Graduate Student Poster Competition

Photosensitized Initiation of Lipid Oxidation in Ground Pork

K. Whang and I.C. Pēng
Food Science Dept.
Purdue University
B-11 Smith Hall, State Street
West Lafayette, IN 47907

The involvement of singlet oxygen ($^1O_2$) through a photosensitization mechanism in initiating the production of hydroperoxides in ground pork was investigated using $^1O_2$ quenchers and electron paramagnetic resonance (EPR) spectroscopy. Singlet oxygen quenchers, N,N,N',N'-tetramethyl-ethylenediamine (TMEDA) and β-carotene, a free radical scavenger, butylated hydroxyanisole (BHA) and TMEDA + BHA were added to ground pork. These samples and control ground pork samples were formed into patties and either illuminated with fluorescent light at 350 foot candles or stored in a dark place. The temperature of the samples was kept at 4°C. Lipid oxidation was determined by measuring peroxide value. Compared to the samples kept in the dark, light exerted a drastic promotive effect on the production of peroxides during 6 days of illumination. Treatment with TMEDA, BHA, and TMEDA + BHA reduced the peroxide level. These results indicate that the $^1O_2$ mechanism and the free radical pathway were both involved in initiating production of hydroperoxides. The photosensitized formation of $^1O_2$ in ground pork was monitored by EPR using a spin trapping technique. A stable nitroxide radical, 2,2,6,6-tetramethyl-4-piperidone-N-oxyl (TAN) was generated by reacting the $^1O_2$ formed in ground pork with a spin labelling agent, 2,2,6,6-tetramethyl-piperidone (TMPD). Identically treated ground pork samples as described above were alternatively exposed to a higher intensity light (500 foot candles) to enhance $^1O_2$ generation. The formation of TAN was detected by EPR in a control sample which was illuminated for 6 days. This study suggests the involvement of singlet oxygen in initiating the production of hydroperoxides in ground pork.

Lactate Dehydrogenase Activity in Bovine Muscle as Influenced by Electrical Stimulation, Aging, Freezing, Thawing and Heating

S.S. Collins, S.B. Smith and J.T. Keeton
Texas A&M University
Department of Animal Science
Texas A&M University
College Station, TX 77843

As a means of developing a rapid, simple assay to identify endpoint cooking temperatures in beef, the activity of lactate dehydrogenase was measured in extracts of cooked bovine muscle samples. Top round muscles (*semimembranosus* and *adductor*) from six Angus steers were assigned one of four treatments: fresh, held at 4 C for 24 hr; aged 7 d at 4 C; frozen at −24.5 C for 5 d and thawed 2 to 3 d at 4 C and 1 d at 25 C; aged 7 d at 4 C and then frozen at −24.5 C and thawed 2 to 3 d at 4 C and 1 d at 25 C. Each treatment was applied to rounds from carcass sides that did or did not receive electrical stimulation (ES) (200-300 v, 3 amps, 60 Hz, 19 pulses/min). Muscle samples were heated in a water bath to endpoints of 60, 63 or 66 C or to 55 C and held for 121 min. The heated and unheated samples of each muscle treatment were analyzed for lactate dehydrogenase (LDH) activity after 3 wk vacuum packaged storage at 4 C. Treatments other than heating showed little effect on LDH activity rates. A major portion of LDH activity was lost upon heating to 63 C and only marginal activity was detectable upon heating to 66 C. LDH activity in fresh top round muscles decreased from 684.8 ± 37.6 μmol/min × g in raw muscle to 131.4 ± 14.1 and 24.5 ± 2.7 μmol/min × g in samples heated to 63 and 66 C, respectively. Extractable protein also decreased with increasing temperature. Thus, the decrease in LDH activity with increasing temperature may result from heat denaturation of the enzyme and/or from decreased amounts of extractable protein. Regardless, the data indicate that LDH activity may serve as an indicator of endpoint cooking temperatures.

Specific Gene Expression in Longissimus Dorsi Muscle of Angus Steers Fed Clenbuterol

D.K. Garcia, S.K. Davis and S.B. Smith
Texas A&M University
Department of Animal Science
Texas A&M University
College Station, TX 77843

