



## An Investigation on the Influence of Various Biochemical Tenderness Factors on Eight Different Bovine Muscles

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**Abstract:** This study's objective was to understand the relationships between biochemical tenderness components and objective/sensory measure of tenderness of 8 muscles from beef carcasses. *Longissimus thoracis* (LT), *pectoralis profundus* (PP), *supraspinatus*, *triceps brachii* (TB), *gluteus medius* (GM), *rectus abdominus* (RA), *rectus femoris*, and *semitehdinosus* (ST) were collected from 10 USDA upper 2/3 Choice beef carcasses and assigned to a 2- or 21-d aging period (n = 160). Troponin-T degradation, desmin degradation, sarcomere length, collagen content, mature collagen crosslink density, intramuscular lipid content, pH, Warner-Bratzler Shear Force (WBSF), and trained sensory panel analyses were measured. A Pearson correlation analysis was conducted to determine the relationship between each tenderness contributor measured in this study with WBSF or the overall tenderness evaluated by the trained panelist for each of the 8 muscles. In addition, multivariate regression models were constructed to confirm this relationship. The results showed that muscle anatomical locations and physiological functions driven by muscle fiber types may explain some of the biochemical/tenderness differences found in this study. The correlation analysis showed that each muscle had a specific tenderness factor(s) that contributed to the overall tenderness. For instance, tenderness for LT, TB, GM, RA, and ST may be influenced more by proteolytic degradation, while the collagen characteristics may primarily influence tenderness for PP. Also, lipid content has a significant influence on GM tenderness. Finally, the multivariate regression model showed that almost all of the biochemical measurements conducted in this study contributed to overall tenderness to some degree across muscles. Increasing the knowledge base on the various tenderness components' level of contribution will allow end-users to develop specific tenderness management strategies to ensure consistent tenderness in beef products.

**Key words:** beef, tenderness, trained panel, proteolysis, collagen, lipid

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## Introduction

Historically, tenderness has been identified as the most important palatability trait (Miller et al., 1995; Huffman et al., 1996; Egan et al., 2001). Consequently, this has resulted in a large focus on tenderness research resulting in significant improvement in beef tenderness over the past 25 years (O'Quinn et al., 2018). The most recent Beef Tenderness Survey

conveyed that ~95% of the middle meat (ribeye roll, striploin, tenderloin, and top sirloin) is considered “tender” or “very tender” (Martinez et al., 2017). However, middle meat only accounts for ~12% of the weight of the entire beef carcass (NCBA, 2020), and many cuts that reside in other portions of the carcass, such as the round and chuck, are still considered tough and lower in quality (Anderson et al., 2012). In addition, these “lower quality” beef cuts lack popularity among consumers due to the

additional time and/or knowledge required to prepare these cuts to optimize palatability (Pfeiffer et al., 2005).

Defined as the resistance to shear or the toughness of meat (Chandraratne et al., 2006), meat tenderness is influenced by 3 different basic mechanisms: (1) the actomyosin effect; (2) the background effect; and (3) the bulk density or lubrication effect (Berry et al., 1974). Past studies have shown that each of the 3 tenderness mechanisms is further influenced by the various biochemical components, which differed from muscle to muscle (Koochmaraie, 1992; Sullivan and Calkins, 2011; Roy et al., 2015). Chun et al. (2020) found that both tri-tip and heel are similar in the overall perception of tenderness, but the tenderness perception for tri-tip was driven by collagen content, while the tenderness of heel was driven by proteolysis of myofibrillar proteins. Anderson et al. (2012) also concluded that observing proteolysis alone is not a good indicator for meat tenderness without knowing supporting factors such as pH, connective tissue content, sarcomere length, and muscle fiber types. These studies demonstrated that a holistic exploration of each muscle's biochemical and physical properties is necessary to fully understand the reason for deviations in tenderness that occur from muscle to muscle (Rhee et al., 2004). Therefore, the objective of this study was to understand the relationships of various tenderness contributing components to the overall tenderness of 8 different muscles from beef carcasses in order to expand knowledge in tenderness management strategies for different beef cuts.

## Materials and Methods

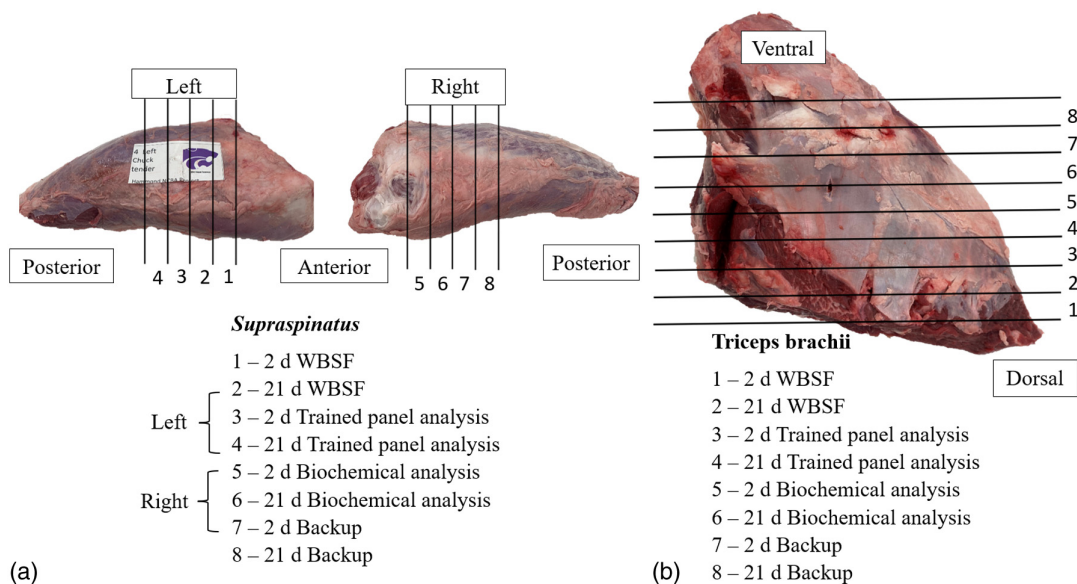
### Sample collection

Ten USDA upper 2/3 Choice beef carcasses at 1 d postmortem were selected from a Midwest beef packing plant. These 2/3 Choice grade carcasses were selected based on the kill date, in which these carcasses were the most recently harvested group in the carcass cooler (1 d postmortem), which would provide greater contrasts for the biochemical analysis performed in this study. Ribeye roll (NAMP #112), brisket (NAMP #120), shoulder clod (NAMP #114), top sirloin butt (NAMP #184), knuckle (NAMP #167), and eye of round (NAMP #171C) were collected only from the left side of the carcass, while the mock tenders (NAMP #116B) and flanks (NAMP #193) were collected from both sides of the carcass. Again, these muscles were selected because of their economic

importance and inherent differences in sensorial and biochemical properties. The selected cuts of beef were vacuum packaged and transported to the Kansas State University meat laboratory. The cuts were further fabricated the following morning at 2 d postmortem. Each cut was initially fabricated to remove the accessory muscles not included in this study, leaving only *longissimus thoracis* (LT) from the ribeye roll, *pectoralis profundus* (PP) from brisket, *triceps brachii* (TB) from shoulder clod, *gluteus medius* (GM) from top sirloin butt, *rectus femoris* (RF) from knuckle, *semitendinosus* (ST) from eye of round, *supraspinatus* (SS) from chuck tender, and *rectus abdominus* (RA) from flanks. Eight 1-in (2.54 cm) steaks were cut anterior to the posterior end or dorsal to ventral of each muscle depending on the muscle fiber direction (achieving cuts perpendicular to muscle fiber direction). Representative figures of how each cut was fabricated for analysis are demonstrated for SS and TB in Figure 1. Steaks were vacuum packaged and assigned to 1 of the 2 aging periods at  $2 \pm 2^\circ\text{C}$ : 2 or 21 d, and 3 analysis groups: Warner-Bratzler Shear Force (WBSF), trained sensory analysis, and biochemical analysis. Each analysis group consisted of 160 samples. At the end of the allotted aging period, all the samples were frozen at  $-40^\circ\text{C}$  (WBSF and trained panel samples) or  $-80^\circ\text{C}$  (biochemical samples) until analysis.

