Technical note: Sampling methodology for relating sarcomere length, collagen concentration, and the extent of postmortem proteolysis to beef and pork longissimus tenderness[1,2]

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ABSTRACT: The objective of this study was to determine the effect of sampling methodology on the relationship between longissimus tenderness and measures of biochemical meat traits. Sampling methodology included measurements of sarcomere length, collagen concentration, and postmortem desmin proteolysis on raw samples and measurements of these same traits on the same cooked meat used for shear force measurement. Twenty crossbred steers and 20 crossbred barrows were used for these studies. The beef longissimus thoracis were vacuum-packaged, stored at 2°C until 14 d postmortem, then frozen and stored at –30°C. The pork longissimus thoracis et lumborum were vacuum-packaged, stored at 2°C until 7 d postmortem, then frozen and stored at –30°C. Trained sensory panel tenderness rating ranged from 3.1 to 7.6 for beef and 4.1 to 7.4 for pork. The coefficient of variation was lower for sarcomere length than for all other traits. Simple correlation coefficients between measurements on raw and cooked samples were 0.58 (beef) and 0.11 (pork) for sarcomere length, 0.66 (beef) and 0.59 (pork) for collagen, and 0.74 (beef) and 0.76 (pork) for desmin degradation. Simple correlation coefficients between biochemical traits and measures of tenderness (Warner-Bratzler shear force and trained sensory tenderness rating) were higher or not different for cooked compared to raw samples. Correlation coefficients between biochemical traits and tenderness rating were 0.38 (raw) and 0.22 (cooked) for sarcomere length, –0.12 (raw) and –0.45 (cooked) for collagen, and 0.48 (raw) and 0.80 (cooked) for desmin degradation in beef longissimus and 0.14 (raw) and 0.15 (cooked) for sarcomere length, –0.38 (raw) and –0.33 (cooked) for collagen, and 0.53 (raw) and 0.67 (cooked) for desmin degradation in pork longissimus. The coefficients of determination for explaining variation in tenderness rating using sarcomere length, collagen concentration, and desmin degradation for raw and cooked samples were 0.43 and 0.73 (beef) and 0.48 and 0.57 (pork), respectively. This study indicates that measurements of biochemical traits on the same cooked meat as used for shear force determination account for more of the variation in measures of tenderness than biochemical measurements made on a separate raw sample.

Key Words: Beef, Methodology, Prediction, Pork, Quality, Tenderness

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Introduction

There is a large body of literature on investigations into associations of biochemical traits with variation in meat tenderness (Ouali, 1990; Koohmaraie, 1996) and prediction of meat tenderness with biochemical traits (Seideman et al., 1987; Shackelford et al., 1991; Wheeler et al, 2000). Samples for measurements of cooked longissimus tenderness and samples for measurements of biochemical traits have almost always come from different steaks/chops. These samples sometimes were from adjacent steaks/chops and sometimes were from steaks/chops 5 to 15 cm or more from the location used for tenderness measurements (McKeith et al., 1985; DeVol et al., 1988; Wheeler et al., 2000). However, it would seem likely that in order to most accurately determine the relationship between tenderness and other traits, samples for the measurements of all traits should be from the same location and treated in the same manner. Thus, the objective of this study was to determine whether a greater proportion of the

[1]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2]Anti-desmin (clone D3) was developed by D. A. Fischman and obtained from the Developmental Studies Hybridoma Bank maintained by Univ. of Iowa, Dept. of Biol. Sci., Iowa City, IA 52242, under contract N01-HD-7-3263 from the NICHD.

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variation in tenderness could be explained by sarcomere length, collagen concentration, and postmortem proteolysis if these traits were measured on the same cooked meat as shear force was measured on rather than on a separate raw sample.

Materials and Methods

Animals

The Roman L. Hruska U.S. Meat Animal Research Center (**MARC**) Animal Care and Use Committee approved the use of animals in this study. Twenty steers were humanely slaughtered and processed at the MARC abattoir. Beef carcasses were not electrically stimulated and were chilled for 48 h at 0°C. Barrows were electrically stunned and humanely slaughtered and processed at a commercial facility. Pork carcasses were chilled for 24 h at 0°C.