As a means of monitoring protein synthesis in growing animals, procedures were developed to quantify MLC-1/3 in bovine skeletal muscle. RNA was extracted from *longissimus dorsi* muscle (obtained at slaughter and frozen in liquid nitrogen) by homogenization and subsequent centrifugation through cesium chloride. The mRNA was eluted from an oligo d(T) column and used to generate a cDNA library. A synthetic oligonucleotide corresponding to a portion of MLC-1 was made and used to screen the library, in which one positive colony was identified. The positive cDNA clone was used to quantify MLC-1/3 mRNA in *l. dorsi* muscle obtained at slaughter from Angus steers slaughtered at 250 kg (initial group), 385 kg (fed finishing diets $\pm$ 7 mg·head^{-1}·d^{-1} clenbuterol for 50 d) and 456 kg (fed an additional 90 d after withdrawal of clenbuterol from the diet of the treated steers). Clenbuterol treatment for 50 d increased muscle fiber diameters by 24% ($P<.05$), relative to control animals. Carcass data indicated that *l. dorsi* muscle cross-sectional area was increased by 28% ($P<.05$) by clenbuterol, relative to control animals, and was still 11% greater after the 90 d post-treatment period. After 50 d on trial, poly (A)$^+$mRNA was higher in the *l. dorsi* of control ($P<.025$) and clenbuterol-treated steers ($P<.005$) than in the initial slaughter group. Furthermore, mRNA from steers fed clenbuterol was higher ($P<.10$) than mRNA from the control steers. The putative MLC-1/3 cDNA was hybridized to 5 μg total RNA and 1 μg mRNA using a commercial slot-blot apparatus. When data were analyzed as relative absorbance units/g muscle, clenbuterol increased expression of MLC-1/3 ($P<.10$) in both total and messenger RNA. After withdrawal of the compound from the diet, the expression of MLC-1/3 returned to control levels. The results suggest that clenbuterol either increased the transcription of the MLC-1/3 gene or increased the stability of MLC-1/3 mRNA in bovine *l. dorsi* muscle, either of which could result in specific myofibrillar protein synthesis.

Fate of *Listeria monocytogenes* During Manufacture and Storage of Hard Salami

J.L. Johnson, M.P. Doyle and R.G. Cassens
University of Wisconsin
1805 Linden Drive West
Madison, WI 53706

A study was done to determine the fate of *Listeria monocytogenes* during manufacture and storage of fermented hard salami. Three sausage treatments were examined: control sausage (C), "naturally" contaminated sausage (NC) and "spiked" sausage (S) made from inoculated beef trim. Inoculum levels for the spiked sausage were adjusted to approximate *Listeria* numbers found in the NC meat; for Trial 1, this was 1×10^3 CFU/g meat and for Trial 2, 1×10^4 CFU/g. Sausage was made by coarse grinding the meat, mixing in a spice pre-mix, dextrose, $NaNO_2$ – containing cure mixture and starter culture, and grinding the mixture through a 3/16-in. grinder plate. The batter was then stuffed into 2-in. fibrous casings and fermented for 24 h before undergoing a 9-day drying period. *Listeria* numbers decreased approximately 1 $\log_{10}$/g during fermentation and pH dropped to 4.3-4.5. By 2 weeks after manufacture, *Listeria* was present at <50 CFU/g and at times could not be detected (<10 CFU/g) except by enrichment. This behavior was observed throughout a 6-week holding period at 4°C. The organism appeared to behave similarly in "naturally" and artificially contaminated sausage. *L. monocytogenes* was recovered from enrichment of control meat in both trials and from one sausage on day 10. It appears there is a major reduction in numbers of *L. monocytogenes* as a result of fermentation and drying, but the organism survives at very low levels for several weeks of storage at 4°C.

Reduction of Metmyoglobin by Extracts of Bovine Liver and Cardiac Muscle

C. Faustman, R.G. Cassens and M.L. Greaser
University of Wisconsin
Muscle Biology Laboratory
1805 Linden Drive
Madison, WI 53706

Maintenance of color is a key factor in prolonging the shelf life of fresh meat. Though many studies have concentrated on preventing pigment oxidation, far less work has dealt with the reduction of ferric myoglobin to ferrous myoglobin. In order to investigate the problem with a new approach, we isolated a crude extract of metmyoglobin reductase from bovine cardiac muscle and characterized its properties with respect to temperature, pH and mediator. All preparative steps were carried out at 4°C and included tissue homogenization, ammonium sulfate fractionation, cation exchange chromatography and anion exchange chromatography. Enzyme activity was followed at 580nm with an assay which included EDTA, buffer, mediator (potassium ferrocyanide or a cytochrome b_5 preparation), horse heart metmyoglobin, enzyme, and NADH. Three separate trials were run and initial velocities of metmyoglobin reduction were recorded for both mediators of pH 6.3, 7.0 and 7.3 and at 22°C and 37.5°C. A least squares difference test for significance was used to analyze data under a general linear models procedure. Greater substrate reduction ($P<.05$) occurred at pH 6.3 versus 7.0 or 7.3 and at 37.5°C compared to 22°C, using either partially purified cytochrome b_5 or potassium ferrocyanide. Significant differences between the two mediator's effects on the reaction were highly dependent on the specific temperature/pH conditions. Control investigations revealed that, in the absence of metmyoglobin reductase, the cytochrome b_5 preparation effectively reduced substrate.

