NIR Prediction Of Pork Tenderness[1]

Steven D. Shackelford, David A. King and Tommy L. Wheeler*

INTRODUCTION

A system for on-line classification of beef carcasses for longissimus tenderness using visible and near-infrared (**VISNIR**) reflectance spectroscopy had been developed and validated (Shackelford et al., 2004a, 2005, 2012bc) and commercially implemented. That system involved evaluation of the cut surface of longissimus of ribbed beef carcasses. As we developed this technology for application to beef carcasses, we also tested its efficacy for application to beef steaks and pork chops. But, because pork carcasses are not normally ribbed commercially, we did not readily see a manner to accurately extend this technology to application to commercial pork production. However, a colleague of ours with extensive knowledge of the pork industry, noted that during commercial boneless loin production, the ventral side of longissimus is frequently exposed, particularly in the production of "meaty" back ribs. Initial tests indicated that the VISNIR system could easily be applied on-line to the exposed ventral side of the longissimus in boneless pork loins. Thus, a series of experiments were conducted to evaluate the efficacy of this measurement for prediction of tenderness and other pork quality traits.

MATERIALS AND METHODS

Experiment 1 (Shackelford et al., 2011).

Boneless pork loins (n = 901) were evaluated either on line on the loin boning and trimming line of large-scale commercial plants (n = 465) or at the U.S. Meat Animal Research Center (USMARC) abattoir (n = 436). Within 2 min of deboning, exposed longissimus on the ventral side of boneless loins was evaluated with VISNIR using a commercial system (Unit 5016) described by Shackelford et al. (2012b). The system is comprised of a laptop computer, a sampling head with tungsten lamp, a fiber optic cable, and a spectrophotometer. Light is reflected off of the object of interest and passes through the fiber optic cable to the spectrophotometer, which relays the data to the laptop for processing. When possible, the measurement was made near the 10th rib region. However, the manner in which the back ribs are removed from the longissimus varies from loin to loin in commercial practice. Thus, the most suitable location along the length of the boneless loin for VISNIR evaluation varied depending on where on each loin longissimus was most cleanly exposed with a smooth cut during the removal of the back ribs.

Boneless loin sections were vacuum-packaged, aged (2°C) until 14 d postmortem and two 2.54-cm thick chops were obtained from the 11th rib region. Fresh (never frozen) chops were cooked (71°C) with a belt grill and longissimus slice shear force (**SSF**) was measured on each of the two chops (Shackelford et al., 2004b). The duplicate SSF values were averaged and that value was used for all analyses.

Experiment 2 (Shackelford et al., 2013).

Boneless pork loins (n = 1,208) were evaluated on the loin boning and trimming line of four large-scale commercial plants (n = 300 or 304/plant) as described above. Although it was not possible for one plant, an attempt was made to evaluate each loin with 2 identical spectroscopy systems. Boneless loins were vacuum-packaged, boxed, and transported (-2.8ºC) to USMARC and processed as in Exp 1, except that SSF was measured at 15 d postmortem for Exp 2.

Experiment 3 (Shackelford et al., 2012a).

To evaluate models developed in Exp. 2, boneless pork loins (n = 599) were evaluated on the loin boning and trimming line of 3 large-scale commercial plants on 2 sampling days. The 3 different commercial facilities differed in regards to two factors that may contribute to variation in meat quality traits. Plant A utilized CO_2 stunning and conventional spray chilling, Plant B utilized CO_2 stunning and blast chilling, and Plant C utilized electrical stunning and blast chilling. All of the plants utilized conventional scalding and dehairing systems (i.e., carcasses were not skinned). Loins were processed in a similar manner to

Steven D. Shackelford, Ph.D.
USDA -ARS
Roman L. Hruska US Meat Animal Research Center
Clay Center, NE 68933-0166
steven.shackelford@ars.usda.gov

[1]Mention of trade names, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

Exp. 2 and details of loin selection and processing and data collection (including sarcomere length and proteolysis) procedures are reported elsewhere (Shackelford et al., 2012a). Because loin evaluation took place in multiple plants at the same time, for 2 of the 3 plants, only a single instrument was tested.

Statistical analysis.

Analysis of variance for mean SSF differences among data sets (Figure 1) or plants (Figure 2) was conducted using the GLMMIX procedure of SAS (SAS Inst., Cary, NC) and differences among least-squares means were determined with the DIFF and LINES options. Variances were compared among data sets (Figure 1) or plants (Figure 2) using the F-Test Two-Sample for Variances analysis tool of Microsoft Excel 2007.

