An investigation on the influence of various biochemical tenderness factors on eight different bovine muscles

by

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Abstract

Tenderness is defined as the amount of force required to bite through a piece of meat. Despite its simple definition, three factors underlie the complexity of tenderness: the actomyosin, the background, and the bulk density or lubrication effects. However, past studies concluded that no single tenderness component could predict beef tenderness for all cuts. Therefore, this study's objective was to understand the relationships of biochemical tenderness components and the perception of overall tenderness of eight muscles from the forequarter and the hindquarter of beef carcasses. Longissimus thoracis (LT), pectoralis profundus (PP), supraspinatus (SS), triceps brachii (TB), gluteus medius (GM), rectus abdominus (RA), rectus femoris (RF), and semitendinosus (ST) were collected from 10 UDSA upper 2/3 choice beef carcasses and assigned to a two or 21 day aging period (n = 160). Troponin-T (TNT) degradation, desmin degradation, sarcomere length, muscle fiber cross sectional area (CSA) and diameter, collagen content, mature collagen crosslink density, intramuscular lipid content, pH, trained panel analyses, and Warner-Bratzler Shear Force (WBSF) were measured. A Pearson correlation analysis was conducted to determine the relationship between each tenderness contributor measured in this study and the overall tenderness evaluated by the trained panelist and WBSF. 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 and/or tenderness differences found in this study. The correlation analysis showed that all of the biochemical measurements conducted (except for pH) in this study played a small but important role as an overall tenderness predictor ($P < 0.01$); however, each muscle has a specific tenderness factor(s) that contributed to the overall tenderness evaluated by trained panelists. For instance, tenderness for LT, TB, GM, RA, and ST

may be influenced by proteolytic degradation, while the collagen characteristics may primarily influence tenderness for PP. Also, lipid content has a significant influence on GM tenderness. 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.

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Dedication

I would like to dedicate this thesis to my late brother Michael Hammond. I am heartbroken we could not share this memory together. Still, I could not have asked for a more supportive, admirable older brother.

Chapter 1 - Literature Review

Introduction

Historically, tenderness has been identified as the most critical palatability attribute for beef among consumers (Huffman et al., 1996; Egan et al., 2003), and research has shown that consumers are willing to pay more for guaranteed tenderness (Miller, 2002). The most recent national beef tenderness survey noted that the majority of cuts considered "tender" or "very tender" are from the middle meat portion of the carcass (ribeye roll, striploin, tenderloin, and top sirloin) (Martinez et al., 2017), which only account for approximately 12% of the weight of the entire beef carcass (NCBA, 2020). Unfortunately, many other cuts that reside in the other portions of the carcass, such as the round and chuck, are traditionally considered less tender and lower in quality (Anderson et al., 2012).

Tenderness is a universal term describing the amount of force required to bite through a piece of meat (Kerth, 2013). Despite this simple definition, tenderness qualifies as one of the more complex quality parameters (Kerth, 2013). The complexity lies in the three different mechanisms that account for meat tenderness: 1) the actomyosin effect or the influence of muscle fibers; 2) the background effect or the influence of connective tissue; and 3) the bulk density or lubrication effect, or the influence of intramuscular fat (Koohmaraie et al., 2002). This is further complicated as each mechanism is influenced by different biochemical components, which all contribute to the overall perception of beef tenderness. For instance, the actomyosin effect's contribution to overall tenderness depends on the extent of sarcomere shortening during rigor development and the proteolysis of muscle proteins during postmortem aging (Anderson et al., 2012). In contrast, the background effect's contribution to tenderness is mainly driven by the amount of connective tissue present and the thermal stability of the collagen crosslinks (Chun et

al., 2020; Wu et al., 2021). Finally, the bulk density effect seeks to explain the perception of tenderness that results from intramuscular fat within the muscle (Savell and Cross, 1988). By improving the knowledge base on the various mechanisms that influence tenderness, strategies can be developed to improve tenderness from cuts that have been historically merchandised as lower in value. Therefore, this literature review aims to elucidate the various biochemical components that contribute to tenderness and highlight pertinent findings.

Proteolysis/Enzymes

Past research has revealed that postmortem proteolysis of critical structural proteins in muscle fibers is the major contributor to meat tenderness (Weaver et al., 2008). Proteolysis in meat refers to the enzymatic breakdown of key cytoskeletal proteins in the Z-disk, i.e., titin, desmin, and vinculin, resulting in the structural collapse of the sarcomere, thus increasing the overall tenderness of meat during aging (Kemp et al., 2010).

Some of the major myofibrillar proteins susceptible to proteolysis are troponin-I, troponin-T (TNT), desmin, vinculin, meta-vinculin, dystrophin, nebulin, and titin (Koohmaraie and Geesink, 2006). The enzymatic breakdown of these proteins results in the degradation of three major cytoskeletal structures during postmortem proteolysis: 1) Z-line to Z-line attachments via intermediate filaments; 2) Z-line and M-line attachments to the sarcolemma through costameric proteins; and 3) the elastic filament protein titin, which also attaches to the Z-line and M-line (Koohmaraie and Geesink, 2006). Koohmaraie et al. (1995) conducted a study monitoring the detachment of Z- and M-lines from the sarcolemma during the aging process using sheep muscle. At 14 days postmortem, the Z- and M-lines were almost completely detached from the sarcolemma, with an increase in tenderness compared to the structurally intact samples. The results from this study suggested that cytoskeletal proteins such as titin and desmin are likely

determinants of meat tenderness during postmortem aging. However, many studies have also found that TNT exhibited a strong relationship between its proteolytic degradation and meat tenderness even though TNT is not cytoskeletal in nature (Penny and Dransfield, 1979; Szalata et al., 2005; Marino et al., 2013).

One of the main enzyme groups that have been studied extensively regarding postmortem aging is the calpains. These enzymes are ubiquitously found within the muscle cells. Caplains require calcium for activation, and greater calcium concentration can accelerate the activities of these enzymes (Koohmaraie et al., 1989; Wheeler et al., 1997). There are two major types of calpains, the μ-calpain, and the m-calpain, which are activated by differential calcium concentrations (Khorchid and Ikura, 2002). The μ-calpain is activated at lower concentrations (5-50 μM), and the m-calpain is activated at higher concentrations (200-1000 μM; Khorchid and Ikura, 2002). Considerable evidence supports calpains being the main enzyme groups responsible for proteolysis and postmortem tenderization as calpains have been shown to degrade many cytoskeletal proteins during postmortem aging (Koohmaraie et al., 1991; Huff-Lonergan et al., 1996). In an *in vitro* study, Mohrhauser et al. (2011) showed that beef samples incubated with only the proteolytic enzyme caspase-3 did not show any change in abundance of intact cytoskeletal proteins in samples. However, samples incubated with both μ-calpain and caspase-3 showed significant degradation in the samples, indicating that μ-calpain is the causative agent of postmortem proteolysis.

Many factors can impact the rate of proteolysis in meat and its ability to improve meat tenderness. Several studies have concluded that the optimal pH for μ-calpain is around 6.2 to 6.5 (Geesink and Koohmaraie, 2000; Maddock et al., 2005). Furthermore, both μ-calpain and m-calpain display lower rates of enzymatic activity in the pH range of 5.4-5.8 compared to pH 7

(Kendall et al., 1993; Geesink and Koohmaraie, 1999). The low pH values may cause conformational changes, increasing aggregation and hydrophobicity (Huff-Lonergan and Lonergan, 1999), which makes the substrate proteins less susceptible to cleavage via calpains (Huff-Lonergan and Lonergan, 2005).

In addition to pH, temperature has been known to impact the rate of proteolysis in meat. Pomponio and Ertbjerg (2012) determined the effect of temperature on calpains. They found that calpain activity increased as temperature increased. This study found a more significant amount of myofibril fragmentation during the first 24 hours of incubation at 15°C compared to samples incubated at 2°C. Similarly, White et al. (2006) compared the rate of proteolysis of pre-rigor bovine longissimus dorsi in water baths set at 5°C and 15°C for eight hours, and the samples were stored at 2°C for 21 days postmortem following the designated water bath chilling. They found the 30 kDa degraded TNT bands of the samples from the 15°C treatment to be more intense than the 5°C ones, demonstrating that temperature could be directly used to manipulate calpain activity.

In addition, prolonged exposure of meat to higher temperatures can result in a faster decline of pH and decrease sarcoplasmic reticulum Ca2+-ATPase (SERCA) activity, which would increase the concentration of free calcium ions in meat (Jeacocke, 1993; Zhang et al., 2010). Higher calcium concentrations ($\geq$ 500µm) allow for the activation and autolysis of m-calpain (Pomponio and Ertbjerg, 2012). Interestingly, a study conducted by Doumit and Koohmaraie (1999) noted µ-calpain and m-calpain are capable of hydrolyzing calpastatin in vitro. Therefore, the increased activity of µ-calpain and m-calpain at higher temperatures may allow for the degradation of calpastatin, thereby reducing the activity of this calpain inhibitor.

Furthermore, muscle fiber types can also influence the rate of proteolysis. For instance, Muroya et al. (2010) conducted a study to evaluate the effect of muscle fiber type on postmortem protein degradation. Their results indicated a greater rate and extent of proteolysis in type IIb fibers than in type I fibers. Interestingly, although type I muscles tend to display lower proteolytic enzyme activity, they contain higher amounts of calpains. However, type I fibers also display higher amounts of calpastatin, which inhibits the proteolytic activity from the calpains. In addition, Ryu and Kim (2006) found that the percentage of type IIb fibers is negatively correlated to muscle pH. Type IIb fiber types rely more on glycolytic metabolism and utilize glucose as a substrate; therefore, these muscles have larger stores of glycogen compared to the other muscle fiber types (Kerth, 2013). Consequently, this results in an accumulation of lactate resulting in a more rapid decline in muscle pH, which, as previously mentioned, can also negatively influence proteolysis in meat.

Sarcomere Length and Fiber Size

Previous studies have found that longer sarcomere lengths have lower resistance to shear force (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996; Grayson and Lawrence, 2013). By definition, contraction results in a shortening of the muscle fiber. Shorter, contracted fibers consequently increase the toughness of the cut due to the increased overlap of thick and thin filaments (Kerth, 2013). Battaglia et al. (2016) utilized ten beef striploins from Nellore young bulls to evaluate if sarcomere length can predict instrumental tenderness of beef at 14- and 21-days postmortem. When evaluating the correlation between WBSF and sarcomere length, a strong negative correlation was found for 14 days (-0.73, $P < 0.05$), and a weaker but still significant correlation was found for the 21 day samples (-0.16, $P < 0.05$). These results indicate that as sarcomere length increases, the values for shear force decrease.

