DEVELOPMENT OF EARLY POSTMORTEM TUMBLING METHODS TO IMPROVE TENDERNESS AND PROTEOLYSIS OF FRESH BEEF LOINS

by

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A Thesis

Submitted to the Faculty of Purdue University
In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Animal Sciences

West Lafayette, Indiana

December 2021

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Dedicated to my supportive fiancé, family, and friends.

ACKNOWLEDGMENTS

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ABSTRACT

Historically, the meat industry has struggled to provide consumers with consistent beef tenderness. Various post-harvest technologies have been used in industry; however, there is still a need to develop a natural and safe post-harvest processing system that can be used to create consistently tender products for consumers. In addition to postmortem aging being a time-consuming process, literature has suggested that it is not a sufficient method to achieve tenderization in certain cull cow muscles. This has resulted in the large supply of cull cow beef to be underutilized due to its inferior quality, specifically tenderness. Applying a combination of mechanical tenderization with additional postmortem aging may be an effective strategy to overcome deficiencies in beef tenderness. Recent studies have found that tumbling without brine addition can be successful at improving instrumental tenderness and consumer liking of tenderness of fresh beef loin. The physical disruptions of muscles, which likely occur during tumbling, may enhance activity of proteolytic enzymes and thus induce more tenderization. The overall objective of this thesis was to investigate the effects of fresh beef tumbling methods and postmortem aging times on the tenderness and proteolysis of loin muscles from both A maturity cattle and cull cows.

The first chapter of this thesis is a literature review that will address the factors affecting tenderness and the methods used by the industry to improve tenderness, specifically focusing on meat tumbling and cull cow beef. The second chapter is a study that investigated the effects of fresh beef tumbling at different postmortem times on meat quality attributes and proteolytic features of loins. The results from this study suggest that early postmortem tumbling coupled with aging can synergistically impact the improvements of beef loin tenderness and proteolysis, shortening the necessary aging period. The third and final chapter of this thesis is a study that aimed to determine the effect of fresh beef tumbling and postmortem aging on the quality and proteolysis of loins from cull cows. The results from this study indicate that aging would be effective at improving the quality and palatability of cull cow beef loins, although tumbling could improve consumer liking of tenderness at earlier postmortem times.

CHAPTER 1.   LITERATURE REVIEW

1.1   Meat Quality

Meat quality is typically defined as the sensory acceptability of a product (Miller, 2020). Palatability is one of the most important factors of beef quality to consumers, and it is determined by the tenderness, juiciness, and flavor of the product (Miller, 2020; O'Quinn *et al.*, 2018). Tenderness is considered to be one of the most important palatability traits to beef consumers (Shackelford *et al.*, 2001; Miller, 2020). Consumers can detect differences between beef tenderness levels and are often willing to pay a higher price for more tender beef (Boleman *et al.*, 1997; Miller *et al.*, 2001; Shackelford *et al.*, 2001).

Despite its importance, the industry has historically struggled with providing consumers with consistently tender beef products, especially for inherently tough cuts (Morgan *et al.*, 1991; Voges *et al.*, 2007; Guelker *et al.*, 2013; Martinez *et al.*, 2017). The considerable variability in beef tenderness can be explained by the various intrinsic and extrinsic factors that can affect this attribute (Wood *et al.*, 1999; Koohmaraie *et al.*, 2002). These traits can include animal age, sex, weight, genetics, nutrition, among others. It is well-known that there are considerable intermuscular variations in tenderness, and some cuts may exhibit locational differences even within the same muscle (Jeremiah *et al.*, 2003). Consequently, this inconsistency has sparked many studies aimed to understand the underlying factors affecting tenderness, how this attribute can be measured and predicted, as well as pre-and post-harvest processes that can optimize quality.

This literature review will address the factors affecting and methods used by the industry to improve tenderness, specifically focusing on meat tumbling and cull cow beef.

