use of hoop structures. Hoops are large, tent-like shelters with cornstalks or straw for bedding (Honeyman et al., 2001). Gilts were fed a two-phase diet ad libitum for a standard period of 45 days. At 45 days, gilts were weighed and allocated into pre-slaughter groups stratified by weight. One gilt was randomly chosen to represent each weight group to total six pigs per treatment for observations and measurements.

Growth and Performance: Beginning weight, 21-day weight and live weight prior to slaughter were obtained for each pig. Average daily gain (ADG, g/day), feed conversion (g:f) and shrink (%) during transport and lairage were calculated for each pig.

Slaughter and Sampling: Pigs were slaughtered and placed in a 0°C cooler and chilled for 24 hours. Two 20 g samples of adipose tissue from the blade end of the loin were obtained for fatty acid analysis and fat firmness measurement. Four 2.54 cm chops were obtained for star probe analysis; the first two chops were assigned an aging period of 24 hours and the second two were assigned an aging period of 120 hours. Three 2.54 cm chops were obtained for objective color and drip loss analysis. Sirloin ends of pork loin were obtained for purge analysis. All samples were vacuum packaged and held until analysis was conducted.

Pork Quality: pH and temperature measurements were taken at 1, 6 and 24 hours postmortem on right side loins by a penetration probe. Carcasses were ribbed between the 10th and 11th ribs and were subjectively analyzed for color and appraised for firmness, wetness and marbling. Objective measurements of tenth and last rib backfat and loin eye area were taken. Percent fat free lean (% FFL) was calculated using the NPB percent fat free lean calculation (National Pork Board, 2005). Four 2.54 cm chops from right side loins were stored in a vacuum bag at 4°C for 24 or 120 hours postmortem. After aging, chops were frozen in a -20°C blast freezer until needed for star probe analysis. All procedures were done in accordance to Lonergan and Prusa (2002). Hunter L^* , a^* and b^* values were determined at 1-d postmortem on 2.54cm thick chops using a calibrated Hunter Labscan colorimeter (Hunter Association Laboratories Inc.; Reston, VA). Drip loss was determined using 2.54-cm-thick boneless chops by similar method to Lonergan et al. (2001). Purge Loss was measured on the sirloin and of the loin after 120 hours of storage at 4°C .

Fatty Acid Composition and Total Lipid: Approximately 3 g samples from inner layer only were weighed into a 50 mL test tube for total lipid analysis by the method of Folch et al. (1957). Crude lipid analysis was conducted from the method of 10 ml of folch extract from lipid extraction. Total lipid percentage was calculated as: percentage total crude lipids = lipid weight * lipid layer volume (ml) / 10 (ml) / sample weight (g) * 100. Fatty acids were methylated and separated using the method of Morrison and Smith (1964). Methylated fatty acids were used for gas chromatographic analysis according to the method of Jo and Ahn (2000).

Firmness: Adipose samples were cut into 5 x 3 cm squares and analyzed for firmness using a method modified from Nishioka and Irie (2005). Samples were evaluated using TA-XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY) with a ¼” diameter ball shaped probe. Sample height was noted by the testing machine, and the probe was

driven downward at 2mm/sec to a depth of 20% of the sample height. Peak force exerted (kg) and sample height (cm) were recorded for three positions on the square, and were averaged by sample for statistical analysis.

Results & Discussion

Allocating greater area of space did not influence performance (Table 1). Space differences in previous reports may be due to seasonal variation (Honeyman & Harmon, 2003). Greater space allocation had minimal influence on fresh pork quality attributes (Table 2). Temperature and pH decline did not differ between the two treatment groups ($P>0.05$). Space allocation did not affect lean marbling, firmness or wetness. ($P>0.05$). High treatment pigs had significantly lower ($P<0.05$) degree of muscling in the loin (44.71cm^2 v 42.19cm^2), and produced pork appearing significantly darker ($P<0.05$) than low pigs. There were no measurable differences between L^* , a^* or b^* between the two groups of pigs. Although our subjective color measurements were not congruent with objective color measurements our results are similar to Gentry et al. (2002a), who reported pigs finished with larger space allowance had pork obtaining higher color scores than highly stocked pigs. Drip and purge loss were not affected by space allocation. Space allocation variation altered the fatty acid composition of inner layer adipose tissues (Table 3). Greater space allocation resulted in higher ($P<0.05$) amounts of C14:0 and significantly lower ($P<0.01$) amounts of C18:2 in adipose. These differences in concentration led to significant differences in total saturation (SAT) and polyunsaturation (PUFA) overall. These results are interesting in that there were no differences in feed intake or g:f between treatments. Factors affecting fatty acid composition are diet, fatness, age/body weight, gender, breed, environmental temperature, depot site and maintenance (Wood & Enser, 1997). Replications spanned the months of August to November, with temperatures ranging from -2° - 32°C within the hoop. Lebret et al. (2002) reported decreasing outdoor environmental temperature from 24°C to 17°C during finishing affected the fatty acid composition of the back fat of pigs leading to higher MUFA and lower SAT and PUFA contents ($P<0.001$). SAT, MUFA and PUFA varied by replication group in the current experiment. As environmental temperature declined, adipose tissue decreased in SAT and PUFA. In agreement with Lebret et al. (2002), an increase in MUFA occurred as temperature decreased. Therefore, replication responses might have been dictated by temperature, leading to differences in fatty acid profile. Fat firmness and height did not vary between treatments. Pigs with greater space allocation had significantly higher total lipid. Paralleling these differences was an increase in PUFA incorporation in low space pigs. Bee et al. (2004) noted a similar response between indoor and outdoor finished pigs, where outdoor pigs displayed increased PUFA with a lower total lipid in the outer layer of backfat. It has been established that when lipid content is reduced, the proportion of unsaturated phospholipids is higher, driving an increase in overall PUFA content (Bee, 2002). Therefore, changes in fatty acid composition in this study were predominately due to depositional changes within the adipose tissue.

Conclusions

The results showed that allocating larger space during finishing in hoop structures did not affect swine growth, performance or pork quality. Variations in fatty acid composition and lipid percentage of adipose tissue were observed when space allocation was changed within hoop structures. These results were related to depositional changes in adipose tissue, and may also be dependant on ambient temperature fluctuations. These results indicate the influence of ambient temperature in alternative production scenarios and its resulting effect on items such as backfat deposition and consistency, intramuscular and subcutaneous adipose fatty acid composition need to be further investigated.

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Table 1 The effect of space allocation within hoops on swine growth and carcass performance

	L ¹	H ¹	L v H ²	SE ³	Group ⁴	INT ⁵
BW (kg)	73.82	73.98	NS	3.58	***	NS
LW (kg)	106.09	106.66	NS	5.19	**	***
CW (kg)	79.15	78.60	NS	3.92	***	***
FFL (%)	56.86	56.18	NS	0.53	***	***
ADG (kg/day)	0.80	0.82	NS	0.09	NS	NS
G:F	0.42	0.43	NS	0.03	NS	NS
DP (%)	74.20	74.04	NS	0.62	NS	NS
10 th rib BF (mm)	13.72	12.70	NS	0.02	**	NS
LRBF (mm)	17.02	15.49	NS	0.05	***	NS

- 1 Low = 0.70m²/pig, High = 1.13 m²/pig
- 2 ** P<0.05 *** P<0.01
- 3 Standard Error of Treatment
- 4 Group 1-5
- 5 Interaction of treatment and repetitive group

Table 2. The effect of space allocation within hoops on fresh pork quality attributes

	L ¹	H ¹	L v H ²	SE ³	Group ⁴	INT ⁵
Temp – 1 °C)	36.48	36.36	NS	0.43	NS	NS
Temp – 6 °C)	9.05	8.64	NS	0.42	NS	NS
Temp – 24 °C)	1.39	1.46	NS	0.43	NS	NS
pH – 1	6.21	6.16	NS	0.56	NS	NS
pH – 6	5.61	5.52	NS	0.53	NS	NS
pH – 24	5.32	5.37	NS	0.52	NS	NS
LEA (cm)	44.71	42.19	**	0.20	***	**
Color	1.91	2.12	**	0.10	**	NS
Marbling	1.41	1.47	NS	0.14	***	NS
Firmness	1.90	1.91	NS	0.06	NS	NS
Wetness	1.84	1.83	NS	0.08	NS	NS
L*	54.58	54.74	NS	0.68	**	NS
a*	8.05	8.34	NS	0.26	NS	NS
b*	14.16	14.53	NS	0.33	NS	NS
DL (%)	3.67	3.59	NS	0.35	NS	NS
Purge (%)	2.74	2.64	NS	0.33	NS	NS

- 1 Low = 0.70m²/pig, High = 1.13 m²/pig
- 2 ** P<0.05 *** P<0.01
- 3 Standard Error of Treatment
- 4 Group 1-5
- 5 Interaction of treatment and repetitive group

Table 3. The effect of space allocation within hoops on fatty acid composition and total lipid of adipose tissue

FA	L ¹	H ¹	L v H ²	SE ³	Group ⁴	INT ⁵
C14:0	1.88	3.09	**	0.55	***	**
C16:0	15.23	15.06	NS	0.59	**	**
C16:1, n7	9.41	10.22	NS	0.39	**	**
C17:0	0.93	0.83	NS	0.11	NS	NS
C17:1, n10	0.73	1.14	NS	0.32	NS	NS
C18:0	11.16	11.59	NS	0.81	NS	NS
C18:1, n9	40.03	39.33	NS	1.59	NS	NS
C18:1, n7	0.74	1.49	NS	0.46	NS	NS
C18:2, n6	16.76	12.74	***	0.82	***	***
C18:3, n3	0.84	0.96	NS	0.14	NS	NS
C20:0	0.67	1.74	NS	0.19	NS	NS
C20:4, n6	0.64	0.73	NS	0.11	**	**
C20:5, n3	0.44	0.51	NS	0.15	**	**
C22:0	0.30	0.32	NS	0.10	NS	NS
C22:5, n3	0.18	0.19	NS	0.08	**	**
C22:6, n3	0.06	.11	NS	0.04	**	**
SAT	30.17	32.48	**	0.82	**	**
MUFA	50.77	51.57	NS	1.44	NS	NS
PUFA	17.63	15.24	***	0.88	**	***
% Lipid	81.55	85.52	**	1.89	NS	NS

1 Low = 0.70m²/pig, High = 1.13 m²/pig

2 ** P<0.05 *** P<0.01

3 Standard Error of Treatment

4 Group 1-5

5 Interaction of treatment and repetitive group

EXIT VELOCITY EFFECTS ON GROWTH, CARCASS CHARACTERISTICS, AND TENDERNESS IN HALF-BLOOD BONSMARA STEERS

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Key Words: Bonsmara, carcass characteristics, exit velocity, growth, tenderness

Introduction

One of the goals of the beef industry has been to produce a more palatable, uniform, tender and cost efficient product. Live animal stress has been shown to impact live animal growth, carcass characteristics and tenderness. It has been hypothesized, that flighty or stressed cattle have undesirable performance and carcass characteristics. One method of classifying cattle, proposed by Burrow et al. (1988), is to classify cattle by exit velocity leaving the chute. Curley (2004) reported a relationship between stress and exit velocity. Steers expressing flighty behaviors in response to handling stress had lower average daily gains, higher feed to gain ratios, lower USDA Quality and Yield grades and higher Warner-Bratzler shear force values (WBS) (Brown et al., 2002, Vann et al., 2004).

By evaluating animal behavior and temperament during stressful periods these measurements may be used as a method to classify and sort cattle. Not only could it help to identify cattle that produce more desirable carcasses containing more tender meat, but it could also help producers make production decisions. Producers could select animals that either respond more positively or adapt to stress.

The Bonsmara breed developed in the late 1930's and early 1940's in South Africa, is a composite of 5/8 Afrikaner (tropically adapted breed), and 3/8 Hereford /Shorthorn. The breed was developed with the goal of adaptability, tenderness, and productivity in the subtropical region. Bonsmara-influenced cattle have been shown to produce comparable carcasses to British cattle that are considered tender under US production systems (Holloway et al., 2000, Miller et al., 2005). Research in South Africa has suggested that carcass characteristics and WBS values were similar to those of British cattle breeds produced under the same production conditions (Strydom, 1994). Bonsmara germplasm was first imported into the United States in 1996 to Amarillo, TX. Bonsmara-influenced cattle are being produced in the southwest and Colorado. These cattle provide a unique population to evaluate the relationship between stress adaptation and temperament traits with average daily gain in the feedlot, carcass characteristics and cooked beef tenderness.

Objectives

The objective was to examine the relationship between exit velocity as a measurement of temperament and stress at weaning, entering the feedlot, and leaving the feedlot on live animal growth, carcass characteristics and beef tenderness in half-blood Bonsmara steers.

Methodology

Experiment 1

Bonsmara X Beefmaster (BM) steers (n=139) from Dos Amigos Ranch near Roswell, NM were used to evaluate exit velocities (EV) at weaning and entering the feedlot and the effect it had on growth and carcass performance and WBS. At weaning, steers were evaluated for EV and live animal weight (BW). Exit velocity was an adapted procedure described by Burrow et al. (1988). Exit velocity was measured electronically as the time (m/sec) between two sets of timers placed 1.83 m apart and .9 m in front of a squeeze chute. Twenty-two weaned Bonsmara cross (BONX) steers from the Harris Ranch (Cline, TX) were also used in this study. Sixty-two BM steers and 22 BONX steers were randomly assigned to Uvalde (semi-arid) and 77 BM steers assigned to Overton (humid), TX for winter grazing (Table 1). Prior to the initiation of grazing, Uvalde steers were fed hay *ad libitum* and 0.9 kg/head/d of a 20% protein range cube. Steers in Uvalde were allowed to graze irrigated 'TAM 90' annual ryegrass pasture. Steers assigned to Overton remained in a dry lot and were allowed *ad libitum* Coastal bermudagrass hay and 0.9 kg/hd/d of a 4:1 (corn:SBM) ration until initiation of a winter pasture ('Maton rye' + 'TAM 90' ryegrass) experiment. The experiment at Overton used a 2x2x2 factorial arrangement to examine the effects of stocking rate, stocking method, and stocking strategy on grazing performance.. Upon completion of the winter grazing, all steers entered the Liveoak Feedlot on May 13, 2002. One week after entering the feedlot, EV and BW were measured during routine feedlot processing (defined as EV and BW entering the feedlot). Exit velocity was determined as described earlier. Approximately 50 d after entering the feedlot, an estimate of subcutaneous fat was taken using ultrasound. Animals were harvested in five groups on 23, 68, 90, 126, and 153 d on feed as steers were targeted to be harvested at 7 mm subcutaneous fat thickness based on visual and ultrasound measurements. The BW was recorded prior to leaving the feedlot to calculate ADG. Average daily gain was calculated by subtracting the weight taken when the cattle left the feedlot minus the weight taken during processing or when the cattle entered the feedlot, divided by the number of days on feed. The animals were harvested at Sam Kane Packing Plant in Corpus Christi, TX when a group reached approximately 7 mm of backfat. At approximately 36 h post-harvest, hot carcass weight, 12th rib fat thickness, estimated percentage of kidney, pelvic and heart fat, ribeye area, and marbling score were determined as defined by USDA (1989). The USDA Yield and Quality grades were calculated according to USDA (1989) using these factors. A 2.5 cm steak was removed from the 13th rib for Warner-Bratzler shear force determination at 14 d post-harvest. For WBS, steaks were removed from the -80°C freezer and allowed to thaw at 2°C for 48 h. The steaks were cooked on Faberware Open-Hearth broilers (Faberware

Co., Bronx, NY) to an internal temperature of 70°C (monitored by copper constantan thermocouples and a recording potentiometer). When the desired internal temperature was reached, steaks were removed and cooled at room temperature (20°C) before testing. Six, 1.27 cm diameter cores were removed parallel to the longitudinal orientation of the muscle fibers. Each core was sheared using an Instron Universal Testing Instrument (Instron, Canton, MA) equipped with Warner-Bratzler shearing device. The average force (kg) required to segment the six cores was reported as the WBS for each steak.

Experiment 2

Bonsmara X Angus spring born steers (n=207) from near Dalhart, TX were weaned and grazed on winter ryegrass pasture. The animals were evaluated for EV and BW were measured after 27 d on feed (DOF), to provide measurements near entry in the feedlot. The cattle were evaluated after either 93 or 119 d of high concentrate feeding in the feedlot for exit velocity and live animal weight. Exit velocity and ADG was determined as described in Experiment 1. Steers were harvested at 2 times so that steers had approximately 7 mm of visually assessed subcutaneous fat. Steers were harvested (97 and 126 d, respectively) at Excel Corporation in Plainview, TX. At approximately 48 h post-harvest USDA Yield and Quality grades (1=Prime; 2=Choice; 3=Select; 9=Standard) were obtained and carcass characteristics of hot carcass weight, 12th rib fat thickness and ribeye area were determined as defined by USDA (1989). A 2.5 cm steak was removed from the 12th rib, aged at 2°C for 21 d and placed in a -80°C freezer until used for WBS evaluation as described in Experiment 1.

Statistical Analysis

To determine the effect of pre-finishing background treatments on carcass characteristics, data were analyzed by Analysis of Variance using the general linear model (GLM) procedure of SAS (Version 6.12, Cary, NC, 1998) with a predetermined significance level of $P \leq 0.05$. Pre-finishing background treatment was defined as a main effect. For carcass characteristics that were affected by pre-finishing background treatment, least squares means were calculated and differences between means were determined using the standard error pdiff function (Table 1). Descriptive statistics were calculated for Experiment 1 and 2 (Table 2). Simple correlation coefficients were determined between exit velocity measures from Experiments 1 and 2 and live animal and carcass characteristics (Table 3). Partial regression coefficients were calculated using the manova function of GLM where the effect of pre-finishing background treatment was defined as a fixed effect (Table 4). Exit velocity data were converted to discrete data that was defined as exit velocity groups of slow, medium and fast based on <0.5 SD, ± 0.5 SD, and >0.5 SD, respectively, from the mean. Weaning EV categories from Experiment 1 and in feedlot EV categories from Experiment 2 were analyzed by Analysis of Variance as previously described using pre-finishing background treatment as a block and the EV category as a main effect. Least squares means were calculated and if differences in EV category were reported ($P < 0.05$) then least squares means were separated using the standard error pdiff function.

Results & Discussion

Experiment 1

Pre-finishing background treatments affected feedlot average daily gain (ADG) and hot carcass weight (HCW) measurements (Table 1). Steers that were fed at a low stocking rate had lower ADG compared to steers fed on other pre-finishing treatments. Pre-finishing treatments have been shown to impact live animal growth and carcass characteristics of steers fed high concentrate diets (Miller et al., 1987). As steers in our study were fed from 23 to 153 days to a projected fat constant endpoint of 7 mm during finishing, nutritional and management practices for steers prior to entering the finishing phase would expectantly impact ADG and HCW. While understanding the effect of pre-finishing treatments on live animal growth and carcass characteristics is important, cattle will vary in nutritional management and pre-finishing treatments prior to entering the finishing phase of beef production. Therefore, variation in pre-finishing management of steers provided necessary variation to understand the relationship between EV on live animal growth, carcass characteristics, and beef tenderness. Although ADG and hot carcass weight were influenced by pre-finishing treatments, EV at weaning was correlated with ADG, Yield Grade (YG), and WBS ($P < 0.05$), but EV for cattle entering the feedlot was not correlated (Table 3). When pre-finishing treatment was used as a fixed effect, ADG and WBS were still correlated with weaning EV, and correlations were not found with EV for animals entering the feedlot (Table 4). To help discover the differences between the EV groups, pre-finishing treatment was used as a block and weaning EV category was defined as a main effect (Table 5). Steers with fast weaning EV had higher YG than steers from the other weaning EV groups.

Experiment 2

Experiment 2 was a second set of cattle used to further examine the relationship between EV, growth, carcass characteristics and tenderness. This group of cattle was not evaluated at weaning, but cattle were evaluated entering and exiting the feedlot. Experiment 2 did not follow the same trends as Experiment 1. When cattle entered the feedlot, backfat and QG were correlated with EV ($P < 0.05$) (Table 3). Although EV and backfat were found to be significant entering the feedlot, the fast EV group had more backfat when compared to the slow EV group (Table 5).

Conclusions

Exit velocity at weaning had the highest relationship, even though the relationships were not always significant, between live animal growth, carcass characteristics, and tenderness. It appears that as cattle are either fed in production systems that incorporate more exposure to humans or as they get older, exit velocity is not as good of a predictor of average daily gain, carcass characteristics or tenderness.

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Table 1. Effects of grazing treatment on average daily gain in the feedlot (p=0.051) and hot carcass weight (p=0.024) due to stocking treatment.

Stocking treatment ^a			Average daily gain, kg/d		Hot Carcass weight, kg	
Method ^b	Strategy ^c	Level ^d	LSMean	SE	LSMean	SE
Continuous	Fixed	Low	1.13 ^c	0.101	333.1 ^c	10.55
Continuous	Variable	Low	1.33 ^{efg}	0.096	333.7 ^{ef}	14.92
Rotational	Fixed	Low	1.26 ^{efg}	0.107	321.6 ^{ef}	9.77
Rotational	Variable	Low	1.26 ^{ef}	0.096	310.3 ^e	11.56
Continuous	Fixed	Medium	1.35 ^{efg}	0.083	331.8 ^{ef}	9.77
Continuous	Variable	Medium	1.46 ^{fg}	0.107	337.1 ^{ef}	11.56
Rotational	Fixed	Medium	1.47 ^{fg}	0.114	348.3 ^f	12.92
Rotational	Variable	Medium	1.55 ^g	0.107	332.5 ^{ef}	11.56
Continuous	Fixed	Medium	1.44 ^{fg}	0.034	310.4 ^e	3.81

^aThe first 8 treatments were stocking treatments applied at the Texas Agricultural Research and Extension Center in Overton, TX and last treatment was the stocking treatment at the Texas Agricultural Research and Extension Center in Uvalde, TX.

^bContinuous: steers were continual access to grass on the same pasture during the stocking treatment; Rotational: 8-paddock rotation with an approximate 2-day residence and 14-day rest.

^cFixed: the stocking rate was not changed the entire grazing period; Variable: stocking rate at initiation (both 0.9 and 1.7 hd/ac) were fixed until March 4, 2003, and then both low and medium stocking rates, respectively, were increased to approximately 3 hd/ac for the duration of the grazing period.

^dLow: approximately 0.9 steer/ac at initiation of grazing; Medium: approximately 1.7 steers/ac at initiation of grazing.

^{efg}Least squares means with different superscripts within a column differ, P<0.05.

Table 2. Exit velocity characteristics, average daily gain, carcass characteristics and Warner-Bratzler shear force descriptive statistics.

Variable	Experiment 1			Experiment 2		
	N	Mean	SD	N	Mean	SD
Weaning exit velocity, m/sec	138	3.53	0.79	-	-	-
In feedlot exit velocity, m/sec	156	2.91	0.94	205	2.85	0.90
End feedlot exit velocity, m/sec	-	-	-	207	2.38	0.92
Average daily gain, kg/d	152	1.39	0.310	207	1.42	0.311
Hot carcass weight, kg	88	319.8	27.43	207	328.6	28.66
Backfat, mm	88	6.99	0.247	207	9.83	0.347
Quality grade ^a	88	692.56	31.14	207	2.91	1.53
Yield grade	88	2.16	0.40	196	2.07	0.37
Warner-Bratzler shear force, N	146	26.46	6.251	204	27.84	5.517

^a600 = Select.

Table 3. Simple correlations coefficients for average daily gain, carcass characteristics and Warner-Bratzler shear force and exit velocity measurements.

Trait	Experiment 1		Experiment 2	
	Weaning	In Feedlot	In Feedlot	Out Feedlot
	Exit Velocity	Exit Velocity	Exit Velocity	Exit Velocity
Average daily gain, kg/d	-0.25 ^a	0.05	0.09	-0.07
Hot carcass weight, kg	-0.17	-0.08	-0.05	-0.13
Backfat, mm	0.02	-0.03	0.14 ^a	-0.05
Quality grade ^c	0.03	-0.02	0.18 ^a	0.03
Yield grade	0.29 ^a	-0.03	-0.08	-0.15 ^a
Warner-Bratzler shear force, N	0.23 ^a	0.02	0.10	0.02

^aP<0.05

Table 4. Partial correlations coefficients for average daily gain, carcass characteristics and Warner-Bratzler shear force and exit velocity measurements adjusted for the effect of prefinishing background treatment.

Trait	Experiment 1	
	Weaning	In Feedlot
	Exit Velocity	Exit Velocity
Average daily gain, kg/d	-0.28 ^a	0.06
Hot carcass weight, kg	-0.22	-0.22
Backfat, mm	-0.07	-0.03
Quality grade ^c	-0.19	0.09
Yield grade	0.20	-0.05
Warner-Bratzler shear force, N	0.29 ^a	0.13

^aP<0.05

Table 5. Least squares means, standard errors and p-values for average daily gain, carcass characteristics and Warner-Bratzler shear force as effected by exit velocity groups at weaning and entering the feedlot for Experiment 1.

Variable	Experiment 1 Weaning exit velocity group				Experiment 2 In feedlot exit velocity group					
	Slow	Medium	Fast	P-value	RMSE ^d	Slow	Medium	Fast	P-value	RMSE ^d
Average daily gain, kg/d	1.47	1.36	1.30	0.058	0.297	1.40	1.40	1.47	0.36	0.309
Hot carcass weight, kg	341.6	326.8	325.9	0.11	25.74	329.8	329.6	327.6	0.89	28.56
Backfat, mm	8.8 ^b	6.5 ^a	8.1 ^b	0.002	2.14	9.4 ^a	9.4 ^a	10.9 ^b	0.019	3.44
Quality grade ^c	692.7	681.2	690.5	0.39	30.15	2.61	3.03	3.13	0.109	1.52
Yield grade	2.20 ^{ab}	2.06 ^a	2.38 ^b	0.015	0.354	2.08	2.04	2.07	0.80	0.369
Warner-Bratzler shear force, N	25.04	27.52	28.07	0.108	6.113	27.15	28.00	28.65	0.30	5.519

^{ab}Least squares means with different superscripts within a row and velocity group differ, P<0.05.

^cUSDA Beef Quality Grade for Experiment 1: 600=Select; USDA Beef Quality Grade for Experiment 2: 1=Prime; 2=Choice; 3=Select; 9=Standard

^dRMSE: Root Mean Square Error from Analysis of Variance table.

**INFLUENCE OF ANIMAL TEMPERAMENT ON THE TENDERNESS OF BEEF
M. LONGISSIMUS LUMBORUM STEAKS**

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Key Words: Beef; Tenderness; Temperament

Introduction

Research on human subjects suggests that individuals neurologically respond to stressful stimuli differently (Rosenkranz et al., 2003). Neurological response seemed to be related to variation in the activation of the adrenal axis, which subsequently adversely affected immune response. Clearly, animals, like humans, do not react equally to stressors.

Previous research has indicated strong relationships between animal temperament and stress responsiveness (Curley, Neuendorff, Lewis, Cleere, Welsh, & Randel, 2004). Cattle with more excitable temperaments also had more extensive responses to a CRH challenge. Elevated stress responsiveness has been linked to decreased animal growth and efficiency, as well as reduced immune function.

Voisinet, Grandin, O'Connor, Tatum, and Deesing (1997) reported that a greater percentage of excitable cattle displayed borderline dark cutting lean compared to less temperamental animals. Those investigators also found that steaks from the carcasses of excitable cattle had higher Warner-Bratzler shear force values than those from the carcasses of calmer animals. Additionally, a low-to-moderate relationship has been observed between these measures of temperament and Warner-Bratzler shear force (Vann, Paschal, & Randel, 2004). We hypothesize that animals exhibiting excitable temperament characteristics during common management practices may produce carcasses that possess less merit and consequently, meat that is less tender than animals that exhibit a lesser response.