Exp. 1. For model development and validation, loins were blocked by plant (n = 3), production day (n = 24), and observed SSF and one-half of the loins (n = 451) were assigned to a calibration data set, which was used to develop regression equations, and one-half of the loins (n = 450) were assigned to a prediction data set, which was used to validate the regression equations (Neter et al., 1989). Models were developed using the PLS1 procedure of The Unscrambler v9.8 (CAMO Software AS; OSLO, Norway). Spectra were not pretreated. Model validation used the test set method using the prediction data set as described above. The number of principal components was set at 20, which meant that model selection could have included up to 20 principal components. The X-variable weights were set to 0 for 350-449 nm and 1,001-1,050 nm. Carcasses with VISNIR-predicted SSF ≤ 14 kg were classified as VISNIR predicted tender and carcasses with VISNIR-predicted SSF > 14 kg were classified as VISNIR not predicted tender. One-way ANOVA (Figure 3) for differences among VISNIR tenderness classes in observed SSF at 14 d postmortem was conducted using the GLM procedure of SAS. The frequency of carcasses with SSF values > 20 kg was calculated for each VISNIR class. Differences in these frequencies among VISNIR classes were compared using the DIFFER program of PEPI (version 2; USD, Inc., Stone Mountain, Ga.).

Exp. 2. To facilitate statistical analysis, the data were divided in half. Specifically, within each plant and pooled across plants, those loins with VISNIR-predicted SSF values less than or equal to the median for that data set were classified as VISNIR-predicted tender and those loins with VISNIR-predicted SSF values greater than the median were classified as VISNIR-not-predicted tender. One-way ANOVA for mean SSF differences among VISNIR tenderness classes (Figure 4) was conducted using the Single Factor ANOVA tool of Microsoft Excel 2007. Differences in the frequency of loins with SSF values > 25 kg were compared with chi-square analysis and Bonferroni correction for multiple comparisons using the categorical data option of the Compare2 program of WinPepi (v. 11.4; www.brixtonhealth.com/pepi4windows.html). Cor-

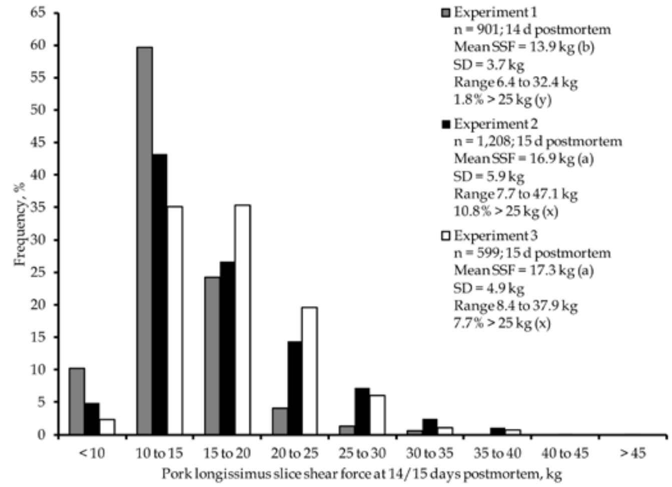

Figure 1. Frequency distributions of pork longissimus slice shear force at 15 d postmortem for Exp. 2 and 3 (Shackelford et al., 2012) as compared to the frequency distributions of pork longissimus slice shear force at 14 d postmortem for Exp. 1 (Shackelford et al., 2011). Adapted from Shackelford et al. (2013).

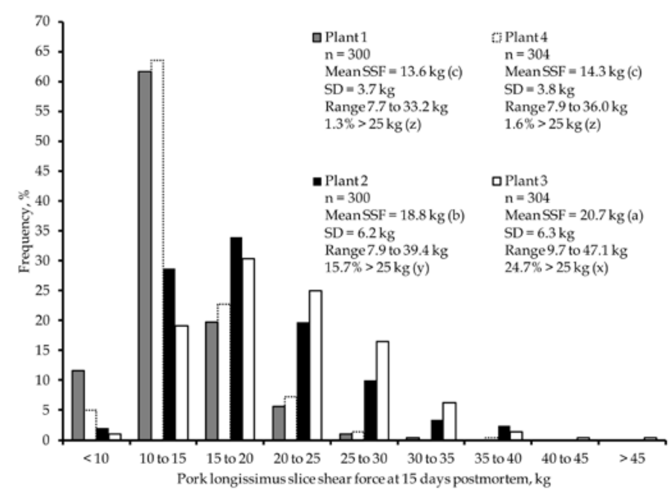

Figure 2. Frequency distributions of longissimus slice shear force at 15 d postmortem for the 4 plants sampled in Exp. 2. Adapted from Shackelford et al. (2013).