1.1.1   Tenderness

The conversion of muscle to meat results in substantial changes to myofibrillar protein structures, which in turn leads to differences in tenderness (Wood *et al.*, 1999). The conversion of muscle to meat is a cascade of events that begin with a near immediate switch to anaerobic glycolysis to metabolize glycogen and maintain homeostasis (Matarneh *et al.*, 2017). Glycolysis supplies adenosine triphosphate (ATP) and causes an accumulation of protons, leading to

acidification of the muscle. In the final step, lactate is produced from pyruvate in attempt to buffer the acidification. As the process progresses, several notable changes occur including the depletion of ATP, accumulation of calcium ions in the sarcoplasmic reticulum, formation of actomyosin cross-bridges, and subsequently the completion of rigor mortis in the muscle (Matarneh *et al*., 2017).

Meat toughness increases with the onset of rigor, though as rigor is resolved, tenderness begins to increase through postmortem proteolysis (Maltin *et al*., 2003). Postmortem aging allows the endogenous proteolytic enzymes to degrade the myofibrillar proteins, which in turn results in increased tenderness through a weakening of key structural components.

1.1.2 Measuring Tenderness

Various methods are used to measure meat tenderness including Warner-Bratzler shear force (WBSF), slice shear force (SSF), or sensory analysis using a trained or consumer panel, among others (Warner *et al*., 2021). Both WBSF and SSF are standard methods to measure instrumental tenderness (AMSA, 2015), but sensory panels can provide vital information regarding how that may translate to an eating experience. However, as it has been seen in several other studies, WBSF values do not always properly reflect the sensory analysis of tenderness of various beef muscles, including the loin (Strydom *et al*., 2015; Rhee *et al*., 2004; AMSA, 2015). Discrepancies between WBSF values and sensory analysis could potentially be related to the connective tissue and myofibrillar properties of the muscles (Strydom *et al*., 2015), which may be especially true with cull cow beef.

Researchers have continuously sought to develop novel methods to accurately predict meat tenderness. Several studies have employed various instruments to assess and predict tenderness, though the results are often not as reliable as shear force (Wheeler *et al*., 2002; Hocquette *et al*., 2012). To further explain changes and variations in tenderness, biochemical measures including myofibrillar fragmentation index (MFI) and western blotting are also commonly performed, though doing so is often time and labor intensive. Measuring MFI shows the extent of fragmentation in the myofibrils, which can determine the extent of postmortem proteolysis that has occurred, presumably affecting tenderness (Davey & Gilbert, 1969). While many methods are effective and feasible in research settings, most are not practical for the industry.

1.1.3   Factors of Tenderness

The three primary factors that determine meat tenderness are the amount of connective tissue, sarcomere length, and postmortem proteolysis (Warner *et al*., 2021). The importance of these three factors depends on the specific muscle type.

Background Toughness

Background toughness of meat is primarily affected by the amount of connective tissue and the extent of chemical cross-linking of proteins such as collagen and elastin. These attributes are well-known to be affected by animal maturity (Purslow, 2014; Purslow, 2018). However, even for animals of similar maturity, there still exists large variations in background toughness. Muscle type is another factor of background toughness that plays an important role influencing meat quality potential. Muscle type and location influence tenderness specifically. Muscles from the chuck and round primals are generally tougher due to larger amounts of connective tissue. The type of muscle will affect the tenderization process that occurs postmortem (Guillemin *et al*., 2011).

Sarcomere Length

Sarcomere length is another major factor of meat tenderness. A sarcomere is the basic contractile unit in striated muscle, and it contracts as muscle undergoes rigor postmortem. As previously discussed, the conversion of muscle to meat and subsequent onset of rigor mortis includes multiple biochemical events. At the end of the cascade during the completion phase of rigor mortis, irreversible cross-links between myosin and actin form, resulting in the complex of actomyosin. The formation of actomyosin leads to stiffness of the muscle and loss of elasticity due to the shortening of the sarcomeres (Eino & Stanley, 1973; Ertbjerg & Puolanne, 2017). In beef, this toughening phase occurs during the first 24 hours after slaughter. The toughening phase is largely similar for all carcasses, while most of the variation in tenderness comes from the tenderization phase (Koohmaraie & Geesink, 2006).