In addition to sarcomere length, muscle fiber size may also be indicative of tenderness. Past studies have found a correlation between fiber size and shear force, with meat having larger muscle fiber CSA displaying less tenderness than meat with smaller muscle fiber area (Tuma et al., 1962; Berry et al., 1971). Seideman et al. (1982) found that meat from bulls containing larger muscle fibers was less tender than meat from steers. In addition, Seideman et al. (1988) found correlations between muscle fiber size and sensory panel tenderness (r =-0.29; $P < 0.01$) / shear force (r =0.36; $P < 0.01$). Based on their results, they speculated as the number of myofibrils per unit of mass increased, the perception of tenderness decreased, which ultimately resulted in a less satisfying experience (Seideman et al., 1988). Finally, Herring et al. (1965) studied the interrelationships of fiber diameter, sarcomere length, and tenderness of 12 bovine muscles from horizontally placed and vertically suspended carcasses, and they discovered an inverse relationship between sarcomere length and shear force values (r =-0.80; $P < 0.01$), a positive correlation between fiber size and shear force values (r =0.73; $P < 0.01$) and an inverse relationship between sarcomere length and fiber diameter (r =-0.82; $P < 0.01$). They concluded that as muscle fibers shorten, sarcomere lengths decrease while muscle fiber diameters increase, thus decreasing overall meat tenderness (Herring et al., 1965).

Lastly, recent approaches have sought to explore the connection between postmortem degradation and sarcomere length as they relate to meat tenderness. In one example, England et al. (2012) examined how an increase in sarcomere length may contribute to susceptibility to postmortem proteolysis. They found that degradation of titin occurred more quickly in those bovine muscles with longer sarcomeres. This finding led to speculation that the fiber overlap caused by contracted muscles may reduce or even prevent the enzyme-substrate interactions

necessary for successful proteolysis, indicating a link between sarcomere length and proteolysis influencing tenderness (England et al., 2012).

Connective Tissue amount and crosslinks

As previously mentioned, the background effect's contribution to tenderness is mainly driven by the amount of connective tissue present and the thermal stability of the collagen crosslinks (Chun et al., 2020; Wu et al., 2021). Connective tissue serves as a scaffold for the growth and development of muscle tissue and allows for the transmission of forces generated by muscle contractions (Nishimura et al., 2002; Purslow, 2010). The amount, composition, and distribution of connective tissue provide one of the most variable phenotypic differences among muscles (Purslow, 2005; Nishimura, 2010). Connective tissues possess an extensive extracellular matrix composed of collagen, proteoglycans, and glycoproteins (Roy et al., 2015). Of the three proteins mentioned, collagen fibers are the most abundant, forming connective tissue's main constituent (Roy et al., 2015).

The functionality of muscles may explain the variation in connective tissue amounts among different muscles. For instance, muscles with locomotive functions tend to display greater amounts of collagen content than muscles with a more postural or support function (Rhee et al., 2004; Chun et al., 2020). Torrescano et al. (2003) found psoas major to have significantly less collagen content and was significantly more tender than muscles with high amount of collagen content such as flexor digitorum superficialis. Similarly, Rhee et al. (2004) found that psoas major displayed lower shear force values and collagen content than various locomotive muscles from the chuck and round.

In addition to overall collagen content, the density and type of collagen crosslinks play a role in meat tenderness. Collagen fibrils become stabilized by several posttranslational

modifications allowing the formation of intermolecular and interfibrillar collagen crosslinks (Depalle et al., 2018). Initially, immature crosslinks link two collagen molecules together; and with time, the crosslinks further react with another collagen molecule and mature into a trivalent form (Depalle et al., 2018). Therefore, younger animals tend to contain more heat-labile divalent crosslinks, whereas, in older animals, the same fibers mature into heat-stable trivalent crosslinks (Roy et al., 2015). In addition to age, studies have found that locomotive muscles tend to display more mature collagen crosslinks and less collagen solubility than postural muscles (Torrescano et al., 2003; Chun et al., 2020).

Allain et al. (1978) tested the thermostability of two thermolabile divalent crosslinks - hydroxylysinorleucine (HLNL) and dihydroxylysinorleucine (DHLNL). They found that HLNL and DHLNL are completely destroyed when meat is heated to 80°C for 10 minutes. Due to their heat labile nature, divalent crosslinks are usually dismissed when collagen crosslinks are being measured (Allain et al., 1978). In contrast, pyridinoline (PYD) and Ehrlich chromogen crosslinks are examples of mature, trivalent, and thermostable crosslinks. Horgan et al. (1991) found that PYD and Ehrlich Chromogen crosslinks densities did not change, even when the meat was heated to 80°C for 45 min. Similarly, Dubost et al. (2013) conducted a study found that cooked various muscles to 55°C and found PYD remained intact. In addition, they found increased PYD density negatively affects the sensory tenderness of cooked muscles. Wu et al. (2021) further confirmed that mature collagen crosslinks' mechanical strength and thermal stability contributed to connective tissue texture and meat toughness, which negatively affected the consumers' overall liking. Lastly, it is important to note that the relationship between collagen crosslink content and meat toughness is only meaningful in meat cuts containing a high amount of connective tissue (Lepetit, 2007).

Fat

Several studies have found negative correlations between shear force and increased intramuscular fat deposition (Jones and Tatum, 1994; Wheeler et al., 1994). However, the contribution of intramuscular fat to tenderness is debated. May et al. (1992) found a negative correlation between quality grade and shear force values (r =- 0.61) using Angus x Hereford steers. In addition, a positive relationship between sensory panel perception of tenderness and quality grade was also found (r =0.51) in the same study. This finding of reduced shear force values may be attributed to the bite theory, which postulates that the occurrence of intramuscular fat lowers the mass per unit of volume in a bite-sized piece of meat (Smith and Carpenter, 1974). Ueda et al. (2007) corroborated this postulation. 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. In contrast, Armbruster et al. (1983) found that intramuscular fat content had a minor effect on sensory perception tenderness; however, it was noted that the meat used in this study had much lower fat content compared to the Ueda et al. (2007) study. Therefore, intramuscular fat may increase tenderness only if presented in high amounts (Smith and Carpenter, 1974).

Rather than focusing on the proximate analysis, a study conducted by Nishimura et al. (1999) focused on the influence of intramuscular fat deposition on the structure of intramuscular connective tissue. With scanning electron microscopy, they found that adipose tissue development appeared to disrupt the structure of intramuscular tissue, contributing to the tenderization of highly marbled longissimus dorsi from Japanese black cattle. In addition, they found that the increase of adipose tissue resulted in decreased shear force values. The results found in this study align with the strain theory, which states that marbling deposited in the

perivascular cells of the walls of the perimysium or endomysium disrupts the structure of connective tissues. Consequently, the structural disruption of intramuscular connective tissue from marbling decreases the width, thickness, and strength of connective tissues, rendering them more susceptible to heat penetration and solubilization (Smith and Carpenter, 1974). However, it is important to note that Nishimura et al. (1999) did not find disruptions in intramuscular connective tissue from marbling deposition in the semitendinosus muscle, a muscle known to be relatively lower in fat content than longissimus. Therefore, it is likely that the disruption of connective tissue via adipocyte deposition may only be applied to highly marbled cuts.

Finally, it has been speculated that marbling itself does not directly affect objective tenderness, but indirectly affects consumers' perception of tenderness due to the increase in the sensation of juiciness (Špehar et al., 2008). For example, Thompson (2004) examined the relationship between intramuscular fat content and consumer sensory scores, and they found curvilinear relationships between sensory scores for tenderness, juiciness, and intramuscular fat percentage. This finding aligns with the lubrication theory, which implies that intramuscular fat surrounds and lubricates the muscle fibers when heated, which the intramuscular fat will solubilize and become integrated into the meat juices, increasing juiciness and perceived tenderness (Smith and Carpenter, 1974; Jeremiah, 1996).

Conclusion

Countless studies have evaluated the impact of various individual tenderness components' contribution to meat tenderness over the past three decades. However, the overall perception of beef tenderness depends on all the tenderness components and the interaction among them; therefore, only evaluating one or two tenderness components may not provide an accurate representation. Consequently, a better understanding of the biochemical mechanisms of various

components affecting tenderness will allow end-users to develop specific tenderness management strategies to ensure consistent quality of tenderness in meat products while increasing the quality of traditionally considered lower-quality cuts. For example, aging is a common practice used in the industry to increase tenderness. Rather than freezing the meat, it is left at refrigerated temperatures for a period of time to allow naturally occurring proteolytic enzymes to break down the structural proteins in meat, resulting in a more tender meat product (Kerth, 2013). On the other hand, cuts high in connective tissue should be prepared with moist heat cookery to gelatinize collagen with mature crosslinks, creating a more tender product (Jeremiah and Gibson, 2003). Therefore, this research aims to understand better the relationships of various biochemical tenderness contributing components to the overall tenderness perception of eight different beef muscles of various anatomical locations

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Chapter 2 - An investigation on the influence of various biochemical tenderness factors on eight different bovine muscles

Abstract

This study's objective was to understand the relationships of biochemical tenderness components and the perception of overall tenderness of eight muscles from the forequarter and the hindquarter of beef carcasses. Longissimus thoracis (LT), pectoralis profundus (PP), supraspinatus (SS), triceps brachii (TB), gluteus medius (GM), rectus abdominus (RA), rectus femoris (RF), and semitendinosus (ST) were collected from 10 USDA upper 2/3 Choice beef carcasses and assigned to a two- or 21-day aging period. Troponin-T (TNT) degradation, desmin degradation, sarcomere length, muscle fiber cross sectional area (CSA) and diameter, collagen content, mature collagen crosslink density, intramuscular lipid content, pH, trained sensory panel analyses, and Warner-Bratzler Shear Force (WBSF) were measured. A Pearson correlation analysis was conducted to determine the relationship between each tenderness contributor measured in this study and the overall tenderness evaluated by the trained panelist and WBSF. 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 all of the biochemical measurements conducted (except for pH) in this study may play a small but important role as an overall tenderness predictor ($P < 0.01$); however, each muscle had a specific tenderness factor(s) that contributed to the overall tenderness evaluated by trained panelists. For instance, tenderness for LT, TB, GM, RA, and ST may be influenced by proteolytic degradation, while the collagen characteristics may primarily influence tenderness for PP. Also, lipid content has a significant influence on GM tenderness.

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

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 of the other 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 properly (Pfeiffer et al., 2005).

Defined as the resistance to shear or the toughness of meat (Chandraratne et al., 2006), meat tenderness is influenced by three different basic mechanisms: 1) the actomyosin effect; 2) the background effect; and 3) the bulk density or lubrication effect (Koohmaraie et al., 2002). Past studies have shown that each of the three tenderness mechanisms is further influenced by the various biochemical components, which differed from muscle to muscle (Koohmaraie, 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 (Anderson et al., 2012; Chun et al., 2020). Therefore, the objective of this study is to understand the relationships of various tenderness contributing components to the overall tenderness perception of four different beef muscles from the forequarter, and four different beef muscles from the hindquarter, to expand knowledge in tenderness management strategies of lower quality beef cuts.