Objectives

(1) To determine if a relationship exists between the behavioral response of cattle to common management practices and the tenderness of the *M. Longissimus lumborum*, and
(2) To characterize the biological mechanisms by which stress response impacts muscle tenderness of feedlot cattle.

Methodology

Two trials were conducted under typical industry conditions to test the stated hypotheses. Trial 1 consisted of Bonsmara-sired ($n = 32$) and Angus-sired ($n = 49$) yearling steers that had been grown on grass prior to entering the feed yard. Forty-eight Angus-sired steers were backgrounded for 40 d post-weaning and then fed for Trial 2. With the exception of data and sample collection as described, these steers were managed identically to other cattle in the feed yard.

In both trials, temperament-indicating traits were measured on the farm before transport to the feed yard, upon arrival at the feed yard, and after approximately 70 d on feed. At each time, exit velocity was measured as the rate at which cattle left the working chute (Burrow, Seifert, & Corbet, 1988). As the animals moved through the facilities during the pre-shipment data collection, chute score (scale of 1 to 5; 1 = calm, no movement; 5 = rearing, twisting, and struggling violently; Grandin, 1993) and pen score (scale of 1 to 5; 1 = not excited by human presence; 5 = excited by human presence, runs over anything in its path; Hammond et al., 1996) were assigned subjectively to each animal. Each time the animals were handled, serum samples were collected via tail venipuncture and subsequently assayed for cortisol concentrations using radio immuno assay (RIA) procedures as an indicator of hypothalamic-pituitary-adrenal axis status (Carroll, Willard, Bruner, McArthur, & Welsh Jr., 1996).

The cattle were fed to a target endpoint determined by the feed yard manager and slaughtered using standard procedures. Days on feed were 120 (Trial 1) and 200 (Trial 2). Carcasses were subjected to high-voltage electrical stimulation immediately before evisceration. Carcasses were chilled for 48 h in a 0°C cooler with intermittent spray chill for 8 h. Muscle pH and temperature decline was monitored during chilling in the caudal portion of the *M. longissimus lumborum*. Due to equipment failure, pH readings were not taken after 12 h on the carcasses in Trial 2. Forty-eight h postmortem, carcasses were ribbed at the 12th-13th rib interface and allowed to bloom for at least 15 min before carcass quality and yield grade characteristics were determined. At this time, CIE L^* , a^* , and b^* values were measured. Following grading, the carcasses were fabricated and the strip loin was removed from the left side of each carcass.

At 72 h postmortem, the strip loins were sliced into 2.54-cm steaks, which were assigned to laboratory analyses. Calpastatin activity was determined at 72 h postmortem by the protocol of Koochmaraie, Shackelford, Wheeler, Lonergan, and Doumit (1995). Sarcomere length was measured by the method of Cross, West, and Dutson (1981). Additional steaks were randomly assigned to be aged for 3, 7, 14, or 21 d and used for Warner-Bratzler shear force determination using the method of McKenna, King, and Savell (2004).

Cattle were segmented into temperament groups based on a temperament index value calculated as the sum of the pre-shipment exit velocity and pen score divided by 2. Cattle with temperament index values more than 1 standard deviation higher or lower than the mean of their contemporary group were included in the Excitable and Calm groups, respectively. The Intermediate group consisted of animals with temperament index values within 1 standard deviation of the mean. Temperament groups were compared using analysis of variance with the PROC MIXED procedure of SAS (SAS Institute, Cary, NC).

Results & Discussion

Table 1. Least-squares means for temperament indicating traits of yearling-fed steers segmented into groups according to temperament traits measured before shipment to the feeding facility (Trial 1)

Trait	Temperament classification			RMSE	<i>P</i> > <i>F</i>
	Calm	Intermediate	Excitable		
n	15	50	16		
Pre-shipment temperament index ^a	1.75c	2.98b	4.20a	0.31	<0.001
Pre-shipment exit velocity (m/s)	1.45c	2.67b	3.47a	0.37	<0.001
Pre-shipment pen score	1.07c	2.56b	3.47a	0.65	<0.001
Pre-shipment chute score	0.68c	1.12b	1.50a	0.50	0.01
Pre-shipment cortisol (ng/mL)	11.92b	12.02b	16.13a	26.86	0.02
Arrival exit velocity	1.30c	2.05b	2.89a	0.74	<0.001
Arrival cortisol (ng/mL)	10.48	12.46	13.20	52.64	0.55
Mid-point exit velocity	1.23c	1.86b	2.75a	0.64	<0.001
Mid-point cortisol (ng/mL)	8.82	12.10	13.50	37.34	0.09
Final cortisol (ng/mL)	9.85	10.90	11.69	32.96	0.67

^aTemperament index = (exit velocity + pen score)/2.

Least-squares means within a row with different letters (a-c) differ (*P* < 0.05).

These cattle were segmented into temperament categories based on exit velocity and pen scores taken before the cattle were transported to the feedlot. Because of this classification, all temperament-indicating variables differed (*P* < 0.05) between the classifications (Table 1). These differences were maintained in subsequent exit velocity measurements, although the magnitude of the differences diminished somewhat as the animals grew larger. This is likely due to a combination of the larger cattle having more difficulty moving through the facility and adaptation with increasing experience to being handled. Serum cortisol was higher (*P* < 0.05) in the Excitable cattle than the other groups at the pre-shipment sampling. Though no differences were observed in later samplings, the Excitable cattle had numerically higher means for cortisol concentration at each sampling.

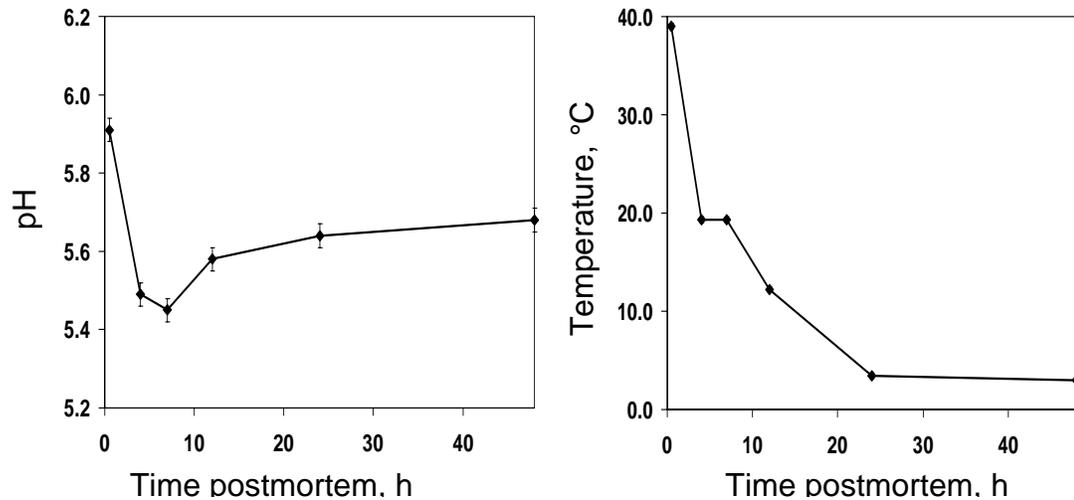


Figure 1. Muscle pH and temperature decline of the *M. longissimus lumborum* of beef carcasses during the chilling process (Trial 1)

The muscle pH and temperature decline for these carcasses during chilling are presented in Figure 1. The pH decline was extremely rapid and had reached a minimum value by 7 h postmortem, which was lower than the values typically seen in muscle. During the next 40 h, the pH increased to a point normally observed in postmortem muscle. Neither pH nor muscle temperature differed between temperament groups during chilling (Table 2).

None of the carcass yield or quality grade characteristics differed between temperament groups. The temperament group \times aging interaction was not significant for Warner-Bratzler shear force in this trial. However, aging resulted in considerable improvements in tenderness (data not shown). Steaks from cattle in the Excitable group produced Warner-Bratzler shear force values that were higher ($P < 0.05$) than the Calm or Intermediate groups, which did not differ. Despite this tenderness difference, sarcomere length, calpastatin activity, and color values were not affected.

Table 2. Least-squares means for carcass traits of yearling-fed steers segmented into groups according to temperament traits measured before shipment to the feeding facility (Trial 1)

Trait	Temperament classification			RMSE	$P > F$
	Calm	Intermediate	Excitable		
pH	5.62	5.66	5.60	0.04	0.10
Temperature, °C	17.4	17.53	17.68	0.19	0.43
L^*	42.49	43.23	43.74	6.13	0.38
a^*	31.81	31.75	31.74	1.24	0.98
b^*	23.30	23.48	23.24	1.99	0.81
Sarcomere length, μm	1.86	1.84	1.82	0.001	0.37
Calpastatin activity	1.01	0.99	0.94	0.25	0.92
Warner-Bratzler shear force, kg	2.88b	2.97b	3.34a	0.53	0.01

Least-squares means within a row with different letters (a-c) differ ($P < 0.05$)

The cattle in Trial 2 were segmented into temperament categories in the same manner as Trial 1, and differences in temperament measurements taken before shipment to the feedlot were similar to those in Trial 1 (Table 3). Similarly, the differences between the temperament groups diminished as the animals aged, though this trend was more pronounced than in Trial 1. Serum cortisol concentrations did not differ between groups; however, the means for excitable cattle were consistently higher numerically than those for the intermediate and calm groups.

Table 3. Least-squares means for temperament indicating traits of calf-fed steers segmented into groups according to temperament traits measured before shipment to the feeding facility (Trial 2)

Trait	Temperament classification			RMSE	P > F
	Calm	Intermediate	Excitable		
n	7	33	9		
Pre-shipment temperament index ^a	1.33c	2.48b	4.02a	0.22	<0.001
Pre-shipment exit velocity (m/s)	1.23c	2.49b	4.15a	0.42	<0.001
Pre-shipment pen score	1.43c	2.48b	3.89a	0.67	<0.001
Pre-shipment chute score	1.57	1.54	2.22	0.64	0.08
Pre-shipment cortisol (ng/mL)	7.24	9.77	10.57	20.18	0.31
Arrival exit velocity	2.10b	2.54b	3.13a	0.62	0.04
Arrival cortisol (ng/mL)	9.75	10.95	13.94	39.25	0.35
Mid-point exit velocity	2.31	2.44	2.87	0.41	0.15
Mid-point cortisol (ng/mL)	13.31	15.17	19.25	37.32	0.13
Final cortisol (ng/mL)	15.68	16.10	18.31	45.47	0.65

^aTemperament index = (exit velocity + pen score)/2.

Least-squares means within a row with different letters (a-c) differ ($P < 0.05$).

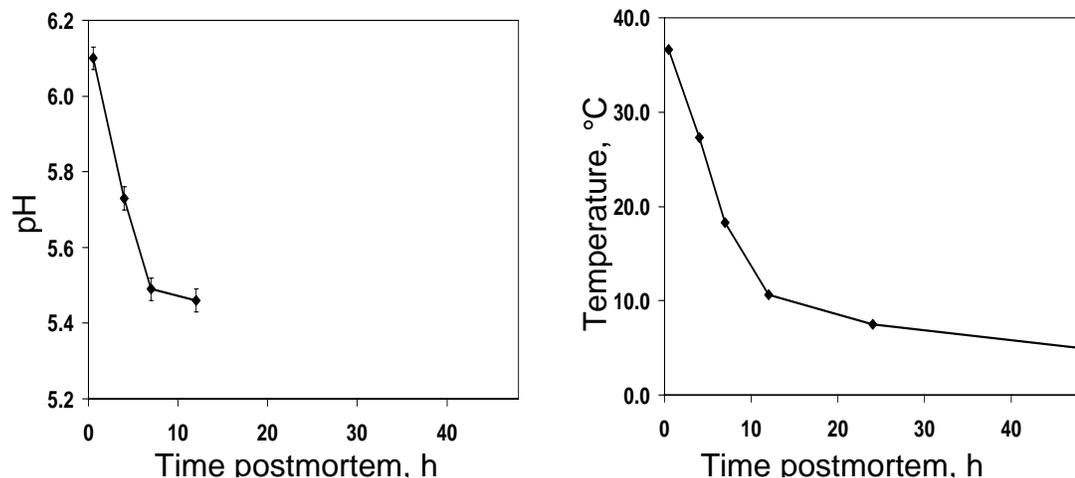


Figure 2. Muscle pH and temperature decline of the *M. longissimus lumborum* of beef carcasses during the chilling process (Trial 2).

Figure 2 presents the pH and temperature decline curves for the *M. longissimus lumborum* in these carcasses. These curves indicate that these carcasses mirrored the rapid pH and temperature declines observed in Trial 1. However, it is unknown if the buffering phenomenon observed in Trial 2 occurred in this trial because these readings were not taken due to equipment failure. Once again, neither pH nor temperature during chilling was affected by temperament.

Table 4. Least-squares means for carcass traits of calf-fed steers segmented into groups according to temperament traits measured before shipment to the feeding facility (Trial 2)

Trait	Temperament classification			RMSE	<i>P</i> > F
	Calm	Intermediate	Excitable		
pH	5.73	5.70	5.65	0.03	0.25
Temperature, °C	16.08	16.00	16.07	0.18	0.74
<i>L</i> *	48.04	48.75	47.34	5.55	0.27
<i>a</i> *	31.25	30.38	31.07	1.65	0.15
<i>b</i> *	22.87	22.18	22.68	2.30	0.44
Sarcomere length, μm	1.79	1.79	1.77	0.003	0.65
Calpastatin activity	0.86	1.15	0.97	0.32	0.41
Warner-Bratzler shear force, kg	2.91	3.04	2.86	0.31	0.62

Least-squares means within a row with different letters (a-c) differ (*P* < 0.05).

Carcass characteristics were not affected by temperament grouping in these steers. Once again, aging improved the tenderness of steaks in this trial (data not shown). In contrast to Trial 1, temperament group did not affect Warner-Bratzler shear force. Perhaps the effects of temperament group on tenderness were related to time on feed, as these cattle were fed much longer than the cattle in Trial 1 (200 versus 120 d). It should be noted that the Warner-Bratzler shear force values for all temperament groups in both trials are very tender. This may be due, in part, to electrical stimulation, which has been consistently reported to improve tenderness. As observed in Trial 1, temperament had no effect on color values, sarcomere length, and calpastatin activity.

Conclusions

Measures of animal temperament appeared to rank animals consistently, and differences observed between temperament groups before shipment to the feedlot remained throughout the feeding period. Temperament affected tenderness in the yearling-fed cattle, but not in the calf-fed cattle. Based on these data, it is not clear what mechanism mediates these tenderness differences, but it appears that time on feed may play a role.

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HEALTH STATUS EFFECTS ON CARCASS QUALITY AND BEEF TENDERNESS OF FEEDLOT STEERS

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Key Words: Beef; Carcass quality; Health; Tenderness

Introduction

Profitability of feedlot cattle is dependent upon two important factors, production efficiency and carcass quality. Any illness that occurs during the feeding period will influence the animal's behavior resulting in a reduced performance in growth and will further inhibit the overall value of the cattle. In addition to the maintenance costs associated with treating affected animals and reduced productive efficiency, additional losses may be incurred by producers due to the reduced value obtained by the carcass. Evidence from the Ranch to Rail program (Gardner, Doelzal, Bryant, Owens and Smith, 1999) sponsored by Texas Cooperative Extension Service demonstrates this conclusion. This program consisted of 775 head from 74 ranches and analyzed various live animal and carcass characteristics. In this report, it suggests that cattle that have encountered sickness while on feed tended to obtain a greater loss in value and produced fewer carcasses grading U.S. Choice and alternatively produced a greater proportion of cattle grading U.S. Standard.

Gardner et al, (1999) reported that steers that had been treated for Undifferentiated Bovine Respiratory Disease (UBRD) had lower carcass weights and higher percentages of carcasses grading U.S. Standard. Furthermore, these authors reported that animals possessing lung lesions at slaughter had slower daily gains, lighter carcass weights, lower marbling scores, and higher Warner-Bratzler shear force values than those that did not have lung lesions at slaughter. With this information, this investigation was conducted to further investigate the effects of health status during the feeding period.

Objectives

To examine the impact of illness during the feeding period on the carcass quality and the tenderness of the *M. Longissimus lumborum* from feedlot steers.

Methodology

Angus-sired steers (n = 48) were weaned and backgrounded for 40d before being placed in the feedlot for this trial. The steers were from a common herd and possessed

similar genetics and were handled identically to all other cattle at the facility. The cattle were marketed at the end-point determined by the feedlot manager (200 d on feed). During this time, records were maintained for treatments received by the animals. Each animal requiring treatment was subsequently classified in the Treated group (n=10). All remaining animals were classified as Non-treated (n=38). Due to a limited population size, no distinction was made for multiple treatments.

Cattle were processed at a commercial abattoir and carcasses were subjected to high-voltage electrical stimulation. Carcasses were chilled for 48 h in a 0°C cooler with intermittent spray chill for 8 h. Forty-eight h postmortem, carcasses were ribbed at the 12th-13th rib interface and allowed to bloom for at least 15 min before carcass quality and yield grade characteristics (USDA, 1996) were determined. At this time, CIE L^* , a^* , and b^* values were measured. Following grading, carcasses were fabricated and the strip loin was removed from the left side of each carcass.

At 72 h postmortem, the strip loins were sliced into 2.54-cm steaks, which were assigned to laboratory analyses. Calpastatin activity was determined at 72 h postmortem by the protocol of Koochmarai, Shackelford, Wheeler, Lonergan, and Doumit (1995). Sarcomere length was measured by the method of Cross, West, and Dutson (1981). Additional steaks were assigned to be aged for 14 d and used for Warner-Bratzler shear force determination using the method of McKenna, King, and Savell (2004). Treated and Non-Treated cattle were compared using analysis of variance with the PROC MIXED procedure of SAS (SAS Institute, Cary, NC).

Results & Discussion

The calf-fed steers used in this trial had beginning and final body weights of 291 ± 34 and 580 ± 61 kg, respectively. Initial weight did not differ between the groups. However, the final weight tended ($P = 0.06$) to be greater in the cattle that remained healthy throughout the feeding period compared to those that required treatment (589 versus 548 kg, respectively). Average daily gain was not affected by health status.

The least-squares means for the carcass characteristics of cattle treated for illness during the feeding period and those not requiring treatment are presented in Table 1.

Table 1. Means for carcass characteristics for steers treated and non-treated for illness during the feeding period

Trait	Treated	Non-treated	$P > F$
Dressing percentage	58.7 ± 0.6	60.1 ± 0.3	0.04
Hot carcass weight, kg	321.9 ± 12.6	354.9 ± 6.1	0.02
Adjusted fat thickness, cm	1.5 ± 0.1	1.8 ± 0.1	0.01
Longissimus muscle area, cm ²	78.1 ± 3.0	83.7 ± 1.4	0.09
Estimated kidney, pelvic, and heart fat, %	2.0 ± 0.1	2.2 ± 0.1	0.11
Yield Grade	3.2 ± 0.2	3.5 ± 0.1	0.11
Marbling score ^a	390.0 ± 29.3	438.9 ± 14.1	0.13
Quality Grade ^b	676.7 ± 14.1	702.9 ± 6.8	0.10

^a300 = Slight⁰⁰; 400 = Small⁰⁰; 500 = Modest⁰⁰

^b600 = Select⁰⁰; 700 = Choice⁰⁰

Those cattle that did not get sick during the feeding period and were not treated for illness had greater ($P = 0.05$) dressing percentages and heavier carcass weights than those that were treated. Additionally, the carcasses of the cattle that got sick had less subcutaneous fat as indicated by adjusted fat thickness. Longissimus muscle area tended ($P = 0.10$) to be larger in cattle that did not require treatment during feeding. The finding that healthy cattle had less fat and tended to be more muscular indicates that the cattle requiring treatment were less advanced in their development, conceivably because of gains lost due to illness. Marbling score did not differ with regard to health status. However, the quality grade of the carcasses from cattle treated for illness tended ($P = 0.10$) to be lower than the grades of healthy cattle. The mean quality grade of cattle that had been sick was the equivalent of U.S. Select while the mean quality grade of carcasses from healthy cattle was equivalent to U.S. Choice.

The least squares means for color values, sarcomere length, 72-h calpastatin activity and Warner-Bratzler shear force values of the treated and non-treated steers are presented in Table 2.

Table 2. Means for tenderness and histochemical traits for steers treated and non-treated for illness during the feeding period

Trait	Treated	Non-treated	$P > F$
L^*	48.2 ± 0.8	48.4 ± 0.4	0.77
a^*	30.8 ± 0.4	30.6 ± 0.2	0.71
b^*	22.7 ± 0.5	22.3 ± 0.2	0.48
Sarcomere length, μm	1.78 ± 0.01	1.79 ± 0.02	0.59
72-h Calpastatin activity	1.07 ± 0.19	1.08 ± 0.09	0.96
Warner Bratzler Shear-14 Day, kg	2.84 ± 0.19	2.82 ± 0.09	0.91

Muscle L^* , a^* , and b^* values were not different between health status groups. Additionally, sarcomere length, 72-h calpastatin activity, and Warner-Bratzler shear force were unaffected by previous illness in these cattle. This may suggest that the differences in growth that affected carcass characteristics did not affect the tenderness of the meat from these animals.

Conclusions

It is evident that there were some differences in quality characteristics between the Treated and Non-treated groups. Cattle identified as sick during the feeding period ultimately produced carcasses that were lighter when compared to those free of illness. Carcasses of healthy cattle were fatter and tended to have larger longissimus muscle areas. Carcasses from healthy steers generally had higher quality grades than those from steers that had been sick. Furthermore, the tenderness attributes did not differ between the treated and non-treated steers. These results further demonstrate that illness during the feeding period can have an effect on carcass characteristics, more specifically with the reduction of carcass weight and overall quality grade in feedlot cattle.

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ORGANIC LAMB FROM AUTOCHTHONOUS BREEDS OF THE APULIAN REGION

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Key Words: organic lamb, autochthonous sheep breed, biodiversity

Introduction

Of the wide range of typical Italian sheep breeds, some are particularly right for the territory features. Some rustic breeds have been selected from the southern regions of the peninsula being characterized by very hard feeding and environmental conditions. This mainly relates to the Apulian area, bordering on the Basilicata region with steppe landscape which is recently under special protection by declaring it National Park of the Alta Murgia. This area, boasting remote shepherd vocation, is the place where sheep breeds have been selected due to their ability to survive the aridity of poor pastures, thus creating since long ago, a whole system based on wool and meat production, which is slowly disappearing following to the specialized breeds spreading.

Among these selected breeds, the Gentile di Puglia one originated, in the XV century, by Merinos breed substitution on Apulian autochthonous breeds, above all on the Apennines mountains population Garfagna (Dellaquila et al., 1995; Sarti, 1996). Today the breed is raised openair, permanent and transhumance, in medium-sized and large flocks in the Apulia, Basilicata and Calabria regions for meat production. The breed is known for standing hard local conditions (hills and mountains). It is characterized by a white elegant wool fleece with a rectilinear profile head. Rams are strong loop horned and the females are polled. They are mainly used for wool and meat production, above all the typical lean lamb, slaughtered at 50-70 days of age (live weight 14-20 kg). Wool production which once equaled the best foreign merinos has by now no market.

Another typical native local breed from the area is the “Altamura”, found in Bari and Foggia provinces. The animals are white and occasionally have dark spots on the face. Adult males weigh on average 52 and females 37 kg with an average wither height of 70 and 65 cm respectively. These sheep have coarse/carpet type wool and all animals are polled. The breed is adapted to live on arid soils. It is raised openair (often with little transhumance) for milk that is employed for the production of a traditional fresh cheese, the “Canestrato”, branded IGP (Protected Geographical Indication) according to E.C. rules.

Both breeds are at risk of extinction and have been inserted in FAO databank protection program DAD-IS (Domestic Animal Diversity - Information System). Numerically, the “Gentile di Puglia” and “Altamura” breeds in 2004 were respectively 4,587 and 409 heads (Asso.Na.Pa., 2005). In the study area the profitability of sheep rearing is mainly connected with milk production, mostly coming from other specialized breeds. A correlated production of remarkable importance is represented by the lamb

meat. It represents a legacy of popular tradition, primarily consumed on occasion of the Catholic celebrations. The possibility of adding milk income, coming from the sale of improved lambs (crossbreeding among breeds with different attitude), can constitute a valid opportunity and, consequently, allow the recovery of autochthonous genotypes such as Altamura, though their low productivity. The preservation of the genetic purity of crossbreeding breeds is ensured by the commercial target of the hybrid lambs, which are never used for flock reconstitution.

Objectives

The aim is to recover and to preserve the biodiversity of autochthonous sheep legacy in the steppe highlands (Murgia) of Apulia, through the productive exploitation of the lean lamb, raised according to the organic system. To improve lamb productive performances we used the effect that meat-type rams (Gentile di Puglia) produce when crossbred with a milk-type sheep (Altamura). At the same time this allows the recovery important genotypes, produced over centuries of selection. In a previous paper (Schiavone et al., 2005) some quanti-quality traits of crossbred lamb have been considered. The present paper shows the real opportunities offered by this type of crossbreeding through the comparison between the genotypes and the evaluation of other parameters.

Methodology

Eighteen male single-birth lambs, six of Altamura breed (A), six half-breed (Gentile di Puglia x Altamura - GA) and six Gentile di Puglia (G), have been naturally suckled from birth to 68 days; their mothers were reared on pasture. Lambs were weighed at birth, before and after slaughtering. The daily mean increase, the slaughtering yield and the cool loss were calculated. pH values were surveyed, using a glass electrode, on shoulder, *Longissimus dorsi* (Ld) muscle and thigh at slaughtering (pH₁), after refrigeration to +4°C for 24 (pH₂₄), 48 (pH₄₈) and 72 hours (pH₇₂). Carcasses were divided in two parts and that right part subdivided in commercial cuts, according to ASPA (1996) indications. The weights of the single cuts were recorded. On a sample of Ld muscle the color was estimated by the Hunter Lab system using a colorimeter (illuminant D 65), which measured the values of Lightness (L), Redness (a) and Yellowness (b) by making 5 readings for each meat sample, approximately 2.5 cm thick. Tenderness was measured using a Warner Bratzler shear device applied to an Instron 5544 and expressed as the cutting force (kg/cm²) required to shear perpendicularly to the direction of the fibres half an inch diameter cylinders of raw and cooked meat, taking three measurements for each subject. Data were analyzed for variance using the GLM procedure of SAS (1999/2000).

Results & Discussion

Weights at birth (Table 1) were greater for GA lambs (4,450 g) than A subjects (3,600 g; P <0.05) and G (4,350 g). This difference in weight was also found at slaughtering, where half-breed showed the highest weights (GA: 21,176 g; G: 20,000 g;

A: 19,379 g). The daily weight increase was higher for GA lambs (242.98 g/d) than A (232.05 g/d) and G (230.00 g/d) subjects. Santos-Silva et al. (2002) recorded different data in the half-breed Merino Branco and Ile de France, while a positive heterosis effect, according to our results, was observed by Teixeira et al. (1996) in the crossbreeding of Brancagnano sheep (a mountain-type breed of northern Portugal) and Suffolk and Merino Precoce rams. The carcass weights have been influenced, though with no statistical significance, by the genetic composition of the animals, according to Boujenane et al. (2002) in the crossbreeding of three genotypes. Major greater weights were measured for F1 animals, with 13,283.33 g, against 12,533.33 g for G and 12,116.67 g in A groups. Yields at slaughtering were almost identical among the groups. The differences emerged for cool loss were not significant, probably because the animals were slaughtered at a low weight and therefore related to a narrow range. pH (Table 2) and color (Table 3) values were always found very close among groups, attesting a substantial preservation of the qualitative peculiarities, typical of crossing genotypes. Particularly the lightness (L) of lamb meat, very appreciated by consumers, was higher ($P<0.05$) in the half-breed meat than that from the two genotypes. The incidence of commercial cuts (Table 4) on the half-carcass weight showed no statistical difference, although the fleshy cut weights of the half-breed animals were close to those of the G subjects, attesting a preservation of the conformational characteristic, as found by other authors (Santos-Silva et al., 2002). Finally the lean/fat/bone relationship (Table 5) of the leg fairly matches values from half-breed lambs and the meat-type breed ones (Gentile di Puglia).