Sarcomere length can influence meat quality traits like tenderness and water-holding attributes (Ertbjerg & Puolanne, 2017); however, it is muscle-dependent (Rhee *et al*., 2004). The degree to which sarcomeres shorten is primarily dependent on pH decline, cooling rate, and any stretching of the muscles. Minimizing shortening of the sarcomeres is widely considered to be

critical for tenderization (Hwang *et al*., 2004). Problems with sarcomere shortening can include cold shortening and heat-induced shortening/toughening. Cold shortening results when the carcass is rapidly chilled immediately during the conversion of muscle to meat. This is in contrast to heat-induced toughening, where the carcass is cooled too slowly (Ertbjerg & Puolanne, 2017).

The degree of tenderization is dependent on the length of the sarcomeres (Ertbjerg & Puolanne, 2017). The relationship between sarcomere length and proteolysis may be muscle and species dependent (Ertbjerg & Puolanne, 2017). For example, in beef loin muscles, sarcomere length is not highly correlated with trained panel tenderness, but it does have a relationship with WBSF values (Rhee *et al*., 2004).

Proteolysis

Postmortem proteolysis is the natural degradation of proteins or peptides that occurs during the aging period (Kim *et al*., 2018; Warner *et al*., 2021). Generally, more proteolysis during aging results in more tenderization (Kim *et al*., 2018; Warner *et al*., 2021). The tenderization phase can explain a large extent of the variations in tenderness between carcasses. The effect that proteolysis has on tenderization is muscle-specific (Rhee *et al*., 2004). Both the rate and extent of proteolytic degradation varies between muscles (Koohmaraie & Geesink, 2006), and is known to be influenced by muscle fiber type, where glycolytic muscles typically exhibit more proteolysis compared to oxidative (Muroya *et al*., 2010). Further, meat with higher background toughness will typically need greater proteolysis to achieve acceptable tenderness, though proteolysis may be unable to overcome the inherent toughness of certain beef muscles (Stolowski *et al*., 2006; Rhee *et al*., 2004).

Structural weakening resulting in tenderization mainly comes from the degradation of the Z- to Z-line attachments (mostly made of desmin) by intermediate filaments, Z- and M-line attachments to the sarcolemma, and the elastic filament protein titin (Koohmaraie & Geesink, 2006; Davey & Gilbert, 1969; Eino & Stanley, 1973; Taylor *et al*., 1995). Proteins that are degraded in postmortem proteolysis include desmin, troponin-T, troponin-I, nebulin, titin, vinculin, and dystrophin (Koohmaraie & Geesink, 2006; Huff Lonergan *et al*., 2010), which are classified as myofibrillar or cytoskeletal proteins. Most proteolysis occurs three to fourteen days postmortem in beef muscle (Boehm *et al*., 1998).

There are two main protease systems considered to be relevant to meat tenderness: the calpains and the cathepsins. Koohmaraie (1992), stated that the calpains are the proteolytic system that is mainly responsible for the degradation of protein that happens postmortem. The calpain system includes: calpain-1, calpain-2, and calpain-3. The calpains are calcium activated and are present in the sarcoplasm. After activation, the calpains degrade proteins and then undergo autolysis (Koohmaraie & Geesink, 2006). Within the calpain system, the tenderization process is primarily attributed to the activity of calpain-1 (Koohmaraie & Geesink, 2006; Boehm *et al*., 1998). The other two proteases, calpain-2 and calpain-3, are not generally considered as relevant to most postmortem proteolytic activity (Koohmaraie & Geesink, 2006).

Calpastatin, an endogenous calpain inhibitor, is the main regulator of the calpain proteases. As a result, calpastatin has an inverse relationship with meat tenderness (Shackelford *et al*., 1994). According to Pörn-Ares *et al*. (1998), calpastatin can be cleaved by caspases, which leads to more proteolysis and tenderization of the muscle. It is evident that calpastatin activity can cause differences in the rate and extent of postmortem proteolysis in meat (Koohmaraie & Geesink, 2006), and this can help to explain why there are variations in meat tenderness. Mature beef animals may have reduced proteolytic potential, in addition to greater connective tissues as previously discussed. Cruzen *et al*. (2014) found that mature beef cattle had greater calpastatin activity compared to the younger growing cattle in all muscles evaluated (*longissimus dorsi, semimembranosus,* and *triceps brachii*). As a result, muscles from the older cattle also had less calpain-1 autolysis and less protein degradation at 1d postmortem (Cruzen *et al*., 2014). Proteolytic potential seems to be dependent on both animal age and muscle, resulting in increased toughness of certain cuts from cull cows even after aging (Cruzen *et al*., 2014).