### Proteolysis analysis

The steaks designated for lab analysis were cubed, frozen under liquid nitrogen, and pulverized using a commercial blender (model 51BL32, Waring Commercial, Torrington, CT). Myofibrillar proteins were extracted according to the method described by Pietrzak et al. (1997) with modifications. Pulverized meat was homogenized in ice-cold ultrapure water using a bead homogenizer (D2400 Homogenizer, Benchmark Scientific, Sayreville, NJ) for 30 s. After homogenization, the homogenate was transferred into microcentrifuge tubes and centrifuged at  $4,000 \times g$  for 5 min. The pellet was washed in ultrapure water 3 times. After the third wash, the pellet was resuspended in protein extraction buffer (0.1 M Tris-hydrochloride, 1.25 mM ethylenediamine tetraacetic acid, 2% sodium dodecyl sulfate [SDS]). Samples were centrifuged for 5 min at  $4,000 \times g$ , and the supernatant was removed and transferred to new microcentrifuge tubes. Protein concentration was determined by using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA), and the protein stock was adjusted to 2 mg/mL using the protein extraction buffer.



**Figure 1.** Representative images of fabrication map for (A) *supraspinatus* and (B) *triceps brachii*.

Level of proteolysis was measured by troponin-T (TNT) and desmin degradation according to the method described by Chao et al. (2017) and Kondo et al. (2018) with modifications. Protein samples were mixed 1:1 with 2x Laemmli SDS sample buffer (Alfa Aesar, Haverhill, MA) and was heated on a heat block at 95°C for 5 min. SDS-polyacrylamide gel electrophoresis was conducted using an Invitrogen precast 10% Tris-glycine gel (Thermo Fisher Scientific) in a Mini Gel Tank Electrophoresis System (Thermo Fisher Scientific). Five micrograms of the protein from each sample was loaded into the wells of the precast gels and run for 1 h at 180 V. The gel was removed from the gel assembly and transferred onto the polyvinylidene difluoride membrane (iBlot 2 Transfer Stack) using an iBlot 2 Gel Transfer Device (Thermo Fisher Scientific) with settings of 20 V for 1 min, 23 V for 4 min, and 25 V for 2 min.

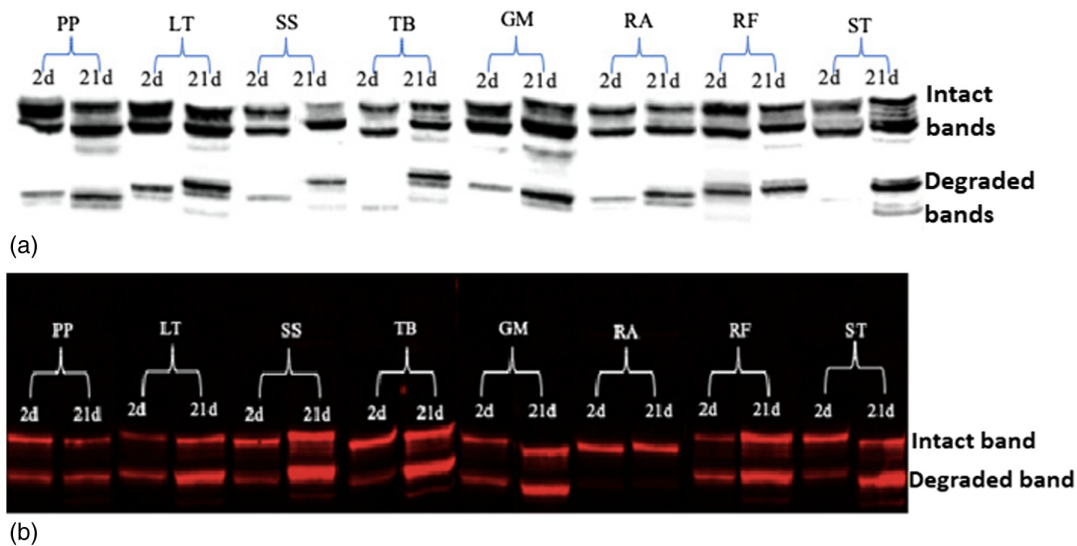
For TNT degradation, the membrane was blocked with 5% nonfat dry milk (NFDM) in Tris-buffered saline with 0.1% Tween 20 (TBS-T) for 90 min at room temperature. The blot was incubated in primary antibody, anti-troponin-T IgG1 from mouse (JLT-12; Boster Bio, Pleasanton, CA), at 1:1,000 dilution in 5% NFDM and TBS-T for 60 min. The membrane was washed 3 times in TBS-T and incubated in secondary antibody, Peroxidase Conjugated Goat Anti-Mouse IgG1 (BA 1050; Boster Bio) at 1:1,000 dilution in 5% NFDM and TBS-T for 60 min at room temperature. The blot was again washed 3 times for 5 min each with 1x TBS-T. Prior to imaging, the membrane was incubated in Amersham ECL Prime Western Blotting

Detection Reagent (GE Healthcare, Chicago, IL) for 3 min, and an image was captured using the iBright FL1500 imager with a chemiluminescent mode (Thermo Fisher Scientific).

For desmin degradation, the membrane was blocked with fluorescent detection compatible blocking buffer (OneBlock Western-FL Blocking Buffer, Genesee Scientific, El Cajon, CA) for 1 h at room temperature. The blot was incubated in primary antibody, anti-desmin from rabbit (M01948-1; Boster Bio), at 1:2,000 dilution for 60 min. The membrane was washed 3 times for 5 min each in TBS-T and incubated in secondary antibody, Alexa-Fluor Plus 647 goat anti-rabbit H&L (Thermo Fisher Scientific), at 1:10,000 dilution in blocking buffer for 60 min at room temperature. The blot was again washed 3 times for 5 min each in TBS-T. An image was captured using the iBright FL1500 imager with a fluorescence mode (Thermo Fisher Scientific). The band intensities of intact TNT and desmin and degraded TNT and desmin were quantified using the iBright Analysis software, and the percent of TNT and desmin degradation was calculated by dividing the band signals of the identified degraded bands by the signals of all the bands within the same lane (Figure 2).

### Sarcomere length

Sarcomere lengths were measured using the method described by Mohrhauser et al. (2011) with modifications. Approximately 50 µg of pulverized sample was lightly dusted onto a microscope slide.



**Figure 2.** Representative images for (A) troponin-T degradation and (B) desmin degradation of *pectoralis profundus* (PP), *longissimus thoracis* (LT), *supraspinatus* (SS), *triceps brachii* (TB), *gluteus medius* (GM), *rectus abdominus* (RA), *rectus femoris* (RF), and *semitendinosus* (ST) after aging for either 2 or 21 d.

Samples were allowed to air-dry, and a hydrophobic pen was used to trace the sample area to create a barrier for the wash buffer and antibodies. The samples were incubated overnight at 4°C with a monoclonal anti- $\alpha$ -actinin antibody (A7811, Sigma-Aldrich, St. Louis, MO), diluted 1:5,000 with 10% horse serum and 0.2% TritonX-100 in phosphate-buffered saline (PBS). After incubation with primary antibody, sections were washed with PBS 3 times and incubated with a secondary antibody diluted at 1:1,000 (Alexa-Fluor Plus 488 goat anti-mouse H&L; Thermo Fisher Scientific) in 10% horse serum and 0.2% TritonX-100 in PBS for 30 min. Finally, samples were washed and covered with a drop of 9:1 glycerol in PBS. A coverslip was placed on the samples, and the slide was imaged using a Zeiss LSM700 upright confocal microscope with a 63x/1.4 oil differential interference contrast objective (Zeiss, Oberkochen, Germany). Three images were captured per sample using the Zen Black software (Zeiss), and 30 sarcomeres were measured and averaged for each sample using ImageJ software (version 1.52k; National Institutes of Health) with the LSM Toolbox plugins.