Beef. At 48 h postmortem, the Institutional Meat Purchase Specifications (IMPS) #112 ribeye roll (containing longissimus thoracis) was obtained from the right carcass sides, vacuum-packaged, stored at 2°C until 14 d postmortem, then frozen at –30°C. Six steaks, 2.54-cm thick, were cut from the caudal end of the frozen ribeye roll with a band saw. From the caudal end, steaks three and four were used for trained sensory evaluation, steak five was used for Warner-Bratzler shear force measurement, and steak six was used for measurements of sarcomere length, collagen concentration, and postmortem proteolysis of desmin on raw sample. Steaks one and two were not used in this experiment. Measurements of sarcomere length, collagen concentration, and postmortem proteolysis on cooked sample were made on the Warner-Bratzler shear force cores (from steak five). All measurements were conducted on the longissimus thoracis.

Pork. The IMPS #410 bone-in pork loin (containing longissimus thoracis et lumborum) was obtained from the right carcass sides at 24 h postmortem, stacked into a plastic-lined, cardboard "combo," and shipped to MARC. At 48 h postmortem, the loins were cut at the 10th rib and a 4-cm-long section was removed from the cranial end of the longissimus section and used in another experiment. The remainder of the longissimus was vacuum-packaged, stored at 2°C until 7 d postmortem, and then frozen at –30°C. Five chops, 2.54 cm thick, were cut with a band saw from the cranial end of the frozen loin section. From the cranial end, chops one and two were used for slice shear force, chops three and four were used for trained sensory evaluation, and chop five was used for measurements of sarcomere length, collagen concentration, and postmortem proteolysis of desmin on raw sample. Measurements of sarcomere length, collagen concentration, and postmortem proteolysis on cooked sample were made on the slice shear force samples. All measurements were conducted on the longissimus.

Sampling

For measurements on raw samples, three cubes ($7 \times 7 \times 7$ mm), one each from lateral, central, and medial locations within the transverse sections (steak six or chop five), were removed for sarcomere length measurement. The remainder of the steak or chop was trimmed of epimysium and powdered in liquid nitrogen for immunoblotting to detect proteolysis and collagen determination. For measurements on cooked beef (steak five), one cube ($7 \times 7 \times 7$ mm) was cut from one half of each Warner-Bratzler shear force core (n = 6) after shearing (adjacent to the shear cut). For measurements on cooked pork (chops one and two), two cubes ($7 \times 7 \times 7$ mm) were cut from each half of the slice shear force slice (n = 4) after shearing (adjacent to the shear cut). The remainder of the cooked sample (half cores or half slices) was trimmed to remove the crusted cooked surface and powdered in liquid nitrogen for immunoblotting to detect proteolysis and collagen determination.

Cooking

Steaks and chops were thawed and cooked as described by Wheeler et al. (1998) with the following modifications. The preheat platen on the belt grill was set at 149°C, rather than disconnected. Cooking time was 5.5 min for beef and 5.7 min for pork.

Sarcomere Length

Raw and cooked cubes were fixed according to Koolmees et al. (1986). From each cube, sarcomere length of eight fiber samples was determined (24 [raw], 48 [cooked beef], or 32 [cooked pork] total measurements per observation) by helium neon laser diffraction (model 05-LHR-021, Melles Griot, Carlsbad, CA) as described by Cross et al. (1981).

Total Collagen

Total collagen concentration was calculated from HPLC measurement of hydroxyproline as described by Wheeler et al. (2000).

Immunoblotting

Extraction, electrophoresis, Western blotting, and quantification of desmin were conducted as described by Wheeler and Koohmaraie (1999) with the following modifications. Whole muscle extracts were diluted to 2 mg/mL protein. For electrophoresis, 10 μg of protein per lane were loaded. Gels were transferred for 1 h at room temperature using Bio-Rad Mini Trans-Blot Electrophoretic Transfer Cell (Hercules, CA) with 15% methanol in the transfer buffer. Membranes were blocked overnight at 4°C. Primary antibody was diluted 1:20. After primary antibody incubation, membranes were washed once for 15 min and then twice more for 5 min each. After secondary antibody incubation, mem-

Table 1. Means and variability for meat quality traits for beef and pork longissimus