relation analysis was conducted with the correlation procedure of SAS. For new model development, correlation analysis was conducted for each plant (Exp. 2) to identify the wavelength range at which reflectance was most highly related to SSF. Then, simple linear regression (Microsoft Excel 2007) was conducted for all samples in Exp. 2 to predict SSF from reflectance at 822 nm.

Exp. 3. Pooled across plants, those loins with VISNIR-predicted SSF values less than or equal to the median for that data set were classified as VISNIR-predicted tender and those loins with VISNIR-predicted SSF values greater than the median were classified as VISNIR-not-predicted tender. One-way ANOVA and difference analysis (Figure 5) was conducted as for Exp 2.

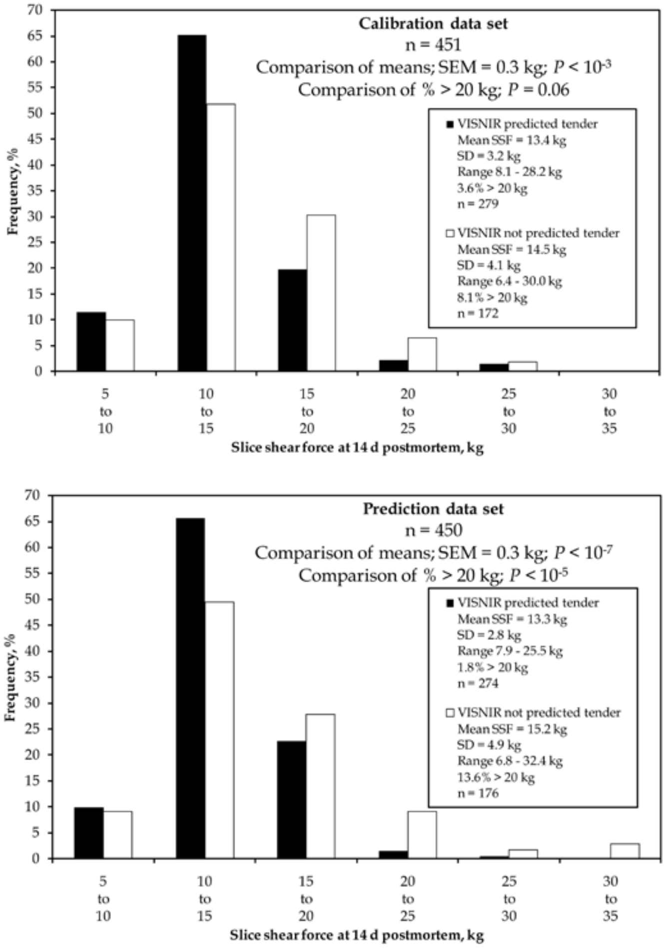

Figure 3. Effect of sorting pork loins immediately after deboning into predicted tenderness classes using visible and near infrared (VISNIR) spectroscopic evaluation of the ventral side of longissimus on longissimus slice shear force at 14 d postmortem. Loins with VISNIR-predicted SSF ≤ 14 kg were classified as VISNIR predicted tender. The left panel shows the calibration data set (n = 451), which was used to develop the model, and the right panel shows the prediction data set (n = 450), which was used to validate the model. Adapted from Shackelford et al. (2011)

RESULTS AND DISCUSSION

The mean SSF, the level of variation in SSF, and the percentage of samples with SSF > 25 kg differed greatly among these three experiments (Figure 1). The sample used for the development of the system (Exp. 1; Shackelford et al., 2011) to predict pork longissimus SSF with VISNIR was much more tender than the sample used for Exp. 2 and 3. For Exp. 2, there was a high degree of difference among plants in mean SSF, the level of variation in SSF, and the percentage of samples with SSF > 25 kg ($P < 0.05$; Figure 2). Shackelford et al. (2012a) objectively compared tenderness differences among 3 plants and ascribed a large portion of the variation in tenderness among plants to differences in chilling rate. Specifically,