Materials and Methods

Sample Collection

Ten USDA upper 2/3 Choice beef carcasses at one day postmortem were selected from a Midwest beef packing plant. For the forequarter, ribeye roll (NAMP #112), brisket (NAMP #120), and shoulder clod (NAMP #114) were collected only from the left side of the carcass, while the mock tenders (NAMP #116B) were collected from both sides of the carcass. For the hindquarter, top sirloin butt (NAMP #184), knuckle (NAMP #167), and eye of round (NAMP #171C) were collected only from the left side of the carcass. The flanks (NAMP#193) were collected from both sides of the carcass. The selected cuts of beef were vacuum packaged and transported to the Kansas State University (KSU) meat laboratory. The cuts were further fabricated the following morning at two days postmortem. Each cut was initially fabricated to remove the accessory muscles not included in this study, leaving only pectoralis profundus (PP), longissimus thoracis (LT), supraspinatus (SS), and triceps brachii (TB) for the forequarter, and

gluteus medius (GM), rectus femoris (RF), rectus abdominus (RA), and semitendinosus (ST) for the hindquarter. Eight 2.54 cm steaks were cut from each muscle perpendicular to the muscle fiber direction. Steaks were vacuum packaged and assigned to one of the two aging periods: two or 21 days, and four analysis groups: Warner-Bratzler Shear Force (WBSF), trained sensory analysis, biochemical analysis, and backup. At the end of the allotted aging period, all the samples were frozen at –40°C (WBSF and trained panel samples) or -80°C (biochemical samples) until analysis.

Proteolysis Analysis

Prior to all lab analysis, steaks designated for lab analysis were cubed, frozen under liquid nitrogen and pulverized using a commercial blender (model 51BL32, Waring Commercial, Torring, CT, USA). Myofibrillar proteins were extracted according the method described by Pietrzak et al. (1997) with modifications. Pulverized meat was homogenized in ice-cold ultrapure water using a bead homogenizer (Benchmark Scientific D2400 Homogenizer; Sayreville, NJ) for 30 seconds. After homogenization, the homogenate was transferred into microcentrifuge tubes and centrifuged at 4,000 x g for five minutes. The supernatant was decanted, and the pellet was resuspended in one mL of ultrapure water. This process was repeated three times. After the third wash, the pellet was re-suspended in one mL of protein extraction buffer (0.1 M Tris-HCl, 1.25 mM EDTA, 2% SDS). Samples were centrifuged for five minutes at 4,000 x 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 two mg/mL using the protein extraction buffer.

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 five minutes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS – PAGE) was conducted using an Invitrogen precast 10% tris-glycine gel (Thermo Fisher Scientific) in a Mini Gel Tank Electrophoresis System (Thermo Fisher Scientific). Five μg of the protein from each sample was loaded into the wells of the precast gel and ran for one hour at 180 V. Gel electrophoresis continued until the tracking dye reached the bottom of the gel – approximately one hour. The gel was removed from the gel assembly and transferred onto the polyvinylidene difluoride membrane (PVDF; iBlot 2 Transfer Stack), using an iBlot 2 Gel Transfer Device (Thermo Fisher Scientific) with settings of 20 V for one minute, 23V for four minutes, and 25 V for two minutes. For TNT degradation, the membrane was blocked with ten mL of 5% non-fat dry milk (NFDM) in 10 mM Tris Base, 150 mM NaCl, 0.1% Tween-20 (TBS-T) for 90 minutes at room temperature. The blot was incubated in primary antibody – anti-troponin-T IgG1 from mouse (JLT-12; Boster Bio, Pleasanton, CA) at 1:1000 dilution in 5% NFDM and TBS-T for 60 minutes. The membrane was washed three times for five minutes each using 15 mL of TBS-T and incubated in secondary antibody – a Peroxidase Conjugated Goat Anti-Mouse IgG1 (BA 1050; Boster Bio.) at 1:1,000 dilution in 5% dry milk and TBS-T for 60 minutes at room temperature. The blot was again washed three times for five minutes each with 15 mL of 1x TBS-T. Prior to imaging, the membrane was incubated in five mL of Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare; Chicago, IL) for three minutes, 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 ten mL of One block Western-FL Blocking Buffer (Genesee Scientific; San Diego, CA) for one hour 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 minutes. The membrane was washed three times for five minutes each using 15 mL of TBS-T and incubated in secondary antibody – Alexa-Fluor Plus 647 goat anti-rabbit H&L; Thermo Fischer Scientific at 1:10,000 dilution for 60 minutes at room temperature. The blot was again washed three times for five minutes each with 15 mL of 1x 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 intensities of the identified degraded bands by all the bands within the same lane.

Sarcomere Length

Sarcomere lengths were measured using the method described by Mohrhauser et al. (2011) with modifications. Approximately ten µg of pulverized sample was lightly dusted onto a microscope slide. Samples were air-dried for ten minutes. The samples were traced with a hydrophobic pen to prevent leaking and drying. After drying, the samples were incubated for 12 hours at room temperature with a monoclonal anti-α-actinin antibody (A7811 Sigma, St. Louis, MO), diluted 1:5,000 with 10% horse serum and 0.2% TritonX-100 (Thermo Fisher Scientific) in 1X PBS. After incubation with primary antibody, sections were washed with PBS three times and incubated with a secondary antibody diluted at 1:1,000 (Alexa-Fluor Plus 488 goat anti-mouse H&L; Thermo Fischer Scientific) in 10% horse serum and 0.2% TritonX-100 in 1X PBS for 30 minutes. Finally, samples were washed and covered with a drop of 9:1 glycerol in 1X PBS. A coverslip was placed, and samples were sealed with nail polish to prevent drying. Samples were imaged by using a Zeiss LSM700 upright confocal microscope with a 63x/1.4 oil objective. The images were captured using the Zen Black software (Zeiss, Oberkochen,

Germany), and 30 sarcomeres were measured and averaged for each sample using ImageJ (version 1.52k; National Institute of Health) using the LSM Toolbox plugins.

Muscle Fiber CSA and Muscle Fiber Diameter

A 2.54cm^3 cube of muscle was collected from the geometric center of each steak for cross sectional analysis. The cubes were further fabricated to ensure the slice face is perpendicular to the muscle fiber. The fabricated cube is placed with slice face down into an embedding mold and were inundated with optimal cutting temperature tissue embedding media (Thermo Fisher Scientific). Samples were frozen in a 2-methyl butane bath cooled by liquid nitrogen. For each sample, two 10-µm cryosections were sliced using a cryostat (Microm HM 550; Thermo Fisher Scientific) and transferred to frost resistant slides (Thermo Fisher Scientific).

Cross-sectional area and diameter were measured following the method described by Phelps et al. (2016) with modifications. The cryosections were traced with a hydrophobic pen to prevent leaking during the washing and incubation. Nonspecific antigen binding sites were inhibited by blocking cryosections in 5% horse serum and 0.2% TritonX-100 in 1X PBS for 30 minutes. All sections were incubated with 1:50 anti dystrophin rabbit polyclonal (PA1–37587; Thermo Fisher Scientific) in blocking solution for 60 minutes. After primary antibody incubation, sections were washed with 1X PBS and incubated with the secondary antibody (Alexa-Fluor 594 goat anti-rabbit H&L; Thermo Fisher Scientific) at 1:1,000 in blocking solution for 30 minutes. Finally, sections were washed with 1X PBS and a small drop of 9:1 glycerol in 1X PBS was applied to the samples prior to imaging. Cryosections were imaged using a Nikon Eclipse TI-U inverted microscope with 10x working distance magnification (Nikon Instruments Inc., Melville, NY). Five representative photomicrographs per section were

captured using a Nikon DS-QiMc digital camera (Nikon Instruments Inc.) that was calibrated to the 10x objective. An average of 400 fibers per samples were analyzed for muscle fiber area and diameter for each sample using NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc.).

Collagen Extraction

Collagen was hydrolyzed according the method described by Sims et al. (2000) with modifications. Five hundred mg of each powdered sample was placed into 15 x 125 mm glass tubes with polytetrafluoroethylene (PTFE) coated caps. Ten mL of 6N hydrochloric acid (HCl) was added to each sample and placed in a forced-air oven (Isotemp, Thermo Fisher Scientific) set for 24 hours at 115°C to hydrolyze the samples. After hydrolysis, samples were cooled to room temperature 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). Finally, the dried samples were rehydrated with 0.5 mL of ultrapure water and were 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 µL of the rehydrated samples were diluted 1:800 with ultrapure water, and two mL of diluted sample was transferred to 15 x 125 mm glass tubes. One mL of six 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-proponal) was added to each sample following a 20 minute incubation time at room temperature. One mL 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 minutes. Samples were cooled down in a cold-water bath for three minutes. Hydroxyproline concentration was determined using a spectrophotometer equipped with a microplate reader (BioTek Eon; BioTek Instruments Inc., Winooski) with wavelength set at 558 nm. A Hydroxyproline standard curve and the samples were plated in duplicates on each plate. A conversion factor of 7.14 for hydroxyproline to collagen ratio was used. Collagen content was displayed as mg of collagen per g of wet tissue.

Crosslink Determination

Collagen crosslinks were purified following the method described by Viguet-Carrin et al. (2009) with modifications. The hydrolyzed samples were diluted 1:25 using ultrapure water. Diluted sample was mixed with sample buffer (6:1 acetonitrile and acetic acid) and injected into Bond Elut Cellulose cartridge 300 mg, three mL (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 four times with 2.5 mL of the wash buffer (8:1:1, acetonitrile, acetic acid, and ultrapure water). The crosslinks were eluted by loading 0.6 mL of 1% heptafluorobutyric acid (HFBA) twice. The cleaned samples were transferred into two mL amber vials (*P*/N 5188-6535, Agilent Technologies) capped with a nine mm pre-slit PTFE screw cap (*P*/N 5185-5865, Agilent Technologies).

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 degasser, quaternary pump, sample manager, and an Acquity UPLC Fluorescence Detector (Waters Corporation). A PYD/DPD standard (*P*/N 4101, Quidel Co., San Diego, CA) was used to generate a standard curve to determine the linearity

range of assays and detection limits. Briefly, the crosslinks were separated using an HSS T3 2.1 x 100 mm, 1.8 μm column (Waters Corporation). The column flow rate was 0.5 mL/min, and the column temperature was set at 60°C. Solvent A (0.2% HFBA in ultrapure water) and solvent B (100% acetonitrile) were used as a gradient solution. After a ten-minute 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. In between each sample, 100% of Solvent B was used to rinse off the previous samples' residues, followed by 100% Solvent A to equilibrate the column. The total run time for each sample was 20 minutes. 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 ppm. 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 Analysis

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 minutes. The weight of the pre-labeled and pre-dried tubes was recorded. One g of powdered sample was measured into a fifty ml polypropylene conical tube. Ultrapure water (3.2 mL) and chloroform/methanol (1:1 v/v; 16 mL) was added to the sample. Samples were shaken for ten minutes using a Wrist Action Shaker (Model; 75 Burrel Corporation, Pittsburg, PA). Four mL of 0.74% KCl solution in ultrapure water was added, and samples were centrifuged at 1,000 x g for five minutes. One mL of chloroform was extracted from the bottom layer and transferred into the pre-dried 16x100 mm glass tubes. Chloroform was

evaporated using a nitrogen evaporator (REACTI-VAP III #TS-18826, Thermo Fisher Scientific). Test tubes were transferred back into the oven set to 100°C for 30 minutes to evaporate any potential moisture picked up from the surrounding air. Percent lipid was calculated by dividing the calculated lipid weight (difference of final weight and glass tube) over the sample weight.

pH Analysis

The pH analysis was conducted by weighing out five g of pulverized muscle sample into 100 mL beakers. Fifty mL of ultrapure water added to each sample and homogenized for 20 seconds 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 (Meter 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.