The second main protease system is the cathepsins. Cathepsins are cysteine proteases that are present in lysosomes. Numerous studies have researched the effects of the proteolytic enzymes, calpains and cathepsins, on postmortem meat tenderization (Warner *et al*., 2021; Koohmaraie & Geesink, 2006; Etherington *et al*., 1987). Chéret *et al*. (2007) found evidence that both protease systems have the ability to function in a synergistic relationship together to enhance postmortem tenderization.

1.1.4 Methods to Improve Tenderness

There are many methods used by the industry in attempt to improve tenderness of meat products post-harvest. A common method to improve meat tenderness is allowing the meat to age naturally during the postmortem period, especially for beef (Kim *et al*., 2018; Warner *et al*., 2021). Some other methods use mechanical tenderization to disrupt the muscle fibers, while others may add non-meat ingredients like a brine or exogenous proteases to the meat to try to improve tenderness. Methods that penetrate the meat can cause microbial contamination by introducing spoilage and pathogenic bacteria into the product (Luchansky *et al*., 2008, Bhat *et al*., 2018a). If a tenderization method were able to avoid penetrating the muscle, then it is reasonable to expect that aging could be coupled with another tenderization method to improve tenderness even further.

Aging

Aging allows endogenous proteolytic enzymes to naturally degrade myofibrillar protein for a specified amount of time after slaughter under controlled refrigerated conditions (Kim *et al*., 2018). According to the 2015 National Beef Tenderness Survey (Martinez *et al*., 2017), beef steak aging durations at retail establishments averaged about 26 days. The main disadvantages of postmortem aging include the amount of time necessary and muscle-specific aging potential (Nair *et al*., 2019). As mentioned previously, aging potential is also dependent on animal maturity (Cruzen *et al*., 2014). Even after a long aging period, cull cow beef may still remain tough due to the amount of intramuscular connective tissue or a high amount of insoluble collagen (Purslow, 2014; Purslow, 2018). Physical disruptions from another tenderizing method may be necessary to combine with an aging period for cull cow beef to reach acceptable tenderness levels. Enzymatic tenderization can be increased when muscle structure is disrupted (Koohmaraie, 1994; Jayasooriya *et al*., 2004; Koohmaraie & Geesink, 2006; Kemp *et al*., 2010). However, these effects could be dependent on the postmortem time of application considering differences in protease activity during the course of aging (Nair *et al*., 2019).

Alternative Carcass Suspension

Alternative carcass suspension are methods used in an attempt to lengthen the sarcomeres of certain muscles. Pelvic suspension (also known as Tender-Stretch) is a method of carcass

stretching that is done by hanging the carcass by the aitch bone instead of the Achilles tendon. The stretching that occurs physically lengthens the sarcomeres within the muscles and results in improved meat tenderness (Ertbjerg & Puolanne, 2017). The disadvantage of using pelvic suspension methods is that only certain hind limb and vertebral muscles are tenderized, rather than throughout the entire carcass (Ertbjerg & Puolanne, 2017). As previously discussed, proteolytic activity is also increased with longer sarcomeres (Ertbjerg & Puolanne, 2017).

Brine Enhancement

Brine enhancement is another common method that is used in the industry to improve meat palatability, including tenderness. Most commonly, this is performed by pumping brine into the meat via needle injection. Molina *et al*. (2005) found that needle pumping brine improves palatability more than just marinating the meat in the solution. A brine is a liquid mixture of ingredients usually comprised of water, salt, sugar, and sodium tripolyphosphate (Boles & Shand, 2001; McDonald *et al*., 2001). Brine enhancement can also be done in addition to a meat tumbling application. One disadvantage with the addition of non-meat ingredients is that the product is no longer considered fresh meat (USDA, 2013).

