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Relationships among Instrumental Tenderness Parameters, Meat Quality Traits, and Histochemical Characteristics in Porcine *Longissimus dorsi* Muscle

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Abstract The objective of this study was to examine the relationship between instrumental tenderness parameters and histochemical characteristics in the porcine *longissimus dorsi* muscle, and to investigate a comparison between tenderness parameters such as the Warner-Bratzler shear-force (WBS) and texture profile analysis (TPA). A negative relationship between WBS and fiber area was observed. However, there was no significant relationship between hardness and muscle fiber area. The percentage of fiber type IIb exhibited a positive correlation with hardness. There was a negative relationship between the type IIa composition percentage and hardness. This study showed that some muscle fiber characteristics were related to WBS and TPA parameters, especially hardness.

Keywords: tenderness, Warner-Bratzler shear-force, texture profile analysis, muscle fiber, pork quality

Introduction

The palatability of meat is defined as the interaction between several factors, including tenderness, juiciness, and flavor. Although consumers can recognize flavor, juiciness, and tenderness as they evaluate meat palatability, tenderness has been identified as the most important characteristics for influencing the satisfaction of consumers (1). The tenderness of meat can be affected by the animal's genetics, the feeding regimen, and the physiological maturity of the animal when it is slaughtered (2). Boleman *et al*. (3) and Savell and Shackelford (4) reported that tenderness was the primary economic factor for beef palatability. Moreover, not only can consumers differentiate between tenderness groups, they are also willing to pay a premium for tender meat (3). Tenderness characteristics consist of chemical composition, structural organization, and integrity, which in turn are physiological properties of the skeletal muscle. Tenderness is often associated with the amount of marbling, or the amount of fat in the muscle.

Fiber size is an important factor in determining meat tenderness (5). Muscles with larger fiber size, especially type IIb fiber, exhibit tougher meat than muscles of smaller fiber size in pigs (6). In addition, for the texture parameters, muscles with greater hardness are related to a larger fiber area (7). Muscle with higher numbers of low or medium size fibers tend to exhibit good meat quality, without significant differences in muscle mass. On the other hand, selecting for leaner livestock animals can result in large muscle fiber, especially type IIb fiber, which seems to be associated with poorer meat quality (8).

Numerous attempts have been made to create an accurate instrument to measure meat tenderness (9). Inconsistency in meat tenderness has been identified as one of the major problems facing the beef industry (10). One method to limit the consequences of inadequate tenderness is to objectively evaluate tenderness, and market cuts appropriately. However, Warner-Bratzler shear-force (WBS) has remained the most popular and accurate instrumental measure of meat tenderness, despite its critics (11). Numerous studies have evaluated various factors influencing WBS values since Warner invented the apparatus (12). These investigations first led to refinements in blade thickness and sharpness, as well as the size and shape of the hole in the shear blade (13), and most recently, identified the combined effects of several cooking, coring, and shearing factors (14). Previous research indicates that shear-force values are influenced by varying the following shear-force determination parameters: core orientation with respect to muscle fiber (15), end point temperature (16), steak and core location (10), the hand or machine to obtain the cores (17), heating rate (18), and chilling time after cooking (19).

There are correlations between fiber type and quality traits in cattle. However, it is controversial to state the relationship between fiber type and meat quality in pig. In addition, WBS and texture profile analysis (TPA) have similar capabilities to predict the sensory measurement of tenderness and other subjective traits. However, there is limited information comparing these two instrumental methods (20). The objective of the present study was to examine the relationship between meat tenderness and histochemical characteristics in the porcine *longissimus dorsi* muscle. In addition, this study investigated a comparison between the tenderness parameters of WBS and TPA.

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Materials and Methods

Animals and muscle samples A total of 103 crossbred pigs (103 gilts) were used in this study. The pigs were slaughtered using electrical stunning, during spring. The abattoir utilized the traditional scalding-singeing process. At 45 min postmortem, a total of 103 muscle samples were collected from the *longissimus dorsi* muscles (at the 8th *thoracic vertebra*) for histochemical analysis and postmortem metabolic rate. After 24 hr of chilling, the pork loins (9th-13th) were collected in order to evaluate the meat quality measurements and tenderness parameters.