Trained Sensory Panel Evaluation

Sensory panelists were trained according to the American Meat Sci. Association (AMSA) sensory guidelines (AMSA, 2015) using scale anchors previously described by Vierck et al. (2018) and Lucherk et al. (2016). Sensory panelists were trained six times over a two-weeks period prior to evaluating myofibril tenderness, connective tissue amount, lipid flavor intensity, and overall tenderness from various muscles (biceps femoris, longissimus dorsi, semitendinosus, and psoas major). Twenty panels were conducted with eight samples per session served in a random order to seven to eight panelists. Sensory panel steaks were cooked according to procedures described for WBSF analysis. 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 deionized 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 for 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 Software, 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 (WBSF) Objective Tenderness

Objective tenderness was measured with WBSF. Procedures were conducted according to the American Meat Sci. Association (AMSA) Meat Cookery and Sensory Guidelines (AMSA, 2015). All WBSF steak samples were thawed at 4°C for 24 hours and grilled on a Cuisine Art Griddle Deluxe Clamshell (Cuisine Art; Stamford, CT) to an internal temperature of 71°C (medium). The internal temperature was monitored with a Thermapen MK4 thermometer (Thermoworks; American Fork, Utah) by inserting the thermometer into the geometric center of each steak. Once cooking was completed, the samples were covered with plastic wrap and refrigerated at 4°C to cool 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 Elec. Mfg., Manhattan, KS). The mean shear force (kgf) values of the six 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 meat cut, the sub-plot factors of aging time, and the cut × 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 between all tenderness contributors measured in this study to the overall tenderness evaluated by the trained panelists. The correlation analysis was determined among the variables for each muscle individually. Furthermore, a correlation analysis was conducted across all four muscles from the forequarter, all four muscles from the hindquarter, and all eight muscles together. Finally, multivariate regression models were constructed using PROC REG and the stepwise selection procedure, with variable required to be significant ($P < 0.05$) to enter the model and remain in the final model to determine the significance of chemical factors influencing the overall tenderness and WBSF for the four muscles in the forequarter, the four muscles in the hindquarter, and all eight muscles combined.

Results and Discussion

Proteolysis Analysis

Representative images of immunoblot from each muscle for TNT and desmin are shown in figure 2-1 and 2-2 respectively. No muscle x aging interaction was found for either TNT or desmin degradation for the forequarter muscles ($P > 0.10$). However, there was an aging effect ($P < 0.01$; Table 2-1) and a muscle effect ($P < 0.05$; Table 2-2) for TNT and desmin degradation for the forequarter muscles. LT and SS displayed the largest amounts of degradation for TNT, and PP displayed the lowest amount of TNT degradation ($P < 0.05$), while TB did not differ

from any muscle ($P > 0.10$). Desmin degradation followed a similar trend, with LT displaying the highest desmin degradation ($P < 0.05$), while the other three muscles did not differ from one another ($P > 0.10$). Similarly, the hindquarter muscles did not display a muscle x aging interaction for TNT degradation ($P > 0.10$), but displayed an aging effect ($P < 0.01$; Table 2-3) and a muscle effect ($P < 0.01$; Table 2-4) for TNT degradation for the hindquarter muscles. RF had the highest amount of TNT degradation, while RA and ST displayed the lowest amounts of TNT degradation ($P < 0.05$), and GM did not differ from any muscle ($P > 0.10$). On the other hand, a muscle x aging interaction for the hindquarter muscles for desmin degradation ($P < 0.01$; Table 2-4). All retail cuts studied increased in desmin degradation from two to 21 days of postmortem storage ($P < 0.05$), except for RA ($P > 0.10$). At both two and 21 days postmortem, RF, ST, and GM all displayed a greater 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 (Koohmaraie, 1992; Anderson et al., 2012). The results for the degradation pattern of TNT and desmin for LT, TB, SS, GM 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, however, no proteolysis data of TNT and desmin could be found for PP, RA and RF. It is well established that muscle fiber types strongly influence the rate of postmortem aging, and muscles with greater relative percentages of glycolytic muscle fibers are more susceptible to 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), who reported PP, SS, and TB all contain a relatively higher percentage of oxidative muscle fibers than LT for the forequarter muscles, and RA contains a relatively higher percentage of oxidative muscle fibers than the rest of the muscles in the hindquarter portion of the study. This can be explained by muscles that contain 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, increasing the concentration of free calcium ions ($\geq$ 500 μM) in meat (Jeacocke, 1993; Zhang et al., 2010), allowing for the early activation and autolysis of m-calpain (Pomponio and Ertbjerg, 2012). Lastly, oxidative fibers tend to display higher levels of calpastatin, a known inhibitor of the calpains (Ouali and Talmant, 1990; Koohmaraie, 1992), which could also play a role in the differences in the level of proteolysis among the muscles examined.

Sarcomere Length

A representative image of an immunohistochemically stained muscle fiber depicting individual sarcomeres is shown in figure 2-3. There was no muscle x aging interaction found for sarcomere length ($P > 0.10$), but there was a muscle effect for both forequarter and hindquarter muscles ($P < 0.01$; Table 2-2 and 2-4, respectively). For the forequarter muscles, PP displayed the longest sarcomere lengths, followed by TB, SS, with LT displaying the shortest sarcomere length ($P < 0.05$). For the hindquarter muscles, RA displayed the longest sarcomere lengths, followed by ST, then RF, with GM displaying the shortest sarcomere lengths ($P < 0.01$).

However, the sarcomere lengths for PP and RA in these analyses were longer than those reported by Grayson and Lawrence (2013) and Lee et al. (2017), respectively.

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 due to reduced amounts of glycogen/ATP content, resulting in muscles containing mainly oxidative muscle fibers displaying 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 how beef carcasses are typically hung, which is by the Achilles tendon. This hanging method results in stretching muscles in the round 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 improved 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.

Muscle Fiber CSA and Diameter

A representative image of immunohistochemically stained muscle fiber CSA is shown in figure 2-4. There was no muscle x aging interaction found for muscle fiber CSA or muscle fiber diameter for the forequarter or hindquarter muscles ($P > 0.10$; Table 2-2 and 2-4, respectively). Also, there was a muscle effect for muscle fiber CSA and muscle fiber diameter for the forequarter and hindquarter muscles ($P < 0.01$; Table 2-2 and 2-4, respectively). For the

forequarter muscles, LT, SS, and TB all had similar muscle fiber CSA, and they all exhibited greater muscle fiber CSA than those for PP ($P < 0.05$). Muscle fiber diameter for the forequarter muscles followed a similar trend as muscle fiber CSA, which LT displayed the largest muscle fiber diameter followed by TB, with PP displaying the smallest muscle fiber diameter ($P < 0.05$), while SS was not different from LT or TB ($P > 0.10$). For the hindquarter muscles, RA displayed the greatest muscle fiber CSA, with GM and RF displaying the smallest muscle fiber CSA ($P < 0.05$), and ST did not differ from any of the hindquarter muscles ($P > 0.10$). Again, muscle fiber diameter for the hindquarter muscles followed a similar trend to muscle fiber CSA. RA and ST displayed the greatest muscle fiber diameter, and RF displayed the lowest ($P < 0.05$), while GM muscle fiber diameter did not differ from ST or RF among the hindquarter muscles evaluated in this study ($P > 0.10$). Finally, there was an aging effect for the hindquarter muscles, which the muscle fiber CSA increased in size after 21 d of postmortem aging ($P < 0.05$; Table 2-3).

The results from muscle fiber CSA and diameter follow an inverse trend to the sarcomere length results from this study. Our finding agrees with the results reported by Herring et al. (1965) in which, they discovered an inverse relationship between sarcomere length and muscle fiber diameter (r =-0.82; $P < 0.01$). However, the differences in muscle fiber CSA and diameter among the muscles evaluated in this study could again be attributed to differences in muscle fiber types. Research has determined that oxidative muscle fibers tend to be smaller in muscle fiber diameter than those from glycolytic fiber types (Anne et al., 2016). The muscle fibers CSA and diameter evaluated in this study followed that general trend based on the muscle fiber typing data from (Ozawa et al., 2000; Kirchofer et al., 2002; Chriki et al., 2012), with the only exception being RA. The RA contains a relatively higher percentage of oxidative fibers (Oury et

al., 2010). However, Oury et al. (2010) found that RA had larger mean fiber areas than LT or TB, which they contain more glycolytic muscle fibers than RA. Interestingly, they also found that the oxidative muscle fibers in RA displayed larger CSA than the glycolytic muscle fibers. There is more worked needed to further understand the relationship among muscle fiber types, sarcomere length and CSA for muscles in the abdominal region in beef. Furthermore, the aging effect detected in the hindquarter muscles may be explained by the enzymatic breakdown of key cytoskeletal proteins under the sarcolemma during postmortem aging (Koohmaraie and Geesink, 2006). Consequently, the collapse in these structures can cause the muscle fiber CSA to become more irregular in shape resulting in the imaging system measuring a larger area.

Collagen Content

There was no muscle x aging interaction for collagen content ($P > 0.10$), but there was a muscle effect for the forequarter and hindquarter muscles ($P < 0.01$; Table 2-2 and 2-4, respectively). As expected, PP, SS, and TB all displayed greater collagen content than LT in the forequarter muscles ($P < 0.05$). On the other hand, RF and ST displayed greater collagen content compared to RA ($P < 0.05$), while GM did not differ in collagen content compared to ST and RA in the hindquarter muscles evaluated in this study ($P > 0.10$). It was interesting to note that there was an aging effect for the collagen content in the hindquarter muscles ($P < 0.05$; Table 2-3).

For both forequarter and hindquarter muscles, 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, 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), as connective tissue layers specifically support and provide the framework for the transmission of contractile forces (Purslow, 2010).

Finally, Chun et al. (2020) also found collagen content decreased for various beef muscles after 21 days of aging, and Koulicoff et al. (2021) further demonstrated Collagenase type matrix metalloproteases (MMP) activity in beef and their ability to hydrolyze collagen in extended aged beef. The collagenase MMPs have been speculated to play a role in the tenderization of meat due to their ability to degrade collagen (Nishimura et al., 1998; Veiseth-Kent et al., 2018). Perhaps, native beef collagenase MMPs played a role in the collagen degradation in this study as well, but future study is needed to further confirm this speculation.