Species and trait	n	Mean	SD	Minimum	Maximum	CV, %
Beef (14 d postmortem)	20					
Raw						
Sarcomere length, μm		1.77	0.08	1.68	2.01	4.5
Collagen, mg/g		3.0[b]	0.5	2.2	3.9	16.7
Desmin, % degraded		65.1[b]	18.6	18.4	87.7	28.6
Cooked						
Sarcomere length, μm		1.72	0.09	1.51	1.90	5.2
Collagen, mg/g		4.1[b]	0.8	2.9	5.9	19.5
Desmin, % degraded		89.0[b]	13.4	47.0	100.0	15.1
Warner-Bratzler shear force, kg		3.6	0.7	2.4	5.5	19.4
Tenderness rating[a]		6.4	1.1	3.1	7.6	17.2
Pork (7 d postmortem)	20					
Raw						
Sarcomere length, μm		1.69[b]	0.07	1.57	1.87	4.1
Collagen, mg/g		4.6[b]	0.7	3.5	6.1	15.2
Desmin, % degraded		83.5[b]	12.3	57.8	97.4	14.7
Cooked						
Sarcomere length, μm		1.49[b]	0.07	1.34	1.58	4.7
Collagen, mg/g		5.2[b]	0.8	4.2	6.9	15.4
Desmin, % degraded		91.4[b]	7.3	75.5	99.5	8.0
Warner-Bratzler shear force, kg		3.5	0.9	2.9	4.9	25.7
Tenderness rating[a]		6.0	0.8	4.1	7.4	13.3

[a]1 = extremely tough, 8 = extremely tender.
[b]Means within species differ between raw and cooked state ($P < 0.05$).

branes were washed once for 15 min and then four more times for 5 min each. Antibody binding was detected by incubating membranes for 5 min with SuperSignal West Dura Extended Duration chemiluminescence substrate (Pierce, Rockford, IL), removing substrate then waiting 1 min, and finally exposing membranes for 5 min. Each blot included three lanes of at-death longissimus samples (beef or pork) that were averaged as a reference standard. Desmin data were expressed as percentages of the reference standard desmin that was degraded (calculated on a within-blot basis).

Shear Force

Warner-Bratzler shear force of beef longissimus was determined as described by Wheeler et al. (1998). Slice shear force of pork longissimus was determined as described by Shackelford et al. (1999) with the exception that two slices, one from each of two chops, were sheared and the slice shear forces from the two chops averaged. Slice shear force was used instead of Warner-Bratzler shear force because it is easier to perform, quicker, and is a slightly more repeatable test (Shackelford et al., 1999). However, to simplify interpretation of the data, slice shear force values were converted to Warner-Bratzler shear force values based on an equation from 1,561 observations (our unpublished data).

Trained Sensory Panel Evaluation

An eight-member trained descriptive attribute panel evaluated cooked steaks or chops as described by Wheeler et al. (1998).

Statistical Analyses

Data from the two experiments (beef and pork) were analyzed by analysis of variance for a completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The main effect was cook state in both experiments. The CORR procedure of SAS was used for simple correlations. The RSQUARE procedure of SAS was used for multiple linear regression of biochemical traits on measures of tenderness. Significant differences between correlation coefficients were determined using a homogeneity of correlation coefficients test (Steel and Torrie, 1980).

Results and Discussion

To characterize the sample used in this study, means and variability in tenderness and biochemical traits are shown in Table 1. Tenderness variation in these samples was relatively large for aged beef and pork and should be sufficient for studying factors potentially associated with that variation. In beef, collagen concentration and desmin degradation were lower ($P < 0.05$) in raw than in cooked samples; however, sarcomere length was not different between raw and cooked samples ($P > 0.05$). In pork, collagen concentration and desmin degradation were lower ($P < 0.05$) and sarcomere length was longer ($P < 0.05$) in raw than in cooked samples. In contrast to the beef sarcomere length results, but similar to the pork results, Lewis et al. (1977) reported that sarcomere length of 7-d-postmortem, raw beef longissimus was 0.35 μm longer than in cooked longissimus. Koohmaraie et al. (1996) and Wheeler and