Postmortem metabolic rate The muscle pH of the 45 min ($pH_{45\ min}$) and 24 hr ($pH_{24\ hr}$) postmortem samples was measured with a spear type pH meter (290A; Orion Research Inc., Boston, MA, USA). The *R*-value was measured according to the procedure reported by Calkins *et al*. (21). Briefly, the 45 min postmortem samples, which had been placed in 6% perchloric acid, were homogenized at 5,000 rpm for 90 sec, and then centrifuged at 3,000×g for 10 min at 4°C. The absorbance was measured with a calibrated spectrophotometer (Model Du-64; Beckman Co., Fullerton, CA, USA). All the adenine nucleotides showed an absorption maximum at 259-260 nm, whereas the maximum absorption of hypoxanthine was found at 250 nm. The *R*-value (R_{250}) was defined as the ratio of A_{250}/A_{260}.

Meat quality measurements The meat color was assessed at 24 hr postmortem, using a Minolta chromameter (CR-300; Minolta Camera Co., Osaka, Japan) after exposing the surface to air for 30 min at 4°C. An average of 3 measurements was recorded, and the results were expressed as C.I.E. (Commission International de l'Eclairage) lightness (L^*), redness (a^*), and yellowness (b^*). Drip loss was determined by suspending the muscle samples standardized for surface area in an inflated plastic bag at 4°C for 48 hr (22). Filter paper fluid uptake (FFU) was also measured in accordance with the method described by Kauffman *et al*. (23). Subjective measures of color (1=pale pinkish gray to white; 6=dark purplish red) and marbling [1=1.0% intramuscular fat (IMF); 10=10% IMF] were evaluated according to the National Pork Producers Council (NPPC, 24). The NPPC scores were measured by 20 panelists. Cooking loss was determined by assessing the value of exudation after thermal treatment. The pork samples from each treatment were cooked to a final core temperature of 71°C, and the cooking loss was estimated by weighing before and after cooking (25).

Protein solubility was utilized as an indicator of protein denaturation (26). The muscle samples, taken at 45 min postmortem from the *longissimus* muscle at the 8th *thoracic vertebra,* were immediately frozen in liquid nitrogen for the evaluation of protein solubility. The muscle samples were then powdered using a motor-based homogenizer (HGBSS, Waring Products Inc., Torrington, CT, USA). The sarcoplasmic protein solubility was determined via the dissolution of 1 g of muscle powder in 10 mL of ice-cold 25 mM potassium phosphate buffer (pH 7.2). The samples were minced, homogenized on ice with a polytron set at the lowest setting, and allowed to stand in a shaker at 4°C overnight. Then, the samples were centrifuged for 20 min at 1,500×g, and the protein concentrations in the supernatants were determined via the Biuret method (27). Total protein solubility was determined via the homogenization of 1 g of muscle powder in 20 mL of ice-cold 1.1 M potassium iodide in a 100 mM phosphate buffer (pH 7.2). The procedures employed for homogenization, shaking, centrifugation, and protein determination were the same as described above. The myofibrillar protein solubility was determined by measuring the differences between the total and sarcoplasmic protein solubilities.

Warner-Bratzler shear-force (WBS) WBS was determined using an Instron Universal Testing Machine (Model Series IX; Instron Co., Norwood, MA, USA) equipped with a Warner-Bratzler shearing device, where the WBS is used as an indicator of meat tenderness. Six cores (1.27 cm diameters), parallel to the longitudinal orientation of the muscle fibers, were taken from each steak. The samples were sheared perpendicular to the long axis of the core.

Texture profile analysis Pork samples from each treatment were cooked to a final core temperature of 71°C. After cooking, 6 cores (1.27 cm diameters), parallel to the longitudinal orientation of the muscle fibers, were taken from each steak. Six to 10 strips for each sample were measured for TPA. Each sample underwent 2 cycles of 80% compression (relative to sample width, 200 mm/min crosshead speed) using the overhead probe fitted to a texture analyzer (Model 3353; 25 kg load cell, Series 12 Cyclic Testing software, Stable Micro Systems, Surrey, England). Two separate TPA were done per strip, for a total of 6 measurements per steak. The force-by-time data from each test were used to calculate the mean values for the TPA parameters of each steak. Values for hardness (peak force of the first compression cycle in kg), cohesiveness (ratio of the positive force area during the second compression to that during the first compression, or area 2/area 1), springiness (ratio of the time duration of force input during the second compression to that during the first compression, or length 2/length 1), gumminess (hardness multiplied by cohesiveness), and chewiness (gumminess multiplied by springiness) were determined as described by Bourne (28).