Mature Collagen Crosslinks

There was no muscle x aging interaction for PYD density for the forequarter muscles evaluated in this study ($P > 0.10$; Table 2-2). However, there was a muscle effect for PYD density for the forequarter muscles ($P < 0.01$; Table 2-2). The PP and SS had greater PYD densities compared to those from TB and LT ($P < 0.05$). On the other hand, there was a tendency for muscle x aging interaction for PYD density for the hindquarter muscles, which RA had greater PYD density than all the other hindquarter muscles evaluated in this study at 2 days postmortem ($P = 0.08$). However, this trend was observed after 21 days of postmortem aging ($P > 0.10$).

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 interactions or main effects were found for the forequarter nor the hindquarter muscles for DPD ($P > 0.10$).

Lipid Content

There was no treatment x aging interaction for lipid content ($P > 0.10$). but there was a muscle effect for lipid content for both forequarter and hindquarter muscles ($P < 0.01$; Table 2-2 and 4, respectively). For the forequarter muscles, LT displayed the greatest lipid content, followed by PP and TB, with SS having the lowest lipid content among the four muscles evaluated ($P < 0.01$). For the hindquarter muscles, RA displayed the greatest lipid content, followed by GM and RF, with ST displaying the lowest lipid content ($P < 0.01$).

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 a muscle 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; Hocquette et al., 2012; dos Santos Silva et al., 2019).

pH

There was a muscle x aging interaction for pH for both the forequarter and hindquarter muscles ($P < 0.05$; Table 2-2 and 2-4, respectively). The SS displayed a greater pH value than the rest of the forequarter muscles ($P < 0.05$), while RA displayed a greatest pH value compared to all the hindquarter muscles evaluated in this study ($P < 0.05$). 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, what was not expected was that the pH remained constant from 2 to 21 days of postmortem storage for all retail cuts except for LT and GM, which decreased after 21 days of postmortem aging ($P < 0.05$). Perhaps the LT and GM had larger glycogen stores due to their glycolytic nature compared to the other muscles evaluated in this study, which potentially prolonged the completion of rigor mortis.

Trained Sensory Panel

There was no muscle x aging interaction for myofibrillar tenderness ratings ($P > 0.10$), but there was an aging effect for the forequarter and hindquarter muscles ($P < 0.01$; Table 2-1 and 2-3, respectively), in which all the muscles increased in myofibrillar tenderness rating from 2

to 21 days of postmortem storage ($P < 0.01$). In addition, there was a muscle effect for both forequarter and hindquarter muscles ($P < 0.01$; Table 2-5 and 2-6, respectively). In general, LT had the greatest myofibrillar tenderness ratings, followed by TB, SS, with PP rated with the least myofibrillar tenderness in the forequarter muscles ($P < 0.01$). On the other hand, RA and RF had the greatest myofibrillar tenderness ratings, while ST had the lowest rating ($P < 0.01$), and GM did not differ from RF or ST in hindquarter muscles evaluated in this study ($P > 0.10$).

There was a muscle x aging interaction for connective tissue amount detected by trained panelists for both forequarter and hindquarter muscles evaluated in this study ($P < 0.01$; Table 2-5 and 2-6, respectively). At two days postmortem, PP had the highest ratings for connective tissue amount, followed by SS, with LT and TB rated with the least amount of connective tissue for the forequarter muscles ($P < 0.05$). The muscles followed a similar trend for the 21 day samples, with PP again rated with the most connective tissue, followed by SS and TB, with LT rated with the least amount of connective tissue by trained panelists for the forequarter muscles ($P < 0.05$). Likewise, at 2 days postmortem, RA and ST had the highest, and RF had the lowest connective tissue amount ratings for the hindquarter muscles ($P < 0.05$), while GM did not differ from any of the other muscles ($P > 0.10$). At 21 days, the samples rated RA and RF with the most connective tissue and GM with the least amount of connective tissue ($P < 0.05$), while ST was not different from RF or GM ($P > 0.10$). 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 ($P > 0.10$; Table 2-5 and 2-6, respectively). It was 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 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 while it contained the greatest collagen content. This again can be explained by the low PYD density in RF, which most likely RF experienced more collagen solubilization during cooking compared to the muscles with high PYD densities.

There was no muscle x aging interaction for lipid flavor ratings for the forequarter and hindquarter muscles ($P > 0.10$). In addition, there was no main effects for lipid flavor ratings for the forequarter muscles ($P > 0.10$). However, there was a muscle effect for hindquarter muscles evaluated in this study ($P < 0.01$; Table 2-6). The RA had the greatest rating for lipid flavor, followed by RF, with GM and ST ranked with the lowest ratings for lipid flavor intensity in the hindquarter muscles ($P < 0.05$). The lack of differences for lipid flavor intensity in the forequarter muscles was unexpected, as the muscles varied in lipid content 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 the muscles in the forequarter may have had differences in lipid content, but not lipid-derived volatiles, and thus no discernable differences were found in lipid flavor. For the hindquarter muscles, the results for lipid flavor intensity largely followed the lipid content from the chemical analysis. It was 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 psoas 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.

There was no muscle x aging interaction for overall tenderness ratings detected by the trained panels for the forequarter muscles ($P > 0.10$), but there was an aging and a muscle effect ($P < 0.01$; Table 2-1 and 2-5 respectively). The LT had the greatest overall tenderness ratings, followed by TB, SS, with PP being the toughest in the forequarter muscles ($P < 0.05$). In addition, the muscles increased in overall tenderness rating from two to 21 days of postmortem storage ($P < 0.01$). For the hindquarter muscles, the trained panel results showed a muscle x aging interaction for overall tenderness ($P < 0.05$; Table 2-6). The RF had a greater overall tenderness rating than ST at two days of aging ($P < 0.05$), while RA and GM did not differ from the other two muscles ($P > 0.10$). However, at 21 days postmortem, all the muscles did not differ in tenderness ratings ($P > 0.05$).

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 and the smallest muscle fiber CSA, 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 as this muscle contained a high amount of collagen and the highest 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 and one of the largest muscle fiber CSA of all the muscles investigated 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, which 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.

Objective Tenderness

There was no muscle x aging interaction for WBSF values in the forequarter and hindquarter muscles ($P > 0.10$), but there was an aging effect ($P < 0.01$; Table 2-1 and 2-3, respectively), in which all the muscles decreased in WBSF from two days to 21 days of postmortem storage ($P < 0.01$). Also, there was a muscle effect for both forequarter and hindquarter muscles ($P < 0.01$; Table 2-5 and 2-6, respectively). As expected, PP had the greatest WBSF value, followed by SS, with TB and LT exhibiting the lowest WBSF values among all forequarter muscles ($P < 0.05$). For the hindquarter muscles, ST had the greatest WBSF value, followed by GM, with RF displaying the lowest WBSF values ($P < 0.05$), while RA did not differ from GM or RF ($P > 0.10$). The results from WBSF agreed with the results from the

overall tenderness ratings perceived by the trained panelists for muscles from both the forequarter and the hindquarter.

Correlations of Different Tenderness Factors to Overall Tenderness and WBSF

The correlation coefficients (*r*) of tenderness contributors and overall tenderness evaluated by the trained panelists and WBSF of the four muscles from the forequarter are shown in table 2-7 and 2-8, respectively. 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; $P < 0.10$). The WBSF for LT followed a similar trend with a positive correlation for pH (r =0.58; $P < 0.01$) and a negative correlation for desmin degradation (r =-0.63; $P < 0.01$). In contrast, the overall tenderness for PP showed a positive correlation with PYD density (r =0.52; $P < 0.05$) and a negative correlation with collagen content (r =-0.48; $P < 0.05$). The WBSF for PP displayed a tendency for positive correlation with DPD (r =0.39; $P < 0.10$), muscle fiber CSA (r =0.41; $P < 0.10$), and muscle fiber diameter (r =0.40; $P < 0.10$). Furthermore, WBSF for SS displayed a positive relationship with muscle fiber CSA and diameter (r =0.59 and r =0.59, respectively; $P < 0.01$). The overall tenderness for TB showed a positive correlation for TNT degradation (r =0.55; $P < 0.05$). The WBSF for TB displayed a positive correlation for muscle fiber CSA (r =0.50; $P < 0.05$) and a negative correlation for desmin degradation (r =-0.56; $P < 0.01$). There was also a tendency for positive correlation between WBSF and muscle fiber diameter for TB (r =0.44; $P < 0.10$). The overall tenderness for the combination of all four muscles from the forequarter (n = 80) showed positive correlations for TNT degradation (r =0.36; $P < 0.01$), lipid content (r =0.23; $P < 0.05$), muscle fiber CSA *(*r =0.34; $P < 0.01$), muscle fiber diameter (r =0.40; $P < 0.01$), and a negative correlations for collagen content (r =-0.34; $P < 0.01$), PYD density (r =-0.36; $P < 0.01$), and

sarcomere length (r =-0.70; $P < 0.01$). Also, there were negative tendencies for desmin degradation (r =0.21; $P < 0.10$), DPD density (r =-0.19; $P < 0.10$) and pH (r =-0.21; $P < 0.10$). In comparison, the WBSF values for the combination of all four muscles from the forequarter (n = 80) showed positive correlations for sarcomere length (r =0.58; $P < 0.01$), collagen content (r =0.33; $P < 0.01$), PYD density (r =0.31; $P < 0.01$), DPD density (r =0.26; $P < 0.05$), and pH (r =0.26; $P < 0.05$), and negative correlations for TNT (r =-0.32; $P < 0.01$), desmin (r =-0.28; $P <0.05$) and lipid content (r =-0.26; $P < 0.05$). Finally, there was a tendency for negative correlation between WBSF and muscle fiber diameter for all four muscles from the forequarter (r =-0.21; $P < 0.10$).

The correlation coefficients (r) of tenderness contributors and overall tenderness and WBSF for the four muscles in the hindquarter are shown in tables 2-9 and 2-10, respectively. 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; $P < 0.01$). In addition, there was a tendency for GM to have positive correlations with muscle fiber CSA (r =0.43; $P < 0.10$) and muscle fiber diameter (r =0.42; $P < 0.10$). 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 positive correlation for overall tenderness and TNT degradation (r =0.45; $P < 0.05$), and a negative correlation for WBSF and TNT degradation (r =-0.61; $P < 0.01$). 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 positive correlations between the overall tenderness of ST and muscle fiber CSA (r =0.39; P

< 0.10), muscle fiber diameter (r =0.42; P < 0.10), and a tendency for negative correlation with lipid content (r =-0.43; P < 0.10). The WBSF for ST has positive correlation with collagen content (r =0.52; P < 0.05), and a negative correlation with degraded TNT (r =-0.50; P < 0.05), muscle fiber CSA (r =-0.64; P < 0.01), and muscle fiber diameter (r =-0.63; P < 0.01). There was also a tendency for positive correlation between WBSF for ST and sarcomere length (r =0.43; P < 0.10). The overall tenderness for the combination of all four muscles from the hindquarter (n = 80) showed positive correlations for TNT degradation (r =0.31; P < 0.01) and desmin degradation (r =0.30; P < 0.01). In comparison, the WBSF for the combination of all four muscles from the hindquarter (n = 80) showed a positive correlation with DPD density (r =0.23; P < 0.05) and a negative correlation with TNT degradation (r =-0.39; P < 0.01).