### Collagen hydrolysis

Collagen was hydrolyzed according to the method described by Sims et al. (2000) with modifications. Five hundred milligrams of each powdered sample was placed into 16 × 125 mm glass tubes with polytetrafluoroethylene (PTFE) coated caps. Ten milliliters of 6N hydrochloric acid (HCl) was added to each

sample and placed in a forced air oven (Isotemp, Thermo Fisher Scientific) set for 24 h at 115°C to hydrolyze the samples. After hydrolysis, samples were cooled to room temperature and then placed in a –80°C freezer to freeze the samples. The HCl from the samples were evaporated and captured in a –84°C cold trap using a vacuum evaporator with a full vacuum setting and vortex speed set at 53% (RapidVap, Labconco, Kansas City, MO). Finally, the dried samples were rehydrated with 0.5 mL of ultrapure water and stored at –80°C until analysis.

### Collagen content determination

Collagen content was determined by the hydroxyproline assay described by Chun et al. (2020) with modifications. Fifty microliters of the rehydrated samples were diluted 1:800 with ultrapure water, and 2 mL of diluted sample was transferred to 16 × 125 mm glass tubes. One milliliter of 6 mM of chloramine-T hydrate in buffer solution (140 mM citric acid monohydrate, 37.5 mM sodium hydroxide, 660 mM sodium acetate trihydrate, and 29% 1-propanol) was added to each sample following a 20-min incubation period at room temperature. One milliliter of dimethylaminobenzaldehyde (DMBA) color reagent (60 mM DMBA in 21% perchloric acid, 65% two-propanol, and 14% ultrapure water) was added to each tube and placed in a water bath set at 60°C to incubate for 90 min. Samples were cooled down in a cold-water bath for 3 min. Hydroxyproline concentration was determined using a spectrophotometer equipped with a microplate reader (BioTek Eon,

BioTek Instruments Inc., Winooski, VT) with wavelength set at 558 nm. A hydroxyproline standard curve and the samples were plated in duplicate on each plate. A conversion factor of 7.14 for hydroxyproline to collagen ratio was used. Collagen content was displayed as milligram of collagen per gram of wet muscle tissue.

### **Collagen crosslink determination**

The hydrolyzed samples were cleaned following the method described by Viguet-Carrin et al. (2009) with modifications. Briefly, the hydrolyzed samples were diluted 1:200 in sample buffer (6:1 acetonitrile and acetic acid) and injected into Bond Elut Cellulose cartridges (12102095; Agilent Technologies, Santa Clara, CA) through a PrepSep 24-port solid-phase extraction vacuum manifold (Thermo Fisher Scientific). After the samples were loaded into the cartridge, the cartridge was washed 4 times with 2.5 mL of the wash buffer (8:1:1, acetonitrile, acetic acid, and ultrapure water, respectively). The collagen crosslinks were eluted by loading 0.6 mL of 1% heptafluorobutyric acid (HFBA) twice. The cleaned samples were transferred into 2 mL amber vials capped with a 9 mm pre-slit PTFE screw cap.

Mature crosslinks pyridinoline (PYD) and deoxypyridinoline (DPD) were determined by an ultra-high-pressure liquid chromatography system (Acquity UPLC H-Class, Waters Corporation, Milford, MA) equipped with a Fluorescence Detector as described by Wu et al. (2021). Briefly, the crosslinks were separated using a high-strength silica T3 2.1 × 100 mm, 1.8 μm column (Waters Corporation) with a flow rate of 0.5 mL/min and column temperature at 60°C. Solvent A (0.2% HFBA in ultrapure water) and solvent B (100% acetonitrile) were used as a gradient solution. After a 10-min isocratic step at 100% solvent A, PYD/DPD were eluted with 85% solvent A and 15% solvent B. The PYD and DPD were measured for fluorescence at an emission of 395 nm and excitation of 297 nm. The total run time for each sample was 20 min. The PYD and DPD were quantified using a calibration curve by plotting the peak area ratio (crosslink area/standard area). The concentration of PYD and DPD were multiplied by the dilution factors to get the final concentration in parts per million. The molar masses of 428.44, 412.44, and 300,000 g/mol were used to calculate the levels of crosslinks in mol/mol of collagen for PYD, DPD, and collagen, respectively.

### **Lipid content determination**

Lipid content was quantified using the method described by Folch et al. (1957) with modifications.

Glass tubes were pre-labeled and dried in a forced air oven (Isotemp, Thermo Fisher Scientific) set at 100°C for 30 min. The weight of the pre-labeled and pre-dried tubes was recorded. One gram of powdered sample was measured into a 50 mL polypropylene conical tube. Ultrapure water (3.2 mL) and chloroform/methanol (1:1 v/v; 16 mL) were added to the sample. Samples were shaken for 10 min using a Wrist Action Shaker (Model; 75 Burrell Corporation, Pittsburgh, PA). Four milliliters of 0.74% potassium chloride solution in ultrapure water was added, and samples were centrifuged at 1,000 × g for 5 min. One milliliter of chloroform was extracted from the bottom layer and transferred into the pre-dried 16 × 100 mm glass tubes. Chloroform was evaporated under nitrogen using a nitrogen evaporator (REACTI-VAP III #TS-18826, Thermo Fisher Scientific). The glass tubes containing the lipid were transferred back into the oven set to 100°C for 30 min to evaporate any potential moisture picked up from the surrounding air. Percent lipid was calculated by dividing the calculated lipid weight over the sample weight × 100.

### **pH analysis**

The pH analysis was conducted by weighing out 5 g of pulverized muscle sample into 100 mL beakers. Fifty milliliters of ultrapure water added to each sample and homogenized for 20 s at 10,000 rpm using a bench top homogenizer with a medium probe (Homogenizer 850, Thermo Fisher Scientific). An InLab Science Pro-ISM probe connected to a Seven Compact pH meter (Mettler Toledo, Columbus, OH) was calibrated using a pH 4.0 and 7.00 standard solution prior to pH measurement. The ultimate pH of each sample was measured by placing the probe into sample homogenate under constant stirring using a magnetic stirrer. All pH measurements were conducted in duplicate.

### **Trained sensory panel evaluation**

Sensory panelists were trained according to the American Meat Science Association (AMSA) sensory guidelines (AMSA, 2015) using scale anchors previously described by Vierck et al. (2018) and Lucherik et al. (2016). Sensory panelists were trained 6 times over a 2-wk period prior to evaluating myofibrillar tenderness, connective tissue amount, lipid flavor intensity, and overall tenderness of various muscles (*biceps femoris*, *longissimus dorsi*, *semitendinosus*, and *psoas major*). All trained panel steak samples were thawed at 4°C for 24 h and grilled on a Cuisineart Griddler Deluxe Clamshell (Cuisineart, Stamford, CT) to an

internal temperature of 71°C (medium). The internal temperature was monitored with a ThermoMap MK4 thermometer (ThermoWorks, American Fork, UT) by inserting the thermometer into the geometric center of each steak. Twenty panels were conducted with 8 samples per session served in a random order to 7 to 8 panelists. The 8 samples served on each session consisted of one of each muscle from the same carcass of the same aging time. Steaks were cubed into 1.27 by 1.27 by 2.54 cm pieces. Two cubes of steak were served to each panelist along with filtered water, apple slices, and unsalted crackers to serve as palate cleansers between samples. At the beginning of each session, a warmup sample was provided before each panel to allow the panelists to calibrate. Panelists evaluated samples in individual booths under a low intensity red incandescent light (<107.64 lumens) to avoid visual bias. Panelists evaluated samples on continuous line scales using a digital survey (Qualtrics, Provo, UT) on electric tablets (Model TB-8505F, Lenovo, Quarry Bay, Hong Kong). Each scale was anchored at both end and midpoints with the descriptive terms (0 = extremely tough/none/bland, 50 = neither tough nor tender, 100 = extremely tender/abundant/intense).

### Warner-Bratzler Shear Force

The WBSF procedures were conducted according to the AMSA Meat Cookery and Sensory Guidelines (AMSA, 2015). All WBSF steak samples were thawed and grilled following the same procedure as described in the “Trained Sensory Panel Evaluation” section. Once cooking was completed, the samples were covered with plastic wrap and refrigerated at 4°C and cooled overnight. Six 1.27 cm cores were drilled parallel to the muscle fibers from each steak. Each core was sheared once through the center, perpendicular to the muscle fibers at 250 mm/min using an Instron testing machine (Model 5569, Instron, Norwood, MA) with a WBSF blade attachment (G-R Electric Manufacturing, Manhattan, KS). The mean shear force (kilogram-force) values of the 6 cores were calculated for each steak.