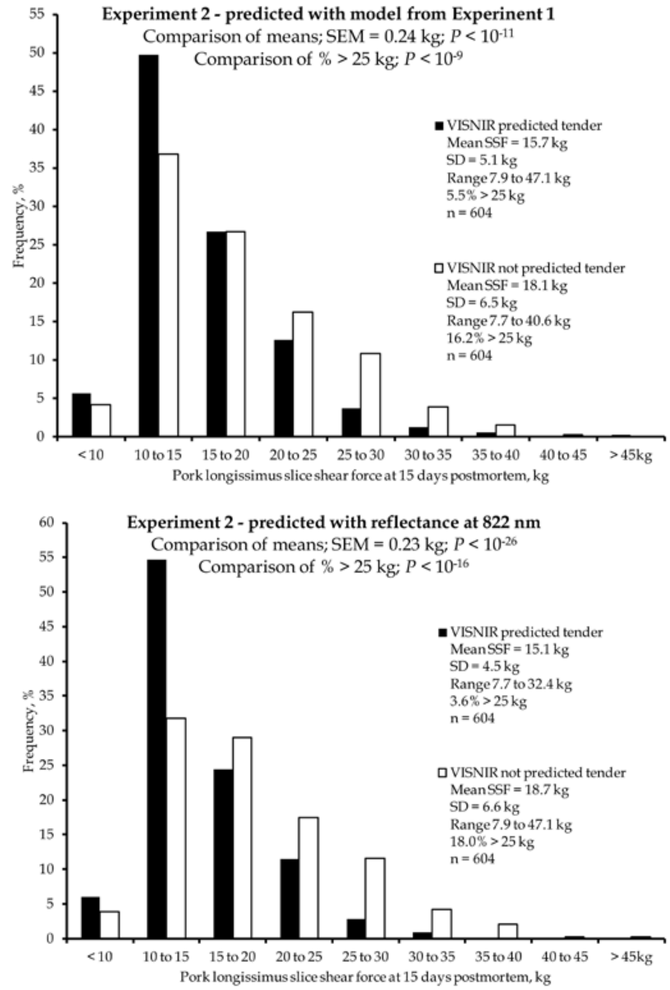

Figure 4. Exp. 2. Prediction of pork longissimus slice shear force using visible and near infrared (VISNIR) spectroscopic evaluation of the ventral side of longissimus and either the model from Exp. 1 (top panel) or reflectance at 822 nm (bottom panel). Adapted from Shackelford et al. (2013).

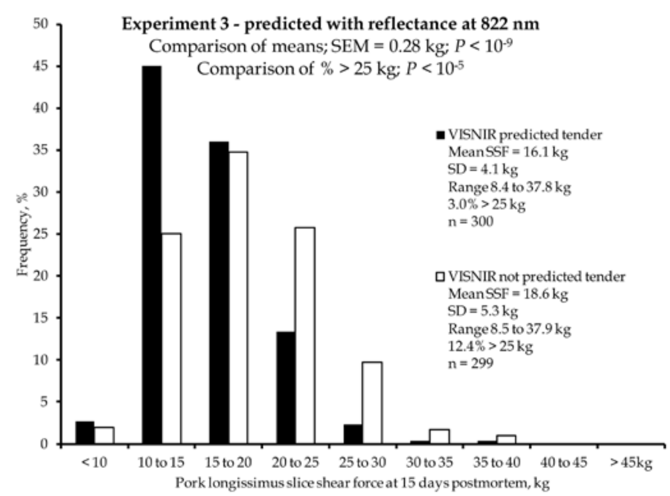

Figure 5. Validation of the relationship between reflectance at 822 nm, from visible and near infrared (VISNIR) spectroscopic evaluation of the ventral side of longissimus, and pork longissimus slice shear force (Exp. 3). Adapted from Shackelford et al. (2013).

blast chilling resulted in a greater frequency of loins with excessively high (> 25 kg) longissimus SSF values. This is particularly relevant to the present discussion because the data set (Shackelford et al., 2011) that was used to develop the VISNIR technology for pork longissimus SSF prediction did not include any carcasses from plants that employed blast-chilling.