Mechanical Tenderization

Mechanical tenderization methods use blades, needles, or other mechanical processes to improve meat tenderness (Yang *et al*., 2021). Blade tenderization uses sharp and thin blades to puncture the meat and cut the muscle fibers (Pietrasik & Shand, 2004). Blade tenderization has been shown to decrease shear force values and decrease hardness of beef roasts (Pietrasik & Shand, 2004). Blade tenderization in combination with tumbling may further improve tenderness of beef roasts (Pietrasik & Shand, 2004). While mechanical tenderization is an effective method for certain inherently tough beef cuts, these methods have a potential risk of introducing pathogenic bacteria into the interior of the muscle (Luchansky *et al*., 2008, Bhat *et al*., 2018a). Consequently, mechanically tenderized beef must be labeled to inform consumers of how it was processed and it requires a higher cooking temperature to reduce the biological hazard (USDA, 2019; USDA, 2015), which would be expected to decrease palatability.

Electrical Stimulation

Electrical stimulation (ES) uses an electric current passed through the carcass to improve meat tenderness, especially in beef cattle. This stimulation increases the onset of rigor mortis by accelerating the rate of glycolysis (Adeyemi & Sazili, 2014). Muscle disruption from ES activates the calpain system earlier postmortem, causing an increased rate of glycolysis and calcium ion release, thus resulting in more proteolysis (Hwang *et al*., 2003; Adeyemi & Sazili, 2014). The rapid acidification of the muscle while the carcass is not fully chilled also causes lysosomal membranes to rupture and release cathepsins (Troy & Tarrant, 1987; Hwang *et al*., 2003). According to Adeyemi & Sazili (2014), ES might negatively impact color stability and water-holding capacity. Hwang *et al*. (2003) found that ES would not improve beef tenderness that was "beyond baseline toughness" and that under- or over-stimulation would not improve the tenderness either.

Sonication

Sonication can be used to improve meat tenderness by disrupting muscle tissue with high frequency ultrasound waves (Awad *et al*., 2012; Turantaş *et al*., 2015; Barekat & Soltanizadeh, 2017). The sound energy created from the ultrasound enters the meat and physically changes the muscle structure via acoustic vibrations (Awad *et al*., 2012; Turantaş *et al*., 2015). Ultrasound can be applied to meat by using a probe or an ultrasonic bath, and the effects of ultrasound can differ with varying intensity, frequency, and duration (Awad *et al*., 2012, Turantaş *et al*., 2015). Jayasooriya *et al*. (2004) postulated that sonication could be used to make the postmortem aging period shorter while still achieving improved meat tenderness. Sonication is often considered a superior method of tenderization as it is not a mechanical or chemical application. However, some studies were not always capable of producing a more tender product, and there are thermal effects associated with the ultrasound technique (Jayasooriya *et al*., 2004; Smith *et al*., 1991). Unlike mechanical tenderization, ultrasonication may have some antimicrobial properties (Awad *et al*., 2012; Turantaş *et al*., 2015). Similar to other tenderization methods, the effects on tenderness are typically muscle-dependent (Turantaş *et al*., 2015).

1.2 Tumbling

1.2.1 What is Tumbling?

The tumbling process involves meat being physically disrupted when it is rotated in a large metal drum, thereby improving tenderness. As the drum circulates, the meat falls onto the drum walls, paddles, and the other pieces of meat. Tumbling has been typically used only for processed meat applications to improve meat quality, form restructured products, and incorporate non-meat ingredients (Cheng *et al*., 2011). Though brine is usually added with tumbling, some recent studies have considered tumbling without brine enhancement as a potential method to improve beef quality and palatability (Morrow *et al*., 2019; Tuell & Kim, 2021; Tuell *et al*., 2021).

With tumbling, there are different factors and combinations that can be modified. These may include the presence or absence of a vacuum, whether tumbling is applied continuously or intermittently, the duration, and the speed of tumbling. These factors will be discussed in greater detail in the subsequent sections.