### Statistical analysis

All data were analyzed as a split-plot using PROC GLIMMIX of SAS (version 9.4, Cary, NC). The model included the whole-plot factor of muscle, the sub-plot factors of aging time, and the muscle × aging time interaction. For all analyses, Satterthwaite approximation was used to estimate the degrees of freedom. Differences among means were detected at the 5% level using

the least significant difference. The PROC CORR procedure of SAS was used to determine Pearson’s correlation coefficients among all tenderness contributors measured in this study to the overall tenderness evaluated by the trained panelists and WBSF for each of the 8 muscles evaluated in this study. Finally, multivariate regression models were constructed using PROC REG and the stepwise selection procedure, with the variable required to be significant ( $P < 0.05$ ) to enter the model and remain in the final model to determine the significance of biochemical factors influencing overall tenderness evaluated by the trained panelists and WBSF for all 8 muscles combined.

## Results and Discussion

### Proteolysis analysis

Representative images of immunoblot from each muscle for TNT and desmin are shown in Figure 2. No muscle × aging interaction was found for TNT degradation ( $P > 0.05$ ). However, there was an aging effect ( $P < 0.01$ ; Table 1) and a muscle effect ( $P < 0.01$ ; Table 2) for TNT degradation for the 8 beef muscles evaluated. As expected, all muscles studied increased in TNT degradation from 2 to 21 d of postmortem storage ( $P < 0.01$ ). Among the muscles, LT, RF, and SS displayed the greatest amounts of degradation for TNT, while PP and RA displayed the lowest amount of TNT degradation ( $P < 0.05$ ). TB, GM, and ST were not different in TNT degradation from SS or PP ( $P < 0.05$ ). A muscle × aging interaction was found for desmin degradation for the 8 beef muscles evaluated ( $P < 0.01$ ; Table 2). All muscles studied increased in desmin degradation from 2 to 21 d of postmortem storage ( $P < 0.01$ ), except for RA ( $P > 0.05$ ). At 2 d postmortem, LT and GM displayed the greatest amount

**Table 1.** Main effect of aging time for troponin-t (TNT) degradation, myofibrillar tenderness evaluated by trained panelists, and Warner-Bratzler Shear Force (WBSF) of 8 beef muscles aged for 2 or 21 d

Measurements	Aging time		SEM <sup>1</sup>	P value
	2 d	21 d		
<b>TNT degraded, %</b>	32.93	56.22	3.03	<0.01
<b>Myofibrillar tenderness<sup>2</sup></b>	55.66	62.99	0.95	<0.01
<b>WBSF, kgf</b>	5.20	4.45	0.13	<0.01

<sup>1</sup>Standard error of the mean.

<sup>2</sup>Sensory scores: 0 = extremely tough; 50 = neither tough nor tender; 100 = extremely tender.

**Table 2.** Least squares means of troponin-T (TNT) degradation, desmin degradation, sarcomere length, collagen content, pyridinoline (PYD) collagen crosslink density, lipid content, and pH of 8 beef muscles evaluated in this study. A muscle  $\times$  aging interaction ( $P < 0.05$ ) was identified only for desmin degradation and pH in the 8 beef muscles

Measurements	Age	LT	PP	SS	TB	GM	RA	RF	ST	SEM <sup>1</sup>	P value
<b>TNT degraded, %</b>		50.83 <sup>ab</sup>	37.90 <sup>de</sup>	48.91 <sup>abc</sup>	44.62 <sup>bcd</sup>	43.66 <sup>bcd</sup>	33.80 <sup>e</sup>	54.62 <sup>a</sup>	42.27 <sup>cd</sup>	3.93	<0.01
<b>Desmin degraded, %</b>										4.90	<0.01
	2	21.63 <sup>Bab</sup>	8.46 <sup>Bcd</sup>	13.48 <sup>Bc</sup>	11.45 <sup>Bc</sup>	27.19 <sup>Ba</sup>	3.00 <sup>Bd</sup>	16.19 <sup>Bbc</sup>	8.96 <sup>Bcd</sup>		
	21	53.87 <sup>Aa</sup>	49.84 <sup>Aa</sup>	36.31 <sup>Ab</sup>	44.25 <sup>Aab</sup>	54.70 <sup>Aa</sup>	11.34 <sup>Bc</sup>	44.47 <sup>Aab</sup>	48.80 <sup>Aa</sup>		
<b>Sarcomere length, <math>\mu</math>m</b>		1.75 <sup>e</sup>	2.47 <sup>a</sup>	1.88 <sup>d</sup>	2.09 <sup>c</sup>	1.65 <sup>f</sup>	2.47 <sup>a</sup>	2.01 <sup>c</sup>	2.24 <sup>b</sup>	0.04	<0.01
<b>Collagen, mg/g wet tissue</b>		4.97 <sup>d</sup>	7.89 <sup>ab</sup>	8.14 <sup>ab</sup>	6.68 <sup>bc</sup>	6.34 <sup>cd</sup>	4.87 <sup>d</sup>	8.29 <sup>a</sup>	6.86 <sup>ab</sup>	0.54	<0.01
<b>PYD density, mol/mol collagen</b>		0.15 <sup>d</sup>	0.34 <sup>a</sup>	0.36 <sup>a</sup>	0.20 <sup>bcd</sup>	0.22 <sup>bcd</sup>	0.28 <sup>ab</sup>	0.19 <sup>cd</sup>	0.24 <sup>bc</sup>	0.03	<0.01
<b>Lipid content, %</b>		9.50 <sup>b</sup>	7.33 <sup>c</sup>	6.95 <sup>cd</sup>	7.47 <sup>c</sup>	7.25 <sup>c</sup>	11.12 <sup>a</sup>	7.47 <sup>c</sup>	6.06 <sup>d</sup>	0.37	<0.01
<b>pH</b>										0.03	<0.01
	2	5.56 <sup>AcD</sup>	5.60 <sup>Ac</sup>	5.80 <sup>Ab</sup>	5.60 <sup>Ac</sup>	5.58 <sup>AcD</sup>	5.92 <sup>Aa</sup>	5.62 <sup>Ac</sup>	5.52 <sup>Ad</sup>		
	21	5.39 <sup>Be</sup>	5.56 <sup>Abc</sup>	5.79 <sup>Aa</sup>	5.56 <sup>Abc</sup>	5.46 <sup>Bde</sup>	5.86 <sup>Aa</sup>	5.64 <sup>Ab</sup>	5.54 <sup>AcD</sup>		

<sup>A,B</sup>Within a column of the same measurement, means without a common superscript differ at  $P < 0.05$ .

<sup>a-f</sup>Within a row, means without a common superscript differ at  $P < 0.05$ .

<sup>1</sup>Standard error of the mean.

GM = *gluteus medius*, LT = *longissimus thoracis*, PP = *pectoralis profundus*, RA = *rectus abdominus*, RF = *rectus femoris*, SS = *supraspinatus*, ST = *semitendinosus*, TB = *triceps brachii*.

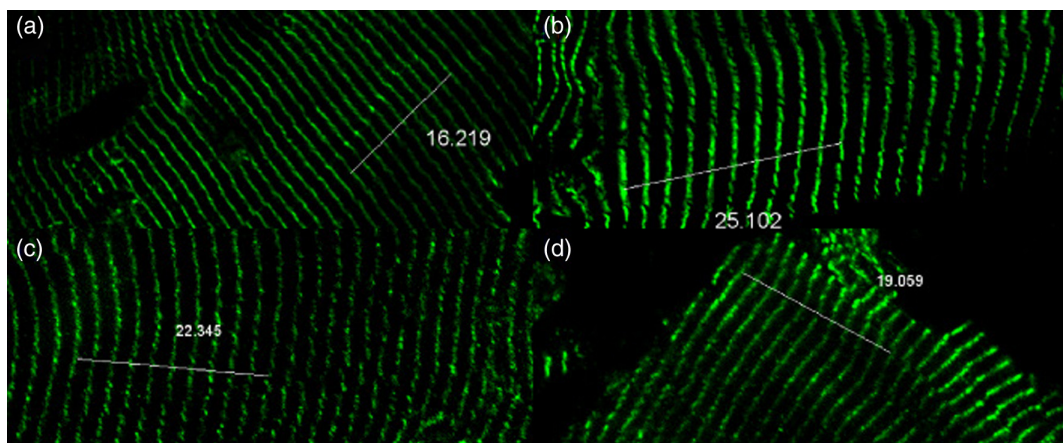
of desmin degradation, while SS, TB, ST, PP, and RA displayed the lowest amount of desmin degradation ( $P < 0.05$ ), with RF not differing from either group ( $P > 0.05$ ). However, at 21 d postmortem, LT, PP, SS, TB, GM, RF, and ST all had a more significant amount of desmin degradation compared to RA ( $P < 0.05$ ).