Conclusions

More attention to autochthonous breed/populations, even if low productive, have to be paid in order to protect genetic variability. Crossbreeding, then, is to be considered a valid tool to find solutions and meet with breeders' needs. The preservation of the Altamura breed genetic pool, otherwise destined to a slow decline and to extinct, can well rely on "commercial" crossbreeding. The introduction of Gentile di Puglia genes, also risking extinction, positively weighs on lamb production, because of the heterosis effect, and offers an additional income for breeders. In conclusion, the characters of the crossing meat-type breed, i.e. the fast growth and the favorable muscular development, have been manifest in the first generation Gentile di Puglia x Altamura half-breed and have not produced substantial modifications of the quality characters of the meats.

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Tables and Figures

Table 1. Productive performance and physical traits

	A	GA	G	D.S.E.
Birth weight (g)	3,600.00 b	4,450.00 a	4,350.00	645.497
Live weight (g)	19,379.50	21,176.33	20,000.00	3,627.896
Daily Mean Increase (g/d)	232.05	242.98	230.12	48.102
Carcass weight (g)	12,116.67	13,283.33	12,533.33	1,981.330
Yield (%)	62.52	62.73	62.67	2.637
Cool loss (%)	2.05	2.45	2.32	1.060
Cooking loss (%)	36.09	38.59	37.44	2.3260
Raw meat tenderness (kg/cm ²)	1.745	1.567	1.611	0.4112
Cooked meat ten.ss (kg/cm ²)	2.132	2.167	2.045	0.7581

Table 2. pH

	A	GA	G	D.S.E.
pH ₁ Shoulder	6.45	6.36	6.44	0.283
Ld	6.41	6.36	6.33	0.181
Thigh	6.39	6.30	6.19	0.206
pH ₂₄ Shoulder	5.54	5.61	5.62	0.321
Ld	5.46	5.44	5.54	0.293
Thigh	5.40	5.62	5.65	0.390
pH ₄₈ Shoulder	5.73	5.68	5.61	0.221
Ld	5.48	5.57	5.37	0.206
Thigh	5.48	5.41	5.39	0.254
pH ₇₂ Shoulder	5.76 a	5.97 a	5.47 b	0.239
Ld	5.54	5.64	5.53	0.217
Thigh	5.55	5.60	5.55	0.231

Table 3. Ld muscle color

	A	GA	G	D.S.E.
Color ₂₄ L	38.69 b	43.36 a	41.43	2.888
a	13.28	16.73	11.03	9.542
b	13.57	14.49	14.78	1.212
Color ₄₈ L	36.69	39.92	41.77	2.032
a	12.17	11.23	11.52	0.816
b	15.52	16.28	14.83	1.214
Color ₇₂ L	39.71	39.40	42.52	2.776
a	12.35	11.65	11.42	1.311
b	16.42	16.52	16.75	1.734

Table 4. Commercial cuts (% on reconstituted half-carcass weight)

	A	GA	G
Neck	12.06	11.48	11.52
Ribs	21.99	21.82	21.28
Shoulder	19.15	19.59	20.48
Loin	3.51	4.04	3.53
Brest	4.84	4.91	5.05
Leg	35.64	35.35	35.23
Shins	2.81	2.81	2.91

Table 5. Leg tissue composition (% on reconstituted leg weight)

	A	GA	G
Lean	62.50	62.37	62.61
Fat	7.07	6.73	6.25
Bone	30.43	30.90	31.15

SEASONAL VARIATION IN CARCASS QUALITY OF REINDEER (*RANGIFER TARANDUS TARANDUS*) FROM THE SEWARD PENINSULA, ALASKA

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Key Words: Reindeer, meat, venison, seasonal effects, carcass composition, yield, ultimate pH

Introduction

During much of its history in Alaska the reindeer industry has produced meat for subsistence and local use (Stern *et al.*, 1980). Today, reindeer producers wish to commercialize their reindeer operations by selling meat in upscale local markets and exporting a high quality product to both national and international markets. State regulation allows Alaskan Native producers to sell field slaughtered, non-inspected carcasses to local retail outlets (D.E.C., State of Alaska, 2003). Under these regulations reindeer can be field slaughtered if ambient air temperature is less than 32 F and there is snow on the ground. The market potential for this product is limited because field slaughtered meat cannot be added to other meat products *i.e.* sausage, cannot be sold to restaurants or exported out of the state and must remain frozen immediately after field slaughter through purchase by the consumer. Also, wholesale distributors, *i.e.* Fred Meyer and Safeway, are reluctant to market reindeer meat on a seasonal basis. They demand a nearly year round supply of premium quality meat before offering the product to the consumer (Krieg, 1991). Reindeer producers on the Seward Peninsula want to enhance their operations by reliably delivering a high quality product from animals slaughtered outside the mid-winter months. The steady supply of a high quality product over an extended period of time will be the key to successful commercialization and profitability of the reindeer industry in Alaska.

The reindeer (*Rangifer tarandus tarandus*) is a seasonal animal and, like other Nordic cervides, is known to have a low capacity to gain weight during winter vis-à-vis summer (White & Fancy, 1986). Reproductive status and season have been demonstrated to be important factors influencing carcass yield and composition in red deer (*Cervus elaphus*) (Drew, 1991). Seasonal changes in appetite (Ryg & Jacobsen, 1982), body condition (McEwan & Whitehead, 1970; Reimers, 1983) and thus carcass composition occur rapidly in reindeer, however these changes are asynchronous between sex and age classes (Finstad & Prichard, 2000). Supplemental feeding may help to improve the nutritional status of the reindeer, both by increasing body condition and thereby carcass conformation and by increasing the glycogen levels in the muscles, which is of critical importance for several meat quality attributes (Jacobsen, Bjarghov & Skjenneberg, 1977; Wiklund *et al.*, 1996; Wiklund *et al.*, 2000a).

The most important factor contributing to future stability and profitability of reindeer producers is the ability to consistently slaughter animals with a carcass type that will yield the greatest quantity of high quality product. There are no published studies that have systematically evaluated carcass yield and quality across animal categories of Alaskan reindeer slaughtered through an extended season.

Objectives

Determine reindeer body condition dynamics (weight gain and loss), carcass characteristics, composition and yield of adult reindeer bulls and steers (castrated bulls) through a nine -month slaughtering season, July through March. In addition, ultimate pH values of *M. longissimus* were recorded.

Methodology

A total of 42 reindeer were included in the study (19 bulls and 23 steers). All animals came from the same herd out in the Seward Peninsula, Alaska, and were slaughtered at three different times; mid July (group 1), late November (group 2) and mid March (group 3). Group 1 were gathered and herded with helicopter and snow machine before entering the corral, the animals were positioned in a squeeze chute and stunned with a captive bolt. Animals in groups 2 and 3 were gathered with snow machine and, while still free-ranging in the herd, they were shot with a rifle. All carcasses were gutted and dressed out in the field, and then transported as soon as possible to a meat processing facility for further sampling and boning. Carcass cutting followed a protocol developed for this study but based on earlier moose and reindeer carcass studies (Hansson & Malmfors, 1978; Wiklund *et al.*, 2000b). Ultimate pH was recorded at boning in *M. longissimus* (at the last rib) using a portable pH meter (Knick Elektronische Messgeräte GmbH & Co, Germany) equipped with a Xerolyte electrode (InLab[®] 427, Mettler Toledo, Switzerland).

Definitions of the cuts presented in Table 1 are as follows: semiboneless leg (major part of the hind-quarter of the carcass without shank, including the *M. gluteus medius*, *M. quadriceps femoris*, *M. biceps femoris*, *M. semitendinosus* and *M. semimembranosus*), saddle (*M. longissimus* with bone), striploin (*M. longissimus*), topside (*M. semimembranosus*) and shoulder (*M. triceps brachii*, *M. supra spinatus* and *M. infra spinatus* with bone and shank).

Results & Discussion

Reindeer slaughtered in July (group 1) had the highest body weights; however the carcass weights (Cw) did not differ much over the season (Table 1). The exception was the low weights of carcasses from bulls (47.2 kg) in group 2. This result could not only be related to the effects of the rutting season, but also to that these bulls were estimated to be younger (2 ½ years old) than the rest of the animals in the study. Overall, the carcass weights in the present study were much higher than those presented for Swedish reindeer bulls by Wiklund *et al* (2000b). This may be due to differences in animal age, slaughter strategies and herd composition between the two studies.

The present study provides important information on the yield of carcass cuts as a percentage of Cw from both bulls and steers over a 9 month season and comparisons within a time period between the two sexes (Table 1). As expected, the reindeer bulls showed a bigger seasonal variation in body composition than the steers. However, at the last slaughter occasion (group 3 in March) it was clear that both animal categories had started to regain the fat and protein reserves lost during the winter. The fat content (% of Cw) in bulls and steers in November was 1.6 and 6.4 respectively, while these figures had increased to 4.4 and 10.6 in March (Table 1). The proportion (% of Cw) and total weight (kg) of two of the most valuable cuts; striploin and topside, did not show a great variation between the sexes or over the season. Earlier studies have demonstrated the effects of pre-slaughter stress and nutritional status on muscle glycogen content and ultimate pH values in reindeer meat (Wiklund, 1996). The present results are in good agreement with previous studies, and we could conclude that the present high pH values recorded were clearly related to pre-slaughter stress (animals in group 1) and poor nutritional status (bulls in group 2) (Table 2).

Further studies within this project will relate the cutting data to the economical value of the carcasses. All together, this data could provide important information for the Alaskan reindeer industry and promote new management and slaughtering strategies focused on high animal productivity and optimal meat quality.

Conclusions

Seasonal effects in reindeer carcass composition were demonstrated in the present study, although the carcasses from the late slaughter occasion (March) had higher weights and better composition (proportion of valuable cuts) than expected. Reindeer bulls were more affected by the season than the steers, and showed the largest variation in carcass weights and fat content.

The present project will continue with evaluation of eating quality (trained sensory panel and consumer preference tests) of reindeer meat from bulls and steers as well as chemical analyses to give a complete sensory and nutritional profile of reindeer meat with the seasonal effects included. We expect that the results from the present study will generate information necessary for Alaskan reindeer producers to develop an operational plan that will increase the value and expand the delivery of reindeer products demanded and accepted by upscale markets and consumers.

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Table 1. Carcass characteristics and composition (least-squares means \pm standard errors) in reindeer bulls and steers included in the study

Trait	Bulls July (n=7)	Steers July (n=7)	Bulls Nov (n=5)	Steers Nov (n=9)	Bulls March (n=7)	Steers March (n=7)	Degree of sign. ¹
Body weight, kg	137.7 ^a ± 5.1	124.6 ^{ac} ± 5.1	88.4 ^b ± 6.1	101.3 ^{bc} ± 4.5	113.2 ^c ± 5.1	110.9 ^c ± 5.1	***
Carcass weight (Cw), kg	61.0 ^{ac} ± 2.7	60.0 ^{ac} ± 2.7	47.2 ^b ± 3.3	53.9 ^{ab} ± 2.4	59.6 ^c ± 2.8	62.3 ^c ± 2.8	**
Semiboneless leg, kg	16.7 ^a ± 0.8	15.8 ^{ac} ± 0.7	12.5 ^b ± 0.8	14.9 ^a ± 0.7	15.0 ^{ac} ± 0.7	14.5 ^c ± 0.7	*
Semiboneless leg, % of Cw	27.7 ^a ± 0.6	26.5 ^{ab} ± 0.6	26.6 ^{ab} ± 0.7	27.6 ^a ± 0.5	25.2 ^b ± 0.6	23.4 ^c ± 0.6	***
Saddle, kg	7.9 ^a ± 0.5	7.4 ^a ± 0.5	5.6 ^{bc} ± 0.6	7.2 ^a ± 0.5	4.6 ^c ± 0.5	5.1 ^c ± 0.5	***
Saddle, % of Cw	12.8 ^{ab} ± 0.4	12.3 ^{ab} ± 0.4	11.8 ^b ± 0.5	13.3 ^a ± 0.4	7.7 ^c ± 0.4	8.2 ^c ± 0.4	***
Topside, kg	3.3 ^{ac} ± 0.1	3.2 ^a ± 0.1	2.8 ^b ± 0.2	2.9 ^{ab} ± 0.1	3.6 ^c ± 0.1	3.3 ^{ac} ± 0.1	***
Topside, % of Cw	5.4 ^a ± 0.1	5.4 ^a ± 0.1	6.0 ^b ± 0.1	5.5 ^a ± 0.1	6.1 ^b ± 0.1	5.3 ^a ± 0.1	***
Striploin, kg	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	n.s.
Striploin, % of Cw	3.1 ^{ac} ± 0.1	3.1 ^{ab} ± 0.1	3.5 ^b ± 0.2	3.4 ^{ab} ± 0.1	2.8 ^c ± 0.1	2.9 ^c ± 0.1	**
Shoulder, kg	11.0 ^{ab} ± 0.4	10.7 ^a ± 0.4	8.3 ^c ± 0.5	8.9 ^c ± 0.4	12.2 ^b ± 0.4	11.7 ^{ab} ± 0.4	***
Shoulder, % of Cw	15.2 ^a ± 1.1	17.9 ^{abc} ± 1.1	17.6 ^{abc} ± 1.3	16.6 ^{ac} ± 1.0	20.5 ^{bc} ± 1.1	18.8 ^c ± 1.1	*
Bone, kg	12.2 ± 0.6	14.2 ± 0.6	12.6 ± 0.8	13.2 ± 0.6	14.5 ± 0.6	13.9 ± 0.6	n.s.
Bone, % of Cw	20.0 ^a ± 0.8	23.8 ^b ± 0.8	26.7 ^c ± 1.0	24.7 ^{bc} ± 0.8	24.3 ^{bc} ± 0.8	22.4 ^{ab} ± 0.8	***
Fat, kg	4.1 ^a ± 0.8	2.3 ^{ab} ± 0.9	0.8 ^b ± 0.9	3.7 ^a ± 0.7	2.7 ^{ab} ± 0.8	6.7 ^c ± 0.8	***
Fat, % of Cw	6.5 ^a ± 1.1	3.1 ^b ± 1.1	1.6 ^{bc} ± 1.3	6.4 ^a ± 0.9	4.4 ^{ab} ± 1.1	10.6 ^d ± 1.1	***

In.s. = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. Means in the same row having the same superscript are not significantly different ($p > 0.05$).

Table 2. Ultimate pH values (least-squares means \pm standard errors) in *M. longissimus* from reindeer bulls and steers included in the study

Trait	Bulls July (n=7)	Steers July (n=7)	Bulls Nov (n=5)	Steers Nov (n=9)	Bulls March (n=7)	Steers March (n=7)	Degree of sign. ¹
Ultimate pH	6.28 ^a \pm 0.08	6.26 ^a \pm 0.08	5.88 ^b \pm 0.09	5.70 ^{bc} \pm 0.07	5.61 ^c \pm 0.08	5.68 ^{bc} \pm 0.08	***

¹*** = $p \leq 0.001$. Means in the same row having the same superscript are not significantly different ($p > 0.05$).

THE EFFECT OF GENOTYPE, DURATION OF FEED WITHDRAWAL AND ELECTRICAL STIMULATION ON MEAT QUALITY

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Key Words: Electrical stimulation, feed withdrawal, ageing, genotype, tenderness

Introduction

Extrinsic factors, such as chilling rate, electrical stimulation and ageing, proved to have major influences on tenderness development of red meat (Ouali, 1990; Olsson *et al.*, 1994; Koochmaraie, 1996). The variation in tenderness, due to the development of *rigor* (peak toughness), is related to the conditions (pH and temperature) under which the muscle fibres enter *rigor* and, subsequently, settle into *rigor mortis* when all supplies of ATP are exhausted (Review: Hwang, Devine & Hopkins, 2003). Early and recent studies show that minimal shortening occurs above 12-15°C resulting in optimum tenderness (Locker & Hagyard, 1963; Tornberg, 1996). Below this temperature, pre-*rigor* contracture takes place until *rigor* is completed resulting in higher *rigor* toughness (Tornberg, 1996). The historical reason for the development of electrical stimulation was the acceleration of *post-mortem* glycolysis so that when the muscle entered rigor it was prevented from excessive shortening (Swatland, 1981). Further studies indicated that electrical stimulation may also contribute to the acceleration of proteolysis (Uytterhaegen, Claeys, & Demeyer, 1992). However, high temperature combined with low pH values could result into *rigor* contracture (termed heat shortening) has a concurrent reduction in ageing potential leading to less tender meat both at *rigor mortis* and when fully aged (Devine, Wahlgren & Tornberg, 1999). Stress, independent of ultimate pH seems to have a toughening effect on meat which is enhanced by electrical stimulation according to Morton, Bickerstaffe, Le Couteur & Keeley, (1997), although Geesink, Mareko, Morton & Bickerstaffe (2001), could not confirm these results.

Objectives

Considering an optimum pH/temperature ratio for maximum tenderness development, management of these ratios is not simple when animals with different muscle energy levels are slaughtered due to variation in pre-slaughter practice. In addition, differences in duration of stimulation together with different carcass sizes and therefore chilling rates enhance the problem of optimum rigor management further.

In the present study, the effect of breed, which also represented different carcass weights, and duration of stimulation and pre-slaughter stress on meat quality characteristics were investigated in order to find an optimum stimulation treatment for different types of carcasses and different levels of stress.

Methodology

From three groups sixty Simmental cross, Brahman cross and Nguni cross bulls raised under intensive conditions and aged 12 months, thirty animals were withdrawn from feed for 20 hours pre-slaughter. The remaining thirty of each breed cross were withdrawn from feed for only three hours pre-slaughter. Within each group of thirty animals, 10 carcasses were not stimulated while the remaining twenty were either stimulated (ES) for 15 (n=10) or 120 seconds (n=10) (400 V peak, 5 ms pulses at 15 pulses per second). Carcasses were chilled directly after dressing at room temperature before loading at 0 – 4°C. Sampling of the *M.longissimus* (LD) for measurement of Warner Bratzler shear force (WBS), sarcomere length and drip loss took place 24 hours *post mortem*. The pH and temperature of the LD were measured with a digital handheld meat pH meter (Sentron, Model 1001) between the 11th and 12th rib at 1, 2, 3, 4, 6, 8 and 20 hours *post mortem*. every hour for 4 hours *post-mortem*, and thereafter at 24 hours. LD of both sides were sampled. Samples destined for WBS were vacuum packaged and aged at 2°C ± 2 °C for 1 days and 14 days *post-mortem*. The sarcomere lengths were measured by using a Video Image Analyser (Kontron, Germany) after preparation of a fresh sample (24 hours *post-mortem*), according the method of Hegarty & Naudé (1970), by using distilled water instead of Ringer Locke solution (Dreyer, van Rensburg, Naudé, Grouws, & Stiemie, 1979). Fifty grams of fresh meat (24 hours) sliced into cubes of 10 x 10 x 20 mm were suspended on a pin inside a sample bottle (200 ml. Duplicate samples were stored for three days at 4°C ± 2 °C. The amount of drip was expressed as a percentage of the starting mass. LD aged for one or 14 days were processed into 30 mm steaks by means of a band saw before being thawed at 4°C for 24 h and prepared according to an oven-broiling method using direct radiant heat (AMSA, 1978). The steaks were broiled at 260°C (pre-set) to 70°C internal temperature. Core samples of 12.5 mm were removed along the fibre and sheared perpendicular to the fibre with a Warner Bratzler shear device attached to an Instron Universal Testing (Instron, 1990). Shear force was measured as the peak force (kg) average for eight cores per sample. The data were subjected to a three way analysis of variance. Means for the main effects and their were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level (Snedecor & Cochran, 1980).

Results & Discussion

Carcasses with pH values higher than 6 were excluded from the analysis. Furthermore, no combination of breed, feed withdrawal or ES treatment resulted in muscle temperatures lower than 12 °C before pH values reached 6. Therefore, cold shortening according to the rule of thumb (Tornberg, 1996) was not likely to take place. However, pH values declined faster for stimulated carcasses than for non-stimulated ones and initially faster for longer stimulation times (data not presented). For the latter, the unfavourable temperature/pH ratio coincided with shorter sarcomeres (Table 1) probably indicating some heat shortening (Devine, Wahlgren & Tornberg, 1999).pH decline was also faster for shorter feed withdrawal times.

Meat tenderness was significantly influenced by breed, feed withdrawal and ES ($P < 0.05$) (Table 1). These effects occurred at both one day and 14 days *post mortem*. Breed, feed withdrawal and ES also interacted at day one ($P = 0.044$) (Figure 1) and day 14 ($P = 0.08$) (Not shown). On average, the Simmental had the tougher meat than the Nguni and Brahman ($P < 0.05$), which is in contrast with the findings of Shackelford *et al.*, (1995) showing a decrease in tenderness as percentage *Bos indicus* inheritance increased. On the other hand, De Bruyn (1991) reported tougher meat for Simmental compared to other breeds such as the Bonsmara, Charolais and Hereford. The difference between breeds was most evident for long feed withdrawal periods combined with no ES. The general effect of ES was also more pronounced with longer feed withdrawal periods. These patterns were still the same even after prolonged ageing. Regarding stress (duration of feed withdrawal in the present trial) and ES the results of the present trial contradict the findings of Morton *et al.*, (1997). They report a toughening effect of stress on sheep meat which was enhanced by electrical stimulation. Morton *et al.* (1997) concluded that μ -calpain worked less efficiently when ES is applied to stressed animals, hence the tougher meat. With regard to sarcomere length, the shorter sarcomere length (in general) of the Nguni does not reflect its low shear force values. However, sarcomere length of the Simmental was less than $1.6 \mu\text{m}$ (not shown) when animals were on long feed withdrawal and not stimulated, which could partly explain the high shear force values. Improvement in shear force due to ES was reflected by longer sarcomere lengths but could also have been due to an enhanced rate of proteolysis (Uytterhaegen, Claeys, & Demeyer, 1992). Longer feed withdrawal coincided with higher shear force values in general, although it was more evident for non-stimulated carcasses. Longer feed withdrawal also coincided with higher final pH values for Nguni carcasses in particular ($\text{pH} > 5.8$). Purchas (1990) reported high shear force values for ultimate pH values between 5.8 and 6.2 (mildly stressed), which could explain the higher shear force values of the Nguni (1 and 14 days) with long feed withdrawal, but does not explain why ES improved the tenderness.

Despite the benefit of ES with regard to tenderness, the amount of drip loss increased by ~ 0.5 of a percent between no stimulation and 15 seconds stimulation and a further 0.9 of a percent with 120 seconds stimulation (Table 1). Conditions of low pH and high temperatures in *post-mortem* muscle reduce the water binding capacity of meat, an effect attributed to the denaturation of muscle proteins, particularly myosin (Offer & Knight, 1988, Offer, 1991). Electrical stimulation, by accelerating pH decline, contributes to reduced water binding capacity in beef, though the magnitude of the effect depends on the chilling rate (Babiker & Lawrie, 1983). As the pH decline of the 120 second ES was initially faster than that of the 15 second ES treatment, the differences in drip loss was expected. Drip loss was significantly higher for the Brahman compared to the other two breeds. The lower rate of pH decline and faster chilling rate of the Nguni could explain the difference in drip loss between the Nguni and Brahman. The carcass weights of the Simmental and Brahman was almost 70 kg higher than that of the Nguni. A shorter duration of feed withdrawal was associated with a faster rate of pH decline and higher drip loss.

Conclusions

Electrical stimulation was beneficial for LD tenderness even after 14 days of ageing when compared to no stimulation. Electrical stimulation had a greater advantage when the LD had a high inherent toughness and when animals were moderately stressed due to long feed withdrawal periods. Stimulation for fifteen seconds and two minutes had equally favourable effects on tenderness, but the longer stimulation time had a detrimental effect on drip loss.

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Tables and Figures

Table 1. The effect of breed, feed withdrawal period and electrical stimulation treatment on shear force resistance, sarcomere length and drip loss.

Breed	Brahman	Nguni	Simmental	SEM²	P-value
Shear force day 1 (kg)	6.9 ^{ab}	6.7 ^a	7.3 ^b	0.1627	0.027
Shear force day 14 (kg)	4.3 ^a	4.4 ^a	4.9 ^b	0.1266	<0.001
Sarcomere length (µm)	1.72 ^b	1.65 ^a	1.70 ^b	0.0152	0.005
Drip loss (%)	2.7 ^b	1.8 ^a	1.9 ^a	0.1146	<0.001
Feed withdrawal	18 hours	4 hours		SEM²	P-value
Shear force (kg)	7.3 ^a	6.7 ^b		0.1329	0.004
Shear force day 14 (kg)	4.8 ^a	4.3 ^b		0.1034	<0.001
Sarcomere length (µm)	1.66 ^a	1.72 ^b		0.0124	0.003
Drip loss (%)	2.0	2.2		0.0936	0.096
Electrical stimulation	None	15 sec.	120 sec.	SEM²	P-value
Shear force (kg)	8.7 ^b	6.3 ^a	6.0 ^a	0.1627	<0.001
Shear force day 14 (kg)	5.4 ^b	4.0 ^a	4.1 ^a	0.1266	<0.001
Sarcomere length (µm)	1.66 ^a	1.74 ^b	1.68 ^a	0.0152	<0.001
Drip loss (%)	1.5	2.0	2.9	0.1146	0.001

^{a,b}

Means within a row with different superscripts differ significantly (P<0.05)

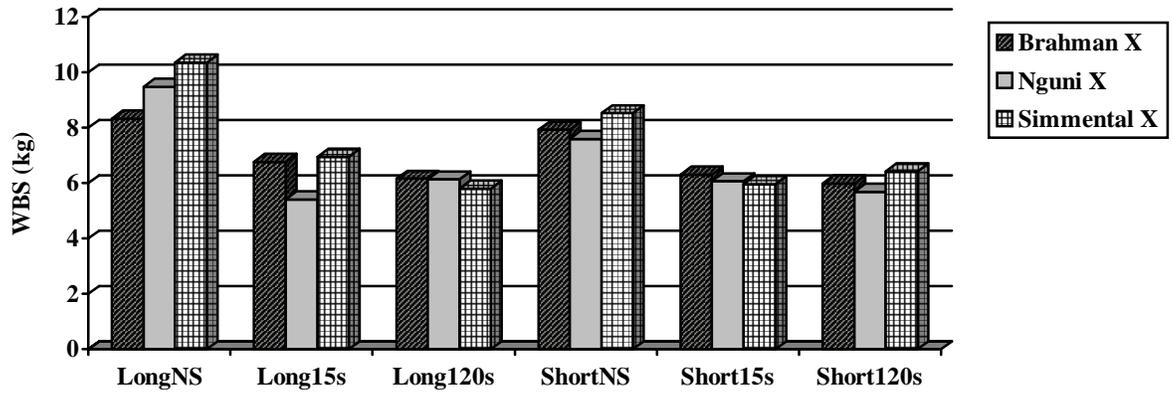


Figure 1: Interaction between breed, feedwithdrawal and electrical stimulation with regard to tenderness (1 day post mortem)

(Long and short – feed withdrawal period; NS, 15s and 120 s – no stimulation, 15 seconds and 120 seconds stimulation respectively; WBS – Warner Bratzler shear force)

**EFFECT OF FEED WITHDRAWAL TIME ON PRE-SLAUGHTER CHICKEN
WEIGHT LOSS, CARCASS YIELD AND TECHNICAL QUALITY
PARAMETERS**

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Key Words: Chicken quality, Feed withdrawal

Introduction

Feed withdrawal for chicken prior to slaughter has a significant effect on meat hygiene and safety. It has been well documented that the prime source of pathogenic contaminations of chicken meat such as Salmonella and Campylobacter are originated from gastro intestinal content and its excrement. The pathogenic microorganisms reside in gastro intestinal track, and cause cross contamination of meat during the process of slaughter (Corrier et al., 1999). In addition, feed restriction affects chicken quality. Chicken contains a limited glycogen in muscle tissue compared to cattle and pig. If feed withdrawal is enforced too far extend, that results in depletion of energy source in muscle. This consequently shortens time cause of rigor onset and results in unfavorable meat quality (Lyon and Buhr, 1999). Feed withdrawal also affects carcass yield through its effect on moisture loss and fat decomposition. In general, weight loss caused by feed withdrawal is in proportion to the length of fasting time, its loss in earlier period is related to excrement of gastro internal content and does not have a significant effect on final yield. However, extended feed restriction can lead to reduction in final weight and economic loss due to moisture loss and fat decomposition(Veercamp, 1986). Weight loss is influenced by age, sex, energy content in feeds, length of time, and transport condition(Duke et al., 1997; Randall et al., 1994). For the reason, chicken industry across world has determined the optimum length of feed withdrawal to compromise the least detrimental effect on quality and weight loss. On the other hand, feed withdrawal program between countries varies due largely to difference in final weight, feed composition, feeding program and regulation.