Despite the relatively low level of variation in SSF in Exp. 1, classification of pork loins based on spectroscopic evaluation of the ventral side of longissimus resulted in VISNIR tenderness classes that differed in mean longissimus SSF values at 14 d postmortem in the calibration ($P < 10^{-3}$) and prediction ($P < 10^{-7}$) data sets (Figure 1). Relative to loins predicted to be tender by spectroscopic evaluation of the ventral side of longissimus, loins that were not predicted to be tender were more likely to have SSF > 20 kg in the calibration ($P = 0.06$) and prediction ($P < 10^{-5}$) data sets.

Exp. 2 was conducted to provide a large-scale field test of application of this technology and the models developed in Exp. 1. Plants were selected to represent a diversity of processing systems with two plants sampled that utilized blast-chilling and two plants sampled that utilized conventional spray chilling systems. Application of the model developed in Exp. 1 to the loins tested in Exp. 2 resulted in VISNIR tenderness classes that differed in mean longissimus SSF values at 15 d postmortem ($P < 10^{11}$; Figure 4). Relative to loins predicted to be tender by spectroscopic evaluation of the ventral side of longissimus, loins that were not predicted to be tender were more likely to have SSF > 25 kg ($P < 10^{9}$). While these findings were favorable, further examination of the data showed that the prediction model did not properly account for tenderness differences among plants. Variation in the plant means for predicted SSF accounted for approximately one-half of the variation in plant means for observed SSF (r = 0.73). Thus, a new model was developed from the Exp 2. data set.

Because the VISNIR system measures reflectance across a wide range of wavelengths, it can detect very subtle differences among data sets which might not have been directly related to the trait of interest. For instance, because of differences among plants in the layout of the loin boning and trimming lines, the time interval between deboning and application of VISNIR was not the same in each plant. A difference between plants of as little as 2 min in the time interval (i.e., 3 vs. 5 min) between deboning and application of VISNIR will result in a large enough difference in the proportion of myoglobin in the oxymyoglobin state that the spectra will differ significantly among plants. Given that there were very large tenderness differences among plants confounded with differences in bloom time before VISNIR, development of a multifactor partial least squares regression model would likely have resulted in an over specified model. To develop a robust model, correlation analysis was conducted for each plant to identify the wavelength range at which reflectance was most highly related to SSF. For each plant, the strongest correlation was found at or near 822 nm. For plants 1, 2, and 3, less reflectance at 822 nm was associated with greater SSF ($P < 0.01$; Table 2), and the degree of SSF difference among predicted tenderness classes was greater when loins were sorted based on reflectance at 822 nm than using the model of Shackelford et al. (2011). Also, variation in the plant means for reflectance at 822 nm accounted for virtually all of the variation in plant means for SSF (correlation of plant means for reflectance at 822 nm with plant means for SSF was r = -0.99). That is, reflectance at 822 nm was indicative of variation in tender-

Table 1. Exp. 2. Slice shear force (kg) as affected by sorting of pork loins, for each plant and pooled across plants, into 2 visible and near-infrared (VISNIR) predicted tenderness classes based on either the model from Exp. 1 (Shackelford et al., 2011) or reflectance at 822 nm

	VISNIR-predicted tender		**VISNIR-not-predicted tender**			
Plant	**n**	**Slice shear force, kg**	**n**	**Slice shear force, kg**	**SEM**	**P < F**
		VISNIR-predicted tenderness classes based on the model from Exp. 1				
Plant 1	150	13.13	150	14.14	0.30	< 0.05
Plant 2	150	18.77	150	18.92	0.51	ns
Plant 3	152	19.74	152	21.61	0.50	< 0.01
Plant 4	152	13.99	152	14.71	0.31	< 0.1
All plants pooled	604	15.71	604	18.05	0.24	< 10-11
		VISNIR-predicted tenderness classes based on reflectance at 822 nm				
Plant 1	150	12.92	150	14.36	0.29	< 0.001
Plant 2	150	17.89	150	19.79	0.50	< 0.01
Plant 3	152	19.14	152	22.21	0.50	< 10-4
Plant 4	152	14.07	152	14.64	0.31	ns
All plants pooled	604	15.09	604	18.67	0.23	< 10-26

ness both among and within plants. Consequently, when samples were classified for tenderness across all samples based on reflectance at 822 nm (VISNIR-predicted tender = 604 loins with reflectance at 822 nm > median and VISNIR-not-predicted tender = 604 loins with reflectance at 822 nm < median), the resulting classes differed in mean longissimus SSF values at 15 d postmortem ($P < 10^{26}$) and the classes differed in the percentage of loins with SSF > 25 kg ($P < 10^{16}$; bottom panel of Figure 3). Comparison of the results from sorting loins for tenderness based on the model of Shackelford et al. (2011) with the results based on reflectance at 822 nm (top and bottom panels of Figure 3, respectively) indicated that use of reflectance at 822 nm would result in a more effective classification system.