It is well established that proteolysis of key proteins can dictate the development of tenderness during postmortem storage; therefore, it was expected to see an increase in myofibrillar protein degradation as aging time progressed (Koochmarai, 1992; Anderson et al., 2012). The results for the degradation pattern of TNT and desmin for LT, TB, SS, GM, RF, and ST are largely in agreement with the degradation pattern from other studies (Rhee et al., 2004; Cruzen et al., 2014; Phelps et al., 2016; Vierck et al., 2020). Unfortunately, no proteolysis data of TNT and desmin could be found for PP and RA. Muscle fiber types strongly influence the rate of postmortem aging, and muscles with greater relative percentages of glycolytic muscle fibers are more capable of early postmortem proteolytic degradation than muscles with greater percentage of oxidative muscle fibers (Muroya et al., 2010; Xiong, 2014). Although we did not conduct muscle fiber typing in this study, the proteolysis data from this study followed closely with the above discussed trend based on the muscle fiber types data reported by Kirchofer et al. (2002) and Oury et al. (2010). These studies showed that PP, SS, TB, and

RA all contain a relatively higher percentage of oxidative muscle fibers than LT. It has been shown that muscles containing more glycolytic muscle fibers have greater glycogen stores than those containing more oxidative muscle fibers, consequently resulting in a more rapid decline of muscle pH during rigor mortis (Huff-Lonergan and Lonergan, 2005). A rapid decline of pH during rigor mortis can decrease sarco/endoplasmic reticulum calcium transport ATPase activity, potentially increasing the concentration of free calcium ions in meat (Jeacocke, 1993), thus allowing for greater m-calpain activity during extended postmortem aging (Pomponio and Erbjerg, 2012). Lastly, oxidative fibers tend to display higher levels of calpastatin, a known inhibitor of the calpains (Ouali and Talmant, 1990; Koochmarai, 1992), which could also play a role in the variable level of proteolysis among the muscles examined in this study.

### Sarcomere length

A representative image of an immunohistochemically stained muscle fiber depicting individual sarcomeres is shown in Figure 3. There was no muscle  $\times$  aging interaction found for sarcomere length ( $P > 0.05$ ), but there was a muscle effect for the 8 beef muscles evaluated ( $P < 0.01$ ; Table 2). PP and RA displayed the longest sarcomeres, followed by ST, RF, TB, and SS, with LT and GM displaying the shortest sarcomeres among the 8 muscles evaluated ( $P < 0.05$ ).



**Figure 3.** Representative images (63× magnification) of sarcomere length measurements (μm) for (A) *gluteus medius*, (B) *pectoralis profundus*, (C) *triceps brachii*, and (D) *rectus abdominus*.

Most of the results from this study are similar to the sarcomere lengths from the 11 beef muscles reported by Rhee et al. (2004). However, the sarcomere lengths for PP and RA found in this study were longer than those reported by Grayson and Lawrence (2013) and Lee et al. (2017), respectively. Kobayashi et al. (2000) found that oxidative muscle fibers can reach rigor mortis much faster than glycolytic muscle fibers because oxidative muscle fibers contain less glycogen/adenosine triphosphate than the glycolytic muscle fibers, resulting in less shortening during rigor mortis. Therefore, muscles containing mainly oxidative muscle fibers typically display longer sarcomeres than muscles containing mainly glycolytic muscle fibers. In addition to the influence from muscle fiber types, the ultimate sarcomere lengths of different muscles from beef carcasses are further affected by variation in tension induced by vertical suspension. This hanging method results in stretching muscles in the knuckle and shortening muscles in the loin (Herring et al., 1965). Finally, it is important to point out all of the referenced studies utilized the laser diffraction method described by Cross et al. (1981) for sarcomere length measurement, while we utilized an immunohistochemical method in this study. Perhaps, besides animal and experimental condition differences, measurement technique differences may also contribute to this slight deviation in sarcomere lengths from study to study.

### Collagen content

There was no muscle × aging interaction for collagen content ( $P > 0.05$ ), but there was a muscle effect for the 8 beef muscles evaluated ( $P < 0.01$ ; Table 2). As expected, PP, RF, SS, ST, and TB all displayed greater collagen content compared to LT and RA

( $P < 0.05$ ), while GM did not differ in collagen content compared to ST, TB, LT, and RA ( $P > 0.05$ ). The collagen content of LT, TB, RA, and RF was similar to the collagen content data reported in other studies (Torrescano et al., 2003; Rhee et al., 2004). In contrast, PP, SS, GM, and ST showed slight numerical variations. However, it is noted that these studies utilized meat from Swiss Brown and Charolais cattle, respectively, and Dubost et al. (2013) reported collagen content differences could exist among cattle breeds. Considering the region where our samples were collected (Nebraska, USA), it is speculated that they came from cattle of predominantly *Bos taurus* influence. Despite these potential differences, the collagen results from this study followed the trend that muscles with locomotive functions tended to display greater amounts of collagen content than muscles with a more postural or support function (Rhee et al., 2004; Chun et al., 2020). This is because connective tissue layers specifically support and provide the framework for the transmission of contractile forces in muscles (Purslow, 2010).

### Mature collagen crosslinks

There was no muscle × aging interaction for PYD density for the forequarter muscles evaluated in this study ( $P > 0.05$ ; Table 2). However, there was a muscle effect for PYD density for the 8 muscles evaluated in this study ( $P < 0.01$ ; Table 2). PP and SS had greater PYD densities compared to LT, RF, ST, TB, and GM ( $P < 0.05$ ), while RA did not differ from PP, SS, TB, GM, and ST in PYD density ( $P > 0.05$ ). The PYD density of LT, GM, and ST from this study was similar to the PYD density of those muscles from Chun et al. (2020), Roy et al. (2015), and



Roy et al. (2021), respectively. The results in this study followed a similar trend in which the locomotive muscles tended to contain higher density of mature collagen crosslinks compared to muscle with supportive functions (Torrescano et al., 2003; Chun et al., 2020). Interestingly, RA displayed a relatively high PYD density even though it plays a more postural role, and Palokangas et al. (1992) found PYD crosslinks to be higher in postural muscles that displayed more oxidative fiber types, which could explain this unexpected finding. Finally, no interaction nor main effects were found for the 8 muscles for DPD ( $P > 0.05$ ; data not shown).

### Lipid content

There was no muscle  $\times$  aging interaction for lipid content ( $P > 0.05$ ), but there was a muscle effect for lipid content for the 8 beef muscles evaluated ( $P < 0.01$ ; Table 2). RA and LT displayed the first and second greatest lipid contents, followed by GM, RF, TB, and PP, with SS and ST having the lowest lipid content among the 8 muscles evaluated ( $P < 0.05$ ). The lipid data presented in this study largely agreed with other previous studies (Jones et al., 2004; Garcia et al., 2006; Duvall et al., 2011; Hunt et al., 2016). Hwang et al. (2010) found a positive relationship between intramuscular fat content and the relative abundance of oxidative muscle fibers in muscles due to the oxidative muscle fibers' preference to use fat as a substrate for metabolism. The greater lipid content in RA can be explained by it containing a greater relative percentage of oxidative muscle fibers than other muscle cuts in the hindquarter evaluated in this study (Oury et al., 2010). However, like many previous studies have found, almost all locomotive muscles containing more oxidative muscle fiber types tend to have lower lipid content than the *longissimus* muscles (Nyquist et al., 2018; Chun et al., 2020), which contains more glycolytic muscle fibers (Kirchofer et al., 2002). Genetic selection may explain this discrepancy as many studies have focused on enhancing intramuscular fat deposition in muscles from the loin area over the years (Wang et al., 2005; dos Santos Silva et al., 2019).