Lastly, the overall tenderness and WBSF values for the combination of all eight muscles used in this study is shown in table 2-11. The overall tenderness showed positive correlations for TNT degradation (r =0.33; P < 0.01), desmin degradation (r =0.23; P < 0.01), muscle fiber CSA (r =0.29; P < 0.01), muscle fiber diameter (r =0.34; P < 0.01), and lipid content (r =0.19; P < 0.05), and a negative correlation for sarcomere length (r =-0.39; P < 0.01), collagen content (r =-0.23; P < 0.01), and PYD density (r =-0.24; P < 0.01). In comparison, the WBSF for the combination of all eight muscles showed a positive correlation with sarcomere length (r =0.37; P < 0.01), collagen content(r =0.21; P < 0.01), PYD density (r =0.23; P < 0.01), DPD density (r =0.24; P < 0.01) and a negative correlation with TNT degradation (r =-0.33; P < 0.01), desmin degradation (r =-0.22; P < 0.01), muscle fiber CSA (r =-0.16; P < 0.05) and muscle fiber diameter (r =-0.21; P < 0.01).

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 decreases. 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, Grzes et al. (2017) also found a strong relationship (r =0.80 – 0.94) between various myofibrillar protein 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 x Hereford steers. Ueda et al. (2007) further corroborated this postulation, which 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). In addition, Wu et al. (2021) and Zimmerman et al. (1993) both found PYD 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. Therefore, it is premature to speculate any potential mechanism for this specific relationship.

Interestingly, a negative correlation was found for overall tenderness and sarcomere length and a positive correlation was found between overall tenderness and muscle fiber CSA and diameter. 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. Furthermore, our results confirmed that the relationship between muscle fiber CSA/diameter and meat tenderness is muscle specific. Like many past studies have shown, muscle fiber CSA from muscles like SS and TB from this study

have an inverse relationship with meat tenderness (Tuma et al., 1962; Berry et al., 1971; Seideman et al., 1988). These studies speculated that as the number of myofibrils per unit of mass increased, the amount of force needed to penetrate the meat increased (Seideman et al., 1988). However, some of the muscles in this study displayed the opposite effect. This could be attributed to the fact that the proteolytic degradation resulting in muscle fiber fracturing and tearing leading to the detection of a slightly larger muscle fiber CSA and diameter, while the meat becomes more tender. Although significant, the results from this study conveyed that sarcomere length and muscle fiber CSA are not a good indicator for the overall tenderness of the whole beef carcass, especially without knowledge of other components of tenderness contributing factors.

Also, it was interesting to note there was a relationship of pH to overall tenderness and WBSF. Although pH of meat does not directly influence tenderness, this relation is most likely attributed to the effect pH has on the rate of proteolysis. Both μ-calpain and m-calpain have been found to display lower rates of enzymatic activity in the pH range of 5.4-5.8 compared to pH 7 (Kendall et al., 1993; Geesink and Koohmaraie, 1999). The low pH values may cause conformational changes in the substrate proteins such as aggregation and increased hydrophobicity, making them less susceptible to cleavage via calpains (Huff-Lonergan and Lonergan, 2005).

Multivariate regression model

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 forequarter, muscles from the hindquarter and the combinations of all 8 muscles evaluated in this study (Table 2-12). The overall tenderness model determined for the four muscles from the forequarter was as follow: Overall tenderness = 125.01 + 0.23 x TNT degraded – 33.29 x sarcomere length - 1.89 x collagen – 32.88 PYD with R^2 = 0.63 and the WBSF model determined for the four muscles from the forequarter was deduced as: WBSF = -16.21 - 0.02 x TNT degraded + 2.52 x sarcomere length + 13.96 x DPD + 2.95 x pH with R^2 = 0.50. The overall tenderness model determined for the four muscles from the hindquarter was as follow: Overall tenderness = 24.50 + 0.15 x TNT degraded + 0.18 x desmin degraded + 1.74 x lipid content with R^2 = 0.23 and the WBSF model determined for the four muscles from the hindquarter was deduced as: WBSF = 6.48- 0.02 x TNT degraded - 0.10 x lipid content with R^2 = 0.21. The overall tenderness model determined for all eight muscles utilized in this study was as follow: Overall tenderness = -7.30 + 0.21 x TNT degraded – 12.93 x sarcomere length – 0.01 x muscle fiber CSA + 1.89 x muscle fiber diameter - 1.03 x collagen – 20.24 PYD density + 1.25 x lipid content with R^2 = 0.40 and the WBSF model determined for all eight muscles involved in this study was deduced as: WBSF = 2.96- 0.02 x TNT degraded + 1.22 x sarcomere length + 0.10 x collagen content + 1.85 PYD density – 0.13 x lipid content with R^2 = 0.33.

It is interesting to note that the equations indicated that different factors impacted the tenderness of forequarter muscles, hindquarter muscles, and all 8 muscles combined. Myofibrillar protein degradation, sarcomere length, collagen content, and mature collagen crosslinks were vital in predicting tenderness in the forequarter muscles. In contrast, only myofibrillar protein degradation and lipid content played a role in predicting tenderness in the hindquarter muscles. As seen in the correlation analysis, the forequarter muscles have a better

coefficient of determination conveying the biochemical factors are better indicators in the forequarter muscles than in the hindquarter muscles. This is most likely due to the fact that the 4 muscles selected in the forequarter contains sharper contrast in biochemical features and tenderness differences while those in the hindquarter muscles were too similar. Lastly, the multivariate regression models conducted for all eight muscles confirmed that all of the biochemical measurements performed in this study play a small but essential role as an overall tenderness predictor.

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. By developing a more substantial knowledge base of the different components affecting tenderness, the industry can create specific tenderness management strategies unique to individual muscles to ensure consistent tenderness of meat products. Finally, a more accurate computational model can potentially be generated with a large amount of tenderness/muscle biochemical data to improve the robustness of the current beef tenderness predicting technology.

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Table 2-1. Main effect of aging time for troponin-t degradation (TNT degraded) desmin degraded, myofibrillar tenderness, overall tenderness, and Warner-Bratzler shear force (WBSF) of four retail beef cuts from the forequarter aged for 2 or 21 days (n = 80).

Items	2 Day	21 Day	[1]SEM	P-value
TNT Degraded, %	31.27	59.86	3.63	<0.01
Desmin Degraded, %	13.75	46.07	4.06	<0.01
[2]Overall Tenderness	40.85	50.46	1.29	<0.01
[2]Myofibrillar Tenderness	55.59	61.01	1.13	<0.01
WBSF, kgf	5.45	4.65	0.14	<0.01

[1] Standard error of the mean.
[2] Sensory scores: 0 = extremely tough; 50 = neither tough nor tender; 100 = extremely tender.

Table 2-2. Troponin-t degradation (TNT degraded), desmin degraded, sarcomere length, muscle fiber cross sectional area (CSA), muscle fiber diameter, collagen content, pyridinoline (PYD) collagen crosslink density, lipid content, and pH of four retail beef cuts from the forequarter aged for 2 or 21 days (n=80).

Items	Age	LT	PP	SS	TB	[1]SEM	P-value
TNT Degraded, %		50.83^{a}	37.90^{b}	48.91^{a}	44.62^{ab}	4.22	<0.05
Desmin Degraded, %		37.75^{a}	29.15^{b}	24.89^{b}	27.85^{b}	4.59	<0.05
Sarcomere Length, µm		1.75^{d}	2.47^{a}	1.88^{c}	2.09^{b}	0.04	<0.01
Muscle Fiber CSA, µm^2		3494.06^{a}	2117.02^{b}	3688.04^{a}	3071.06^{a}	224.59	<0.01
Muscle Fiber Diameter, µm		64.90^{ab}	50.95^{c}	66.19^{a}	60.27^{b}	1.86	<0.01
Collagen, mg/g wet tissue		4.97^{c}	7.89^{ab}	8.14^{a}	6.68^{b}	0.48	<0.01
PYD Density, mol/mol collagen		0.15^{b}	0.34^{a}	0.36^{a}	0.20^{b}	0.03	<0.01
Lipid Content, %		9.50^{a}	7.33^{b}	6.95^{b}	7.47^{b}	0.42	<0.01
pH						0.04	<0.05
	2	5.56^{Ab}	5.60^{Ab}	5.80^{Aa}	5.60^{Ab}		
	21	5.39^{Bc}	5.56^{Ab}	5.79^{Aa}	5.56^{Ab}		

$^{A-B}$ Within a column, means without a common superscript differ at $P < 0.05$.
$^{a-d}$Within a row, means without a common superscript differ at $P < 0.05$.
[1] Standard error of the mean.
LT- Longissimus thoracis
PP- Pectoralis profundus
SS- Supraspinatus
TB- Triceps brachii

Table 2-3. Main effect of aging time for troponin-t degradation (TNT degraded), muscle fiber cross sectional area (CSA), muscle fiber diameter, collagen content, myofibrillar tenderness, and Warner-Bratzler shear force (WBSF) of four retail beef cuts from the hindquarter aged for 2 or 21 days (n = 80).

Items	**2 Day**	**21 Day**	**[1]SEM**	**P-value**
TNT Degraded, %	34.59	52.58	2.27	<0.01
Muscle Fiber CSA, µm²	3103.95	3379.74	99.58	<0.05
Muscle Fiber Diameter, µm	61.29	64.20	0.88	<0.05
Collagen, mg/g wet tissue	7.19	5.99	0.45	<0.05
[2]Myofibrillar Tenderness	55.73	64.97	1.19	<0.01
WBSF, kgf	4.95	4.25	0.13	<0.01

[1] Standard error of the mean.
[2] Sensory scores: 0 = extremely tough; 50 = neither tough nor tender; 100 = extremely tender.

Table 2-4. Troponin-t degradation (TNT degraded), desmin degraded, sarcomere length, muscle fiber cross sectional area (CSA), muscle fiber diameter, collagen content, lipid content, and pH of four retail beef cuts from the hindquarter aged for 2 or 21 days (n=80).