1.2.2 Tumbling Impacts on Meat Quality

Tumbling causes physical disruptions to the muscle structure, which would be expected to increase tenderness levels. However, there may be other factors involved in this improvement of meat quality when applied to fresh product. It has been speculated that physical disruption to the muscle tissue influences a greater activation of calpains because of more calcium ions being released from the sarcoplasmic reticulum (Koohmaraie, 1994; Jayasooriya *et al*., 2004; Koohmaraie & Geesink, 2006; Kemp *et al*., 2010). The disruption also causes lysosomes to release cathepsins (Bolumar *et al*., 2014; Troy & Tarrant, 1987; Canonico & Bird, 1970), promoting more proteolytic activity. Previous studies found consumer panelists to detect improvements in tenderness of tumbled fresh beef loins, without affecting the juiciness and flavor of the product (Tuell & Kim, 2021). Several measures of water-holding ability may be affected such as an increase in cook loss (Tuell & Kim, 2021; Tuell *et al*., 2021; Morrow *et al*., 2019). With the combination of physical disruptions and greater proteolysis, tumbling could have strong impacts on meat tenderization, though at present the effects on other quality attributes would be less clear.

1.2.3 Tumbling Processing Factors

As previously mentioned, there are many factors that may impact the tumbling process. There is the option to incorporate various brine solutions (Boles & Shand, 2002; Cheng *et al*., 2011; Gao *et al*., 2014), tumble with or without a vacuum (Ghavimi *et al*., 1986; Solomon *et al*., 1980), tumble continuously or intermittently (Cassidy *et al*., 1978; Krause *et al*., 1978), tumble for different lengths of time (Tuell & Kim, 2021; Tuell *et al*., 2021; Moon *et al*., 2007; Pietrasik & Shand, 2004; Dzudie & Okubanjo, 1999; Krause *et al*., 1978), and tumble at different speeds (Lin *et al*., 1990). At present, few if any studies have considered the postmortem time point at which tumbling would be applied. However, it stands to reason that this would be relevant to product quality, especially in fresh products with the application of further postmortem aging. Muscle type and location may also play an important role in how meat quality is affected (Tuell & Kim, 2021; Tuell *et al*., 2021), similar to postmortem aging (Rhee *et al*., 2004). The following sections aim to explain how these tumbling factors may influence product quality.

Vacuum or Non-vacuum

Vacuum tumbling is when meat is tumbled with the presence of a vacuum. The vacuum uses negative pressure to incorporate brine solution into the meat. It also improves binding potential by eliminating air bubbles on the surface of the meat (Boles & Shand, 2002; Bosse *et al*., 2018; Solomon *et al*., 1980). Ghavimi *et al*. (1986) performed a study comparing meat quality and yield after vacuum and non-vacuum tumbling restructured, cured beef. No differences in shear force were found, but the non-vacuum tumbled meat produced higher yields (Ghavimi *et al*., 1986). Muscle cell disruption may have contributed to the yield loss from processing (Cassidy *et al*., 1978; Ghavimi *et al*., 1986). Cheng *et al*. (2011) investigated the effects of continuous non-vacuum or vacuum tumbling on beef bottom round quality. No differences in shear force values were found between the vacuum and non-vacuum tumbled roasts; however, the tumbled roasts had lower shear forces than the controls after 2, 4, 7, and 14 days of refrigerated storage after tumbling and roasting (Cheng *et al*., 2011). These results suggest that vacuum compared to non-vacuum tumbling does not necessarily affect tenderness though it would be important for restructured products.

Continuous or Intermittent

Continuous versus intermittent tumbling refers to the time patterns used during tumbling. For continuous tumbling, the meat is tumbled constantly for the intended duration. With intermittent tumbling, the tumbling is stopped and started again at specific intervals over the duration. Cassidy *et al*. (1978) observed more disruptions of myofiber structure with intermittent tumbling. Krause *et al*. (1978) found that intermittent tumbling increased cured ham yield. Gao *et al*. (2014) found that the continuously tumbled pork chops had lower shear force values, lower cook loss, and higher yield compared to intermittently tumbled chops. However, other studies (Boles & Shand, 2002) found no differences between continuous and intermittent tumbling when beef *semimembranosus* muscles were tumbled prior to brine injection. These findings suggest that there may be differences in quality, especially tenderness, between tumbling patterns. However, at present, it is unclear which method would be most beneficial.