### pH

There was a muscle  $\times$  aging interaction for pH for the 8 muscles evaluated in this study ( $P < 0.01$ ; Table 2). RA and SS displayed a greater pH value than the rest of the muscles evaluated in this study at both 2 and 21 d postmortem ( $P < 0.05$ ). The pH values of the muscles from both portions of this study agreed with

findings from many previous studies (Purchas et al., 1999; Torrescano et al., 2003; McKenna et al., 2005; Morrow et al., 2019). Ryu and Kim (2006) found that the relative percentage of glycolytic muscle fibers is negatively correlated to the ultimate muscle pH. As mentioned earlier, SS and RA both contain a relatively greater percentage of oxidative fibers than the other muscles evaluated in this study (Kirchofer et al., 2002). However, it was unexpected that the pH decreased after 21 d of postmortem aging for LT and GM ( $P < 0.05$ ) but not for any other muscle evaluated in this study ( $P > 0.05$ ).

### Trained Sensory Panel and Warner-Bratzler Shear Force

There was no muscle  $\times$  aging interaction for myofibrillar tenderness ratings ( $P > 0.05$ ), but there was an aging effect for the 8 beef muscles ( $P < 0.01$ ; Table 1), in which all the muscles increased in myofibrillar tenderness rating from 2 to 21 d of postmortem storage ( $P < 0.01$ ). In addition, there was a muscle effect for the 8 beef muscles evaluated ( $P < 0.01$ ; Table 3). In general, LT and RA had the greatest myofibrillar tenderness ratings, followed by RF, GM, and SS categorized as being slightly tougher, with PP and ST rated with the lowest myofibrillar tenderness among all the muscles evaluated ( $P < 0.05$ ). TB did not differ from RA or RF in myofibrillar tenderness rating ( $P > 0.05$ ).

There was a muscle  $\times$  aging interaction for the connective tissue amount detected by trained panelists for the 8 beef muscles evaluated in this study ( $P < 0.01$ ; Table 3). At 2 d postmortem, PP had the highest ratings for connective tissue amount, followed by SS, RA, ST, and GM, with LT rated with the least amount of connective tissue ( $P < 0.05$ ). RF and TB did not differ from GM and LT in connective tissue amount rating ( $P > 0.05$ ). The muscles followed a similar trend for the 21-d samples with PP again rated with the most connective tissue, followed by RA, RF, SS, ST, TB, and GM, with LT again rated with the least amount of connective tissue by the panelists ( $P < 0.05$ ). Finally, it was interesting to note that trained panelists observed a decrease in connective tissue amount for most of the muscles evaluated in this study ( $P < 0.05$ ) except for PP and RF ( $P > 0.05$ ; Table 3). It was also peculiar that the panelists perceived RA to have one of the highest connective tissue amounts, considering it has one of the lowest collagen contents according to the biochemical analysis. Wu et al. (2021) found that mature collagen crosslink PYD is heat

**Table 3.** Least squares means of trained panel ratings and Warner-Bratzler Shear Force (WBSF) of the 8 beef muscles evaluated in this study. A muscle × aging interaction ( $P < 0.05$ ) was identified only for connective tissue amount and overall tenderness

Measurements	Age	LT	PP	SS	TB	GM	RA	RF	ST	SEM <sup>1</sup>	P value
<b>Myofibrillar tenderness<sup>2</sup></b>		69.55 <sup>a</sup>	42.63 <sup>f</sup>	57.13 <sup>de</sup>	63.89 <sup>bc</sup>	57.94 <sup>de</sup>	66.50 <sup>ab</sup>	61.58 <sup>cd</sup>	55.39 <sup>e</sup>	1.67	<0.01
<b>Connective tissue amount<sup>2</sup></b>										2.84	<0.01
	2	19.95 <sup>Af</sup>	69.33 <sup>Aa</sup>	43.28 <sup>Ab</sup>	25.71 <sup>Aef</sup>	31.05 <sup>Ade</sup>	38.98 <sup>Abc</sup>	25.4 <sup>Aef</sup>	35.08 <sup>Acd</sup>		
	21	9.09 <sup>Be</sup>	67.13 <sup>Aa</sup>	23.70 <sup>Bbc</sup>	18.16 <sup>Bd</sup>	17.20 <sup>Bd</sup>	28.41 <sup>Bb</sup>	26.3 <sup>Ab</sup>	18.69 <sup>Bcd</sup>		
<b>Lipid flavor intensity<sup>2</sup></b>		21.19 <sup>b</sup>	22.25 <sup>b</sup>	22.67 <sup>b</sup>	21.25 <sup>b</sup>	18.22 <sup>c</sup>	29.78 <sup>a</sup>	22.23 <sup>b</sup>	17.52 <sup>c</sup>	0.94	<0.01
<b>Overall tenderness<sup>2</sup></b>										2.87	<0.05
	2	57.61 <sup>Ba</sup>	16.94 <sup>Ac</sup>	37.15 <sup>Bd</sup>	51.69 <sup>Bab</sup>	42.30 <sup>Bcd</sup>	44.84 <sup>Bbc</sup>	50.23 <sup>Aab</sup>	37.95 <sup>Bcd</sup>		
	21	70.23 <sup>Aa</sup>	19.85 <sup>Ad</sup>	51.11 <sup>Ac</sup>	60.64 <sup>Ab</sup>	54.74 <sup>Ac</sup>	61.50 <sup>Ab</sup>	53.08 <sup>Ac</sup>	54.16 <sup>Ac</sup>		
<b>WBSF</b>		3.72 <sup>c</sup>	6.93 <sup>a</sup>	5.37 <sup>b</sup>	4.17 <sup>de</sup>	4.62 <sup>cd</sup>	4.54 <sup>d</sup>	3.97 <sup>de</sup>	5.27 <sup>bc</sup>	0.23	<0.01

<sup>A,B</sup>Within a column of the same measurement, means without a common superscript differ at  $P < 0.05$ .

<sup>a-f</sup>Within a row, means without a common superscript differ at  $P < 0.05$ .

<sup>1</sup>Standard error of the mean.

<sup>2</sup>Sensory scores: 0 = extremely tough/none; 50 = neither tough nor tender; 100 = extremely tender/abundant.

GM = *gluteus medius*, LT = *longissimus thoracis*, PP = *pectoralis profundus*, RA = *rectus abdominus*, RF = *rectus femoris*, SS = *supraspinatus*, ST = *semitendinosus*, TB = *triceps brachii*.

stable, and as PYD density increases, the overall solubility of collagen decreases. Consequently, this results in a tougher connective tissue texture in cooked meat products, which may explain the high amount of connective tissue detected by the trained panels in PP and RA. On the other hand, RF received one of the lowest ratings for connective tissue amount despite the collagen content showing it contains the greatest collagen content. This again can be explained by the low mature collagen crosslink density in RF, which likely allowed for more collagen solubilization during cooking in RF compared to the muscles with high mature collagen crosslink densities.

There was no muscle × aging interaction for lipid flavor ratings for the 8 muscles evaluated ( $P > 0.05$ ). However, there was a muscle effect ( $P < 0.01$ ; Table 3). A had the greatest rating for lipid flavor, followed by LT, PP, RF, SS, and TB, with GM and ST ranked with the lowest ratings for lipid flavor intensity ( $P < 0.05$ ). The results for lipid flavor intensity largely followed the lipid content from the chemical analysis. However, the lack of differences for lipid flavor intensity between LT and PP, SS, TB, and RF was unexpected, as LT has more lipid content than those other muscles based on chemical analysis. Mottram et al. (1982) compared the volatiles of cooked lean meat with and without added adipose tissue and found no differences. They concluded that the addition of adipose tissue does not result in proportional increases in lipid-derived volatiles. The lipid flavors of cooked meat result from the degradation of lipids during cooking (Mottram, 1998). Therefore, it is

possible that these muscles may have had differences in lipid content, but not in lipid-derived volatiles, and thus no discernable differences were found in lipid flavor. It was also interesting to see differences in lipid flavor rankings between RF and GM since these muscles did not differ in lipid content. However, Legako et al. (2015) reported that *psaos major*, *longissimus lumborum*, GM, and *semimembranosus* exhibited similar lipid content but different volatile compound profiles and demonstrated differences in consumer flavor liking. Potentially, the differences in fatty acid profile and lipid/protein interaction during cooking can affect the final lipid flavor intensity detected by the trained panelists.