Objectives

The current study was conducted to identify industrial practice for chicken feed withdrawal prior to transport to slaughter plant and estimate optimum time.

Methodology

Three hundred chickens (1.5 or 2.5 kg) were sampled and enforced feed withdrawal for 0, 3, 6, 9 and 12 hours, without free access to water. Body weight was determined just prior to the treatment. Average body weight after the feed restriction, carcass weight and

gastro intestinal content weight were determined from 15 chickens which were randomly selected for each treatment. Body weight and yield loss were calculated from weight difference after feed withdrawal and expressed as percentage. pH was measured at breast fillet at 1, 3, 12 hours postmortem using a portable pH meter (pH*K21, NWKCo. Germany) and noted as pH1, pH3 and pHu. Meat color was determined using a Chroma meter (CR301, Minolta Co, Japan), standardized on the white board of $Y=92.4$, $x=0.3136$ and $y=0.3196$, on the surface of chest fillet and drum stick. The data was assessed using a SAS package (1999) by applying Duncan procedure at the probability level of 0.05.

Results & Discussion

Body weight and carcass yield loss are presented in Table 1. Weight loss was increased as length of feed withdrawal was extended from 0 to 12 hours. An average loss per hour was approximately 11.5 g for both class of body weight, and resulted in 138 g loss after 12 hours of the experimental period. When length of feed withdrawal is same, higher weight group showed a greater weight loss; 1.5 and 2.5 kg groups resulted in 116 and 153 g loss after 12 hours, respectively. Carcass yield decreased with feed withdrawal time extended regardless of body weight; where 1.5 and 2.5 kg groups showed significant reductions in yield after 9 and 6 hours feed withdrawal, respectively. Weight loss for carcass can be affected by various factors, but previous study showed that reduction occurred after 6 hours treatment (Bigili, 2002). After the time, weight loss is associated with glycogen depletion in muscle tissue takes place, followed by fat decomposition (Reisfeld et al., 1981). Based on the current data, 6-9 hours feed withdrawal for 1.5 kg chicken appeared to be the optimum practice under the Korean industry situation. The prime reason for the assumption was driven from the fact that longer than 9 hours leads to economic loss under the Korea situation. Table 2 presents changes in gastro intestinal content as affected by the length of feed withdrawal. Net weight of crop and gizzard were significantly ($P<0.05$) reduced by 6 hours feed restriction, but there was no noticeable changes after the time. On the other hand, crop and proventriculus showed a considerable reduction by 3 hours, and there were no significant changes after the time. In the case of cecum and rectum, there was a tendency to be decreased in weight over the withdrawal period, but that was not statistically significant. pH of breast fillet within one hour postmortem was not affected by the treatment. A 3 hours feed withdrawal had a significantly higher pH at 3 and 12 hours postmortem than that for control group, but the other treatment showed a similar tendency of resulting in a similar pHs at the time of all measurements. Lyon and Buhr (1999) reported that feed withdrawal reduced the time course of rigor development, and Kotula and Wang (1994) showed that a 36 hour treatment slowed down the rate of rigor development; there results are in consistence with our current data. Objective meat color varied depending on the length of treatment; suggesting that feed withdrawal within 12 hours had a limited effect on meat color. Table 4 presents quadratic and linear regress function and their coefficients of determination between fasting period and weight loss. Coefficients of determination for the developed final models containing quadratic and linear function for fasting period live weight resulted in the higher values, ranging 0.76-0.75 for 1.5 kg chickens, and 0.77-0.73 for 2.5 kg chicken, respectively. It was thought that the prime reason for the improvement of prediction by including quadratic function was related to the greater weight loss at early

period of feed withdrawal, which was largely contributed to weight loss of gastro intestinal content.

Conclusions

The current study demonstrated that an available fasting time for pre-slaughter broiler differentiated to slaughter weight groups. In general, postmortem pH and breast fillet color were not affected until 12 hour. Also, quadratic and linear model functions were presented to predict weight loss during fasting period.

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Table 1. Means and SE of weight loss and carcass yield during fasting periods

	0 hr	3 hr	6 hr	9 hr	12 hr
Weight loss(g)					
Overall	0.00±0.00 ^e	68.37±24.39 ^d	87.77±27.49 ^c	112.8±31.61 ^b	138.4±42.45 ^a
1.5kg	0.00±0.00 ^e	50.37±17.60 ^d	70.43±17.61 ^c	91.46±22.91 ^b	115.96±31.8 ^a
2.5kg	0.00±0.00 ^e	74.48±23.43 ^d	97.10±27.46 ^c	129.12±27.48 ^b	153.14±42.45 ^a
Carcass yield(%)					
Over all	69.5±2.13 ^a	69.39±2.15 ^a	69.38±2.21 ^a	68.53±1.69 ^b	67.84±2.59 ^b
1.5kg	68.24±2.15 ^a	66.93±1.47 ^a	67.40±2.39 ^a	67.76±1.67 ^a	65.21±1.63 ^b
2.5kg	69.98±1.95 ^{ab}	70.00±1.84 ^{ab}	70.19±1.53 ^a	69.01±1.54 ^c	69.19±1.84 ^{bc}

Table 2 Comparisons of gastro intestinal content during fasting periods

	0 hr	3 hr	6 hr	9 hr	12 hr
Crop	12.57±14.18 ^a	11.26±31.56 ^{ab}	0.57±1.58 ^b	0.05±0.12 ^b	0.12±0.43 ^b
Gizzard	19.68±9.22 ^a	16.23±4.61 ^{ab}	14.49±6.51 ^b	13.54±5.72 ^b	11.5±4.08 ^b
Preventriculus	1.15±1.04 ^a	0.52±0.6 ^b	0.38±0.38 ^b	0.18±0.27 ^b	0.31±0.48 ^b
Small intestine	36.04±11.32 ^a	12.71±8.21 ^b	10.57±5.28 ^b	10.4±5.14 ^b	8.75±3.58 ^b
Cecum	4.36±2.84	5.05±3.98	4.76±3.01	3.95±2.42	3.46±2.24
Recum	0.78±0.63	0.81±0.6	0.55±0.25	0.6±0.52	0.58±0.42

Table 3. Influences of fasting periods on chicken breast fillet pH and color

	0 hr	3 hr	6 hr	9 hr	12 hr
pH					
pH ₁	6.00±0.09	6.17±0.10	5.99±0.12	6.00±0.17	6.09±0.25
pH ₃	5.74±0.18 ^b	6.05±0.15 ^a	5.93±0.15 ^{ab}	5.86±0.21 ^{ab}	5.97±0.14 ^{ab}
pH _u	5.72±0.18 ^b	5.97±0.21 ^a	5.96±0.10 ^a	5.77±0.19 ^{ab}	5.77±0.09 ^{ab}
Color					
L	49.56±2.34	49.16±2.73	48.67±2.81	48.45±1.38	47.10±4.20
a	-0.03±1.12	-0.32±0.81	-0.60±1.03	-0.46±1.20	0.75±1.64
b	10.47±1.41	12.06±2.33	11.18±2.69	9.75±2.38	9.07±3.68

Table 4. Regression and coefficients of determination between fasting period and weight loss

	Intercept	x ² (Fasting time)	x1(Fasting time)	x2(Weight)	R-square
Overall	-49.22902	-0.61081	18.53020	0.02376	0.7566
	7.44266	-0.65002	18.52831	-	0.7047
	-39.70899	-	11.19760	0.02457	0.7292
	19.64591	-	10.70795	-	0.6735
1.5kg	26.64381	-0.27995	12.33905	-0.01221	0.7624
	6.30807	-0.27036	12.27174	-	0.7612
	26.16112	-	8.96700	-0.00881	0.7533
	11.36727	-	9.00212	-	0.7527
2.5kg	-34.56649	-0.76086	21.19715	0.01572	0.7757
	6.49634	-0.78029	21.23505	-	0.7650
	-24.03033	-	12.08930	0.01714	0.7379
	11.36727	-	9.00212	-	0.7527

LIPID COMPOSITION OF PDO-BEEF FROM DIFFERENT PORTUGUESE BREEDS

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Key Words: Lipid composition, Cholesterol, Nutrition

Introduction

Maronesa and Barrosã are autochthonous bovine breeds reared in the North of Portugal according to a traditional semi-extensive grazing system using local agricultural resources. Maronesa and Barrosã calves are reared close to their mothers, suckling milk and eating either grass until slaughter, between 6-9 months old.

Mertolenga, another portuguese cattle breed, is produced in the South of the territory, in a semi-extensive system where animals are mainly fed on pasture and forage. Depending on corporal condition and food availability, Mertolenga young bulls are usually finished on concentrate and slaughtered from 15 to 30 months of age.

When obtained according to the required specifications, the meat of these breeds is commercialized under Protected Denomination of Origin (PDO) having an important impact on local economy and sustainability of the environment and providing a raise of the producers income. PDO certification of products is under European Union regulation (Council Regulation n° 2081/92 of 14/07, EEC) and imposes the utilization of traditional rearing methods. PDO meat is supposed to present unique characteristics, especially associated with the properties of its lipid fraction. Cholesterol level and lipid content and composition of the diet have become issues of great concern to consumers due to their possible negative effect on human health (Chizzolini *et al.* 1999). Atherosclerotic lesions appear to be related to an elevated total cholesterol, a lower HDL-cholesterol/LDL-cholesterol ratio in plasma and excess fat consumption. Plasma lipid levels are not only influenced by the amount of fat consumed but by its nature as well (Steinberg & Witztum, 1990). The P/S (polyunsaturated fatty acids/saturated fatty acids) and *n-6/n-3* ratios are also thought to be important nutritional parameters of foods. Health organizations recommend reductions in total and saturated fat intake and at the same time the increasing in consumption of *n-3* PUFA (Polyunsaturated fatty acids) (Department of Health, 1994). High intake of *n-6* PUFA with low negligible intake of *n-3* PUFA, may increase cardiovascular disease risk because of the proinflammatory and prothrombotic effects of *n-6* PUFA (Cunnane, 2003). Recent research in this domain has focused also on the nutritional relevance of conjugated linoleic acid (CLA) in the human diet. Some CLA isomers are considered beneficial to human health, due to anticarcinogenic, antiatherogenic and immune-modulating properties (Mulvihill, 2001).

A number of different factors including anatomical location, production system, sex, breed, age and diet have been reported to influence fat content and intramuscular fatty acid composition in meat (Calkins *et al.*, 1981; Zembayashi *et al.*, 1995; Gandemer, 1998; Deland *et al.*, 2001; Varela *et al.*, 2004). Therefore, it is expectable that differences in intramuscular fat content and composition between Portuguese breeds could exist.

Objectives

The aim of this work was to evaluate and compare the intramuscular lipid composition of PDO beef from Maronesa, Barrosã and Mertolenga breeds.

Methodology

One day after slaughter, about 200 grams of *Longissimus dorsi* muscle (L4-L6) from Maronesa, Barrosã and Mertolenga were excised and stored at -20°C until analysis.

Intramuscular total lipids (ITL) were extracted from duplicate 20 g samples of muscle, trimmed of visible adipose and connective tissues as described by Folch *et al.* (1957). Separation into neutral lipids (NL) and phospholipids (PL) was performed according to the procedure of Juaneda & Rocquelin (1985). Lipid extracts were esterified with KOH (2N) in methanol (ISO 5509, 2000) and resulting fatty acid (FA) methyl esters were analysed by gas-liquid chromatography, using a HRGC 5160, Mega series from Carlo Erba instruments, equipped with a flame ionisation detector and a 60 m long DB 23 capillary column. The oven temperature was raised from $70\text{-}195^{\circ}\text{C}$ at $5^{\circ}\text{C}/\text{min}$ for LN analysis and from $70\text{-}195^{\circ}\text{C}$ (10 min) to 220°C (60 min) at a rate of $5^{\circ}\text{C}/\text{min}$ for LP analysis. Injector and detector temperatures were 220°C and 280°C , respectively.

Identification of FA was based on comparison with standard FA mixtures (Supelco and Nuchek GLC reference standard FAME mixture). FA were expressed as weight percentage.

Cholesterol (mg/g) was quantified according to Roseiro *et al.* (2002), using a HPLC with a Spectra-Physics Model Spectra 100 equipped with variable wavelength UV detector set at 206 nm and a Spherisorb S5W silica cartridge (Waters PSS 845549). The mobile phase was hexane/isopropanol (97:3) at a flow rate of 1.0 mL/min.

Data were analysed using one-way analysis of variance (ANOVA). Analysis of means was performed by the LSD test for 95% of probability (Statistica 6.0-StatSoft Inc., 2001).

Results & Discussion

Intramuscular lipids and cholesterol levels were affected by breed (Table 1). Barrosã presented higher ITL ($P<0.01$) and NL ($P<0.001$) than Maronesa and Mertolenga breeds. This is in agreement with the results obtained by Wagenhoffer & Szabo (2004), whom also reported differences on ITL content among several breeds. Barrosã and Maronesa showed higher PL (polar lipids) ($P<0.01$) and cholesterol ($P<0.001$) contents than Mertolenga. The values found for cholesterol in Barrosã and Mertolenga, respectively 0.52 and 0.44 (mg/g) are coincident to those reported by Quaresma *et al.* (2004) for *Longissimus lumborum*.

Regarding NL, with the exception of SFA (saturated fatty acids), *n-3* PUFA and hypocholesterolaemic/hypercholesterolaemic ratio (h/H), all the other fatty acid proportions in Ld muscle were affected by breed (Table 2). Barrosã and Maronesa presented higher MUFA (monounsaturated fatty acids) proportion than Mertolenga ($P<0.01$). On the other hand, Mertolenga showed higher PUFA ($P<0.001$), P/S ($P<0.01$), *n-6* PUFA ($P<0.01$) and *n-6/n-3* ($P<0.001$) contents in NL fraction than Barrosã and Maronesa. The higher CLA content was found in Barrosã-PDO veal ($P<0.001$). CLA results from ruminal production and by endogenous synthesis of C18:1 *trans-11* by Δ^9 -desaturase enzyme in adipose tissue and is preferentially deposited in NL than in PL. The increase of pasture in diet resulted in higher level of CLA in intramuscular fat (French *et al.*, 2000).

Webb *et al.*, (1998) reported for *Longissimus thoracis* from Belgian Blue breed, lower SFA (45.46%) and PUFA (3.07%) in triacylglycerol fraction than the mean values obtained in Portuguese breeds. These differences could be attributed to breed and diet effects. In contrast, the MUFA content observed by those authors (46.46%) was similar to that found for Barrosã and Maronesa but higher than that detected in Mertolenga breed.

Zembayashi *et al.* (1995) also obtained significant differences on MUFA content in NL among steers from different breeds. According to those authors, some breeds have a genetic predisposition for synthesis and deposition of MUFA in that lipid fraction.

No differences on SFA were observed in PL among breeds, following the same trend observed for NL, which corroborates the results obtained by Zembayashi *et al.* (1995). The SFA content, P/S and h/H indices were also not different among breeds.

Unlike most individual SFA, MUFA have neutral effects on human cholesterol levels (Scientific Review Committee, 1990). Barrosã showed the highest MUFA content among the 3 breeds ($P<0.001$). Deland *et al.* (2001) also reported differences among breeds on MUFA content in PL of Ld muscle. The breeds studied by those authors presented higher MUFA and SFA proportions and lower PUFA content than those used on this work. Laborde *et al.* (2001) studying Simmental and Red Angus finishing steers referred that PL fatty acid profile of Ld muscle included approximately 34% SFA, 26% MUFA, 31% *n-6* PUFA and 8% *n-3* PUFA. Except for MUFA (23.30%) and *n-3* PUFA (9.50%) the proportions referred by Laborde *et al.* (2001) are similar to those found in Maronesa-PDO veal. In addition, those authors reported significant differences among breeds for *n-3* PUFA and *n-6/n-3* ratio but not for SFA, MUFA, PUFA, *n-6* PUFA and P/S ratio in PL. In agreement with these results, differences were found between breeds on *n-6* PUFA proportion and *n-6/n-3* ratio ($P<0.001$) in PL, with Mertolenga young bulls presenting the highest values. In contrast, Mertolenga showed lower *n-3* PUFA than Barrosã and Maronesa ($P<0.001$) and higher PUFA content than Barrosã ($P<0.01$). These differences could be attributed to the diet, age at slaughter, genetic potential and type of finishing among the studied breeds.

According to nutritional recommendations *n-6/n-3* and P/S ratios in diet should not exceed 4.0 and 0.45, respectively (Department of Health, 1994). The *n-6/n-3* ratio hardly exceeded the recommended value in Mertolenga-PDO meat (10.64) but was accomplished in Barrosã and Maronesa-PDO veal (respectively, 3.88 and 3.96). P/S ratio was lower than 0.45 in Barrosã (approximately 0.33) but not in Maronesa and Mertolenga-PDO meat (approximately 0.60 for both). Low P/S values are considered unfavourable since they could induce hypercholesterolaemia. The h/H ratio is at the

present considered a better approach to the nutritional evaluation of fat because is based on individual effects of FA on cholesterol metabolism (Williams, 2000; Santos-Silva *et al.*, 2002). The values obtained in Portuguese breeds ranged from 1.8 to 2.2 and were similar to those referred by Santos-Silva (2002) for light lambs.

Conclusions

Barrosa-PDO veal presented higher ITL and NL than Maronesa and Mertolenga-PDO meat. In respect to PL and cholesterol contents, Barrosã and Maronesa-PDO veal showed the higher values.

In general, the NL and PL FA compositions of Mertolenga-PDO meat were significant different from Barrosã and Maronesa-PDO meat. Mertolenga-PDO meat was the only with an unfavourable *n-6/n-3* ratio whereas Barrosa-PDO veal showed a P/S ratio below the recommended value which was accomplish in Mertolenga and Maronesa-PDO meat. The h/H ratio was similar among breeds. The results suggest that from a nutritional point of view, Mertolenga finished stage based on concentrate could induce unfavourable modifications on FA profile.

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Tables and Figures

Table 1. Age (months±STDEV) and weight (Kg±STDEV) at slaughter and means of intramuscular total lipids (ITL, g/100g), neutral lipids (NL, g/100g) polar lipids (PL, g/100g) and cholesterol (mg/g) contents of Barrosã, Maronesa and Mertolenga PDO-meat.

	Breed			Rsd	P
	Barrosã	Maronesa	Mertolenga		
Carcass characteristics					
n	92	10	44	-	-
Age	7.4±0.9	9.4±3.2	22.0±4.2	-	-
Weight	99.4±16.1	102.0±30.8	249.1±32.4	-	-
Intramuscular lipids and cholesterol					
ITL	2.98 ^a	1.40 ^b	1.58 ^b	0.91	***
NL	2.44 ^a	0.71 ^b	0.99 ^b	0.92	***
PL	0.66 ^a	0.66 ^a	0.60 ^b	0.09	**
Cholesterol	0.52 ^a	0.49 ^a	0.44 ^b	0.08	***

* = P<0.05; ** = P<0.01; *** = P<0.001

Rsd = residual standard deviation of the analysis of variance

Table 2. Fatty acid composition (% w/w) of Barrosã, Maronesa and Mertolenga PDO-meat

n	Breed			Rsd	P
	Barrosã	Maronesa	Mertolenga		
	40	10	20		
Fatty acid composition of NL					
SFA	47.46	48.35	49.02	3.18	ns
MUFA	46.69 ^a	46.84 ^a	43.21 ^b	3.33	**
PUFA	4.77 ^b	3.61 ^b	6.80 ^a	2.13	***
CLA	0.84 ^a	0.51 ^b	0.36 ^b	0.16	***
P/S	0.10 ^b	0.08 ^b	0.14 ^a	0.05	**
<i>n-3</i> PUFA	0.79	0.69	0.62	0.40	ns
<i>n-6</i> PUFA	3.13 ^b	2.96 ^b	5.92 ^a	1.81	***
<i>n-6/n-3</i>	4.16 ^b	4.35 ^b	13.16 ^a	3.79	***
h/H	1.50	1.41	1.54	0.22	ns
Fatty acid composition of PL					
SFA	32.69	34.94	32.45	2.97	ns
MUFA	27.23 ^a	23.30 ^b	22.92 ^b	3.67	***
PUFA	38.50 ^b	41.34 ^{ab}	43.55 ^a	5.73	**
CLA	0.34 ^a	0.31 ^{ab}	0.26 ^b	0.10	*
P/S	1.20	1.19	1.36	0.26	ns
<i>n-3</i> PUFA	10.28 ^a	9.50 ^a	5.36 ^b	2.03	***
<i>n-6</i> PUFA	27.90 ^b	31.81 ^b	37.94 ^a	5.64	***
<i>n-6/n-3</i>	2.85 ^b	3.55 ^b	7.54 ^a	1.61	***
h/H	2.94	2.80	3.26	0.47	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

n-3 PUFA = C18:3 *n-3* + C18:4 *n-3* + C20:3 *n-3* + C20:5 *n-3* + C22:5 *n-3* + C22:6 *n-3*

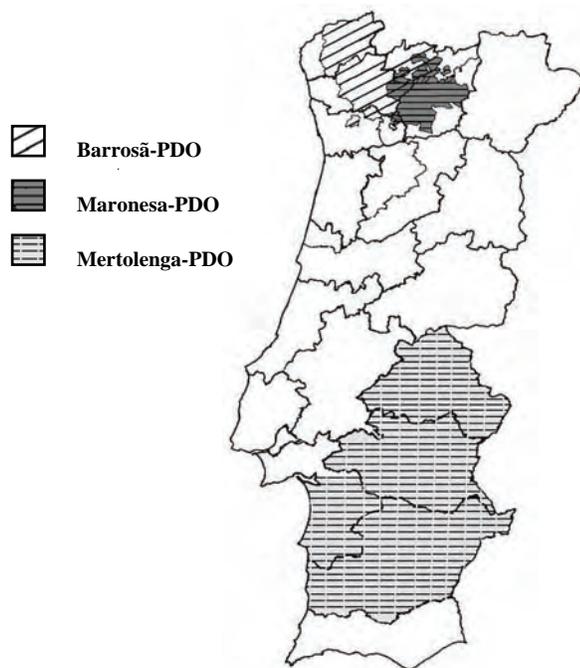
n-6 PUFA = C18:2 *n-6* + C18:3 *n-6* + C20:2 *n-6* + C20:3 *n-6* + C20:4 *n-6* + C22:2 *n-6* + C22:4 *n-6*

h/H = hypocholesterolaemic/hypercholesterolaemic ratio = [(sum of C18:1 *cis-9*, C18:2 *n-6*, C18:3 *n-6*, C18:3 *n-3*, C20:3 *n-3*, C20:4 *n-6*, C20:5 *n-3*, C22:4 *n-6*, C22:5 *n-3* and C22:6 *n-3*)/(sum of C12:0, C14:0 and C16:0)]

ns = not statistically significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Rsd = residual standard deviation of the analysis of variance

Figure 1. Geographical distribution of Barrosã, Maronesa and Mertolenga Breeds.



EFFECT OF TYPICAL PRODUCTION SYSTEM FROM SEVERAL COUNTRIES ON FATTY ACID COMPOSITION OF BEEF

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Key Words: Pasture, Concentrate, Production system, Fatty acid, Beef

Introduction

There are many factors that can affect the fatty acid composition of intramuscular fat such as breed (Robelin, 1986), age (Link et al., 1970), diet (Rhee, 2000), as well as the level of carcass fatness (Nürnberg et al., 1998), all of them make up a production system. Fatty acid composition of meat affects its nutritive value besides its palatability. With regard to the nutritive value, consumption of saturated fatty acids (SFA) has been associated with an increase in plasma of low density lipoprotein and cholesterol, both of them related to a major risk of coronary heart disease. Another fatty acid important in the human diet is the conjugated linoleic acid (CLA), since it exhibits health benefits when is consumed at low levels (French et al., 2000). Moreover, meat flavor is influenced by saturation rate of fatty acids (Purchas et al., 1979). The susceptibility of meat to lipid oxidation increases with the polyunsaturated fatty acid proportion; it could affect the oxidative meat flavor and reduce the acceptability by consumer (Gatellier et al., 2001).

Objectives

The aim of this study was to analyze fatty acid composition in commercial beef types representing typical production system from Spain, United Kingdom, Germany and Uruguay to assess the extent of “natural” dissimilarity in their fatty acid composition.

Methodology

Five groups of 20 beef slaughtered at usual commercial weight were used from four countries: Spain, United Kingdom, Germany and two types of Uruguay, 2 and 3 years old beef, which are representative of their typical production system conditions. Spanish beef were non-castrated males from Frisian breed; they were early weaned and kept on concentrates and cereal straw *ad libitum* until slaughtering. The age of slaughter was between 10 and 11 months and the carcass weight was 228.9 ± 3.0 kg. The cattle from

United Kingdom were castrated males from commercial crossbreed (Simmental, Charolais and Limousine), they were mainly reared on a grass-based system, using strategic concentrate supplementation. The age of slaughter was between 18 and 22 months and the carcass weight was 313.3 ± 5.2 kg. German beef cattle were noncastrated males from Fleckvieh and Limousine crossbreed. They were reared extensively on pasture and finish the last six months with maize silage *ad libitum*, supplemented with soya and cereal meal. The age of slaughter was between 19 and 24 months and the carcass weight was 382.4 ± 9.2 kg. Beef cattle from Uruguay were from castrated males exclusively raised under extensive improved grazing conditions, producing two kinds of beef, one slaughtered at 2 and the other one at 3 years old, with carcasses weights of 224.8 ± 2.8 kg and 282.0 ± 3.4 kg respectively.

Intramuscular fat was extracted from longissimus lumborum muscle (Hanson & Olley, 1963). Methyl esters of the samples were formed according to Morrison and Smith (1964), using nonadecanoic acid (C19:0) prior to saponification as internal standard. Chromatographic analysis of methyl esters was performed using a Perkin-Elmer gas chromatograph (Perkin-Elmer, USA). Fatty acids were identified from standards and quantified using the internal standard.

One-way ANOVA was performed using GLM procedure of SAS version 8.2. (SAS Inst. Inc, Cary, NC) Differences between the means were determined using the Student-Newman-Keuls test. PRINCOMP procedure was used to principal component analysis, the variables were standardized.