Duration

Several studies have found different lengths of tumbling time to affect meat quality attributes differently. Pietrasik & Shand (2004) found that longer tumbling times had lower purge loss, higher cook yield, and decreased shear force values for injected beef *semimembranosus* muscles. The authors concluded that an extended tumbling time of 16 hours improved water-holding ability and thermal stability of the meat product compared to tumbling for 0 or 2 hours (Pietrasik & Shand, 2004). Dzudie & Okubanjo (1999) reported lower cook loss and a decrease in shear force values for goat hams that were tumbled for a longer period of time. For longer tumbling times, Moon *et al*. (2007) observed lower cook loss and improvements in tenderness and juiciness. However, the restructured beef muscles that were tumbled longer had negative effects on binding ability, texture, and overall acceptability (Moon *et al*., 2007). These studies show extended tumbling time as having generally positive effects on water-holding ability and tenderness of meat products, though it is unclear how these may translate to overall acceptability to the consumer.

Speed

Tumbling speed is typically measured in revolutions per minute (rpm). Higher speeds of tumbling would be expected to induce greater physical disruptions to the meat as compared to

slower tumbling. Previous studies have examined the effects of tumbling at different rpm and for different cumulative revolutions. A study done by Lin *et al*. (1990) compared differences between 15 and 25 rpm and 3000, 6000, and 9000 cumulative revolutions. Hams were harder, gummier, and chewier when tumbled at 25 rpm and 3000 cumulative revolutions. At 25 rpm hams were significantly darker and chewier, while the hams at 9000 cumulative revolutions had the best texture. Even though there were differences between shear force values and sensory tenderness results, the authors concluded that both tumbling speed and revolutions impact meat tenderness together (Lin *et al*., 1990).

Brine Incorporation

The addition of a brine, either by injection or into a tumbler, is a common practice to improve meat quality. Pre-tumbling, or tumbling prior to injection, is another method that improves brine pick-up, yield, and tenderness (Boles & Shand, 2002). Molina *et al*. (2005) compared the differences between marinating, needle-injecting, or vacuum tumbling different beef chuck muscles. Only the *subscapularis* muscle had decreased shear force values from vacuum tumbling compared to the control. Needle-injection was generally more effective as it improved the instrumental tenderness for *complexus, subscapularis,* and *triceps brachii* muscles compared to controls. For sensory panel evaluations, *triceps brachii* and *serratus ventralis* muscles improved in overall tenderness with all of the enhancement methods compared to the control (Molina *et al*., 2005). Morrow *et al*. (2019) reported that the greatest improvement to palatability traits was observed in the beef *rectus abdominus* muscles that were injected and tumbled, and there was no improvement of tenderness to the muscles that were tumbled without enhancement.

Muscle Type

Tougher muscles, typically from the chuck or round, may require longer duration of tumbling to improve tenderness to an acceptable level for consumers. In studies that evaluated meat quality after tumbling different muscles, it was observed that each muscle responds differently to the treatments (Molina *et al*., 2005). Molina *et al*. (2005) also found that vacuum tumbling could help decrease the amount of perceptible connective tissue in some of the beef chuck muscles. Morrow *et al*. (2019) observed an increase in cook loss and no effect on shear force or sensory evaluation after tumbling beef *rectus abdominus* without a brine. This lack of

improvement from tumbling might be due to the fact that the flank is a tougher muscle with larger amounts of connective tissue (Jeremiah *et al*., 2003). It may also make a difference whether the muscle type is glycolytic or oxidative, considering differences in proteolysis between fiber types (Muroya *et al*., 2010; Muroya *et al*., 2006).