The trained panel results showed a muscle × aging interaction for overall tenderness ( $P < 0.05$ ; Table 3). At 2 d postmortem, LT, RF, and TB had greater overall tenderness ratings in comparison to GM, ST, and SS ( $P < 0.05$ ), while RA was not different from any of the 6 muscles ( $P > 0.05$ ). Again, PP exhibited the lowest overall tenderness rated by trained panelists ( $P < 0.05$ ). This same trend was also observed at 21 d postmortem with LT, displaying the highest overall tenderness rating. RA and TB displayed the second highest ratings, followed by GM, RF, ST, and SS, with PP again displaying the lowest ratings for overall tenderness ( $P < 0.05$ ). The interaction occurred as RF and PP did not improve in overall tenderness after 21 d of postmortem aging ( $P > 0.05$ ), while the rest of the muscles improved significantly (by 9 to 19 points) in overall tenderness rating ( $P < 0.05$ ), which resonated

with the findings from the connective tissue amount determined by the trained panelists.

The LT was expected to receive the highest overall tenderness rating due to large amounts of proteolysis, low amounts of collagen/PYD density, and a relatively high lipid content compared to the other muscles evaluated in this study. On the other hand, PP displayed the longest sarcomere lengths but still ended with the lowest overall tenderness rating among all the muscles evaluated in this study. This result indicated that PP's perception of tenderness is most likely attributed to the background effect because this muscle contained a high amount of collagen and the greatest PYD density. This background effect was particularly amplified due to the use of dry heat cookery for the trained panels. Historically, dry heat cookery methods have resulted in inferior tenderness for meat products high in connective tissue (Sullivan and Calkins, 2011), and De Smet et al. (1998) further pointed out that when there are significant variations in collagen content among the samples, panelists rely more heavily on connective tissue amount/texture rather than myofibrillar tenderness to discern differences in overall tenderness. One other peculiar muscle to pay special attention to is the RA: the RA had the highest lipid content and one of the longest sarcomere lengths among all the muscles evaluated in this study. However, RA also had the lowest overall proteolysis in this study. Smulders et al. (1990) pointed out that only muscles containing more oxidative fibers exhibit strong positive correlations between sarcomere length and beef tenderness, and RA has been

shown to contain mainly oxidative fibers (Oury et al., 2010). On top of the sarcomere length effect, RA's high rating for overall tenderness was likely also attributed to the bulk density effect from its high intramuscular fat content.

There was no muscle  $\times$  aging interaction for WBSF values in 8 beef muscles evaluated ( $P > 0.05$ ), but there was an aging effect ( $P < 0.01$ ; Table 1), in which all the muscles decreased in WBSF from 2 d to 21 d of postmortem storage ( $P < 0.01$ ). Also, there was a muscle effect for the 8 beef muscles evaluated ( $P < 0.01$ ; Table 3). As expected, PP had the greatest WBSF value, followed by SS, ST, GM, and RA, with RF, TB, and LT exhibiting the lowest WBSF values among all ( $P < 0.05$ ). The results from WBSF agreed with the results from the overall tenderness ratings perceived by the trained panelists.

### Correlation Coefficients and Multivariate Regression Models

The correlation coefficients ( $r$ ) of tenderness contributors and overall tenderness evaluated by the trained panelists of the 8 muscles used in this study are shown in Table 4. The overall tenderness for LT showed a tendency for positive correlation for TNT degradation ( $r = 0.43$ ;  $P < 0.10$ ) and desmin degradation ( $r = 0.44$ ;  $P < 0.10$ ) and a tendency for negative correlation with pH ( $r = 0.40$ ;  $P < 0.10$ ). In contrast, the overall tenderness for PP showed a negative correlation with collagen content ( $r = -0.48$ ;  $P < 0.05$ ) and

**Table 4.** Correlation coefficient ( $r$ ) of overall tenderness evaluated by trained panelists with different biochemical tenderness contributing components for 8 beef muscles evaluated in this study

Measurements	LT	PP	SS	TB	GM	RA	RF	ST
TNT degraded <sup>1</sup>	0.43*	0.37	0.37	0.55**	0.15	0.45**	0.29	0.55**
Desmin degraded	0.44*	0.08	0.36	0.22	0.71***	0.27	0.21	0.67***
Sarcomere length	0.16	-0.16	-0.28	-0.31	0.09	-0.21	-0.28	-0.11
Collagen content	0.37	-0.48**	0.13	0.05	-0.08	-0.17	0.08	-0.29
PYD density <sup>2</sup>	-0.07	-0.52**	0.07	-0.12	-0.12	-0.21	0.34	0.35
DPD density <sup>3</sup>	0.13	-0.11	-0.02	0.35	-0.48**	-0.1	-0.13	0.27
Lipid content	-0.36	0.2	-0.12	-0.07	0.51**	0.05	-0.19	-0.43*
pH	-0.40*	-0.26	-0.29	0.18	-0.74***	0.12	0.04	0.01

<sup>1</sup>Troponin-T.

<sup>2</sup>Pyridinoline.

<sup>3</sup>Deoxypyridinoline.

GM = *gluteus medius*, LT = *longissimus thoracis*, PP = *pectoralis profundus*, RA = *rectus abdominus*, RF = *rectus femoris*, SS = *supraspinatus*, ST = *semitendinosus*, TB = *triceps brachii*.

\* $P < 0.10$ .

\*\* $P < 0.05$ .

\*\*\* $P < 0.01$ .

PYD density ( $r = -0.52$ ;  $P < 0.05$ ). The overall tenderness for TB showed a positive correlation for TNT degradation ( $r = 0.55$ ;  $P < 0.05$ ). The overall tenderness for GM showed positive correlations with degraded desmin ( $r = 0.71$ ;  $P < 0.01$ ) and lipid content ( $r = 0.51$ ;  $P < 0.05$ ) and negative correlations with DPD density ( $r = -0.48$ ;  $P < 0.05$ ) and pH ( $r = -0.74$ ;  $P < 0.01$ ). For RA, there was a positive correlation for overall tenderness and TNT degradation ( $r = 0.45$ ;  $P < 0.05$ ). The overall tenderness for ST showed positive correlations with degraded TNT ( $r = 0.55$ ;  $P < 0.05$ ) and degraded desmin ( $r = 0.67$ ;  $P < 0.01$ ). There was also a tendency for negative correlations between the overall tenderness of ST and lipid content ( $r = -0.43$ ;  $P < 0.10$ ). None of the tenderness contributing factors have a statistically significant relationship with overall tenderness for SS and RF ( $P > 0.05$ ).

The correlation coefficients ( $r$ ) of tenderness contributors and WBSF of the 8 muscles used in this study are shown in Table 5. The WBSF for LT followed a similar trend as the overall tenderness model with a positive correlation for pH ( $r = 0.58$ ;  $P < 0.01$ ) and a negative correlation for desmin degradation ( $r = -0.63$ ;  $P < 0.01$ ). On the other hand, WBSF for PP displayed a tendency for positive correlation with DPD ( $r = 0.39$ ;  $P < 0.10$ ). Furthermore, WBSF for TB displayed a negative correlation for desmin degradation ( $r = -0.56$ ;  $P < 0.01$ ). The WBSF for GM has a negative correlation with degraded TNT ( $r = -0.54$ ;  $P < 0.05$ ) and lipid content ( $r = -0.46$ ;  $P < 0.05$ ). The WBSF for GM also tended to have a positive

correlation with pH ( $r = 0.40$ ;  $P < 0.10$ ) and a negative correlation with degraded desmin ( $r = -0.38$ ;  $P < 0.10$ ). For RA, there was a negative correlation for WBSF and TNT degradation ( $r = -0.61$ ;  $P < 0.01$ ). The WBSF for ST has a positive correlation with collagen content ( $r = 0.52$ ;  $P < 0.05$ ) and a negative correlation with degraded TNT ( $r = -0.50$ ;  $P < 0.05$ ). Finally, there was also a tendency for positive correlation between WBSF for ST and sarcomere length ( $r = 0.43$ ;  $P < 0.10$ ). Similar to the overall tenderness correlation coefficient results, none of the tenderness contributing factors have a statistically significant relationship with WBSF for SS and RF ( $P > 0.05$ ).