To confirm that use of this VISNIR system with reflectance at 822 nm would provide a robust method to classify pork loins for tenderness, Exp. 2 was conducted. As in Exp. 1, loins were classified as VISNIR-predicted tender if the observed reflectance at 822 nm was greater than the median. Again, VISNIR-predicted tenderness classes differed greatly in mean longissimus SSF values at 15 d postmortem ($P < 10^{9}$) and the percentage of loins with SSF > 25 kg ($P < 10^{5}$; Figure 4).

To help elucidate the basis for the VISNIR tenderness sorting, correlation coefficients were determined for the relationships of predicted SSF, based on reflectance at 822 nm, and observed SSF to meat quality traits among and within packing plants for Exp. 2 and Exp. 3 (Table 2). Across all samples in Exp. 3, sarcomere length and intramuscular fat percentage were negatively correlated ($P < 0.001$) with both predicted and observed SSF. For sarcomere length, the negative correlation was significant ($P < 0.01$) for loins sampled from each plant, whereas results for intramuscular fat percentage were inconsistent across plants. For some traits, the direction of the correlation was the opposite for predicted SSF as compared to observed SSF. For example, ultimate pH was positively correlated ($P < 0.001$) with VISNIR-predicted SSF in both Exp. 1 and 2; however, ultimate pH was negatively correlated ($P < 0.05$) with observed SSF in both Exp. 1 and 2. Cooking loss was negatively correlated ($P < 0.001$) with VISNIR-predicted SSF in both Exp. 1 and 2; but, cooking

Table 2. Correlation coefficients for the relationships of predicted slice shear force, based on reflectance at 822 nm, and observed slice shear force to meat quality traits among and within packing plants for Exp. 2 and 3.

	Experiment 2									
	Correlation with predicted slice shear force					Correlation with observed slice shear force				
	All plants	Plant 1	Plant 2	Plant 3	Plant 4	All plants	Plant 1	Plant 2	Plant 3	Plant 4
Purge loss, %	-0.22***	-0.39***	-0.11	-0.09	-0.42***	0.18***	0.06	0.37***	0.24***	0.16**
ultimate pH	0.36***	0.56***	0.23***	0.23***	0.61***	-0.09**	0.04	-0.16**	-0.12*	0.00
L*	-0.49***	-0.59***	-0.34***	-0.37***	-0.56***	-0.16***	-0.02	0.13*	-0.18**	-0.01
a*	0.01	0.05	-0.02	-0.02	-0.02	-0.04	0.00	-0.16**	0.02	0.04
b*	-0.10***	-0.37***	-0.13*	-0.08	-0.22***	-0.01	-0.09	-0.09	-0.07	-0.03
Lean color score[1]	0.21***	0.31***	0.25***	0.24***	0.20***	0.03	-0.05	-0.08	0.10	-0.01
Marbling score[2]	-0.13***	-0.06	-0.22***	-0.28***	0.02	-0.16***	-0.14*	-0.20***	-0.21***	-0.01
Cooking loss, %	-0.36***	-0.59***	-0.26***	-0.16**	-0.63***	0.29***	0.16**	0.53***	0.55***	0.09

	Experiment 3							
	Correlation with predicted slice shear force				Correlation with observed slice shear force			
	All plants	Plant A	Plant B	Plant C	All plants	Plant A	Plant B	Plant C
Purge loss, %	-0.34***	-0.50***	-0.06	-0.42***	0.24***	0.06	0.44***	0.01
ultimate pH	0.47***	0.61***	0.12	0.59***	-0.10*	-0.01	-0.36***	0.21**
L*	-0.49***	-0.53***	-0.19**	-0.58***	-0.02	-0.10	0.26***	-0.33***
a*	-0.17***	-0.10	-0.20**	-0.21**	-0.02	0.13	-0.13	-0.07
b*	-0.06	-0.04	0.02	-0.17*	-0.04	-0.12	0.04	0.02
Lean color score[1]	0.29***	0.32***	0.10	0.34***	0.05	0.15*	-0.15*	0.26***
Marbling score[2]	-0.04	0.11	-0.40***	-0.03	-0.13**	0.02	-0.32***	-0.04
Intramuscular fat, %	-0.18***	-0.01	-0.48***	-0.21**	-0.22***	-0.08	-0.43***	-0.15*
Cooking loss, %	-0.40***	-0.54***	0.08	-0.50***	0.24***	0.16*	0.59***	-0.04
Sarcomere length, µm	-0.29***	-0.36***	-0.29**	-0.28**	-0.60***	-0.65***	-0.57***	-0.62***
Desmin, % degraded	-0.06	-0.15	-0.33***	0.09	-0.42***	-0.39***	-0.42***	-0.37***