Results & Discussion

The least square means of the fatty acid composition (expressed as proportion by weight of total fatty acids) of the beef and fatty acids ratios from typical production systems of the countries studied are showed in table 1. Spanish and Uruguayan 2 years beef showed the lowest intramuscular fat proportion (1.67 % and 1.74 %, respectively) related to the lowest carcasses weights in comparison with British and German beef that had the highest proportion (2.92% and 2.95%, respectively) with highest carcasses weights.

Spanish beef showed the highest proportion of C18:2 and C20:4 and lowest of C16:0 and C18:1, while British and German beef had the highest proportion of C14:0, C16:0. The proportions of C15:0, C18:3 and long chain fatty acids (C20:5, C22:5 and C22:6) were higher in grass fed cattle (Uruguayan) compared with cattle reared intensively using concentrates (Spanish and German beef). The differences in fatty acid composition in beef could be mainly related to differences in the feeding production system (grass or concentrate). Thus, Varela et al. (2004) reported that steers fed on pasture showed higher percentage of C18:3 and C18:0 than steers fed with concentrate. This may be due to the fact that C18:3 is the major fatty acid present in grass (Garton, 1960), precursor of the long chain *n*-3 fatty acids series (C20:5, C22:5 and C22:6) while cereal grain used in concentrated diet had high levels of C18:2 (Barnes, 1983), precursor of *n*-6 fatty acids series (C20:4 mainly, Rhee, 2000). Levels of C15:0 were also higher on grass fed animals due to this fatty acid arise from digestion of ruminant microorganisms encouraged to feed cattle with grass (Smith et al., 1979).

The CLA isomer, *cis-9, trans-11* C18:2, was detected in all beef samples evaluated. However, Uruguayan 2 and 3 years beef had the highest proportions of this fatty acid, 0.57 % and 0.54 %, respectively. British and German groups had lower proportion (0.34 % and 0.33 %, respectively), showing Spanish beef lesser than twofold the proportion of Uruguayan beef (0.22 %). French et al. (2000) showed the linear increment of intramuscular CLA concentration when the proportion of concentrate in the diet decreased. The high concentrations of rapidly fermentable sugar and soluble fiber of forage creates a rumen conditions which promoted a greater production or decreased utilization of CLA by rumen (Kelly et al., 1998).

The highest PUFA/SFA ratio (P/S) was for Spanish beef, due to their lower proportion of SFA and higher proportion of PUFA (40.99% and 17.34 % respectively). It could be due to differences in feed, age and fatness level. Thus, forage stimulated ruminal activity promoting the biohydrogenation of the fatty acids, which in turn, increases the concentration of SFA (Choi et al., 1997). Link et al., (1970) showed in muscle that the proportion of PUFA decreased with increasing animal age and concomitant increases in intramuscular neutral lipid deposition. The increment of SFA with age and decrement of PUFA could be the reason why older animals (German, British and Uruguayan beef) showed low PUFA proportions and P/S ratio. With regard to fatness, Nürnberg et al. (1998) found a negative relationship between fat content and PUFA, and Marmer et al. (1984) found that triacylglycerols, which increased with fatness, are less unsaturated than phospholipids in muscle membranes. The ratio *n-6/n-3* was very high in Spanish beef (14.84) related to the other beef (7.60, 2.62, 1.48 and 1.37 for German, British, Uruguayan 3 years and 2 years, respectively). According to Kemp et al. (1981), the use of concentrate resulted in raised concentrations of *n-6* PUFA and grass diets increased *n-3* PUFA.

Principal component (PC) analysis was performed to study the relationship between fatty acids and examine the relationships between the types of beef compared. Figure 1 displays the projection of the fatty acid data in the plane defined by the two first principal components (PCs). The first PC explained 37.6 % of the variability of the fatty acid composition. PC1 was mainly characterized by PUFA, P/S and C20:4, and in the opposite side by intramuscular fat proportion (fat). The second PC explained the 28.1 % of the total variability, it was defined by long chain *n-3* fatty acids (C20:5 and C22:5), C18:3 and in the opposite direction by C18:2 and *n-6/n-3* ratio. However, Bas and Morand-Fehr (2001), using PCs analysis to study fatty acid composition of lambs, found that the first two PC explained about 45% of the total variance in subcutaneous and intramuscular fat, and about 60% in perirenal adipose tissue. The projection of the fatty acid data in the plane defined by the two first principal components of the five groups studied is shown in figure 2. Spanish beef were clearly separated from rest of beef groups and were placed on the left hand down PC1 axis, close to PUFA, P/S ratio and *n-6* fatty acid (C18:2 and C20:4). The Uruguayan 2 years beef were located up in the figure on the left side, close to long chain fatty acids (C20:5, C22:5 and C22:6), C18:3 and C15:0, whereas Uruguayan 3 years beef are slightly moved on the right, where C17:0, C18:0, and CLA lay. British and German beef were located on the right side of the figure, close MUFA, SFA and fat proportion, both of them clearly separated, British above and German down PC1 axis. This different location was mainly due to German beef had higher *n-6/n-3* ratio than British beef, which is located down in the projection of the fatty acid data.

Conclusions

The Spanish beef fed with concentrate had lowest SFA proportion and higher PUFA proportion, therefore they had a better ratio P/S compared with the rest of the beef groups. The Uruguayan 2 and 3 years beef had the highest proportion of *n*-3 fatty acids (C18:3, C20:5, C22:5 and C22:6), CLA and odd fatty acids (C15:0 and C17:0) and the lowest *n*-6/*n*-3 ratio. British and German beef had the highest intramuscular fat proportion, SFA and MUFA.

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Tables and Figures

Table 1. Fatty acid composition of *m. longissimus lumborum* in percentage by weight of total fatty acids of beef from typical production system of several countries.

	Spain	United Kingdom	Germany	Uruguay 2 years	Uruguay 3 years	RMSE
Fat (%)	1.67 _a	2.92 _c	2.95 _c	1.74 _a	2.35 _b	0.73
C14:0	2.32 _b	2.64 _a	2.78 _a	2.01 _c	2.19 _{bc}	0.39
C15:0	0.38 _b	0.43 _c	0.30 _a	0.47 _d	0.48 _d	0.06
C16:0	22.57 _a	27.29 _d	26.53 _{cd}	24.22 _b	25.24 _{bc}	2.44
C16:1	2.90 _a	3.39 _b	3.63 _b	2.83 _a	3.30 _b	0.48
C17:0	1.05 _b	1.05 _b	0.74 _a	1.11 _b	1.11 _b	0.12
C18:0	14.47 _b	15.00 _{ab}	15.02 _{ab}	16.26 _a	15.20 _{ab}	1.69
C18:1	37.67 _a	41.26 _b	41.96 _b	38.30 _a	40.72 _b	3.49
C18:2	12.22 _a	3.34 _b	4.76 _b	4.68 _b	3.77 _b	1.85
C18:3	0.45 _d	0.88 _c	0.47 _d	2.13 _a	1.70 _b	0.28
CLA	0.23 _c	0.34 _b	0.33 _b	0.57 _a	0.54 _a	0.15
C20:4	3.11 _a	1.16 _c	1.25 _c	2.10 _b	1.54 _c	0.62
C20:5	0.16 _d	0.43 _c	0.14 _d	1.30 _a	0.84 _b	0.25
C22:5	0.45 _d	0.71 _c	0.29 _d	1.73 _a	1.19 _b	0.30
C22:6	0.04 _c	0.07 _c	0.04 _c	0.17 _a	0.12 _b	0.06
SFA	40.99 _c	46.63 _a	45.60 _{ab}	44.27 _b	44.45 _b	2.84
MUFA	41.67 _b	46.11 _a	46.90 _a	42.49 _b	45.44 _a	3.62
PUFA	17.34 _a	7.26 _d	7.50 _d	13.24 _b	10.11 _c	3.02
P/S	0.43 _a	0.16 _d	0.17 _d	0.30 _b	0.23 _c	0.08
<i>n-6/n-3</i>	14.84 _a	2.63 _c	7.60 _b	1.37 _d	1.48 _d	1.29

a, b, c, d: Means in the same row with different letter differ significantly (P<0.05)

RMSE: root of mean square error

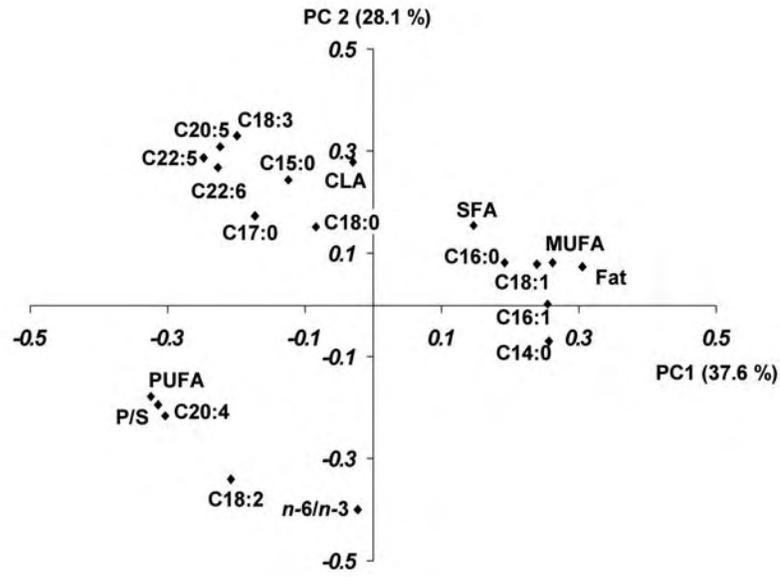


Figure 1. Projection of the fatty acid data in the plane defined by the two first principal components.

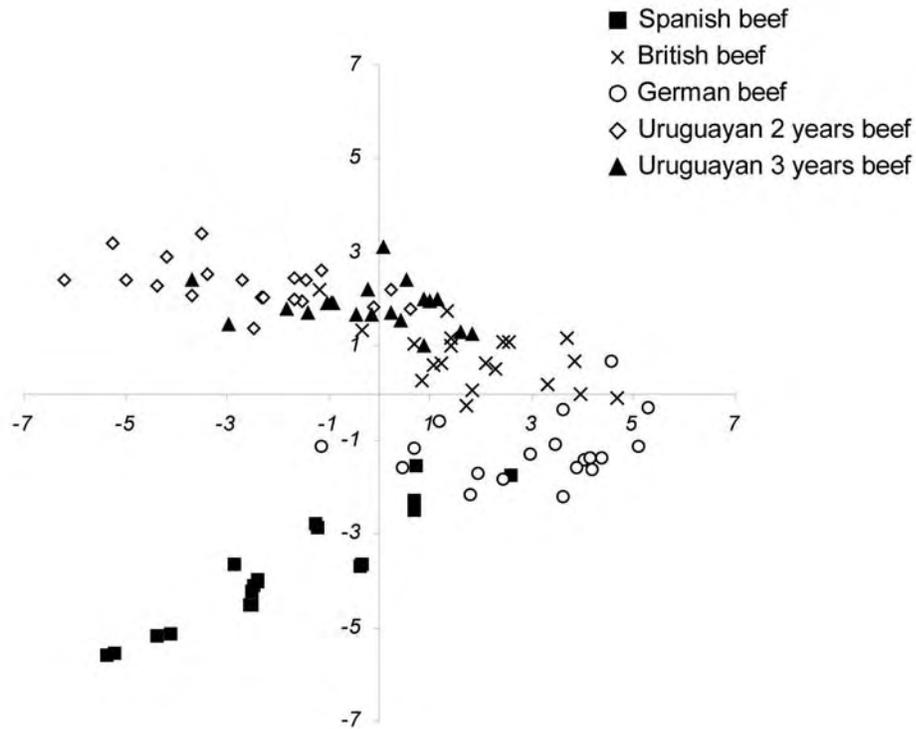


Figure 2. Projection of the fatty acid data of the five groups studied in the plane defined by two principal components.

CHARACTERIZATION OF BEEF MUSCLES STRUCTURE BY HISTOLOGY AND IMAGE ANALYSIS IN DOUBLE-MUSCLED AND NORMAL ANIMALS

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Key Words: muscle, meat, perimysium, double-muscled, image analysis

Introduction

Double-muscled (hereafter referred to as culard) cattle have been studied for many years as a model of the relationship between hyperplasia, collagen and lipid content, and meat quality (Ashmore & Robinson 1969, Bouton et al 1982, Boccard & Dumont 1974, Fiems et al 1995). It is well known that there is an increase in fiber number and a decrease in both collagen and lipid content in these cattle when compared with normal cattle. The decreased collagen content, increased collagen solubility (Ngapo et al 2002, Uytterhaegen et al 1994) and decreased collagen cross-links (Ngapo et al 2002) are reputed to yield extremely tender meat, but several studies have failed to find significant differences between cooked shear force values of culard and normal cattle (Bouton et al 1982, De Smet et al 2000, Wegner et al 2000), or even have found higher cooked shear force values for culard cattle (Uytterhaegen et al 1994, Fiems et al 1995). These discrepancies may be partly explained by differences in post mortem tenderization and myofibrillar toughness (Uytterhaegen et al 1994, Steen et al 1997, De Smet et al 2002), and by differences in the cooking procedures used. However, raw shear force is always significantly lower in culard cattle (Bouton et al 1982, De Smet et al 1998, Ngapo et al 2002), which usually indicates decreased connective tissue content. Taste panel tenderness is also consistently better in culard animals (De Smet et al 2002). Another possible explanation for the tenderness variability is that other connective tissue parameters, especially organization, are also important in determining the role of connective tissue in tenderness, and that the relationship of quality to connective tissue parameters is only detected when collagen is not extensively solubilized at high cooking temperatures. In fact qualitative studies have shown differences in perimysial connective tissue organization between normal and culard cattle (Boccard 1981, Dumont & Schmitt 1973).

Objectives

To better characterize the meat quality of culard cattle we examined by histology the perimysial connective tissue organization in different muscles of culard and normal

Belgian Blue cattle and performed quantitative measures of its organization by image analysis. The results were related to muscle mechanical properties as an index of tenderness.

Methodology

Thirteen bulls of normal conformation (mean age 19.4 months) and ten culard bulls (mean age 21.2 months) of the Belgian Blue breed were slaughtered. Genotyping for the mutation nt821 (del 11) in the myostatin gene responsible for the double-muscling phenotype in the Belgian Blue breed was done according to Grobet et al. (1998) (culard, mh/mh; normal, +/+). At 1 day *post mortem* three muscles: *Gluteobiceps* (GB), *Pectoralis profundus* (PP) and *Semitendinosus* (ST) were excised and samples (1x1x1 cm³) were frozen in isopentane chilled by liquid nitrogen (-160°C) and stored at -80°C. Frozen sections (10 µm thick) were stained using picro-Sirius red coloration (Flint and Pickering, 1984) which reveals the collagen of perimysium and endomysium (Fig. 1).

Histological sections were studied with a Polyvar Reichert microscope, Sony CCD video camera, computer with a Matrox image acquisition card and Visilog 5.4 software (Noesis, France). Automatic thresholding on grey level allowed segmentation of the thickest elements of the perimysium network, thus eliminating the endomysium and the thinnest elements of perimysium (Fig. 2). Measurements of this segmented network allowed calculations of the % area of perimysium. An additional step of skeletization of the segmented network reduced it to a thickness of a pixel allowing the measure of the length of the network (Fig. 2c)

This segmentation only allowed the measurement of length and area occupied by the perimysium and not measures of fascicle size. The fascicles were manually traced (Fig. 3). Measurement of the area of the primary, or smallest, perimysium fascicles of the *Semitendinosus* muscle was performed on all bulls, using Visilog software. Primary fascicles are identified as the smallest group of fiber bundles and are delimited by a continuous connective tissue which is thicker than the endomysium (Fig. 3c).

On images of ST and GB muscles obtained at higher magnification the endomysium was segmented automatically to measure fiber size within the fascicles. Fiber boundaries were manually corrected when necessary.

The shear force and sensory measurements on these animals have been reported in a previous study (De Smet et al 2000). Warner-Bratzler shear force was measured with a Lloyd TA 500 Texture Analyser on cooked (water bath heating at 75 °C) and grilled (until an internal temperature of 80°C) steaks. A 12 members sensory panel evaluated samples of each muscle for tenderness.

Statistical analysis of the measured perimysial parameters was done using the ANOVA "General Linear Model" procedure of SAS. Comparison of fascicle size was done using the Student's T-test.

Results & Discussion

Perimysium network length and area

We found significant differences ($P < 0.001$) in the perimysial surface area and length (Fig. 4) of culard and normal Belgian Blue cattle, with culard cattle having less area and total length of perimysium. The ST and GB were significantly different for both parameters whereas the PP varied slightly for length and not for area. Boccard (1981) found that the total muscle weight of PP actually was more effected by the culard phenotype than ST and Gluteus muscles weights so it is not readily evident why PP perimysium changes much less than GB and ST. As previously reported (Dumont & Schmitt 1973), we also observed that culard perimysium is thinner and has less branching.

Primary fascicle area

The area of primary fascicles in ST muscles was measured for 48 fields and 39 fields for normal and culard animals respectively. This gave a total of 1799 fascicles for normal and 835 for culard cattle with respective means of $0.31 \pm 0.2 \text{ mm}^2$ for normal cattle versus $0.59 \pm 0.44 \text{ mm}^2$ for culard. These differed significantly ($P < 0.001$) with culard fascicles being twice as large. This is the first report of fascicle size for double muscled animals and supports that the tender raw (De Smet et al 1998) and cooked (De Smet et al 2000) meat in these animals is associated with larger fascicles which have thinner perimysium.

Fiber size

Several previous reports have shown differences in fiber size and type between culard and normal cattle (Fiems et al 1995, Holmes & Ahsmore 1972, Wegner et al 2000), with the tendency being slightly smaller fibers in culard animals and more glycolytic IIB fibers. We observed when tracing the perimysium bundles that the endomysium size and shape varied by muscle and animal type (Fig. 5). Therefore were quantified the size distribution of fibers, defined by endomysial area, for GB and ST muscles because they appeared to differ markedly. The fiber size distribution is shown in figure 6 and it is evident that ST fibers are homogeneous in normal and culard with an average size of $4136 \pm 2451 \mu\text{m}^2$ and $5071 \pm 2997 \mu\text{m}^2$ respectively. However, GB fibers have a heterogeneous distribution in culard animals with peaks for small and large fibers. GB normal and culard fibers had a mean size of $3451 \pm 1581 \mu\text{m}^2$ and $4283 \pm 2954 \mu\text{m}^2$ respectively. This has not previously been reported and is not readily explained by the known effects of myostatin mutations (Kambadur et al 1997). Since fiber size is associated with meat texture (Taylor 2004) these differences could influence quality. These fiber sizes are in the range of previous reports but our tendency is slightly larger culard fibers, which may be due to differences in techniques, because we measured endomysium delimited fiber size and not specific myofiber stains such as azorubin.

Conclusions

A recent review by Purslow (ICoMST 2004) discussed the importance of connective tissue organization in meat quality. Our current study is the first to show that primary fascicles are larger in culard ST muscles, which may be associated with the tenderness of meat. This is related to the so-called muscle grain which is a visual image of the coarseness of meat. However, visual inspection would only discriminate the larger secondary fascicles and not the primary as measured herein. We are developing techniques to measure the larger fascicles (Sifre-Maunier et al 2004) which are highly variable. Preliminary results in normal Charolais cattle show that tough muscles have greater proportions of small secondary fascicles.

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Tables and Figures

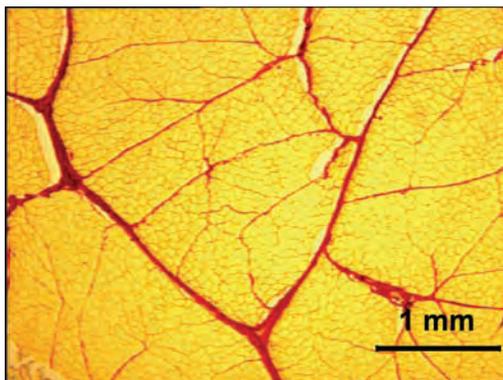


Fig. 1a Normal ST muscle

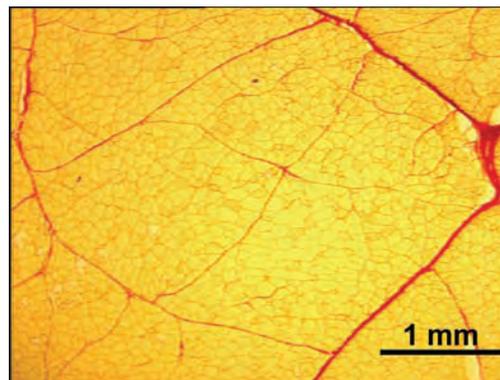


Fig. 1b Culard ST muscle

Figure 1. Picro_Sirius red staining of ST muscle to show perimysium and endomysium organization.

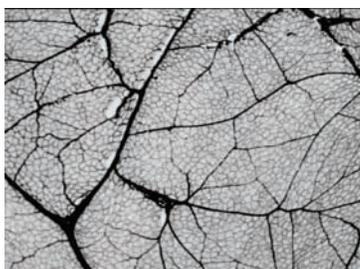
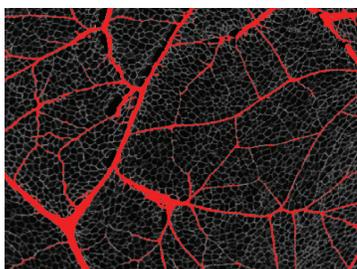
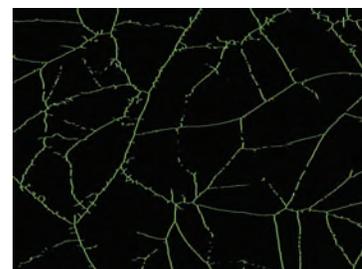


Fig. 2a



2b



2c

Figure 2. Segmentation of normal image (2a) to measure perimysium area (2b) and length (2c).

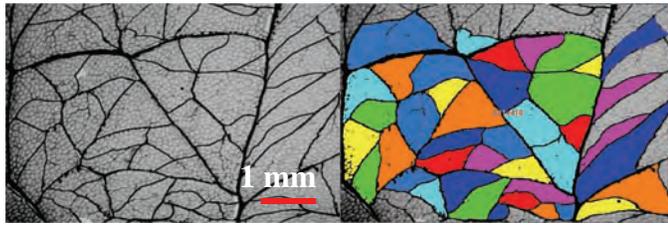


Fig. 3a

Fig. 3b

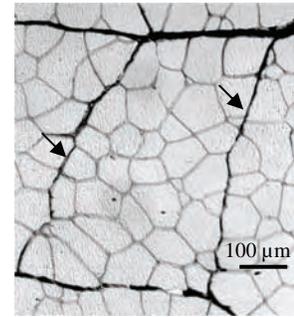


Fig. 3c

Figure 3. Segmented images (3a) were manually traced to give precise boundaries of primary fascicles (3b). Fascicles were identified by thick boundaries (arrows in 3c).

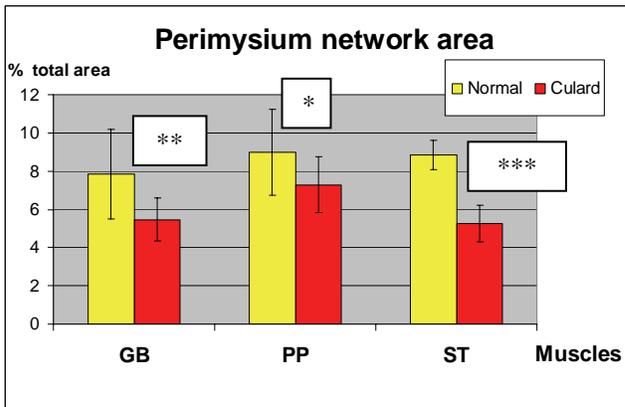


Fig.4a

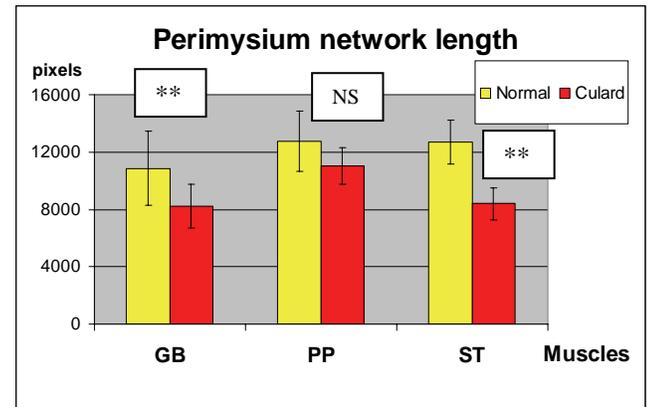


Fig. 4b

Figure 4. The area of perimysium differs between culard and normal cattle for all three muscle types (4a), and length differs for GB and ST (4b).

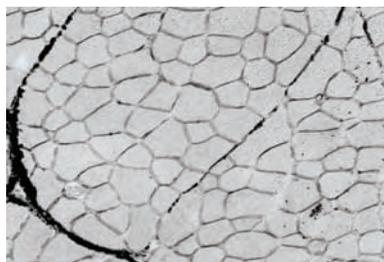


Fig 5a Normal

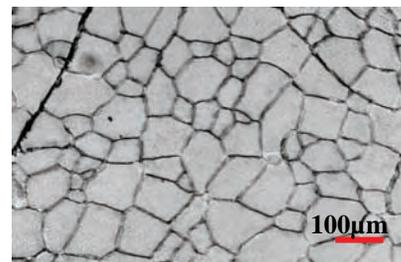


Fig 5b Culard

Figure 5. Endomysial size in GB muscles was homogeneous in normal animals (5a) and heterogeneous in culards (5b)

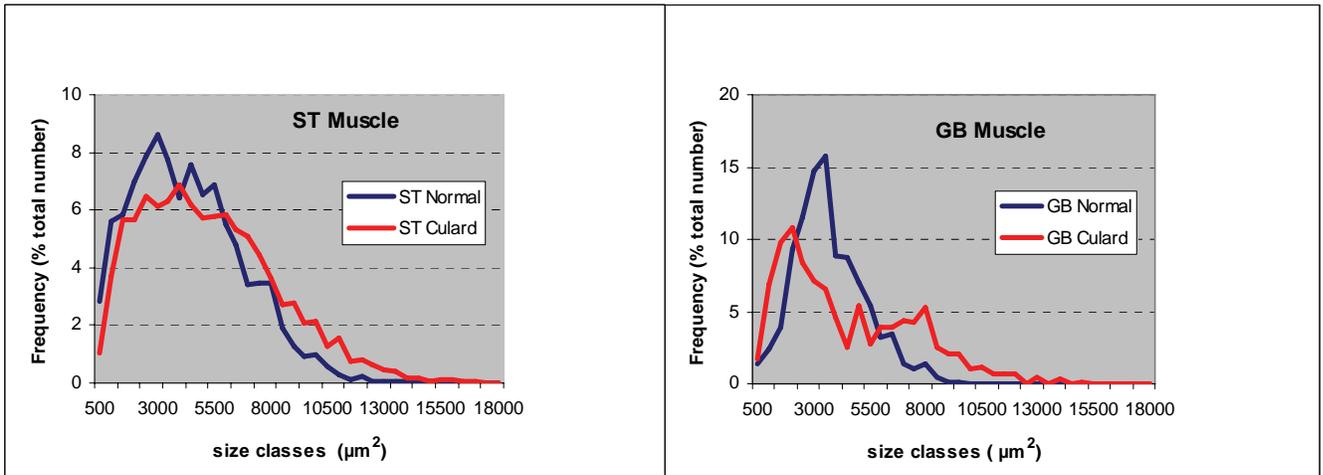


Fig. 6a **Fig. 6b**
Figure 6. Fiber size distribution was similar for normal and culard ST (6a), but heterogeneous for culard GB (6b).