To further confirm the contribution of biochemical tenderness factors to the perception of overall tenderness and WBSF, multiple linear regression analysis was conducted using a stepwise selection procedure to generate linear regression equations to predict the overall tenderness and WBSF for muscles from the combinations of all 8 muscles evaluated in this study (Table 6). The overall tenderness model determined for all 8 muscles utilized in this study was as follows: Overall tenderness =  $75.00 + 0.22 \times \text{TNT degraded} - 15.81 \times \text{sarcomere length} - 1.39 \times \text{collagen content} - 23.65 \text{ PYD density} + 1.40 \times \text{lipid content}$  with final  $R^2 = 0.35$ . The WBSF model determined for all 8 muscles involved in this study was deduced as  $\text{WBSF} = 2.96 - 0.02 \times \text{TNT degraded} + 1.22 \times \text{sarcomere length} + 0.10 \times \text{collagen content} + 1.85 \text{ PYD density} - 0.13 \times \text{lipid content}$  with  $R^2 = 0.34$ .

**Table 5.** Correlation coefficient ( $r$ ) of Warner-Bratzler Shear Force with different biochemical tenderness contributing components for 8 beef muscles evaluated in this study

Measurements	LT	PP	SS	TB	GM	RA	RF	ST
TNT degraded <sup>1</sup>	-0.33	-0.34	-0.32	-0.19	-0.54**	-0.61***	0.01	-0.50**
Desmin degraded	-0.63***	-0.15	-0.20	-0.56***	-0.38*	-0.24	-0.15	-0.21
Sarcomere length	-0.12	0.13	0.22	0.05	-0.33	0.2	-0.11	0.43*
Collagen content	-0.24	0.08	-0.06	0.17	-0.16	0.13	0.21	0.52**
PYD density <sup>2</sup>	-0.01	0.22	-0.14	-0.12	0.27	0.18	-0.18	-0.21
DPD density <sup>3</sup>	-0.22	0.39*	-0.20	-0.18	0.37	0.004	-0.14	0.27
Lipid content	0.02	-0.20	0.24	-0.16	-0.46**	0.005	0.11	0.24
pH	0.58***	-0.06	0.27	0.13	0.40*	-0.06	0.18	0.17

<sup>1</sup>Troponin-T.

<sup>2</sup>Pyridinoline.

<sup>3</sup>Deoxypyridinoline.

GM = *gluteus medius*, LT = *longissimus thoracis*, PP = *pectoralis profundus*, RA = *rectus abdominus*, RF = *rectus femoris*, SS = *supraspinatus*, ST = *semitendinosus*, TB = *triceps brachii*.

\* $P < 0.10$ .

\*\* $P < 0.05$ .

\*\*\* $P < 0.01$ .

**Table 6.** Linear regression equations and coefficients of overall tenderness<sup>1</sup> evaluated by the trained panelists and Warner-Bratzler Shear Force (WBSF) with biochemical tenderness contributing components for all 8 beef muscles evaluated in this study. Stepwise procedure used require all variables in the model to be significant ( $P < 0.05$ )

Locations	Intercept	Regression coefficient	Partial R <sup>2</sup>
<b>Overall tenderness-trained panel</b>	75.00		
TNT <sup>2</sup> degraded		0.22	0.07
Sarcomere length		-15.81	0.15
Collagen content		-1.39	0.03
PYD <sup>3</sup> density		-23.65	0.04
Lipid content		1.40	0.06
<b>WBSF</b>	2.96		
TNT <sup>2</sup> degraded		-0.02	0.07
Sarcomere length		1.22	0.14
Collagen content		0.10	0.04
<sup>3</sup> PYD <sup>3</sup> density		1.85	0.02
Lipid content		-0.13	0.07

<sup>1</sup>Sensory score: 0 = extremely tough; 50 = neither tough nor tender; 100 = extremely tender.

<sup>2</sup>Troponin-T.

<sup>3</sup>Pyridinoline.

As expected, perception of overall tenderness increases and WBSF decreases when proteolysis and lipid content increase and collagen content and a mature collagen crosslink density decrease. It is well established that the extent of postmortem proteolysis and intramuscular lipid content can directly influence the perception of meat tenderness (Lonergan et al., 2001; Lametsch et al., 2003; Smith and Carpenter, 1974). Like the current study, Grze et al. (2017) also found a strong relationship ( $r = 0.80$  to  $0.94$ ) between titin/TNT degradation and pork tenderness. In addition, May et al. (1992) found a negative correlation between quality grade and shear force values ( $r = -0.61$ ) using Angus  $\times$  Hereford steers. Ueda et al. (2007) further corroborated this postulation, wherein they found a negative correlation between fat content and shear force values ( $r = -0.83$ ) in the loins of Wagyu cattle, a breed known for its high levels of marbling. On the other hand, the findings of the current study agreed with Chun et al. (2020), who found an inverse relationship between collagen content and overall tenderness ( $r = -0.42$ ), and Wu et al. (2021) and Zimmerman et al. (1993), who both found mature collagen crosslink density had a positive correlation with different types of

mechanical stress measurement. Finally, this is the first study to the authors' knowledge that reported a statistically significant correlation between overall tenderness and DPD density for GM. Therefore, it is premature to speculate any potential mechanism for this specific relationship. Finally, pH has a negative relationship with overall tenderness and a positive relationship with WBSF for LT and GM. Although pH of meat does not directly influence tenderness, pH typically has a positive relationship on the rate of proteolysis. This is because both  $\mu$ -calpain and m-calpain have been found to have lower rates of enzymatic activity in the pH range of 5.4 to 5.8 compared to pH 7 (Kendall et al., 1993; Geesink and Koohmaraie, 1999). However, this current relationship is likely the result of the unexpected pH drops for LT and GM after 21 d of postmortem aging, which coincides with increased tenderness for these cuts.

The multivariate equations indicated that different factors impacted the tenderness of all 8 muscles combined. Myofibrillar protein degradation, sarcomere length, collagen content, mature collagen crosslinks, and lipid content were vital in predicting tenderness in both the overall tenderness and WBSF models. About 40% of sample variation in overall tenderness can be explained by the multivariate equation, but only 34% of such variation can be explained by the WBSF model. The reason why biochemical factors are slightly better indicators in the overall tenderness model than the WBSF model is most likely because WBSF cannot detect the tenderness perception from fat. Lastly, the multivariate regression models conducted for all 8 muscles confirmed that all of the biochemical measurements performed in this study play a small but important role as tenderness predictor (Table 6). It was particularly noteworthy to mention that sarcomere length has a significant partial R<sup>2</sup> in both equations for the overall tenderness ( $R^2 = 0.15$ ) and WBSF ( $R^2 = 0.14$ ) and shows a negative correlation with overall tenderness and a positive correlation with WBSF. Chun et al. (2020) found the same relationship for sarcomere length and perception of overall tenderness evaluated by trained panelists. This is likely attributed to the fact that some more tender muscles such as the LT displayed shorter sarcomere lengths than those from the other muscles that were rated to be tougher. Although significant, the results from this study conveyed that sarcomere length is not a good indicator for the overall tenderness for the whole beef carcass, especially without knowledge of other components of tenderness contributing factors.

## Conclusion

This study took a novel approach to analyze the tenderness contributing factors of many less extensively studied muscles from different anatomical locations of the beef carcass. Although the results from this study conveyed that every biochemical factor studied played an influential role in the overall tenderness of various beef muscles, the level of contribution to overall tenderness from each biochemical factor varies greatly from one muscle to another. This knowledge can assist the industry to create specific tenderness management strategies unique to individual muscles. For example, cuts like clod heart, top sirloin butt, and eye of round can improve tenderness substantially from extended aging. On the other hand, brisket should be prepared with moist heat cookery or acid marination to ensure the breakdown of connective tissue. Finally, the results showed that top sirloin butt tenderness may also be improved by increasing the intramuscular fat content. More research is needed to biochemically characterize other major beef cuts to provide a comprehensive guide for consistent tenderness management of individual beef cuts.

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