Histochemical analysis The muscle sections were stained for their myosin ATPase reactivity after preincubation at pH 4.7, according to the method reported by Brooke and Kaiser (29). Unfixed sections were pre-incubated at room temperature for 5 min in a buffer consisting of 100 mM potassium chloride in 100 mM sodium acetate that was adjusted to pH 4.7 with acetic acid (30). After pre-incubation, the sections were subjected to the following steps: washing in 4 rinses of distilled water; washing for 30 sec in a 20 mM glycine buffer (pH 9.4) containing 20 mM $CaCl_2$; incubation at room temperature for 25 min in a freshly prepared medium (40 mM glycine buffer containing 20 mM $CaCl_2$ and 2.5 mM ATP disodium salt, pH 9.4); washing in three 30 sec changes of 1% $CaCl_2$; washing in 2% cobalt chloride for 3 min; washing in 3 changes of distilled water; immersing in 1% yellow ammonium sulfide for 30 sec; washing in several changes of distilled water; and mounting in glycerol jelly (31).

All the histochemical samples were examined with an image analysis system. This operational system consisted of a microscope equipped with a charge-coupled device color camera (IK-642K; Toshiba, Tokyo, Japan) and a standard workstation computer, which controlled the image analysis system (Image-Pro Plus; Media Cybernetics, Siver Spring, MD, USA). Approximately 600 fibers per sample were evaluated. The muscle fibers were identified as types I, IIa, and IIb. The average cross-sectional areas (CSA) of the type-identified fibers were also measured. The density of the fibers was calculated from the mean number of fibers/mm^2. The number percentage of muscle fiber type was obtained from the ratio of the number of each fiber type to the total number of fibers counted, and the fiber type area percentage was the ratio of each fiber type's total CSA to the total measured fiber area.

Statistical analysis Descriptive statistics were performed using the MEANS procedure of the SAS PC software (32) to calculate mean values and standard deviations (SD) for all variables. The Pearson correlation coefficients were evaluated using the partial correlation coefficients, in order to determine the relationship existing among the tenderness parameters, the meat quality measurements, and the histochemical characteristics.

Results and Discussion

Relationships between tenderness parameters The mean values, SD and overall ranges for WBS and TPA measured are given in Table 1. Several methods of WBS were conducted to measure the pork tenderness (33). The results of present study were similar to mean and SD of WBS value of the literature. The most widespread method normally used as an indicator of meat sensory tenderness is the WBS test, which is almost the sole methodology used in raw meat, and is referred to in most papers (11). However, several sources of error have been identified that contribute to the error in shear-force assessment within and among institutions (34). Recognizing this limitation, the mechanical process of mastication has been simulated using TPA. This objective method measures the compression force of a probe and the related textural parameters of a test food during 2 cycles of deformation.

There have been few papers describing the relationship between WBS and TPA parameters. However, some papers have reported the relationships among sensory characteristics, TPA parameters, and WBS (21). The correlation of WBS with the sensory assessment of beef tenderness has been variable, ranging from –0.60 to –0.85 (35) and –0.32 to –0.94 (36). Instrument textural parameters accounted for approximately 50 and 30% of the sensory variability in the tenderness and juiciness characteristics, respectively, of warm roast beef (37). In this study investigated the correlation coefficients between these 2 objective tenderness parameters (Table 2), where the WBS parameter was positively related to the hardness (r=0.26, p<0.05) and chewiness values (r=0.25, p<0.05).

Relationships between tenderness and meat quality There has been controversy in the literature with regard to the relationship between ultimate pH and tenderness (38).

Table 1. Warner-Bratzler shear-force (WBS) and texture profile analysis (TPA) in porcine *longissimus dorsi* muscle in pig

	Mean	SD[1)]	Min	Max
WBS (N)	40.63	5.27	24.02	59.83
TPA parameter				
Hardness (N)	2.99	0.59	2.32	3.69
Cohesiveness	0.49	0.04	0.41	0.58
Springiness	0.88	0.04	0.68	1.03
Gumminess	1.48	0.14	1.05	2.05
Chewiness	1.32	0.19	0.91	1.97

[1)]Standard deviation.

Table 2. Correlation coefficients (r) between Warner-Bratzler shear-force (WBS) and texture profile analysis (TPA) parameters in porcine *longissimus dorsi* muscle

	WBS	TPA parameter			
		Hardness	Cohesiveness	Springiness	Gumminess
Hardness	0.26*				
Cohesiveness	-0.13	0.09			
Springiness	0.09	-0.08	-0.90***		
Gumminess	-0.09	0.20	0.99***	-0.89***	
Chewiness	0.25*	0.56***	0.46***	0.65***	-0.38**

*p<0.05, **p<0.01, ***p<0.001.