EFFECT OF PRO-LONGED FATTENING ON CARCASS AND MEAT QUALITY IN PIGS

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Key Words: pig, pork, live weight, gender, carcass composition, meat quality

Introduction

Fattening pigs are usually raised up only to a live weight of at most 120 kg. This is a reasonable compromise regarding the requirements of pig producers and the meat industry. With increasing live weight the relative costs for piglets, slaughter and meat inspection may diminish, but simultaneously the carcass is getting fatter and feed costs per kg carcass weight rise due to a deteriorating feed efficiency. Moreover the common exploitation of a pig carcass relies on certain sizes of the different joints obtained from it, which would be exceeded by slaughtering larger pigs. On the other hand the meat of heavier pigs (live weight up to about 160 kg) is basically preferred for the processing of traditional dry-cured products. In addition it is widely supposed that increasing age at slaughter improves the palatability of pork (Candek-Potokar et al. 1998). However, in order to assess the usability of such carcasses on present-day hogs, detailed knowledge about leanness, sizes and weights of cuts as well as meat quality in different parts of the carcass is essential.

Objectives

It was the aim of the present investigation to contribute objective data on the changes concerning carcass composition, weight of typical joints and meat quality in the course of a pro-longed fattening period up to live weights of 110, 135 and 160 kg. Furthermore the effects of gender and fattening intensity should be studied.

Methodology

A total of 123 crossbred pigs (Piétrain x Landrace, MHS-negative) were allocated to a 3 x 2 x 2 factorial experiment involving three live weights (110, 135, 160 kg), two genders (castrated males, females) and two fattening intensities (high, intermediate). Pigs were housed in straw-littered double-pens. In the growing period (30-80 kg live weight) all animals had free access to the feed. The diet for the finishing period (80 kg to the respective end of fattening) contained less protein and was different in the energy content according to the respective fattening intensity (Table 1). Females received the feed *ad*

libitum and castrates were fed at a level corresponding to the average feed intake of the gilts.

The animals were slaughtered in the abattoir of the Training and Research Centre after a two hours' lairage. Following electrical stunning and exsanguination while hanging carcasses were scalded (62°C for 5 min), eviscerated and split in the usual way before, approximately 50 min p.m., being placed in a chiller (at 2°C). 40 min p.m. the pH₁ (pH-STAR, Matthäus) was measured on *M. longissimus dorsi* and *M. semimembranosus*.

At 24 h p.m., after measuring pH₂₄ and electrical conductivity₂₄ (LF-STAR – Matthäus) of *M. longissimus dorsi*, the right carcass side was cut between 13th and 14th rib to measure backfat thickness, fat and loin area. Afterwards the half carcass was dissected into primal cuts according to the “DLG cutting method” (Scheper & Scholz, 1985), and the samples needed for further investigations were removed (*M. longissimus dorsi*, *M. semimembranosus* and *M. triceps brachii*).

For estimating the lean meat content an extra formula based on the weight of 6 DLG cuts was calculated (R = 0.94, RSD = 1.3) using total dissection data of 338 pigs with carcass weights up to 120 kg.

L*, a*, b* were measured 24 h p.m. (MINOLTA CR 300, light source: D65) after a 10-min blooming period. Additionally the total pigment concentration was determined (Hornsey, 1956).

The intramuscular content of water, protein, and fat was estimated by using Near-Infra-Red spectroscopy. Reference values of intramuscular fat were determined without a previous HCl treatment.

Drip loss was calculated as the proportionate weight loss of 2.5 cm thick slices hanging in narrow plastic containers for 24 hours (24-48 h p.m.) at 2 °C. These samples were subsequently used for cooking loss determination. Each slice was packed in a polyethylene bag and cooked in a pre-heated (75 °C) streaming water-bath. As soon as internal meat temperature had reached 75 °C (50-60 min) the samples were taken out, cooled down in tap water and reweighed. After storage overnight (4 °C) the samples were utilized for shear force measurements eventually. From each slice 6 strips with a cross-section of 1 x 1 cm were excised parallel to the muscle fibres and sheared using an Instron texture analyzer equipped with a Warner-Bratzler shear (Freudenreich and Augustini, 2000).

Samples to be grilled for shear force measurement (*M. long. dorsi*, 2nd lumbar vertebra) and sensory evaluation (*M. long. dorsi*, 4th lumbar vertebra) were cut out 72 h p.m., vacuum-packed and stored at -20 °C until further processing. Samples were thawed at 4 °C for 24 hours before heating. Then they were trimmed, sectioned in 2.5 cm thick chops, covered with tin foil and heated in an iron plate contact grill to an internal temperature of 75°C. In case of shear force measurement the samples were cooled down over-night and then treated in the same way as described above.

Sensory evaluation was carried out on hot samples. From each slice 6 cubes (about 2x2x2 cm) were cut out and presented to the six panellists sitting in single booths. The attributes rated according to 6-point scales were tenderness (1 = very tough, 6 = very tender), juiciness (1 = very dry, 6 = very juicy), and flavour (1 = very poor, 6 = very good).

The effects of weight, sex and fattening intensity and the interaction of sex with weight class were tested with a General Linear Model (Proc GLM of SAS 9.1). If main or interaction effects were significant with $P < 0.05$, between-class differences were tested with least-squares means. As the effect of fattening intensity was generally small and only rarely significant (e.g. some carcass traits), the respective results will not be presented in this paper.

Results & Discussion

The data of growth and carcass composition are shown in Table 2. Fattening the pigs up to a live weight of 160 kg instead of 110 kg increases the average age at slaughter by 55 days. There is no significant difference between the weight classes with respect to the daily gain, and the usual differences between castrates and females become smaller with increasing weight. As expected the feed efficiency of the whole fattening period deteriorates considerably with every stage of live weight and the inferiority of the castrates is most distinctive in the highest class. The average *M. longissimus* area increases from 55.5 to 70.4 cm² so that, in particular in case of the gilts (73 cm²), the pork chops reach a size which might not be accepted by consumers. While the lean meat content of castrates and females, respectively, diminishes between the lowest and highest weight class by 3 and 4.1 percentage points, respectively, the back fat thickness increases from 13.0 to 19.7 mm and from 8.0 to 17.6 mm, respectively. This shift in carcass composition corresponds with the results of Bellof (1991) and Kuhn et al. (1997) obtained for pigs (up to 160 kg) of other breeds. Considering that the meat of heavy pigs is predominately used for dry products, the thicker backfat is rather desired.

In Table 3 the weight dependent shift of the most important primal cuts is expressed as a percentage of side weight. With increasing live weight there is generally a small tendency to a lower proportion of various cuts having a high lean meat content. That concerns especially the ham, the loin, the neck, and the tenderloin. As gilts are basically leaner than barrows, these cuts are represented in female carcasses to a higher percentage. This result could be expected, but the sex dependent differences become smaller with increasing live weight. The adipose layer on the back and the ventral parts of the belly grow more intensive than the leaner cuts mentioned above. Thus the backfat proportion in the carcass of castrates and females increases between 110 and 160 kg by 20 and 35 %, respectively, whereas the loin proportion decreases by 1.5 and 4.8 %, respectively.

Meat quality characteristics for the three muscles (*M. longissimus dorsi* - LD, *M. semimembranosus* - SM, *M. triceps brachii* - TB) are only presented with respect to weight class effects (Table 4). With the exception of electrical conductivity, where the value for LD in the 160 kg group is a little higher, which is possibly caused by a slower chilling of the heavy carcasses, no significant influence of live weight has been found in traits directly connected with the PSE status (pH, drip loss). The brightness (L*) shows a general tendency to lower values with increasing live weight, whereas the colour becomes clearly more red (a*). This finding, which is basically in line with the results of Berry et al. (1970) and Martin et al. (1980), cannot be interpreted as a DFD effect because of the unaltered pH 24 h p.m. The reason has to be sought in the significant weight dependent increase of total pigment concentrations which occurs on different levels in every muscle. The cooking loss and the water content show a small decrease

with higher live weights. But the intramuscular fat content is only in case of the TB slightly affected. This is in contrast to Candek-Potokar et al. (1998), who found a clear rise of IMF when raising pigs up to 130 kg live weight. Not affected is the shear force in LD and SM. However in the SM the values markedly diminish from 110 to 130 and 160 kg live weight, which seems rather unexplainable. Concerning the sensory evaluation, there is only a small deterioration from the medium to the high weight class, being significant only in juiciness.

Conclusions

Increasing the live weight at slaughter of MHS negative Piétrain x Landrace crossbreds from 110 to 160 kg

- does not affect the daily gain but deteriorates the feed efficiency considerably
- diminishes the lean meat content by 3-4 percentage points
- decreases the percentage of lean cuts (loin, ham, neck) slightly, and considerably increases the proportion of adipose tissue on the back and the belly
- does not affect meat quality traits connected with the PSE and DFD problem
- causes a darker and more red meat colour by elevated pigment concentration
- reduces the water content marginally, but does not clearly affect intramuscular fat
- does not influence shear force but slightly impairs sensory quality

Thus, raising heavy pigs only makes sense for the processing of dry cured products but not for the fresh meat market.

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Tables and Figures

Table 1. Ingredients and composition of growing and finishing diets

Ingredient (%)	Growing diet 30 to 80 kg	Finishing diet 80 kg until end of fattening	
		High intensity	Moderate intensity
Barley	48.0	50.0	78.4
Wheat	30.8	36.4	-
Wheat bran	-	-	10.0
Soybean meal (48 % Cr. Prot.)	17.0	11.0	9.0
Acid	0.7	0.5	0.5
Minerals	2.6	2,1	2.1
Soybean oil	0.9	-	-
Composition (%)			
Crude protein	18.5	14.9	13.9
Lysine	1.1	7.4	7.2
Crude fibre	3.9	36.0	51.0
Crude fat	2.8	2.2	2.7
Metabol. energy (MJ/kg)	13.4	12.8	12.0

Table 2: Least square means for growth and carcass data by live weight class (LWC) and sex (castrates/ females)

	Sex	Live weight class			Overall effect ⁴	
		110 kg n ¹ = 19/17	135 kg n ¹ = 27/27	160 kg n ¹ = 17/16	LWC	Sex
Age at slaughter (d)	Castrates	176 ^A	201 ^B	230 ^C	***	*
	Females	181 ^A	210 ^B	235 ^C		
Daily gain (g/d)	Castrates	949 ⁺	898	882	ns	***
	Females	846 ⁺	862	851		
Feed efficiency ²	Castrates	2.69 ^A	2.87 ^B	3.14 ^C	***	ns
	Females	2.65 ^A	2.84 ^A	3.05 ^B		
Carcass weight (kg)	Castrates	89.7 ^A	108.4 ^B	130.5 ^C	***	ns
	Females	88.1 ^A	109.8 ^B	129.0 ^C		
Longissimus area (cm ²)	Castrates	52.9 ^A	63.5 ^B	67.9 ^B	***	***
	Females	58.3 ^A	66.7 ^B	73.1 ^C		
Backfat thickness ³ (mm)	Castrates	13.0 ^{A+}	16.8 ^{B+}	19.7 ^B	***	***
	Females	8.0 ^{A+}	12.9 ^{B+}	17.6 ^C		
Lean content (%)	Castrates	57.1 ^{A+}	56.4 ^{A+}	54.1 ^{B+}	***	***
	Females	61.2 ^{A+}	59.4 ^{A+}	57.0 ^{B+}		

¹) Number of castrates/females within the respective weight class

²) kg feed/kg weight gain

³) Smallest backfat thickness on the cross-sectional area at 13th/14th rib

⁴) ns = not significant, * = <0.05; ** = <0.01; *** = < 0.001

^{A,B,C}) Only LSM estimates with different superscripts within a row differ, P < 0.05

⁺) Only LSM estimates with the superscript “+” (within LWC) differ between castrates and females, P < 0.05

Table 3: Least square means for proportion of cuts¹ in % of the carcass half² by live weight class and sex

Trait	Sex	Live weight class			Overall effect ⁴	
		110 kg n ³ = 19/17	135 kg n ³ = 27/27	160 kg n ³ = 17/16	LWC	Sex
Carcass side ² (kg)	Castrates	43.0 ^A	51.8 ^B	62.4 ^C	***	ns
	Females	42.3 ^A	52.5 ^B	61.7 ^C		
Ham (%)	Castrates	26.4	26.0 ⁺	26.1	*	**
	Females	26.9	26.7 ⁺	26.1		
Loin (%)	Castrates	13.4 ⁺	13.6	13.2	ns	***
	Females	14.5 ⁺	14.1	13.8		
Neck (%)	Castrates	7.5	7.6	7.3	*	*
	Females	7.8	7.7	7.5		
Shoulder (%)	Castrates	13.6	13.6	13.6	ns	ns
	Females	13.6	13.5	13.6		
Belly (%)	Castrates	10.1	10.4 ⁺	10.2	*	**
	Females	9.5	9.9 ⁺	10.2		
Tenderloin (%)	Castrates	1.5 ⁺	1.5	1.4	**	***
	Females	1.6 ⁺	1.6	1.5		
Backfat (%)	Castrates	4.3 ^{A+}	4.7 ^{AB+}	5.2 ^B	***	***
	Females	3.4 ^{A+}	3.9 ^{A+}	4.6 ^B		
Ventral parts of belly (%)	Castrates	3.4	3.7	3.9	***	ns
	Females	3.4 ^A	3.7 ^{AB}	4.1 ^B		

¹) According to DLG cutting method

²) According to EU reference cutting

³) Number of castrates/females within the respective weight class

⁴) Significance level for the effects of live weight class (LWC) and sex

ns = not significant, * = <0,05; ** = <0,01; *** = < 0,001

^{A,B,C}) Only LSM estimates with different superscripts within a row differ, P < 0.05

⁺) Only LSM estimates with the superscript “+” (within LWC) differ between castrates and females, P < 0.05

Table 4: Least square means for meat quality traits in *M. longissimus dorsi*, *M. semimembranosus* and *M. triceps brachii* by weight class

	Muscle	Live weight class			Overall effect ¹
		110 kg n = 36	135 kg n = 54	160 kg n = 33	LWC
pH ₁	LD	6.45	6.53	6.45	ns
	SM	6.65	6.68	6.66	ns
pH ₂₄	LD	5.45	5.43	5.43	ns
	SM	5.62	5.62	5.58	ns
Electrical conductivity ₂₄	LD	3.6 ^A	3.7 ^A	4.8 ^B	***
	SM	2.9	2.9	2.9	ns
L* ₂₄	LD	54.0 ^A	51.9 ^B	52.5 ^{AB}	**
	SM	50.6	51.7	50.3	ns
	TB	45.9 ^A	44.0 ^B	42.2 ^C	***
a* ₂₄	LD	7.3 ^A	7.3 ^A	8.2 ^B	**
	SM	9.1 ^A	9.9 ^{AB}	10.8 ^B	***
	TB	14.1 ^A	13.8 ^A	15.7 ^B	***
b* ₂₄	LD	4.8	4.6	5.1	ns
	SM	5.6 ^A	6.2 ^B	5.9 ^{AB}	*
	TB	6.4 ^A	5.7 ^B	6.0 ^{AB}	*
Total pigment (mg Haemin/100g)	LD	3.4 ^A	3.7 ^A	4.0 ^B	***
	SM	4.0 ^A	4.4 ^B	4.7 ^B	***
	LD	8.5 ^A	9.4 ^B	10.3 ^C	***
Drip loss (%)	LD	1.4	1.2	1.7	ns
	SM	1.6	1.3	1.7	ns
	TB	0.6	0.6	0.7	ns
Cooking loss (%)	LD	33.6 ^A	32.6 ^A	31.5 ^B	***
	SM	33.4 ^A	33.5 ^A	32.3 ^B	**
	TB	34.3	33.5	33.1	ns
Water content (%)	LD	74.4 ^A	74.1 ^B	73.6 ^C	***
	SM	73.7 ^A	73.4 ^A	72.9 ^B	***
	TB	75.6 ^A	75.2 ^B	75.0 ^B	***
Fat content (%)	LD	1.63	1.45	1.57	ns
	SM	3.05	3.06	3.47	ns
	TB	1.78 ^A	2.08 ^{AB}	2.10 ^B	*
Shear force – after boiling (N)	LD	48.6	50.1	49.4	ns
	SM	50.5 ^A	41.7 ^B	39.7 ^B	***
	TB	47.7	48.2	49.2	ns
Shear force – after grilling (N)	LD	32,8	29,8	32,2	ns
Juiciness score ²	LD	3.04 ^{AB}	3.37 ^A	2.87 ^B	**
Tenderness score ²	LD	3.56	3.75	3.46	ns
Flavour score ²	LD	3.41	3.44	3.15	ns

1) ns = not significant, * = <0.05; ** = <0.01; *** = < 0.001

2) 6-point scale, 6 = best rating, 1 = worst rating

A,B,C) Only LSM estimates with different superscripts within a row differ, P < 0.05

DEVELOPMENT OF PREDICTON EQUATIONS FOR BEEF CARCASS YIELD IN COSTA RICA

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Key Words: Prediction equation, Beef carcasses, Cutting out yield.

Introduction

Costa Rican beef carcass grading system is pursuing to establish a primary classification according to cattle class or gender and/or to segregate carcasses according to their quality and potential cutting -out yield (CORFOGA, 2000a).

Nowadays, beef cattle farmers and tradespeople in Costa Rica are more concerned with cutting-out yields than with beef quality. Having said this however, this Central American country does not presently have a reliable system of carcass grading based on yield (CORFOGA, 2003b). Other countries use prediction equation of carcass yield developed by multiple regression techniques to anticipate cutting-out yields and this has been proved to work on young bulls and heifers (Shackelford y col., 1995).

This study, the first of its kind, investigates the possibility of arriving at a prediction equation of carcass yield tailored to Costa Rican beef cattle.

Objectives

The objectives of this study were a) To examine the magnitude and sign of correlation among several carcass characteristics with the cutting-out yield, b) To select carcass characteristics with the greatest potential to predict cutting-out yield, and c) To evaluate multiple linear regression equations of cutting out yield, and choose the best one based on simplicity, ease of measurement and predictive worth.

Methodology

292 Zebu crossed cattle were reared on pasture in different regions of Costa Rica and discriminated by sex (156 bulls, 136 heifers) at the packing plant. Cattle were slaughtered by standard procedures. Warm carcasses were evaluated according to procedures described by CORFOGA (2000b) for linear measurements, conformation profiles and degrees of exterior and intramuscular fat. The weight of the chilled carcass was measured, when possible, before the boning process. Chilled carcasses were evaluated 24h *postmortem* according to several characteristics described by Vargas (2001). The

longissimus muscle was exposed by the transversal cut following USDA (1990) procedures. Subcutaneous fat thickness over the *longissimus* was obtained by averaging three measurements taken at a quarter, half and three quarters of the distance covered by the longitudinal axis of the *longissimus*. All cold carcasses were boned out following butchering procedures described by Vargas (2001). After 24 hours of storage in refrigerated chambers, the carcasses were fabricated into subprimal cuts according to local Costa Rican style. The excess sub-cutaneous fat, where present, was removed leaving a maximum of 2 mm thickness of fat on the cut. Professional butchers carried out the boning process, following precise instructions regarding the style and maximum fat cover on the cuts. The cuts were weighed to determine the individual weight and to calculate the respective proportion (percentage) of the total carcass. Cutting-out yields, absolute (YCKG) and relative (YCP), were defined using boneless value cuts trimmed to a maximum of 2 mm.

An analysis of correlation between the carcass variables measured before fabrication and the relative (percentage) and absolute (kg) yields of valued cuts was conducted. Pearson's simple correlation coefficient (r) was used for measuring closeness of linear association between continuous variables and Spearman's ranges coefficients (r_s) was used when at least one of the variables was discreet. To classify r or r_s values as high, moderate or low, Snedecor's conventional criteria was used (high: > 0.7 ; moderate: from 0.5 to 0.7 and low: < 0.5). To detect multi-colinearity and to evaluate the developed equations, the Variance Inflation Factor (VIF) and Durbin-Watson statistic (DW) of the SAS REG procedure (2000) were used. A residue analysis was also undertaken as selection criteria.

A multiple, linear regression analysis was conducted to develop several prediction equations of the dependent variables (MacNeil, 1983). The data were analyzed using version 8.1 of the Statistical Analysis System (SAS, 2000) statistical package.

Results & Discussion

Table 1 shows the descriptive statistics of the studied variables and the simple linear correlation coefficients for determining correlation between traits of the beef carcasses and absolute yield (YCKG) or relative yield (YCP). The sample under study was made up of lightweight carcasses, with a poor fat cover and small loin eye area (LEA), similar to data reported in American tropical countries like Venezuela where, similar to Costa Rica, Zebu crosses are produced and fattened primarily by pasture (Atencio-Valladares, 2002). The slight variation of fat measurements of the Costa Rican sample can be accounted for by the hot fat trimming, a current practice in that country which consists of removing most of the fat layer at the end of the slaughter line just before the carcass is washed.

Sex condition (SEX) was moderately associated ($P < 0.01$) with YCP. However, previous studies including castrated young bulls and entire bulls have shown medium r -values, indicating that over a quarter of the variation seen in YCP could be explained by its linear regression with SEX (Atencio-Valladares, 2002; Reiling, Rouse & Duello, 1992).

The degree of association of carcass weight (WEIGHT; $P < 0.01$) was high with YCKG but it was unrelated ($P > 0.05$) with YCP. This high association with absolute

yield, in general, indicate that heavier carcasses had higher yield of cuts (Kg) which does not imply they have a better percentage yield.

The tendency observed among the fat indicators, where the internal fat percentage (IFP) is a better estimator of YCP than the covering fat (BACKFAT) is supported by Huerta and Morón (1996) whose study showed that external fat accounted for the 40% of YCP variation. In this sample LEA was a better predictor of YCP.

Prediction equations of absolute and relative yield of valued cuts

None of the equations evaluated in this study achieved sufficient power of YCP prediction. As to predicting YCKG, the equations that best estimated its variation are presented in Table 2. More than 92 percent of the variation of yield of valued cuts in kilograms can be attributed to their linear regression on the variables included in the proposed prediction equations. Particular attention should be paid to equation number 3, for this might be the one that satisfies the requirements of the Costa Rican industry in terms of predicting absolute yield.

The prediction of absolute yield would be of questionable usefulness to the butcher, seeing as when the carcass is boned out the total weight of the cuts would be greater, but the amount of trimmed fat (waste) would also be more. Seeing as the equations in this study are able to predict absolute yield in cuts, these equations must be validated. Possibly, their usefulness might be restricted to a determined weight range within the Costa Rican carcass population. This would limit its recommendation as an official method to design a yield-based grading system.

Conclusions

A large part of the YCP variation cannot be attributed to its simple linear regression with the independent variables studied, whereas these variables on their own manage to explain more than 80% of the variation in YCKG.

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Tables and Figures

Table 1. Costa Rican beef carcass characteristics and its relationship to absolute (Kg) and relative(%) cuts yield.

Variables	N	Average	ED	YCKG ^c	YCP ^c
Sex condition	292			0.674** _r	0.153** _r
Carcass weight, Kg	292	242.03	47.20	0.962**	0.028ns _r
Fattening, score ^a	292	1.52	0.63	-0.156**	-0.325**
Subcutaneous fat, cm	292	0.323	0.14	0.007ns _r	-0.259**
Internal fat percentage, %.	292	1.36	0.73	-0.277**	-0.383**
Loin eye area, In ²	292	9.40	1.85	0.716**	0.116* _r
Carcass length, cm.	292	159.76	9.86	0.479**	-0.214**
Achiles tendon length, cm.	292	19.18	1.54	0.176**	-0.160**
Thigh perimeter, cm.	292	87.29	7.39	0.501**	0.276**
Conformation, puntos ^b	292	2.25	0.75	-0.531**	-0.224**
YGKG	292	98.20	19.89		
YCP	292	40.56	2.22		

N: number of observations; ED: Standard Deviation; ^a: 1=uniform, 3= without; ^b: 1= excelent, 4= canner; ^c: _r: Pearson simple correlation coefficient; ns: Spearman simple correlation coefficient. YCKG: absolut yield in Kg; YCP: relative yield in percentage.

*:P < 0.05, **: P< 0.01, ns: non significant.

Table 2. Predictive equations selected for YCKG.

Coefficient β							
	Intercept	Carcass Weight	Internal Fat Percentage	Length of achiles tendon	R ^{2a}	Cp ^b	CME ^c
1	-2.124	0.416			0.937	94.75	23.12
2	3.870	0.408	-3.092		0.949	27.29	18.88
3	18.741	0.414	-3.254	-0.835	0.953	4.00	17.36

^aDeterminación coefficient; ^bMallows coefficient; ^cSquare means error; Intercept: β_0 . All the variables were significant at 5%.

THE ON LINE MEASUREMENTS OF BEEF CARCASS QUALITY BY MEANS OF THE BIOELECTRICAL IMPEDANCE ANALYSIS

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Key Words: bioelectrical impedance, beef carcasses, resistance, reactance, video image analysis

Introduction

The method of bioelectric impedance analysis has so far been applied in research projects. Among the recent publications, there is the study presented by Balcaen et al. (2002) examining the ability of the BIA method to estimate carcass lean content of Belgian Blue bulls and to examine the impact of time after slaughter and the location of electrodes. The method was considered relatively precise and practically applicable. Allen et al. (2002) examined the accuracy of the BIA method in the estimation of lamb carcass composition. Hegarty et al. (1998) made use of the multifrequency method for the same purposes and found on the contrary that the BIA measurements taken in warm lamb carcasses explained 87% of the variation of saleable yield.

Objectives

The objective of the experiment was to ascertain the quality of the prediction of the commercial grading with the use of a measuring device with flat electrodes. Another objective was to check the reliability of new electrodes in the measurement carried out at an abattoir line with the capacity of 100 carcasses per hour.

Methodology

The experimental measurements were carried out in the company Südostfleisch, Altenburg, Germany, under normal operation. The impedance values were measured always on the left half of moving beef carcasses. Simultaneously, beef carcass classification was in progress on the line, with the use of the installed VBS2000, manufactured by E&V, based on the method of video image analysis.

a) Description of the carcass collection. In total, the evaluation reflected 441 beef carcasses, of this 198 young bulls, 7 bulls, 214 cows and 22 heifers. The physical and electrical characteristics for carcasses of the group of evaluated animals are summarized in TABLE 1.

b) Description of measuring method and device. The measurement of the bioelectric impedance was carried out with the use of an impedance analyzer described in Bohuslavek et al. (2002b). The functional properties are as follows:

Measurement of impedance and phase angle at the frequency of 1 kHz and 100 kHz; Maximum value of measurement current of 5 mA; Measurement with the use of 4-

electrode measuring method; Flat elastic electrodes with a Cu-foil, of the surface area about 30 sq.cm; Galvanic separation of impedance analyzer circuits from the mains; Automatic measurement of the beef carcass temperature; Connection and communication with PC and measurement process control and archiving of measured data.

c) Selection of measuring points. The positions of electrodes were selected in order to ensure simple anatomic definition, easy access and good contact of electrodes. Electrodes were situated at the outsides of the left half-carcasses. All electrodes were placed on the vertical lines going through heel tendon (tendo calcaneus). The upper measuring electrode was placed on the horizontal line going through the broadest part of the leg, i.e. on the musculus vastus lateralis, the lower measuring electrode was on the horizontal line going through tuber olecrani. The drive electrodes were placed 85 mm out of measuring electrodes.

d) Reference values. The carcasses of the examined animals were classified according to the SEUROP standard, according to two methods: "subjective" visual evaluation and "objective" classification with the use of technical facilities. The first method represents the usual subjective commercial grading (Conformation and Fatness) in the EU by the expert classifier, i.e. German Centre for Meat Research (BAFF) Kulmbach. The second method resulted in an objective classification carried out with the automatic classification device VBS2000, based on the method of video image analysis.

e) Output values of the analyzer. The measured impedance and phase angle were for statistical analysis calculated on real and imaginary part of impedance – resistance and reactance of parallel circuit (R_p , X_{cp}) along relationship, which is for example presented in Vrana (1989), Bohuslavek et al.(2002a).