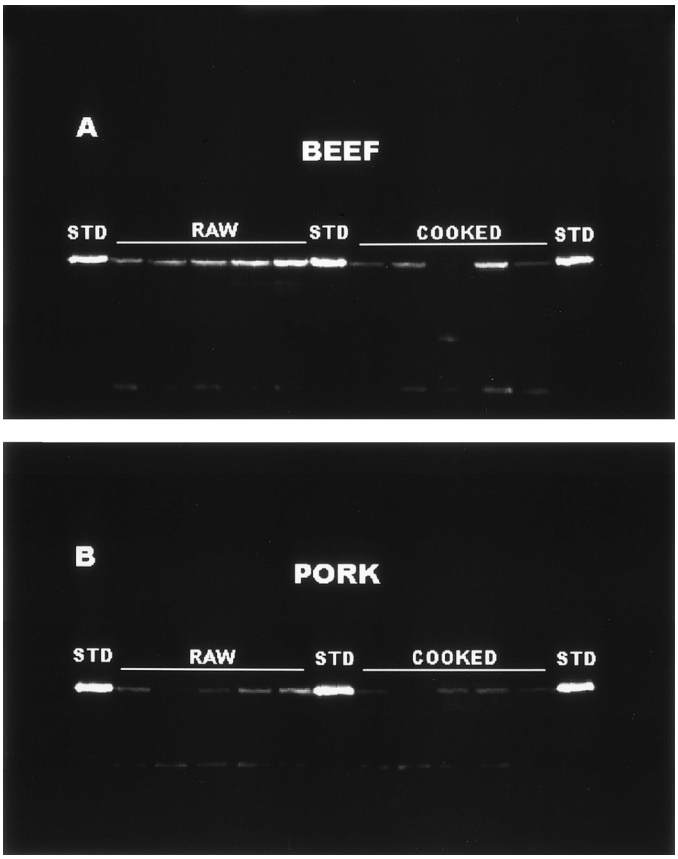

Figure 1. Representative Western blots of desmin degradation in raw and cooked beef (A) and pork (B) longissimus. The five lanes under "raw" and the five lanes under "cooked" contain five representative samples for each treatment. STD = the at-death longissimus sample was used for an internal reference standard to both standardize for among blot variation and to calculate the percentage desmin degraded.

Koohmaraie (1999) found similar differences between raw and cooked sarcomere length in 7- and 10-d-postmortem lamb longissimus, respectively, using the same methodology as in the present study. This effect would be expected due to moisture loss and tissue shrinkage during cooking. Mean values for sarcomere length were similar to previous data for beef longissimus (Lewis et al., 1977; McKeith et al., 1985; Whipple et al., 1990), lamb (Wheeler and Koohmaraie, 1999), and pork (Wheeler et al., 2000). Mean sarcomere length values reported by Koohmaraie et al. (1996) are longer than in other data because they prevented rigor shortening.

We are not aware of any data comparing collagen concentration or postmortem myofibrillar protein degradation in raw and cooked meat. Other estimates of collagen concentration in longissimus range from 2.8 to 5.7 mg/g of wet tissue in beef (Cross et al., 1973; Seideman, 1986; Whipple et al., 1990) and 3.4 to 4.1 mg/g of wet tissue in pork (Nold et al., 1999; Wheeler et al., 2000). We previously reported that 39% of desmin was degraded in 1-d-postmortem pork longissimus (Wheeler et al., 2000). However, we would expect desmin degradation to be highly variable, even in 14-d-postmortem meat. Figure 1 shows a representative blot of beef and pork samples. The top band is desmin and the lower bands are either fragments of desmin or nonspecific binding.