	Age	GM	RA	RF	ST	[1]SEM	P-value
TNT Degraded, %		43.66^{ab}	33.80^{b}	54.62^{a}	42.27^{b}	4.00	<0.01
Desmin Degraded, %						4.20	<0.01
	2	27.19^{Ba}	3.00^{Bc}	16.19^{Bb}	8.96^{Bbc}		
	21	54.70^{Aa}	11.34^{Bc}	44.47^{Ab}	48.80^{Aab}		
Sarcomere Length, µm		1.65^{d}	2.47^{a}	2.01^{c}	2.24^{b}	0.04	<0.01
Muscle Fiber CSA, µm^2		3124.27^{b}	3691.35^{a}	2850.97^{b}	3300.79^{ab}	185.85	<0.01
Muscle Fiber Diameter, µm		61.99^{bc}	67.22^{a}	59.13^{c}	63.44^{ab}	1.43	<0.01
Collagen, mg/g wet tissue		6.34^{bc}	4.87^{c}	8.29^{a}	6.86^{ab}	0.61	<0.01
Lipid Content, %		7.25^{b}	11.12^{a}	7.47^{b}	6.06^{c}	0.36	<0.01
						0.03	<0.05
pH	2	5.58^{Abc}	5.92^{Aa}	5.62^{Ab}	5.52^{Ac}		
	21	5.46^{Bc}	5.86^{Aa}	5.64^{Ab}	5.54^{Ac}		

[A-B] Within a column, means without a common superscript differ at $P < 0.05$.
[a-d] Within a row, means without a common superscript differ at $P < 0.05$.
[1] Standard error of the mean.
GM- Gluteus medius
RA- Rectus abdominus
RF- Rectus femoris
ST- Semitendinosus

Table 2-5. Trained panel ratings and Warner-Bratzler Shear Force (WBSF) of four retail beef cuts from the forequarter aged for 2 or 21 days (n=80).

Items	Age	LT	PP	SS	TB	[1]SEM	P-value
[2]Overall Tenderness		63.92[a]	18.40[d]	44.13[c]	56.17[b]	1.82	<0.01
[2]Myofibrillar Tenderness		69.55[a]	42.63[d]	57.13[c]	63.89[b]	1.60	<0.01
[2]Connective Tissue Amount						2.44	<0.01
	2	19.95[Ac]	69.33[Aa]	43.28[Ab]	25.71[Ac]		
	21	9.09[Bc]	67.13[Aa]	23.70[Bb]	18.16[Bb]		
WBSF		3.72[c]	6.93[a]	5.37[b]	4.17[c]	0.25	<0.01

[A-B] Within a column, means without a common superscript differ at $P < 0.05$.
[a-d] Within a row, means without a common superscript differ at $P < 0.05$.
[1] Standard error of the mean.
[2] Sensory scores: 0 = extremely tough/none; 50 = neither tough nor tender; 100 = extremely tender/abundant
LT- Longissimus thoracis
PP- Pectoralis profundus
SS- Supraspinatus
TB- Triceps brachii

Table 2-6. Trained panel ratings and Warner-Bratzler Shear Force (WBSF) of four retail beef cuts from the hindquarter aged for 2 or 21 days (n=80).

Items	Age	GM	RA	RF	ST	[1]SEM	P-value
[2]Overall Tenderness						3.14	<0.05
	2	42.30^{Bab}	44.84^{Bab}	50.23^{Aa}	37.95^{Bb}		
	21	54.74^{Aa}	61.50^{Aa}	53.08^{Aa}	54.16^{Aa}		
[2]Myofibrillar Tenderness		57.94^{bc}	66.50^{a}	61.58^{ab}	55.39^{c}	1.87	<0.01
[2]Connective Tissue Amount						3.19	<0.05
	2	31.05^{Aab}	38.98^{Aa}	25.41^{Ab}	35.08^{Aa}		
	21	17.20^{Bc}	28.41^{Ba}	26.31^{Aab}	18.69^{Bbc}		
[2]Lipid Flavor Intensity		18.22^{c}	29.78^{a}	22.23^{b}	17.52^{c}	0.87	<0.01
WBSF		4.62^{b}	4.54^{bc}	3.97^{c}	5.27^{a}	0.21	<0.01

[A-B] Within a column, means without a common superscript differ at $P < 0.05$.
[a-c] Within a row, means without a common superscript differ at $P < 0.05$.
[1] Standard error of the mean.
[2] Sensory scores: 0 = extremely tough/none/bland; 50 = neither tough nor tender; 100 = extremely tender/abundant/intense.
GM- Gluteus medius
RA- Rectus abdominus
RF- Rectus femoris
ST- Semitendinosus

Table 2-7. Correlation coefficient (r) of overall tenderness (trained panel) with different tenderness components of four retail beef cuts from the forequarter.

Items	**All forequarter muscles**	**LT**	**PP**	**SS**	**TB**
[1]TNT Degraded	0.36***	0.43*	0.37	0.37	0.55**
Desmin Degraded	0.21*	0.44*	0.08	0.36	0.22
Sarcomere Length	-0.70***	0.16	-0.16	-0.28	-0.31
Muscle Fiber [2]CSA	0.34***	-0.05	-0.28	-0.21	0.26
Muscle Fiber Diameter	0.40***	-0.06	-0.27	-0.18	0.20
Collagen Content	-0.34***	0.37	-0.48**	0.13	0.05
[3]PYD Density	-0.36***	-0.07	0.52**	0.07	-0.12
[4]DPD Density	-0.19*	0.13	-0.11	-0.02	0.35
Lipid Content	0.23**	-0.36	0.2	-0.12	-0.07
pH	-0.21*	-0.40*	-0.26	-0.29	0.18

[1] Troponin-T
[2] Cross Sectional Area
[3] Pyridinoline
[4] Deoxypyridinoline
LT- Longissimus thoracis
PP- Pectoralis profundus
SS- Supraspinatus
TB- Triceps brachii
* *P < 0.10*
** *P < 0.05*
*** *P < 0.01*

Table 2-8. Correlation coefficient (r) of Warner-Bratzler shear force with different tenderness components of four retail beef cuts from the forequarter.

Items	**All forequarter muscles**	**LT**	**PP**	**SS**	**TB**
[1]TNT Degraded	-0.32***	-0.33	-0.34	-0.32	-0.19
Desmin Degraded	-0.28**	-0.63***	-0.15	-0.20	-0.56***
Sarcomere Length	0.58***	-0.12	0.13	0.22	0.05
Muscle Fiber [2]CSA	-0.15	-0.33	0.41*	0.59***	0.50**
Muscle Fiber Diameter	-0.21*	-0.33	0.40*	0.59***	0.44*
Collagen Content	0.33***	-0.24	0.08	-0.06	0.17
[3]PYD Density	0.31***	-0.01	-0.22	-0.14	-0.12
[4]DPD Density	0.26**	-0.22	0.39*	-0.20	-0.18
Lipid Content	-0.26**	0.02	-0.20	0.24	-0.16
pH	0.26**	0.58***	-0.06	0.27	0.13

[1] Troponin-T
[2] Cross Sectional Area
[3] Pyridinoline
[4] Deoxypyridinoline
LT- Longissimus thoracis
PP- Pectoralis profundus
SS- Supraspinatus
TB- Triceps brachii
* *P < 0.10*
** *P < 0.05*
*** *P < 0.01*

Table 2-9. Correlation coefficient (r) of overall tenderness (trained panel) with different tenderness components of four retail beef cuts from the hindquarter.

Items	**All hindquarter muscles**	**GM**	**RA**	**RF**	**ST**
Degraded [1]TNT	0.31***	0.15	0.45**	0.29	0.55**
Degraded Desmin	0.30***	0.71***	0.27	0.21	0.67***
Sarcomere Length	0.01	0.09	-0.21	-0.28	-0.11
Muscle Fiber [2]CSA	0.12	0.43*	-0.12	-0.08	0.39*
Muscle Fiber Diameter	0.12	0.42*	-0.15	-0.12	0.42*
Collagen Content	-0.10	-0.08	-0.17	0.08	-0.29
[3]PYD Density	0.02	-0.12	-0.21	0.34	0.35
[4]DPD Density	-0.002	-0.48**	-0.1	-0.13	0.27
Lipid Content	0.16	0.51**	0.05	-0.19	-0.43*
pH	0.10	-0.74***	0.12	0.04	0.01

[1] Troponin-T
[2] Cross Sectional Area
[3] Pyridinoline
[4] Deoxypyridinoline
GM- Gluteus medius
RA- Rectus abdominus
RF- Rectus femoris
ST- Semitendinosus
* *P < 0.10*
** *P < 0.05*
*** *P < 0.01*

Table 2-10. Correlation coefficient (r) of Warner-Bratzler shear force with different tenderness components of four retail beef cuts from the hindquarter.

Items	All hindquarter muscles	GM	RA	RF	ST
Degraded [1]TNT%	-0.39***	-0.54**	-0.61***	0.01	-0.50**
Degraded Desmin	-0.17	-0.38*	-0.24	-0.15	-0.21
Sarcomere Length	0.13	-0.33	0.2	-0.11	0.43*
Muscle Fiber [2]CSA	-0.13	-0.04	-0.26	-0.30	-0.64***
Muscle Fiber Diameter	-0.12	0.0001	-0.22	-0.29	-0.63***
Collagen Content	0.06	-0.16	0.13	0.21	0.52**
[3]PYD Density	0.06	0.27	0.18	-0.18	-0.21
[4]DPD Density	0.23**	0.37	0.004	-0.14	0.27
Lipid Content	-0.15	-0.46**	0.005	0.11	0.24
pH	-0.03	0.40*	-0.06	0.18	0.17

[1] Troponin-T
[2] Cross Sectional Area
[3] Pyridinoline
[4] Deoxypyridinoline
GM- Gluteus medius
RA- Rectus abdominus
RF- Rectus femoris
ST- Semitendinosus
* *P < 0.10*
** *P < 0.05*
*** *P < 0.01*

Table 2-11. Correlation coefficient (r) of overall tenderness (trained panel) and Warner-Bratzler shear force with different tenderness components of all eight retail beef cuts.

Items	**Overall Tenderness**	**WBSF**
Degraded [1]TNT%	0.33***	-0.33***
Degraded Desmin	0.23***	-0.22***
Sarcomere Length	-0.39***	0.37***
Muscle Fiber [2]CSA	0.29***	-0.16**
Muscle Fiber Diameter	0.34***	-0.21***
Collagen Content	-0.23***	0.21***
[3]PYD Density	-0.24***	0.23***
[4]DPD Density	-0.12	0.24***
Lipid Content	0.19**	-0.20***
pH	-0.07	0.12

[1] Troponin-T
[2] Cross Sectional Area
[3] Pyridinoline
[4] Deoxypyridinoline
* *P < 0.10*
** *P < 0.05*
*** *P < 0.01*

Table 2-12. Regression equations and coefficients between tenderness factors and overall tenderness evaluated by the trained panelists and Warner-Bratzler Shear Force (WBSF) for the four retail beef cuts from the forequarter, hindquarter, and all eight cuts.