Results & Discussion

A number of impedance values to the beef carcass conformation for all categories show correlation dependencies. For separate group of male animals (young bulls + bulls), the strongest correlations ($r = 0.86$) were identified between conformation and the value D^2/X_{cp100k} (relationship between the square of the distance between electrodes and the calculated parallel reactance at 100 kHz). Significant relations were also found for the fatness of all categories and particularly for the female categories (cows + heifers). The highest correlation has revealed in values derived from parallel reactance X_{cp1k} , measured at the frequency of 1 kHz. Also, the difference in reactance $X_{cp1k} - X_{cp100k}$ shows considerable dependence to fatness; e.g. in the category of cows + heifers the correlation coefficient was $r = 0.71$ for the fatness determined by a BAFF expert. The dependence is significantly better than that in fatness determined according to E&V, which is why this value may serve for the improvement of the E&V estimate. Using the outcome of correlation analysis, a regression analysis was carried out in order to identify the best regression models – formulae for the estimation of conformation and fatness. The formulae were examined for the quality commercial grades determined by the BAFF expert, which may be considered to be more precise. TABLE 2 shows the best calculated model for the estimate of conformation.

Conclusions

The results of the experiment confirmed that the BIA method is suitable for practical use in the instrument evaluation of the commercial value of beef carcasses. With respect to the satisfactory results of the fatness estimate, it is also possible to expect that BIA may be applied as a complementary device with the VIA system (video image analysis).

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Tables and Figures

Table 1. Characteristics of the group of evaluated 441 animals

Category	198 Young bulls + 7 Bulls		214 Cows		22 Heifers	
	Mean	S. dev.	Mean	S. dev.	Mean	S. dev.
CW ... warm carcass weight [kg]	366.20	63.70	281.18	58.13	247.25	53.17
D ... distance of electrodes [cm]	159.24	6.57	160.21	6.38	149.67	8.7
Rp1k [ohm]	133.20	16.03	192.5	24.47	189.02	27.02
Rp100k [ohm]	82.08	11.59	121.39	16.94	117.56	18.69
D ² / Xcp1k [cm ² / ohm]	- 7.50	1.66	- 5.74	1.55	- 5.12	1.29
Conformation subclasses* [15]	9.63	2.6	13.13	1.72	12	1.87
Fatness subclasses* [15]	6.28	1.59	6.39	2.59	6.76	2.28

* Grading system EUROP (divided into 15 subclasses) for conformation and fatness respectively

Rp1k ...parallel resistance by frequency 1 kHz , Rp100k ... parallel resistance by frequency 100 kHz

Xcp1k ... parallel reactance by frequency 1 kHz

Table 2. Regression models for beef carcasses

Model no.	Dependent variables	Independent variables	Equations	r ² / r ^{**}	SEE*
1.	Conformation* [15]	CW D D ² /Rp100k D ² /Xcp100k	Conformation = 6.726 - 2.427e ⁻⁰² * CW + 8.487*10 ⁻⁰² *D + 2.034*10 ⁻⁰² * D ² /Rp100k + 9.652*10 ⁻⁰² * D ² /Xcp100k	0.85 / 0.92	1.08

* [SEE]...stand. error of estimation r² / r^{**} ... coefficient of correlation / determination;

all regression coefficients in the equations are significant at P<0.001

TENDERNESS AND COLLAGEN CHARACTERISTICS OF STEERS FINISHED AS CALVES OR YEARLINGS

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Key Words: Beef, Finishing, Management Systems, Tenderness, Collagen

Introduction

Lack of consistency is one of the biggest concerns for today's beef industry. Inadequate tenderness and lack of uniformity in cattle ranked first and second, respectively, as the greatest challenges for all sectors of beef production (Smith, Savell, Morgan, & Montgomery, 2000). The National Beef Quality Audit stated that lack of uniformity in cattle is the concern for purveyors, restaurateurs, and retailers, which has shown the least improvement of the tracked concerns since 1991. Animal management plays a key role in the quality of the final end product. Calf finishing systems in the U. S. use large amounts of grain to feed high concentrate diets to weanlings for extended periods of time. In contrast, yearling systems are more extensive, growing calves for a longer period of time on forage before being fed a high concentrate diet for a short period prior to harvest. This reduction of days on a high concentrate diet may reduce costs; however, decreased days on feed have been associated with lower quality grades and less tender beef (Miller, Cross, Crouse, & Tatum, 1987; May, Dolezal, Gill, Ray, & Buchanan, 1992). Regardless of the management practice used to increase profitability, cattle produced must be of the type that are acceptable for the feedlot and yield a final product desirable to the consumer.

Literature provides varied results when comparing meat quality from finished calves and yearlings. Calves have been reported to produce carcasses with increased fat thickness, higher numerical yield grades and quality grades (Lunt and Orme, 1987), and more tender meat (Dikeman, et al., 1985; Johnson, Huffman, Williams, & Hargrove, 1990). However, others have shown minimal effects on carcass quality grade or meat palatability (Dikeman, Dayton, Hunt, Kastner, Axe, & Ilg, 1985; Huffman, Williams, Hargrove, Johnson, & Marshall, 1990).

Collagen solubility decreases (Hill, 1966; Cross, Carpenter, & Smith, 1973) and collagen cross-linkages increase (Goll, Bray, & Hoekstra, 1964; Goll, Hoekstra, & Bray, 1964; Cross, et al., 1973) in muscle as physiological age increases. Miller, Cross, Crouse, & Jenkins (1987) found decreased insoluble collagen and a higher percentage of newly synthesized soluble collagen in mature cows that had received a high energy diet prior to slaughter. This led to lower Warner-Bratzler shear force values and higher sensory tenderness scores compared to mature cows on a low-energy diet.

Objectives

Therefore, this study was conducted over a two year span to determine if collagen characteristics were responsible for tenderness differences of steers produced in the calf-fed and yearling systems when fed to a constant fat thickness.

Methodology

Steers (3/4 British, 1/4 Continental) were randomly assigned to be finished as calves (CF) or yearlings (YF) at weaning. Thirty-five and 41 calves and 42 and 41 yearlings were designated in year 1 and year 2, respectively.

Each year at weaning, CF steers were implanted (Synovex-S[®]) and were adapted from a 50% concentrate diet to a 92.5% concentrate finishing diet (TDN 84%, CP 12%) fed until harvest. Reimplantation (Revalor-S[®]) occurred after 90 d on feed. All steers were fed to an estimated 12th-rib fat thickness endpoint of 1 cm. To achieve this, Year 1 steers were on feed for 203 d and Year 2 steers were fed for 180 d. The CF steers were approximately 13 to 14 months old at the time of harvest.

The YF steers were drylot for 60 d, until corn stalks became available for grazing. While in drylot, these steers were fed ammoniated wheat straw ad libitum and supplemented with mineral and 2.27 kg/head/d (DM basis) of wet corn gluten feed. Steers then grazed corn stalks for 78 d in Year 1 and 91 d in Year 2. Hay was supplemented during heavy snow cover. After grazing corn stalks, steers were again drylot for the remainder of the wintering period until pasture was available for spring and summer grazing. Spring drylot was 64 d in Year 1 and 50 d in Year 2. Following the spring drylotting period, steers grazed pastures for 96 d in Year 1 and 103 days in Year 2. Steers were implanted (Revalor-G[®]) prior to summer grazing. Spring grazing pastures consisted of smooth bromegrass. Summer grazing pastures consisted of big bluestem, indiangrass, and switchgrass. Following the summer grazing period, steers entered the feedlot, were reimplanted (Revalor-S[®]), blocked by weight, and assigned randomly to one of two pens. Steers were then fed similarly to the CF for receiving and finishing periods. This final finishing period consisted of 93 d in Year 1 and 90 d in Year 2. The YF steers were approximately 19 to 20 months old at the time of harvest.

Steers were harvested in a commercial slaughter facility. Shortly after being bled, carcasses were electrically stimulated with 8 to 10 low voltage (40 V) pulses. Hot carcass weights were obtained from all steers at the time of slaughter. In Year 1, carcasses were chilled for an extended 48 hour weekend chill period. Carcasses in Year 2 were chilled for approximately 42 hours. A marbling score was assigned to the carcass by the USDA grader. Other carcass data were measured and evaluated by experienced University of Nebraska personnel. Carcass measurements were used to calculate yield and quality grades. A boneless beef strip loin (IMPS #180) was collected from the left side of each carcass. Two strip loins from CF cattle were lost during the fabrication process, so additional data analysis continued on 34 and 40 strip loins in Year 1 and Year 2, respectively.

At 7 d postmortem, strip loins were cut into 2.54 cm *M. longissimus dorsi et lumborum* steaks for proximate analysis, Warner-Bratzler shear force (WBS) and sensory

panel evaluation. Individual steaks were designated for aging time (7, 14, and 21 d), shear force, and sensory evaluations, wrapped, and frozen at -22 °C until further analyses.

Frozen steaks were thawed at 4 °C for 24 h prior to cooking. Steaks were cooked to an internal temperature of 70 °C (AMSA, 1995) on a Farberware Open-Hearth broiler (Model 455N, Walter Kidde and Co., Bronx, NY). After cooling, 8 to 10 cores (1.27 cm in diameter) were removed and sheared using a Warner-Bratzler shear attachment to an Instron Universal testing machine (Model 55R1123, Canton, MA). The mean peak shear force (kg) of at least 6 cores was calculated for each steak.

Samples from the CF and YF steers were analyzed for soluble, insoluble, and total collagen according to Hill (1966) and Cross, et al., (1973). Soluble collagen was calculated with a 7.52 constant, and insoluble was calculated with a 7.25 constant (Goll, Bray, & Huekstra, 1963).

Steaks for sensory evaluation were cooked by the same procedure as described for WBS. After cooking the steaks were cut into 1 x 2 x 1 cm pieces for evaluation. Samples were served to a consumer sensory panel ($n \geq 30$) in individual booths under red lighting to mask differences in meat color. An 8-point Hedonic scale (8 = extremely desirable; 1 = extremely undesirable) was used to evaluate tenderness, juiciness, flavor, and overall acceptability.

Carcass traits, chemical characteristics, WBS, and consumer sensory panel data were analyzed using GLM procedure of SAS (1999). Both years were pooled and each steer was an experimental unit for carcass traits and chemical characteristics. Steaks at each aging time were experimental units for shear force and sensory evaluations.

Results & Discussion

Carcass characteristics for CF and YF steers are summarized in Table 1. The YF steers yielded heavier ($P < 0.01$) carcass weights with larger ($P < 0.01$) longissimus muscle areas, and less ($P < 0.01$) internal fat. They also had lower ($P < 0.01$) marbling scores, USDA quality grades, and percentage of carcasses grading USDA Choice or higher when compared to carcasses of CF steers. The differences in marbling scores were confirmed with chemical analysis (8.5 versus 5.5% fat).

As expected, increased aging time from 7 to 14 to 21 d produced steaks with lower ($P < 0.05$) shear force values, regardless of production system (Table 2). Steaks from CF had lower ($P < 0.01$) shear force values at 7, 14, and 21 d of age (Table 2) than steaks from yearlings. The YF steers had steaks that, after aging 21 d, had similar shear force values to CF steaks aged 7 d. They were also rated higher ($P < 0.05$) for tenderness, as well as juiciness, flavor, and overall acceptability (Table 3) after 7 and 14 days of aging. This was also true when comparing all Choice steaks or all Select steaks ($P < 0.05$).

The lower WBS values and higher sensory tenderness scores may be attributed to the collagen differences in the two groups. The CF had a more insoluble ($P < 0.01$), soluble ($P < 0.01$), and total collagen ($P < 0.01$) than the yearlings (Table 4). The higher total and insoluble values are probably due to dilution of collagen by muscle fiber growth. However, the collagen from the CF steers was much more soluble ($P < 0.01$) than from the YF steers (39.7 versus 24.4%, respectively). Hill (1966) demonstrated that collagen solubility decreases in muscle as physiological age increases. Solubility of collagen has been shown to be positively correlated to textural characteristics (Goll, et al., 1963).

Miller, Tatum, Cross, Bowling, and Clayton (1983) did not find these results. However, Aberle, Reeves, Judge, Hunsley, and Perry (1981) hypothesized that beef cattle feed high concentrate diets would have higher protein turnover and thus greater collagen solubility that would allow for less cross-linkages and more tender beef. The CF steers were on a high concentrate diet for a longer period of time which supports that rationale.

Conclusions

Growing steers for a longer period of time on forage with a short finishing period resulted in heavier carcasses with lower quality grades and beef that was less tender than CF steers. Steers finished as calves spent more days in the feedlot and in this study produced beef that was more tender and possessed more acceptable eating characteristics than yearlings. Collagen differences, especially soluble collagen amounts, may help explain the lower shear force values and higher tenderness ratings for the CF steers.

Tables and Figures

Table 1. Comparison of means for carcass characteristics from calf- and yearling-finished steers.

Trait	Calves		Yearlings		P-value
	Mean	SE	Mean	SE	
Hot carcass weight, kg	315.4 ^b	3.37	375.9 ^c	3.18	<0.0001
Fat thickness, cm	1.39	0.045	1.30	0.042	0.1880
Adjusted fat thickness, cm	1.50	0.039	1.42	0.037	0.1479
Longissimus muscle area, cm ²	72.76 ^b	0.67	81.06 ^c	0.63	<0.0001
Kidney, pelvic, heart fat, %	2.33 ^c	0.053	2.07 ^b	0.050	0.0004
Yield grade	3.49	0.054	3.46	0.050	0.6423
Marbling score ^a	454.1 ^c	8.80	346.1 ^b	8.28	<0.0001

^a Marbling score: modest = 500-599; small = 400-499; slight = 300-399.

^{bc} Means in the same row without a common superscript are different ($P < 0.01$)

Table 2. Mean shear force values for steaks aged 7, 14, and 21 days from calf- and yearling-finished steers.

Age, d	Calves		Yearlings		P-value
	Mean	SE	Mean	SE	
7	3.30 ^{b,c}	0.089	4.09 ^e	0.109	<0.0001
14	3.07 ^b	0.089	3.74 ^d	0.109	<0.0001
21	2.79 ^a	0.089	3.40 ^c	0.109	<0.0001

^{abcde} Means without common superscripts are different ($P < 0.05$).

Table 3. Mean sensory panel ratings for steaks aged 7 or 14 days from calf- or yearling-finished steers.

Age, d	Trait	Calves		Yearlings	
		Mean	SEM	Mean	SEM
7	Juiciness	5.08 ^d	0.032	4.88 ^e	0.039
	Tenderness	5.46 ^a	0.034	4.56 ^c	0.043
	Flavor	4.96 ^d	0.033	4.64 ^e	0.041
	Overall Acceptability	5.07 ^d	0.032	4.47 ^e	0.039
14	Juiciness	4.86 ^e	0.032	4.61 ^f	0.039
	Tenderness	5.59 ^b	0.034	4.63 ^c	0.043
	Flavor	4.99 ^d	0.033	4.70 ^e	0.041
	Overall Acceptability	5.03 ^d	0.032	4.49 ^e	0.039

^{abc}Means for a given trait without a common superscript are different ($P < 0.05$).

^{def}Means for a given trait without a common superscript are different ($P < 0.01$).

^zConsumer panel rating for each trait: 8=extremely desirable; 1=extremely undesirable.

Table 4. Collagen (mg/g) in the longissimus muscle of calf- or yearling-finished steers.

Collagen	Calves		Yearlings		<i>P</i> -value
	Mean	SE	Mean	SE	
Insoluble	8.11 ^a	0.391	6.19 ^b	0.357	0.0004
Soluble	6.23 ^a	0.408	2.21 ^b	0.372	<0.0001
Total	14.52 ^a	0.742	8.46 ^b	0.678	<0.0001
% Soluble	39.72 ^a	2.528	24.38 ^b	2.308	<0.0001

^{ab} Means in the same row without common superscript are different ($P < 0.01$).

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TENDERNESS OF MEAT FROM CALVES SLAUGHTERED AT A TIME WITH MAXIMAL MUSCLE PROTEIN DEGRADATION

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Key Words: Beef; Tenderness; Compensatory growth; Fractional breakdown rate; Muscle

Introduction

The association between tenderness and proteolysis in meat is well established and a linkage to muscle protein degradation in the live animal seems also settled. Thus, an optimisation of tenderness could involve slaughter of the animal at a time with maximal muscle protein degradation and thus expected maximal proteolysis *post mortem*. Experiments with steers (Jones et al., 1990) and rats (Millward et al., 1975) have suggested, that compensatory growth may be a way to maximise muscle protein turnover and thus muscle protein degradation. Thus, in a previous experiment we studied if muscle protein degradation reaches a maximum level during compensatory growth of young Friesian bull calves compared with bull calves offered feed ad libitum throughout the experiment (Therkildsen, 2005). We found that when 5 months old bull calves are fed restrictively for 3 months (50% of ad libitum) followed by ad libitum feeding, they respond with maximal fractional muscle protein degradation 5 to 8 weeks after the change to ad libitum feeding, and the level exceeded the level in control calves by 50 to 25% in this period, respectively. Thus, it is expected that if the calves had been slaughtered after 5 to 8 weeks of ad libitum feeding following the 3 months restriction period, the meat tenderness would have been superior to the tenderness of meat from control calves. Thus, a second experiment with similar calves was conducted to test that hypothesis.

Objectives

The aim of the study was to test if slaughter at a time with maximal muscle protein degradation would improve tenderness of beef.

Methodology

The experiment included 12 Holstein Friesian bull calves born after two sires. The calves entered the study at the age of 5 months and were allocated to two feeding strategies either ad libitum (AA) or restricted/ad libitum (RA) with respect to sire and age. The calves on the AA feeding strategy had free access to a concentrate mixture and a total mixed roughage ration (TMR). The calves on the RA feeding strategy were

restricted in energy intake from 6 to 8 months by allowing them free access to the TMR ration. From 6½ months to the end of the restriction period they were supplied with 1 kg of the concentrate mixture each day. From 8 months they were realimentated and given free access to the concentrate mixture and the TMR ration in 6 weeks. The first week of realimentation the calves were gradually adjusted to the free access of concentrate. All calves were slaughtered at the age of 10 months. The calves were weighed at two consecutive days at the beginning of the experiment and at finish of the restriction period and on two consecutive days prior to slaughter.

Urine collection

Two days before slaughter urine was collected from the animals over a 24 h period in order to calculate the fraction breakdown rate of myofibrillar protein on the basis of 3-methylhistidin concentration in urine as described in Therkildsen (2005).

Slaughter procedure

The calves were slaughtered over two days in two following weeks, with 6 calves each day. The calves were slaughtered at the experimental slaughterhouse at Research Center Foulum (500 m), stunned by captive bolt pistol, hung and bled. The carcass was split and weighed. The carcasses were chilled at 12°C for 4 h *post mortem* and then stored at 3°C. Forty-eight hours *post mortem* the carcasses were weighed. *M. semimembranosus* (SM) were cut from both sides, weighed, vacuum-packed and aged until either 7 or 14 days *post mortem* at 3°C before storage at -20°C. The ageing time were switched between the carcass sides. The *M. longissimus dorsi* (LD) from the 2nd to the 5th lumbar vertebra from both sides were cut and aged as described for SM. In addition samples for shear force determination of LD was removed from 10th to 13th thoracic vertebra from both sides and cut in blocks of 7 cm and aged for 2 days (left side, cranial), 7 days (left side, caudal) and 14 days (right side, caudal) before storage as described for samples for tenderness determination.

Shear force

Vacuum-packed samples were heated to 62°C in a water bath and exercised to rectangular blocks of 1.0 x 1.0 cm thick. Shear force was measured with a Texture Analyser HD100 equipped with a Warner Bratzler shear blade with rectangular hole.

Sensory evaluation of tenderness

The SM was prepared as roasts in an oven (160°C) until a core temperature of 60°C was reached. The LD was prepared as 1.5 cm thick steaks placed on a roasting pan in an oven at 160°C and heated for 3 minutes on each side. A 10 member sensory panel evaluated the tenderness of the meat on a 15 cm unstructured line scale.

Statistics

The data was analyzed using a mixed model (SAS[®] System, 1996) with the fixed effects of feeding strategy and sire and the interaction between them. In the analysis of

the sensory determination of tenderness, judge and (animal x feeding strategy) was included in the model as a random effects.

Results & Discussion

The bull calves in the present experiment was slaughtered at an age of 10 months and with a live weight of 327 and 407 kg of RA and AA calves, respectively (see table). The live weight of the RA calves do not represent a typical slaughter group in the Danish beef slaughter categories, and was only a result of the wish to set up a similar experiment as the one, which showed a maximal fractional breakdown rate after 5 to 8 weeks of ad libitum feeding of young bull calves following restrictive feeding in 3 months from the age of 5 months. Thus, for an implementation of the compensatory feeding strategy it should be further developed to fit with the live weights of market slaughter categories.

At the time of slaughter, the RA calves had not shown compensatory growth yet, but the fractional break down rate of muscle protein was higher than in the AA calves, which do suggest a larger muscle protein turnover at the time of slaughter (see table). However, when the shear force of LD was measured no significant effect of the feeding strategy was observed, whereas the sensory evaluation of the tenderness of LD showed a better tenderness of the meat from calves fed ad libitum through out the experiment (AA) both after 7 and 14 days of aging. In contrast, the tenderness of SM was positively affected by the compensatory feeding strategy both after 7 and 14 days of aging.

The explanation of the different response of the muscles to the feeding strategies and the non expected negative effect on tenderness of LD of the compensatory growth strategy is not clear. The different location of the two muscles and thus different functions in the animal most probably play a role, although fiber type frequency and area percentage is very similar in the two muscles according to the results of Kirchofer et al. (2002). However, Andersen (1975) demonstrated a very different growth pattern of the two muscles, i.e. the growth coefficient of LD being around 1.0 whereas the growth coefficient of SM is calculated to 0.78. Also, the oxidative capacity may vary between the muscles, in spite of similar fiber type distribution. The above facts may all result in different protein turn over and especially difference in muscle protein degradation, which lead to different effect on tenderness of the meat.

Conclusions

Slaughter of 10 months old bull calves after 6 weeks of ad libitum feeding following a 3 months period with restrictive feeding resulted in high fractional muscle protein breakdown rate and a positive effect on the tenderness of SM, but a negative effect on the tenderness of LD compared with similar bull calves fed ad libitum throughout the experimental period. The reason for the different response of the muscles is an issue for future studies.

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Tables and Figures

Table 1. Fractional breakdown rate, shear force and tenderness of bull calves slaughtered at a time with maximal muscle protein degradation (RA) and of control bull calves (AA)

Feeding strategy	AA	RA	SEM	P-value
Age at slaughter, days	301	304	3.5	ns
Weight at slaughter, kg	407	327	8.6	0.001
Fractional breakdown rate, %	1.84	2.57	0.24	0.07
Shear force				
Day 2, kg	8.70	8.50	1.10	ns
Day 7, kg	5.33	6.65	0.75	ns
Day 14, kg	4.36	4.82	0.24	ns
Tenderness LD [#]				
Day 7	8.2	6.2	0.62	0.08
Day 14	9.2	7.1	0.41	0.001
Tenderness SM [#]				
Day 7	8.5	10.2	0.69	0.06
Day 14	9.8	11.6	0.68	0.09

[#]Sensory evaluation of tenderness on a scale from 0-15, 15 being extremely tender

EFFECT OF SEX AND GROWTH PATH ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF PELIBUEY SHEEP: PRELIMINARY RESULTS

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Key Words: carcass, color, Pelibuey, texture, meat quality

Introduction

The interest and demand for Pelibuey sheep in Sonora has been increasing. The evidence of this trend is the increase in the production of this species and the incorporation of new producers. The Pelibuey is the most widespread breed of sheep in Cuba and is an animal very tolerant to extreme environmental conditions.

Carcass composition is influenced by weight as well as genetics and breed differences (Fogarty *et al.* 2000), sex of the lamb (Lee *et al.* 1990), and growth path (Murphy *et al.* 1994). Color, texture and flavor are considered the most important meat quality criteria in lamb. Meat texture is considered the most important parameter for consumers and is mainly influenced by pH (Devine *et al.* 1993), the contractile state, state of myofibrillar components and connective tissue properties. Meat characteristics change distinctly due to the age or growth of the animal and, in addition, they are also markedly influenced by the breed and sex of the animal.

This research is a part of an inter-institutional ongoing project aiming to compare growth conditions on carcass and meat quality. Sheep producers need information about the meat quality that is taking place and will provide the basic information to devise breeding programs that enhance quality and take into account regional variations.

Objectives

The purpose of this study was to evaluate the effect of growth rate and sex on carcass composition and meat quality of this breed as a basis to implement a strategy to produce carcass.

Methodology

Carcass

Thirty Pelibuey sheep from the same producer were used. Three groups consisting intact males, castrated males and females were established, and each group consisted of ten sheep with the same average live weight, and the animals were housed in individual pens. All animals were fed *ad libitum* with a commercial diet composed of protein (13%), corn (15%), barley (40%), and the appropriate amount of minerals and vitamins.

After 153 day feeding, the sheep were shipped to the plant of slaughter of the Agriculture and Cattle Department of the University of Sonora. All animals were approximately 215 days of age at the time of the slaughter. All sheep were slaughtered using standard commercial procedures, according to welfare codes of practice. Live and hot carcass weights were collected immediately after of slaughter. Carcasses were chilled at $4 \pm 1^\circ\text{C}$, and at approximately 24 h postmortem cold carcass weight was recorded and the carcasses were ribbed between the 12th and 13th ribs. Dressing percentage was calculated according to the following equation: $100 \times \text{cold carcass weight} / \text{slaughter live weight}$.

The following carcass morphology measurements were assessed according to the methodology described by De Boer *et al.* 1974: carcass length, internal depth of breast, limb length and thickness. The longissimus muscle area at the 12th rib was recorded using a graduate sheet of acetate placed on the surface of the loin; both sides of carcass were recorded. Dorsal fat thickness was recorded with a digital caliper (Mitutoyo, Japan).

The meat pH was measured using a penetrating electrode and thermometer (Hanna, USA) in the *m. longissimus* between the 12th and 13th ribs, 45 min after the slaughter and 24 h *postmortem*, three readings were made. Color was measured using a Minolta Spectrophotometer (CM2600d model), in the *m. longissimus* between the 12th and 13th ribs, 24 h *postmortem*, CIE L*, a* and b* values were recorded.

Meat

At 24 hours after slaughter *Mm. longissimus thoracis* (LT) and *semimembranosus* (SM) muscles were removed from carcass for sarcomere length and texture evaluations.

Sarcomere length was determined by the method described by Torrescano *et al.* (2003). Shear force was measured on raw and cooked meat. Samples were cooked in a water bath at 80°C to an internal temperature of 75°C , then cooled in running tap water for 45 min and stored in a refrigerator for approximately 4 hours. Shear force was measured with a Warner Bratzler device mounted on an Instron 1132 (Instron Corporation, USA), on ten cores with section 1 x 1 cm for each animal.

Statistical analysis

Significance differences ($p < 0.05$) among samples were determined by analysis of variance (ANOVA) using the Least Square Difference method of the General Linear Model procedure of SPSS (SPSS 1995).