Most researchers have observed a curve-linear relationship between tenderness and ultimate muscle pH. Some researchers suggested a negative relationship between sarcomere length and WBS values, up to an ultimate pH of about 6.0 (38). In this study, there was no significant correlation between $pH_{24\ hr}$ and the WBS value (Table 3). The WBS value was positively related to drip loss (p<0.05), FFU (p<0.05), and cooking loss (p<0.001). Drip loss, FFU, and cooking loss indirectly express the water holding capacity (WHC) in meat. WHC influences the profitability of fresh pork products by affecting processing yields and palatability (39). The IMF content is one of the important factors controlling meat tenderness (40). In this study, the NPPC marbling scores were negatively related with WBS (p<0.05) and hardness (p<0.001). In 1970, Rhodes (41) showed that the IMF content had no effect on tenderness; although, Goransson *et al.* (42) did detect an effect of IMF content on tenderness. Flores *et al.* (43) observed a close relationship between IMF and juiciness, although less with hardness.

There were significantly negative relationships between WBS and total protein solubility (p<0.001), and between WBS and myofibrillar protein solubility (p<0.001) (Table 4). However, there were no significant relationships between the TPA parameters and protein solubility. Weinburg and Rose (44) found that an increase in postmortem tenderness was paralleled by an increase in the extractability of the myofibrillar proteins. Dikeman *et al.* (45) supported this result based on the negative relationship shown between myofibril protein solubility and tenderness. In another study, myofibrillar proteins were least extractable at 24 hr postmortem, but solubility significantly increased with aging and paralleled an increase in tenderness (46).

Table 3. Correlation coefficients (*r*) between tenderness parameters and meat quality measurements in porcine *longissimus dorsi* muscle

	WBS[1)]	TPA[2)] parameter				
		Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Postmortem metabolic rate						
Muscle $pH_{45\ min}$	-0.01	0.08	-0.13	0.18	-0.11	0.26*
Muscle $pH_{24\ hr}$	-0.09	0.05	0.10	0.01	0.12	0.14
R-value	0.24*	0.21	0.13	-0.18	0.13	0.09
Meat quality trait[3)]						
Drip loss	0.26*	-0.15	-0.07	-0.02	-0.09	-0.21*
FFU	0.19*	0.12	0.10	-0.13	0.10	-0.06
Cooking loss	0.49***	0.10	-0.26*	0.15	-0.25*	0.11
Lightness (*L**)	0.33*	0.12	-0.13	0.11	-0.13	0.09
Redness (*a**)	-0.03	0.02	0.15	-0.12	0.15	-0.02
Yellowness (*b**)	0.11	0.00	-0.07	0.08	-0.07	0.02
NPPC color score	-0.20*	-0.13	0.18	-0.16	0.16	-0.11
NPPC marbling score	-0.25*	-0.43***	0.03	0.04	-0.02	-0.20*

[1)]WBS, Warner-Bratzler shear-force.
[2)]TPA, texture profile analysis.
[3)]FFU, filter-paper fluid uptake; NPPC, National Pork Producers Council.
*$p<0.05$, ***$p<0.001$.

Table 4. Correlation coefficients (r) between tenderness parameters and protein solubility in porcine *longissimus dorsi* muscle

	WBS[1)]	TPA[2)] parameter				
		Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Protein solubility						
Total protein	-0.35***	-0.17	0.05	-0.09	0.05	-0.09
Sarcoplasmic protein	-0.06	-0.18	-0.11	0.10	0.13	-0.04
Myofibrillar protein	-0.33***	-0.04	0.11	-0.14	0.12	-0.06

[1)]WBS, Warner-Bratzler shear-force.
[2)]TPA, texture profile analysis.
***$p<0.001$.