Treatment	**Equations**	**R^2**
Forequarter muscles		
	[1]Overall tenderness = 125.01 + 0.23 x [2]TNT degraded – 33.29 x sarcomere length - 1.89 x collagen – 32.88 [3]PYD	0.63
	WBSF = -16.21 - 0.02 x [2]TNT degraded + 2.52 x sarcomere length + 13.96 x [4]DPD + 2.95 x pH	0.50
Hindquarter muscles		
	[1]Overall tenderness = 24.50 + 0.15 x [2]TNT degraded + 0.18 x desmin degraded + 1.74 x lipid content	0.23
	WBSF = 6.48- 0.02 x [2]TNT degraded – 0.10 x lipid content	0.21
All muscles		
	[1]Overall tenderness = -7.30 + 0.21 x [2]TNT degraded – 12.93 x sarcomere length – 0.01 x muscle fiber [5]CSA + 1.89 x muscle fiber diameter - 1.03 x collagen – 20.24 PYD density + 1.25 x lipid content	0.40
	WBSF = 2.96- 0.02 x [2]TNT degraded + 1.22 x sarcomere length + 0.10 x collagen content + 1.85 PYD density – 0.13 x lipid content	0.33

Stepwise procedure used require all variables in the model to be significant ($P < 0.05$).
[1] Sensory score: 0 = extremely tough; 50 = neither tough nor tender; 100 = extremely tender.
[2] Troponin-T
[3] Pyridinoline
[4] Deoxypyridinoline
[5] Cross-sectional area

Figure 2-1. Representative images of Troponin-T degradation of pectoralis profundus (PP), longissimus thoracis (LT), supraspinatus (SS), triceps brachii (TB), gluteus medius (GM), rectus abdominus (RA), rectus femoris (RF), semitendinosus (ST).

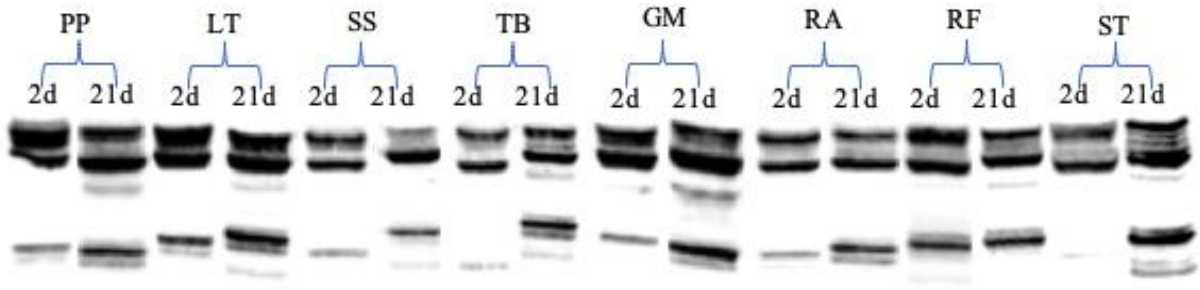


Figure 2-2. Representative images of desmin degradation of pectoralis profundus (PP), longissimus thoracis (LT), supraspinatus (SS), triceps brachii (TB), gluteus medius (GM), rectus abdominus (RA), rectus femoris (RF), semitendinosus (ST).

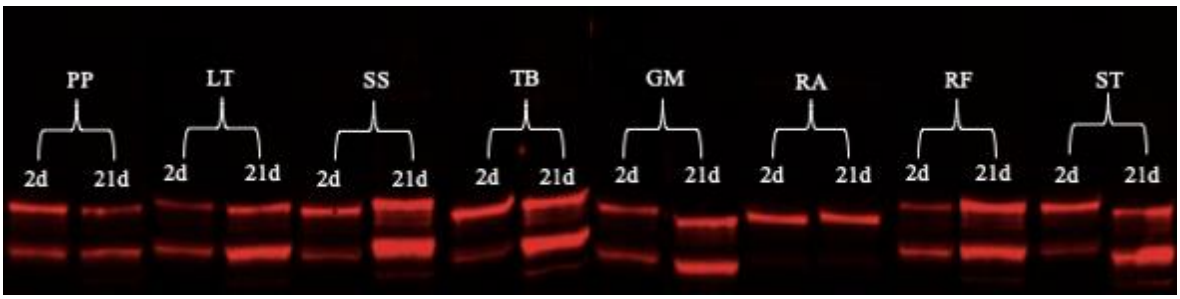


Figure 2-3. Representative images of immunohistochemical staining for sarcomere length measurements.

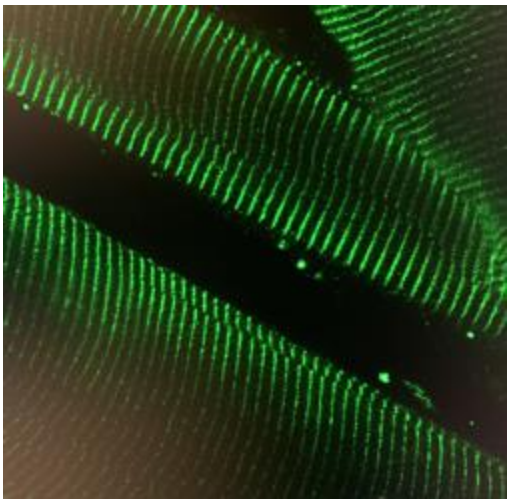
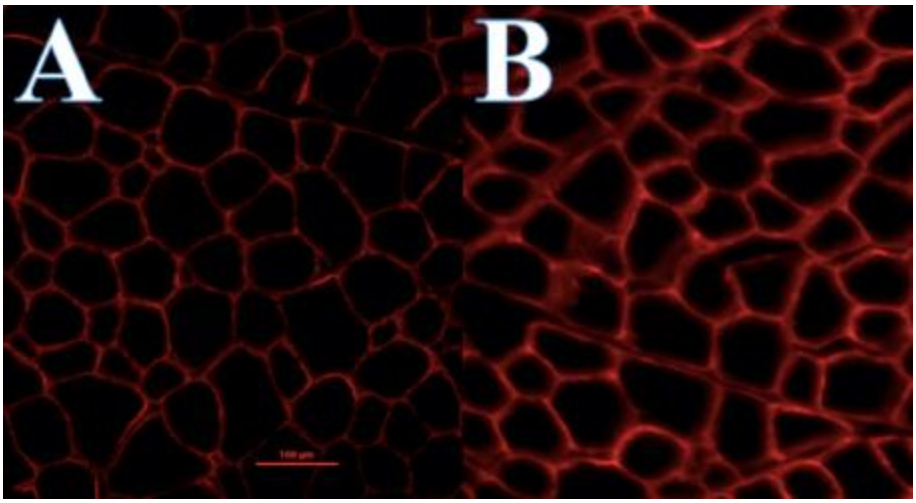
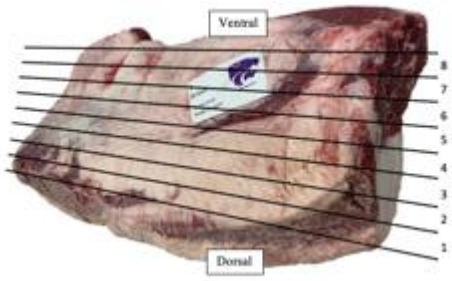


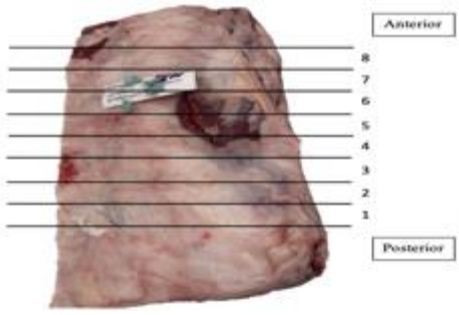
Figure 2-4. Representative images of immunohistochemical staining for muscle fiber CSA aged for 2 days (A) and 21 days (B).



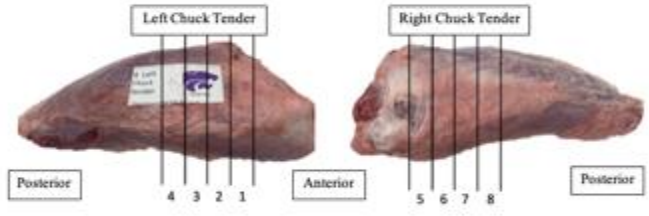
Appendix A - Fabrication of Muscles



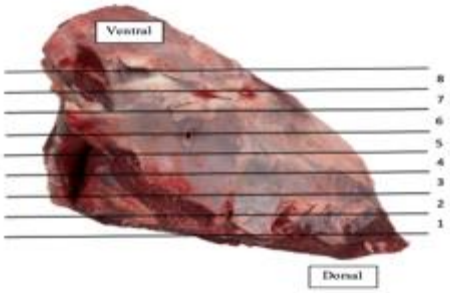
Brisket – Pectoralis profundi
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



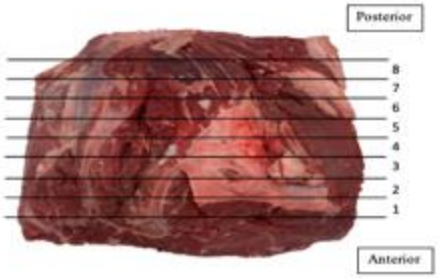
Ribeye roll – *Longissimus thoracis*
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



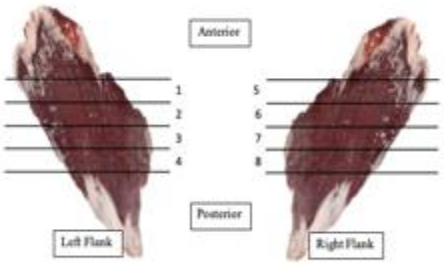
Chuck Tender – *Supraspinatus*
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



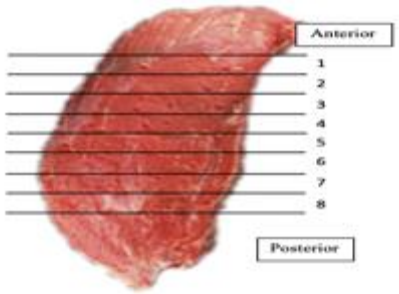
Shoulder – Triceps brachii
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



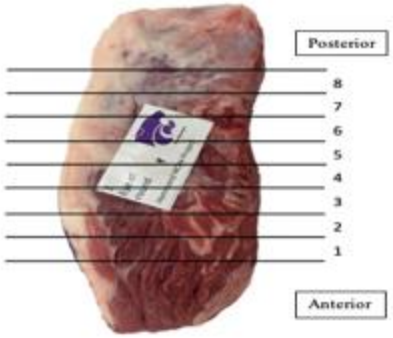
Top sirloin butt – *Gluteus medius*
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



Flank – *Rectus abdominus*
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



Knuckle – *Rectus femoris*
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup



Eye of Round – *Semitendinosus*
1 – 3 d WBSF
2 – 21 d WBSF
3 – 3 d Trained panel analysis
4 – 21 d Trained panel analysis
5 – 3 d Biochemical analysis
6 – 21 d Biochemical analysis
7 – 5 d Backup
8 – 21 d Backup