Results & Discussion

The means of carcass yield and quality measurements in each group are shown in Table 1. Significant differences in live weight were observed between groups, standing out intact male with 43.8 kg ($p < 0.05$), this data is in agreement with that reported by the FAO for this breed, the weight at 300 days is 39 kg and 35 kg, for males and females respectively. On the other hand, hot and cold carcass weights showed no differences ($p > 0.05$) among groups evaluated. Cold carcass weights ranged from 21.5 to 22 kg, being the female carcass the lightest. The lowest dressing percentage corresponded to intact male (49.3%) while castrate male and female had 55.6 y 56 %, respectively. Although no official sheep carcass grading standards exist in Sonora, the sheep carcass in this study had greater body weight than those in most of the studies reviewed by Sañudo *et al.* (2003), which may have resulted in compositional differences.

Morphological measurements on the carcass also showed significant differences among sexes, especially for carcass length, where female carcass showed had longer ($p < 0.05$) carcass than male carcass. Carcasses from female sheep had smaller ($p < 0.05$) internal depth of breast than the rest of the groups. Carcasses from castrate male had longer limb length and limb thickness ($p < 0.05$). No differences in loin area and fat thickness ($p > 0.05$) were observed among groups, averaging 13.5 cm² and 0.15 cm, respectively.

Table 2 shows the values of pH and color of the carcasses evaluated. At 45 min the female carcass had the higher pH value (6.8). At 24 hours the pH was 5.7 for the three sexes, which can be considered as normal. L* values showed that females were lowest ($p < 0.05$), indicating darker samples; a* and b* were more redness and yellowness ($p < 0.05$) in this sex. Intact and castrate males were similar in these parameters, indicating that male castration not have influence in all these color parameters.

Means and standard deviations for sarcomere lengths and texture are presented in Table 3. The data reveal significant differences in sarcomere lengths values in LT muscle ($p < 0.05$) between samples of female (2.1 µm) and males (1.8 µm). The raw meat from castrate males presented a higher value of shear force, in the three muscles evaluated. However, in this sex breed, a lower shear force on cooked meat was observed; the intact males and females were significantly tougher than castrate males.

The advantage of castrate males is primarily in the greater cold carcass weight and meat tenderness.

Conclusions

The sheep sex has an important effect on meat quality characteristics. In particular, the castrate males show very good morphological traits and tenderness. Although there was a tendency to lower tenderness in carcasses, sensory analysis with consumers would be needed to confirm this trend. The information generated by this project will be important for devising breeding strategies to meet the market demands.

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Tables and Figures

Table 1. Means and standard deviations of carcass yield and quality measurements stratified by sex.

Item	Intact male	Castrate male	Female
Live wt, kg	43.8 ± 2.4 ^b	39.5 ± 1.9 ^a	38.2 ± 4.0 ^a
Hot carcass wt, kg	24.3 ± 3.8 ^a	24.1 ± 3.0 ^a	22.2 ± 2.7 ^a
Cold carcass wt, kg	21.6 ± 2.7 ^a	22.0 ± 1.9 ^a	21.5 ± 2.5 ^a
Dressing percentage	49.3 ± 6.4 ^a	55.6 ± 6.0 ^b	56.0 ± 2.6 ^b
Carcass length, cm	49.0 ± 2.5 ^b	36.0 ± 1.3 ^a	57.7 ± 2.1 ^c
Internal depth of breast, cm	20.0 ± 2.1 ^b	22.0 ± 2.3 ^b	17.6 ± 0.4 ^a
Limb length, cm	38.1 ± 3.1 ^a	49.0 ± 3.4 ^b	38.8 ± 2.4 ^a
Limb thickness, cm	20.0 ± 2.1 ^b	22.0 ± 2.3 ^b	17.6 ± 0.4 ^a
LT muscle area, cm ²	13.9 ± 1.3 ^a	13.4 ± 0.9 ^a	13.5 ± 0.4 ^a
Fat thickness, cm	0.10 ± 0.03 ^a	0.19 ± 0.13 ^a	0.18 ± 0.10 ^a

^{a-b}Means on the same row with different superscript differ significantly (P<0.05)

Table 2. Measures of pH and color of carcass stratified by sex.

Item		Intact male	Castrate male	Female
pH	45 min PM	6.2 ± 0.08 ^a	6.2 ± 0.2 ^a	6.8 ± 0.2 ^b
	24 h PM	5.7 ± 0.03 ^a	5.6 ± 0.1 ^a	5.7 ± 0.01 ^a
Color	L*	45.1 ± 3.56 ^b	40.11 ± 11.02 ^b	30 ± 2.3 ^a
	a*	18.8 ± 3.38 ^a	20.25 ± 6.2 ^a	25.3 ± 2.6 ^b
	b*	16.1 ± 3.05 ^a	18.26 ± 3.99 ^a	23.5 ± 2.2 ^b

^{a-b}Means on the same row with different superscript differ significantly (P<0.05)
PM: *post mortem*

Table 3. Measures of sarcomere length and texture 24 h *postmortem*.

Item		Intact male	Castrate male	Female
Sarcomere length (µm)	LT	1.8 ± 0.1 ^a	1.8 ± 0.2 ^a	2.1 ± 0.2 ^b
	SM	2.1 ± 0.2 ^a	2.1 ± 0.2 ^a	2.2 ± 0.2 ^a
Shear force (kgf)	raw			
	LL	2.7 ± 0.5 ^b	3.04 ± 0.76 ^b	1.2 ± 0.3 ^a
	LT	2.0 ± 0.07 ^a	2.38 ± 0.5 ^a	1.2 ± 0.3 ^a
	SM	4.5 ± 1.6 ^b	5.88 ± 1.2 ^b	2.7 ± 1.1 ^a
	cooked			
	LL	2.6 ± 0.7 ^a	2.58 ± 0.24 ^a	2.7 ± 0.7 ^a
	LT	2.5 ± 0.08 ^a	2.33 ± 0.3 ^a	2.6 ± 0.7 ^a
	SM	3.1 ± 0.5 ^b	2.03 ± 0.59 ^a	3.2 ± 0.7 ^b

^{a-b}Means on the same row with different superscript differ significantly (P<0.05)

THE EFFECT OF GENOTYPE AND CARCASS HANGING METHOD ON MEAT QUALITY

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Key Words: genotype, hanging method, meat quality

Introduction

There are many reports that suggest differences in meat quality due to genotype (Chambaz *et al.*, 2003, Monson *et al.*, 2004, and Sinclair *et al.*, 2001), whilst other reports suggest no or limited differences in meat quality due to genotype (Garipey *et al.*, 1999, and Laborde *et al.*, 2001). Currently 53 and 47% of prime beef production in Northern Ireland originates from the beef and dairy herd, respectively. Based on various economic projections from the Department of Agriculture and Rural Development in Northern Ireland (DARDNI), these figures are likely to change in a subsidy free environment, which we have just entered, with beef from the dairy herd having a greater importance to prime beef production.

Lundesjo *et al.*, 2002 reported that with the use of pelvic suspension (tender stretch) young bulls could approach the tenderness of heifers, both through sensory and instrumental evaluation. Hwang *et al.*, 2002 reported tender stretched hung carcasses produced meat with a longer sarcomere length, a lower shear force requirement and a higher tenderness ratings relative to Achilles tendon hung carcasses. The influence of production factors on meat quality are reported in the literature to account for 40% of the variation in meat eating quality (Polkinghorne, 1998). However, a change in post-slaughter treatment such as a change in carcass hanging method may alter the effects of production factors. Indeed, it should be noted that many researchers that have undertaken studies observing the effect of genotype on meat quality have not stated the method of hanging in their methodology.

Objectives

The aim of this study was to investigate the effect of genotype (dairy versus beef genotype) and hanging method (Achilles tendon versus Tender stretch) on meat quality.

Methodology

Animal Husbandry

The study involved 20 Charolais (CH) steers (greater than 75% CH genetics), initial weight range 409-639kg, and 20 purebred Holstein (HOL) steers, initial weight range 440-669kg. The animals were sourced from farms across Northern Ireland and taken to the Agricultural Research Institute of Northern Ireland for finishing. Animals were penned in groups of 3, according to genotype and live-weight and were finished indoors on a common diet, for the duration of the finishing period, which lasted 98 days. The finishing diet consisted of grass silage fed *ad libitum* supplemented with 4.5 kg beef concentrate.

Animal Performance

Animals were weighed on two consecutive days at the start and end of the study, to enable an initial and final live weight be calculated. Growth rate was calculated as the weight difference between the initial and final weight, divided by the duration of the study.

Slaughter procedure

At the end of the study all animals were taken to the abattoir on the night prior to slaughter and after clipping, were randomly penned according to genotype. On the morning of slaughter, after 11 hour in lairage, the animals were put through a cattle crush and allocated a kill number. Immediately afterwards the animals were sent for slaughter. The order of kill was a batch of CH, followed by a batch of HOL, followed by the remaining CH and then the remaining HOL animals. The animals were stunned with a captive bolt stun gun before being stuck, and were dressed according to the European standards. None of the carcasses were electrically stimulated.

Carcass Measurement

The internal kidney, channel and cod (KCC) fat were collected and weighed. The carcasses were graded by a Livestock and Meat Commission (LMC) grader for conformation score and fat classification according to the European scale. For the purpose of statistical analysis the conformation scores (EUROP) were converted to numerical values with E =5, U=4, R = 3, O+ = 2.5, O =2, O- =1.75 and P=1; while fat classification was converted to 1=1 (lean), 2=2, 3 =3, 4L =4, 4H =4.5 and 5 = 5 (fat).

28 hours post-mortem carcasses were split between the 10th and 11th rib (but not quartered) and marbling score assessed according to the USDA marbling scale, also subcutaneous fat measurements were taken at 3 points.

Hanging method

At 40 minutes post-mortem one side of each carcass was hung by the Achilles tendon (AT), while the other side was re-suspended from the pelvis using the tender stretch (TS)

procedure. The first batch of CH and HOL carcasses had their right side hung by the AT and their left by TS, whilst the second batch had their right side hung TS and left side AT. Carcasses entered the chill 45 minutes post-mortem and were chilled under standard commercial conditions.

Meat Quality

The sides were quartered 30 hours post-mortem and a small sample of *longissimus dorsi* (LD) muscle was taken for determination of the sarcomere length using the laser diffraction method as described by Okeudo and Moss (2004). Carcasses were boned out 48 hours post-mortem and the fore-rib joints (6th - 10th ribs) were taken back to the laboratory for meat quality assessment. The LD was removed from the fore-rib joint and sliced into 2.54 cm steaks. These were vacuum packed and stored in a cold room at <4°C, until 7 days post-mortem, when further meat quality assessments were undertaken.

Ultimate pH was measured in the laboratory by inserting a spear point ISFET pH probe (Sentron, Model 1072 LanceFET™) attached to a portable pH meter (Sentron 3001).

Steaks were cooked in the vacuum bag in a hot water bath at 75°C for 50 minutes. The meat was thereafter cooled in a bucket containing ice for 1 hour. The cooked meat was subsequently patted dry with paper towel and placed in a self sealing polythene bag and stored in a refrigerator overnight (<4°C). Cooking loss was the weight loss during cooking divided by fresh sample weight and expressed as a percentage. Ten cores were drilled from each sample along the muscle bundle long axis using a 1.27cm diameter cork borer. The measurement of texture was carried out by shearing the core transversely on a 1kN load cell attached to a Warner-Bratzler shearing device mounted on an Instron Universal Testing Instrument (model 6021). The instrument was calibrated to measure over a range 0-20.38 kg and sheared each core of meat at a speed of 100 mm/minute. Texture was recorded as peak shear force (kg.cm⁻²).

Steaks were removed from the vacuum packs, a 4 mm slice was removed from each steak, discarded and the remaining 2.5 cm slice allowed to bloom with the freshly cut surface uppermost prior to taking the colour measurement. Reflectance spectra of the freshly cut meat surface were measured using a 0°/45° illumination viewing geometry-head, attached to a Monolight 6800 Spectrophotometer (Macam Photometrics, Livingston, Scotland,UK). The instrument was standardized by using black, grey and white reference tiles. The reflectance spectra were recorded continuously from 380nm to 800nm. The spectral data were analysed by computer to provide data in the CIE (Commission Internationale de l'Elairage) tristimulus colour values X,Y and Z and then transformed to CIE L*, a* and b* values.

Statistical Analysis

Data were analysed using analysis of variance for the main effects of genotype (CH vs HOL) and hanging method (AT vs TS) and their interactions. The meat quality data was adjusted by covariance for age at slaughter and carcass weight. In addition relationships of meat quality parameters to carcass parameters were tested for linear relationships.

Results & Discussion

Animal Performance

The main effects of genotype on animal performance are presented in Table 1. Genotype had no effect ($P>0.05$) on live weight gain. CH steers had a higher kill out percentage ($P<0.001$) and produced heavier ($P<0.001$), better conformed ($P<0.001$) carcasses, with a lower KCC fat ($P<0.001$) content and marbling score ($P<0.001$) relative to HOL steers. Although genotype had no significant ($P>0.05$) effect on fat classification, when genotypes were compared at constant carcass weights (Lively et al., 2005) CH carcasses were leaner ($P<0.01$) than HOL carcasses.

Meat quality

The main effects of genotype and hanging method on meat quality are presented in table 2. Neither genotype nor hanging method had any significant effect on meat colour as assessed by L^* , a^* or b^* or ultimate pH. CH meat had a significantly ($P<0.001$) higher cooking loss and Warner Bratzler shear force (WBSF) relative to the HOL meat, but had no significant ($P>0.05$) effect on sarcomere length. Tender stretching significantly ($P<0.001$) decreased WBSF and increased sarcomere length, but did not alter ($P>0.05$) cooking loss. There was a significant ($P<0.001$) genotype by hanging method interaction for WBSF. The TS hanging had a greater improvement on WBSF for the CH genotype (4.3 and 3.0 kg cm^{-2} for AT and TS, respectively) than the HOL genotype (2.8 and 2.3 kg cm^{-2} for AT and TS, respectively).

Linear Relationships

The linear relationships between the meat quality parameters (cooking loss, WBSF) and production / carcass parameters (age at slaughter, carcass weight, conformation score, fat classification, marbling score) are presented in table 3. Cooking loss and WBSF decreased significantly ($P<0.001$) as age at slaughter, carcass weight, fat classification and marbling score increased, regardless of hanging method. Cooking loss was inversely related to conformation score for the AT hanging method, but directly related for the TS hanging method. WBSF increased as conformation score got better (increasing from P to U). For the relationships between WBSF and age at slaughter, carcass weight, conformation score and fat classification the variation for the TS sides was approximately half the variation for the AT sides. This may be due to the reduction in variation of WBSF in the TS sides.

Conclusions

Charolais produced a larger, leaner, better conformed carcass relative to Holstein. LD from Holstein was more tender and had a lower cooking loss than beef from Charolais. Warner Bratzler shear force values were significantly lower in TS sides. Tender stretch hanging reduced the genotype difference for WBSF values. Thus when comparing production effects, we should use post slaughter conditions to optimise meat quality. Highly significant linear relationships were found between cooking loss, WBSF and production / carcass characteristics.

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Tables and Figures

Table 1. The effects of genotype on animal performance and carcass characteristics

	Genotype		sed	Sig
	CH	Hol		
Live weight gain (kg/d)	0.89	0.87	0.059	ns
Carcass weight (kg)	360	281	7.8	***
Kill out percentage (%)	55.5	48.7	0.36	***
Conformation [†]	3.37	1.29	0.106	***
Fat classification [‡]	2.96	3.14	0.114	ns
KCC fat (kg)	10.6	15.6	0.99	***
Marbling score ¹	1.57	2.89	0.156	***

[†] EUROP scale: 5, 4, 3, 2, 1 respectively; [‡] EU fat classification, where 5 = fat, 1 = lean;

¹ 8 point scale : 1=low marbling, 8 = high marbling;

ns = not statistically significant (p>0.05); *** = p<0.001; ** =p<0.01; * =p<0.05

Table 2 The effect of genotype and hanging method on meat quality

	Genotype		Hanging		sem	Significance		
	(G)		Method (H)			(G)	(H)	G*H
	CH	HOL	AT	TS				
L* (lightness)	36.3	34.9	36.6	34.4	1.90	ns	ns	ns
a* (redness)	15.6	18.3	17.3	17.1	0.75	ns	ns	ns
b* (yellowness)	12.3	14.7	13.9	13.5	0.71	ns	ns	ns
Ultimate pH	5.61	5.59	5.61	5.59	0.019	ns	ns	ns
Cooking loss (%)	32.8	27.9	30.1	29.7	0.45	***	ns	ns
WBSF (kg.cm ⁻²)	3.7	2.6	3.4	2.6	0.16	***	***	***
Sarcomere length (µm)	2.4	2.5	2.4	2.6	0.12	ns	***	ns

Table 3. Linear relationship between meat quality and production / carcass parameters

	Cooking loss		WBSF	
	AT	TS	AT	TS
Age at slaughter				
Significance	***	***	***	**
Fit ¹	parallel	parallel	parallel	parallel
Slope ²	-ve	-ve	-ve	-ve
% variance	52.4	48.5	36.4	17.5
Carcass weight				
Significance	***	***	***	*
Fit ¹	parallel	parallel	parallel	parallel
Slope ²	-ve	-ve	-ve	-ve
% variance	46.8	41.3	35.6	16.9
Conformation score				
Significance	***	***	***	**
Fit ¹	parallel	common	common	common
Slope ²	-ve	+ve	+ve	+ve
% variance	43.2	36.7	31.2	17.1
Fat classification				
Significance	***	***	***	*
Fit ¹	parallel	parallel	parallel	parallel
Slope ²	-ve	-ve	-ve	-ve
% variance	59.6	45.0	34.2	16.3
Marbling score				
Significance	***	***	***	***
Fit ¹	parallel	parallel	parallel	common
Slope ²	-ve	-ve	-ve	-ve
% variance	52.8	52.7	39.4	33.2

¹parallel indicates slope not significantly different between genotype;
common indicates both genotypes can be fitted by a single line

²-ve = negative; +ve = positive

**GROWTH, CARCASS TRAITS AND FATTY ACID PROFILES OF
PERCENTAGE BOER WETHER GOAT KIDS RAISED UNDER DIFFERENT
PRODUCTION SYSTEMS**

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Key Words: Goat; production systems; carcass traits; fatty acid profile; cholesterol

Objectives

The objectives of this study were to compare growth performance, carcass traits and longissimus fatty acid composition and cholesterol content from high percentage and low percentage Boer goat kids finished on pasture, browse or concentrate-based diets.

References

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EATING QUALITY OF COMMERCIALY PROCESSED HOT BONED MUTTON

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Key Words: Eating Quality, Hot boned, Mutton

Introduction

The application of hot boning of sheep carcasses for the retail market is not extensive (Waylan & Kastner, 2004), and there is limited literature available on the eating quality of hot boned sheep meat. Hot boning is the removal of muscle from the carcass prior to the completion of rigor mortis in most muscles (Devine et al. 2004). Economic benefits for using hot boning include; increased yield, energy savings, chiller space, labour and time (McPhail, 1995). The use of hot boning can however have major constraints. These include increased risk of shortening in muscles (Devine et al. 2004) which can be minimized by the use of electrical stimulation. Electrical stimulation will accelerate the onset of rigor mortis and reduce cold-induced shortening (Hwang et al., 2003). Shortening is a major contributor to the toughness of meat Tornberg, (1996), and tender meat is one of the most important requirements for consumers (Thompson, 2002). Another constraint of hot boning is the increased risk of bacterial growth Spooncer, (1993), however bacterial growth can be controlled by a combination of drying and cooling of the carcass (Spooncer, 1993).

Objectives

To examine the eating quality of hot boned mutton processed under commercial conditions with the use of high voltage stimulation.

Methodology

Animals

The 60 sheep used in the experiment were sourced from three different farms with an equal sample size (n = 20). The animals were of various ages, breed, sex and had been on various quality pastures prior to slaughter. The animals were slaughtered over two days; day one, group 1 (n = 20) and on day two, groups 2 (n =20) and 3 (n = 20).

Stimulation Treatments

The following electrical inputs were used; an immobiliser (40V, current 1.0-1.5 amps) for 40sec which is applied immediately after stunning, spinal discharge (500-585V, 50Hz) for 3-4sec, which is applied approximately one minute after death and High voltage stimulation (1130V peak, 14Hz the rms V is 800V) for 100sec which is applied approximately 20 minutes after death. These electrical inputs are used routinely by the abattoir

Measurements and sampling

Carcases were trimmed according to the specifications of AUS-MEAT (Anon, 1992). Hot carcass weights were recorded and the GR measured (total tissue depth over the 12th rib, 110 mm from the midline) using a GR knife. Further background information was also collected on each group including where they were sourced from and their transport time.

Carcass pH and temperature measurements were taken at approximately 40 and 120 minutes after death. The pH and temperature measurements were taken in the left portion of the m. longissimus thoracis et lumborum (loin) muscle. The muscle pH was measured using a glass combination pH probe (potassium chloride) Ionode intermediate junction pH electrode, (TPS Pty Ltd., Brisbane, Queensland) attached to a data recording pH meter (TPS WP-80). While muscle temperature was measured using a stainless steel cylindrical probe attached to the same meter. The pH meter was calibrated before use, with two buffers pH 4 and 6.8 at room temperature.

The carcasses were hot boned under the normal commercial procedures of the abattoir. During this process both loins were completely removed. The right side loin was removed with a knife and the caudal portion of the loin was retained for shear force testing and other samples were retained for measurement of sarcomere length and iodoacetate (to determine final pH prior to freezing). Samples for shear testing were prepared into 65-gram blocks and then were frozen (-20°C) at (1230-1600 hours) on the day of preparation and subsequently tested for peak shear force as described by Thompson et al. (2005). Sarcomere length was tested using laser diffraction as described by Bouton et al. (1973). A 1 gram sample of loin muscle was also taken for determination of pH after the sample had been boxed and frozen, using an iodoacetate method adapted from that described by Dransfield et al. (1992). Frozen muscle samples were homogenized using an Ultra Turrax at 19,000 rpm in 6 ml of cold buffer. The buffer contained 5-mM iodoacetate and 150 mM of KCl adjusted to pH 7 with KOH at 4-5°C. Samples were homogenized for 2 bursts of 15 s with breaks of 30-s on ice. The suspensions were then incubated in a water bath at 20°C and the pH measured using a meter (TPS, WP-80, PTS Pty Ltd) with a polypropylene spear-type gel electrode (Ionode IJ 44) which was calibrated at 20°C.

Consumer testing

The opposing loin was removed and samples were prepared on site for consumer testing. The subcutaneous fat, connective tissue and the epimysium were removed. Each sample cut was individually packed and then kept frozen (-22 °C) until testing. Before

testing, the steaks were micro waved to raise the temperature to approximately -4 °C and 5 slices of 15mm thickness prepared. These slices (steaks) were re-stored at -22 °C until thawing at ambient temperature for cooking. Sample preparation for consumer testing has been outlined by Thompson et al. (2005). Each consumer was asked to assess each steak for tenderness, juiciness, liking of flavour and overall liking on a continuous 100 point scale from 0-100. The ten tastings for each muscle sample were averaged to give the final eating quality scores for the muscle. In addition each person was asked to give an overall rating score for each sample as, either, awful (1 star), unsatisfactory (2 star), good every day (3 star), better than every day (4 star) or premium (5 star). The testing regime has been described previously and sample preparation for consumer testing has been outlined by Thompson et al. (2005).

Statistical Analysis

Carcase and meat quality traits were analysed using a residual maximum likelihood (REML) procedure (Genstat 7.1, 2004), which contained a fixed effect for lot to estimate the means and standard errors of the differences. For loin pH immediately post-stimulation muscle temperature was used as a covariate and weight was used as a covariate for GR. Linear regression was used to derive the relationship between overall liking and overall rating score and tenderness and overall rating score.

Results & Discussion

Initial loin pH was not different between lots, however the pH of the loins (LL) before being boxed and frozen was significantly different ($P < 0.05$; Table 1). Lot 2 had a higher average loin pH 5.88 compared to Lot 1 and 3 (Table 1). Sarcomere length was significantly different ($P < 0.05$; Table 1) with lot 2 having the greatest sarcomere length. Irrespective of Lot all samples ($n = 59$) had a shear force above 50 N

A relationship was derived between overall liking and the overall rating score, where; Overall liking score = $-6.01 + 20.45$ (Overall rating score) $R^2 = 0.76$, r.s.d. = 3.9. In addition a relationship was also derived between tenderness and overall rating score, where tenderness score = $-22.66 + 24.18$ (tenderness) $R^2 = 0.64$, r.s.d. = 6.1. Predicted overall liking and tenderness scores at each rating score were derived and these relationships are shown in Fig 1. where it is determined that to achieve a rating score of 3 (good every day) then the overall liking score must be 55 and tenderness score must 50 for this product. Of the samples tested 86.5 % had an overall liking score less than 55 and 84.7% had a tenderness score less than 50

Conclusions

The results show that the initial loin pH was not significantly different between lots however the pH LL prior to boxing and freezing was significantly different between lots with on average one third of the animals sampled having a loin pH greater 5.8. This higher pH can increase the risk of bacterial problems and reduced length of storage life in the product (Jeremiah et al., 1997). Lot 2 had a significantly higher SL compared to lot 1 and 3 however shortening occurred in all three lots. Hwang et al. (2004) determined that

short SL is one factor that causes meat toughness and this was supported in the present study by the shear force and eating quality results. Shorthose et al. (1996) reported the threshold for shear force was 50N, however, these results all exceed this threshold indicating that the sampled product was unacceptable and would be extremely tough for consumers. This was consistent with the eating quality results which showed that an overall liking score of 55 and above was needed to achieve an overall rating of “good every day” and a tenderness score of 50 and above. From this it was determined that a large proportion of the hot boned sheep meat had a overall liking score below 55 and a tenderness score below 50, indicating a critically low compliance rate with consumers. It is concluded that the eating quality of hot boned mutton processed under the conditions of this study needs a significant improvement before it would be acceptable as a table meat. Research into other processing techniques that can be used to enhance this type of product is required, but consumer testing of product must be used to quantify improvements with the aim of achieving higher consumer compliance.

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Tables and Figures

Table 1. Predicted means (av. s.e.d.) of initial pH (post-stimulation), pH LL prior to boxing and freezing, sacromere length(μm), shear force (Newtons), cooking loss (%) and sensory traits for animals according to groups

Traits	Lot			av. s.e.d.
	1	2	3	
Initial pH*	5.92a	5.98a	5.95a	0.145
pH LL (b/f box)	5.67a	5.88b	5.60a	0.070
Sacromere length (μm)	1.66a	1.73b	1.65a	0.028
Shear force (N)	77.2a	83.2a	86.2a	4.99
Cooking loss (%)	19.9a	21.0a	19.9a	0.92
Tenderness	36.4a	43.1b	37.9ab	3.17
Juiciness	35.0a	49.5b	44.1b	2.96
Flavour	48.6a	53.0b	52.2ab	2.19
Overall liking	43.1a	49.5b	46.2ab	2.44

Means followed by a different letter in a row (a, b) are significantly different ($P < 0.05$). *Adjusted to a muscle temperature of 29.3 °C.

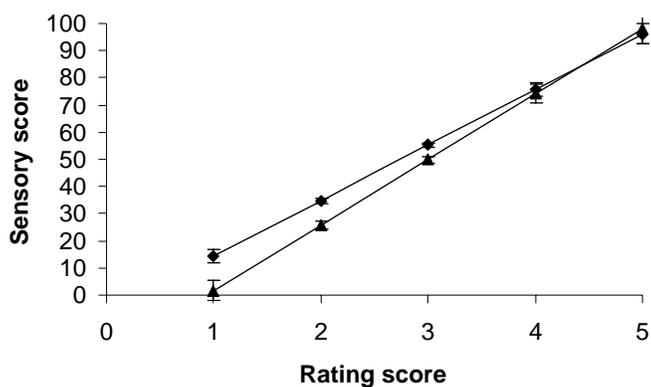


Figure 1. Relationship between tenderness (▲) and overall liking scores (◆) and rating score where 5 is best.

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