Table 5. Correlation coefficients (*r*) between tenderness parameters and histochemical characteristics in porcine *longissimus dorsi* muscle

	WBS[1)]	TPA[2)] parameter				
		Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Muscle fiber area						
Mean	-0.15	-0.03	0.07	0.05	0.07	0.06
Type I	-0.16*	0.02	0.02	0.13	0.05	0.26*
Type IIa	-0.06	-0.23*	-0.07	0.14	-0.10	0.03
Type IIb	-0.17*	-0.10	-0.03	0.15	-0.05	0.05
Muscle fiber density						
Sum	0.14	0.01	-0.10	-0.02	-0.09	-0.04
Type I	-0.08	-0.17	-0.14	0.12	-0.17*	-0.11
Type IIa	0.00	-0.27*	0.07	0.03	0.04	-0.05
Type IIb	0.18*	0.20*	0.02	-0.13	0.05	0.02

[1)]WBS, Warner-Bratzler shear-force.
[2)]TPA, texture profile analysis.
*$p<0.05$.

Relationships between tenderness and histochemical characteristics This study investigated the correlation between histochemical characteristics and meat tenderness parameters such as WBS and TPA (Table 5 and 6). This study found a negative relationship between WBS and the fiber areas of type I (r=–0.16, $p<0.05$) and IIb (r=–0.17, $p<0.05$). However, this study did not find a significant relationship between hardness and muscle fiber area, with

Table 6. Correlation coefficients (*r*) between tenderness parameters and muscle fiber type composition in porcine *longissimus dorsi* muscle

	WBS[1)]	TPA[2)] parameter				
		Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Muscle fiber area percentage						
Type I	-0.19*	-0.14	-0.15	0.16*	0.16*	0.00
Type IIa	0.03	-0.36*	0.02	0.09	-0.02	-0.07
Type IIb	0.12	0.31*	0.08	-0.02	0.11	0.02
Type II (IIa+IIb)	0.20*	0.12	0.15	-0.13	0.15	-0.04
Muscle fiber number percentage						
Type I	-0.14	-0.16*	0.15	0.11	-0.17*	-0.07
Type IIa	-0.05	-0.26*	0.06	-0.12	0.03	0.00
Type IIb	0.15	0.28*	0.07	0.08	0.10	0.05
Type II (IIa+IIb)	0.16*	0.16*	0.15	-0.16*	0.17*	0.07

[1)]WBS, Warner-Bratzler shear-force.
[2)]TPA, texture profile analysis.
*$p<0.05$.

the exception of the type IIa area ($r=-0.23$, $p<0.05$). That fiber size is an important factor determining the toughness of meat, which is supported by results from several different types of studies. In general, when there are clearly more type IIb fibers the meat will be tougher because type IIb fibers are larger and more resistance to chewing and mechanical measures (47-49). Also, in our study, type IIb density was positively related to WBS and hardness. However, a problem in interpreting the results is that fiber size is strongly affected by cold shortening (50).

Postmortem tenderization during the ageing of beef is a variable process depending on a number of biological, as well as external factors. Of all these factors, muscle type plays a major role (51-53). Cattle tend to have stronger correlations between fiber type and quality traits than pigs. In fact, pigs often have no significant relationship between fiber type characteristics and instrumental tenderness parameters (54). In our measurements, the muscle fiber type IIb composition percentage exhibited a positive correlation with hardness ($p<0.05$). This study found a negative relationship between type IIa composition percentage and hardness ($p<0.05$). However, this study discovered a positive relationship between type II (IIa+IIb) and WBS ($p<0.05$). As reviewed by Quali (55), the ageing rate is faster in fast-twitch white muscles than in slow-twitch red muscles. Three different mechanisms were suggested to explain these differences in ageing rate between the different muscle types: levels of proteases and inhibitors, sensitivity of muscle proteins to proteolysis, and osmotic pressure. Of these factors, calpains are primarily involved in the postmortem proteolysis of myofibrillar and associated proteins (56). Similar result was uncovered for lamb muscle by Cena *et al*. (57), who also showed that slow-twitch red fibers in unrestrained muscle had a more intense shortening than fast-twitch white fibers. Quali (55) showed that slow-twitch red muscle fiber such as muscle fiber type I, which exhibit the lowest ageing rate, have the highest calpain content, and suggested that the expression of these proteases is muscle dependent.

Previous reports indicate that TPA and WBS have similar capabilities to predict the sensory assessment of tenderness and the subjective characteristics of beef. Caine *et al*. (37) reported that TPA parameters accounted for 47 and 51% of the variation in initial and overall tenderness, respectively, and prediction equations using WBS accounted for 37 and 36% of the variation in initial and overall tenderness, respectively. This results support the present study. Hence, it is difficult to predict tenderness using one instrumental analysis. This study also showed that some muscle fiber characteristics were related to WBS, and other muscle fiber characteristics exhibited a relationship with the TPA parameters, especially hardness.

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