[1]1 =pale, pinkish gray; 6 = dark purplish-red (NPPC, 1999)
[2]1 = devoid; 10 = abundant (NPPC, 1999)
*P < 0.05, **P < 0.01, ***P < 0.001.

loss was positively correlated ($P < 0.001$) with observed SSF in both Exp. 1 and 2. In summary, these correlations show a moderate relationship between VISNIR tenderness sorting, based on reflectance at 822 nm, and traits related to ultimate pH, lean color, and water-holding capacity, but the relationship does not provide a causative explanation for the mechanism of VISNIR tenderness sorting. Conversely, sarcomere length results were consistent with the observation that beef carcasses predicted to be tender by VISNIR have longer longissimus sarcomeres (Shackelford et al., 2012b).

TECHNOLOGY TRANSFER

There has been widespread interest from U.S. pork processing companies in implementing this technology either as a means to identify loins with superior tenderness for a branded pork product or as a research tool to compare sources of product. We have conducted trials in many commercial plants to help the pork industry to understand how this technology might work in their facilities. Some of these companies tested systems from the company (ASD, Inc., Boulder, CO) that manufactured the system used in these experiments, which also marketed a system, known as the QualitySpecBT, tailored for on-line commercial evaluation of beef tenderness. There are substantial differences in the sampling head design between the system used in these experiments and the QualitySpecBT. The QualitySpecBT was designed to internalize the referencing process inside the sampling head to prevent contamination of the reference material by the plant employees. To do that, the sampling head was made much larger, which made it more difficult to position on beef carcasses and much more difficult to position on pork loins. In contrast, the system that we used in these projects was easy to operate in a variety of on-line situations and was able to operate at commercial boning line speeds. To be useful for application to boneless loins, a technology needs to be able to freely reach across the width of a loin boning line conveyor because the position of loins on the conveyor varies and the best sampling location for a given loin varies. The technology tested in these experiments met that requirement but the QualitySpecBT did not. Because of limited success in marketing the QualitySpecBT, ASD has discontinued production and support of these systems. We have sought other VISNIR systems with potential for on-line application to beef and pork tenderness. Thus, far we have not tested any other systems that were accurate and feasible.

LITERATURE CITED

NPPC. 1999. Pork quality standards. Natl. Pork Prod. Counc. Des Moines, IA.

Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 2004a. Development of optimal protocol for visible and near-infrared reflectance spectroscopic evaluation of meat quality. Meat Sci. 68:371–381.

Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 2004b. Technical Note: Use of belt grill cookery and slice shear force for assessment of pork longissimus tenderness. J. Anim. Sci. 82:238–241.

Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 2005. On-line classification of US Select beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. Meat Sci. 69:409–415.

Shackelford, S. D., D. A. King, and T. L. Wheeler. 2011. Development of a system for classification of pork loins for tenderness using visible and near-infrared reflectance spectroscopy. J. Anim. Sci. 89:3803-3808.

Shackelford, S. D., D. A. King, and T. L. Wheeler. 2013. Field testing of a system for classification of pork loins for tenderness using visible and near-infrared reflectance spectroscopy. J. Anim. Sci. In review..

Shackelford, S. D., D. A. King, and T. L. Wheeler. 2012a. Chilling rate effects on pork loin tenderness in commercial processing plants. J. Anim. Sci. 90:2842-2849.

Shackelford, S. D., T. L. Wheeler, D. A. King and M. Koohmaraie. 2012b. Field testing of a system for on-line classification of beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. J. Anim. Sci. 90:978-988.

Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 2012c. Validation of a model for on-line classification of U.S. Select beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. J. Anim. Sci. 90:973-977.