The simple correlation coefficients between measurements on raw and cooked samples were low and not significant ($P > 0.05$) for sarcomere length in pork but moderate and significant ($P < 0.05$) for all other traits (Table 2). Correlations between measures of longissimus sarcomere length on raw and cooked samples have been reported to be low (r = 0.37) in beef (Lewis et al., 1977) and high (r = 0.97) in lamb (Wheeler and Koohmaraie, 1999). Differences among studies in the strength of the correlation between raw and cooked sarcomere length may be a function of the location sampled (in this study, the raw and cooked chops were about 9 cm apart). Simple correlation coefficients between biochemical traits and measures of tenderness ranged from low to high (Table 2). In seven of eight instances, desmin degradation was significantly correlated ($P < 0.05$) to measures of tenderness regardless of whether measured on raw or cooked sample. Measurements of collagen concentration in beef were significantly correlated ($P < 0.05$) with tenderness rating only if measured on cooked sample. Measurements of collagen concentration in pork were not significantly correlated ($P > 0.05$) with measures of tenderness. Measurements of sarcomere length were not significantly correlated ($P > 0.05$) with measures of tenderness in beef or pork. Measurements of desmin degradation on cooked samples were more highly correlated ($P < 0.05$) with tenderness rating in beef longissimus than were measurements on raw samples. Measurements of collagen on cooked beef samples were more highly correlated ($P < 0.05$) with tenderness rating than were measurements on raw samples. In 9 of the 12 comparisons between raw and cooked meat, the magnitude of the correlations was greater for cooked than for raw meat, but only two of the comparisons between raw and cooked meat were significant, partly due to the low number of observations. In contrast, Lewis et al. (1977) reported significant correlations between sarcomere length and tenderness rating in beef longissimus with similar correlation coefficients for raw and cooked samples (0.35 vs 0.28). Seideman (1986) reported results similar to ours with a correlation coefficient of –0.45 between collagen concentration and tenderness rating in longissimus. In contrast to our results, in d-1-postmortem longissimus, Wheeler et al. (2000) reported correlation coefficients between biochemical traits and tenderness rating of –0.08 (desmin degradation), –0.21 (collagen concentration), and 0.67 (sarcomere length). The differences in the relationships between these traits and tenderness for our data and those of Wheeler et al. (2000) were likely due, in part, to the difference in the length of postmortem storage.

Table 2. Simple correlation coefficients between measurements on raw and cooked samples and between meat quality traits and measures of tenderness for beef and pork longissimus by cook state

Species and trait	Raw vs cooked	Shear force, kg[a]		Tenderness[b]	
		Raw	Cooked	Raw	Cooked
Beef (14 d postmortem)					
Sarcomere length, μm	0.58*	–0.22	–0.38	0.38	0.22
Collagen, mg/g	0.66**	–0.02	0.33	–0.12[c]	–0.45*[d]
Desmin, % degraded	0.74**	–0.54*	–0.69***	0.48*[c]	0.80***[d]
Pork (7 d postmortem)					
Sarcomere length, μm	0.11	–0.40	–0.08	0.14	0.15
Collagen, mg/g	0.59*	–0.12	0.18	–0.38	–0.33
Desmin, % degraded	0.76**	–0.44	–0.61**	0.53*	0.67**

[a]Beef = Warner-Bratzler shear force; Pork = slice shear force.
[b]1 = extremely tough, 8 = extremely tender.
[c,d]For comparisons of raw vs cooked for each biochemical trait, correlations lacking a common superscript letter differ ($P < 0.05$).
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

A three-variable model including sarcomere length, collagen concentration, and desmin degradation explained more of the variation in tenderness rating and shear force value when these traits were measured on the cooked samples used to make the shear force determination than when measured on a separate raw sample (Table 3). Although correlations between measures of tenderness and these individual traits were generally higher for tenderness rating than for shear force, the three-variable model explained more of the variation in shear force than it did the variation in tenderness rating. This difference in the magnitude of correlations with tenderness rating compared to shear force is likely to be because the cooked sample for the biochemical measurements came from the samples used for shear force measurement and tenderness rating was measured on a separate steak/chop. In d-1-postmortem pork longissimus, these same three traits measured on raw samples explained 49% of the variation in tenderness rating, even though at 1 d postmortem proteolysis contributed little to the model (Wheeler et al., 2000). The results of Wheeler et al. (2000) are similar to ours for raw samples. It seems logical that traits would be more closely related to tenderness if measured on the same sample as tenderness was measured. The present study confirms that logic and provides a basis for improved methodology for experiments relating tenderness to other traits.

Table 3. Coefficients of determination for explaining variation in measures of tenderness for beef and pork longissimus by cook state using a three-variable model including sarcomere length, collagen concentration, and desmin degradation

Species and cook state	Shear force, kg[a]	Tenderness
Beef		
Raw	0.43	0.40
Cooked	0.73	0.67
Pork		
Raw	0.48	0.26
Cooked	0.57	0.42

[a]Beef = Warner-Bratzler shear force; Pork = slice shear force.

Implications

We designed experiments to determine the contribution of various biochemical traits to tenderness variation and found that the cooked sample used to make tenderness measurements also could be used for biochemical measurements. This improved results relative to using a separate raw sample for biochemical measurements, increasing the ability of these biochemical measurements to predict tenderness.

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