Effects of Carcass and Postmortem Muscle Characteristics on Beef Palatability

S.G. May, H.G. Dolezal, D.R. Gill, F.K. Ray and D.S. Buchanan
Oklahoma State University
Animal Science Department
Stillwater, OK 74078-0425

Forty-eight steers (M, No. 1) approximately 16 months of age were serially slaughtered at 28-d intervals over a 196-d finishing period to examine the relationships between production, carcass and postmortem characteristics with palatability. Upon slaughter, a randomly selected side of each carcass was trimmed of subcutaneous (SQ) fat in the wholesale rib region. Postmortem *longissimus* (LD) muscle temperature and pH decline were monitored intermittently for each side over a 24-hr chilling period. Grade data were collected and rib steaks were removed for palatability determination. Slaughter weight and most carcass grade traits increased linearly ($P<.05$) while most sensory panel variables and marbling score increased curvilinearly ($P<.05$) at a decreasing rate over days-fed. As time-on-feed increased from 0 to 84-d, palatability attributes improved ($P<.05$); however, once steers had been fed a minimum of 84-d, there was little ($P<.05$) improvement in palatability. Correlations for sensory tenderness with marbling score, days-fed, SQ fat thickness, and carcass weight were .51, .48, .45, and .46, respectively. LD temperature at 2.5 hr postmortem had the highest correlation ($r=.68$) with taste panel tenderness. Sides trimmed of SQ fat had lower ($P<.05$) 2.5 hr postmortem temperatures, smaller ($P<.05$) ribeye areas and lower ($P<.05$) sensory tenderness ratings than untrimmed sides. In order for trimmed sides to achieve comparable tenderness scores (5.0 = slightly tender) to untrimmed sides, the steers would need to be fed an additional 31-d. Results of this study indicate that postmortem chilling rate has the greatest impact on LD tenderness.

Structural Characterization of Nitrosylhemochromogen of Cooked Cured Meat

K. B. Killday, M.E. Bailey and M.S. Tempesta
University of Missouri
Food Science and Nutrition Department
21 Agriculture Building
Columbia, MO 65211

The structure of the cooked cured meat pigment has been a subject of controversy. This pigment has been described as being either a mononitrosyl or a dinitrosyl heme complex and data exist which support both structures. The meat pigment was isolated and completely characterized for the first time by infrared and uv-visible spectroscopies and thin layer chromotography and shown to be identical to synthetic material which was further identified by fast atom bombardment mass spectrometry as mononitrosyliron (II) protoporphyrin. The ^{1}H-NMR spectrum of this paramagnetic complex has been reported for the first time from these studies. It has been shown that this pigment can be formed from chloroiron (III) protoporphyrin by an autoreduction reaction with imidazole and nitric oxide. Analogous results have been obtained on reaction of nitric oxide gas with metmyoglobin followed by protein denaturation. This leads us to propose a new mechanism for the meat curing process involving: 1) oxidation of myoglobin to metmyoglobin with subsequent reduction of nitrite to nitric oxide; 2) formation of nitrosyl metmyoglobin; 3) rapid autoreduction to a nitrosylmyoglobin radical cation; 4) further reduction to nitrosylmyoglobin; and 5) formation of nitrosylheme and incorporation of a second mole of nitrite into the denatured protein on heating.

Chemical and Sensory Characteristics of Precooked Beef Chuck Roasts as Influenced by the Addition of Antioxidants

T.F. Mann, J.O. Reagan, D.A. Lillard and D.R. Campion
Department of Animal Science
University of Georgia
Athens, GA 30602

Precooked beef roasts prepared from two recombined chuck muscles of different types were used to evaluate the effectiveness of a phosphate compound, alone or in combination with other antioxidants, in preventing warmed over flavor (WOF) development over extended refrigerated storage as compared to control (no phosphate) treatments. Treatments without phosphate had significantly different proximate composition due to lower yields and higher cooking losses and received lower tenderness and juiciness scores from the sensory panel. These treatments also had significantly higher WOF sensory scores and TBA values. A correlation coefficent of 0.96 was obtained between these values. Results of this study indicate that phosphate alone was effective in preventing WOF in vacuum-packaged precooked roast beef for a 23-day refrigerated storage period. Adding nitrite or Maillard reaction products (MRP) did not increase antioxidant activity over that of phosphate alone. However, the addition of MRP may contribute to flavor maintenance over extended storage periods.

Index words: Hot-boning, salt level, muscle type, precooked, phosphate, sensory scores, warmed-over flavor, TBA values.