1.3 Cull Cows

Cull cows are bovine animals that are insufficient for their primary form of production. Typically, cows are culled from a dairy or beef herd when the animal has poor performance, poor health, or fails to produce offspring. The cull cows then enter the beef supply chain and are typically processed into products such as ground beef (Alvarenga *et al*., 2021; Streiter *et al*., 2012). According to the USDA's 2020 livestock slaughter summary (USDA, 2021), culled beef and dairy cows accounted for 19.7% of all cattle slaughtered for the year. With 32.8 million head of commercial cattle slaughtered in the United States, cull cows contributed over 6.4 million carcasses (USDA, 2021). Given the high contribution of cull cow beef to the total United States beef industry, it is of importance to identify novel methods to improve palatability and acceptability of cull cow beef to consumers. Further processing is typically done as cull cow beef is often perceived as lower quality and not suitable for whole muscle cuts. This may be due to a decrease in meat tenderness as animal maturity increases (Lucero-Borja *et al*., 2014), as well as the introduction of off-flavors (Stelzleni *et al*., 2007; Stelzleni & Johnson, 2010; Gredell *et al*., 2018). However, the recent study by Alvarenga *et al*. (2021) found that certain cuts from cull cow may be undervalued.

Meat tenderness decreases with animal age due to more connective tissue accumulation and increased collagen cross-linking (Tuma *et al*., 1962; Obuz *et al*., 2014; Miller *et al*., 1987). The recent study by Alvarenga *et al*. (2021) provides some evidence that cull cow beef may be able to reach acceptable tenderness levels for consumers, dependent on the individual muscle. At 2 days postmortem, 13% of cull cow beef loin muscles were considered tender. At 14 days, this percentage increased to 60% (Alvarenga *et al*., 2021). This may be attributed to the loin being a muscle that has relatively low amounts of connective tissue, and tenderization of this muscle mostly depends on the extent of proteolysis (Rhee *et al*., 2004).

1.3.1 Improving Cull Cow Beef

Numerous studies have been conducted in an attempt to improve cull cow beef with various diet and nutritional changes (Gredell *et al*., 2018; Holmer *et al*., 2009; Miller *et al*., 1987; Stelzleni & Johnson, 2010; Minchin *et al*., 2009; Minchin *et al*., 2010) and different meat processing methods (Boleman *et al*., 1996; Bhat *et al*., 2018b; Obuz *et al*., 2014; Vitale *et al*, 2014; Mandell *et al*., 2006; Streiter *et al*., 2012). It is known that the industry has issues with consistency in beef quality and palatability (Guelker *et al*., 2013; Martinez *et al*., 2017), and this is especially true for cull cow beef (Alvarenga *et al*., 2021). Though Jurie *et al*. (2007) found that breed type did not cause variability of meat quality from culled dairy cows and culled beef cows, there still may be quality differences between beef and dairy breed types and between culled cows and bulls.

The majority of the variability of cull cow beef tenderness is due to the amount of connective tissue and percent of soluble collagen in the muscle (Alvarenga *et al*., 2021; Obuz *et al*., 2014). Inherently tender muscles like the *longissimus lumborum et thoracis* from cull cow carcasses have the potential to be sold as fresh meat products (Alvarenga *et al*., 2021); however, individual muscles vary in aging potential which is partially dependent on animal age (Nair *et al*., 2019; Cruzen *et al*., 2014). Extended aging periods may not have a strong tenderizing effect on tougher muscles from cull cows due to the high amounts of connective tissue or the higher percent of insoluble collagen present (Purslow, 2014; Purslow, 2018). Through nutritional or post-harvest tenderization methods, tenderness of cull cow beef may be improved.

1.3.2 Improving Cull Cow Beef with Nutritional Methods

Feeding high grain diets pre-slaughter is typically considered to improve the value of cull cow beef (Gredell *et al*., 2018; Holmer *et al*., 2009). If the cull cows are fed a high concentrate diet prior to slaughter, the percent of soluble collagen would be expected to increase (Miller *et al*., 1987). Another issue with cull cows is the detection of off-flavors in the meat (Stelzleni *et al*., 2007; Gredell *et al*., 2018). In a study by Stelzleni & Johnson (2010), the objective was to evaluate off-flavor production in loin muscles from fed and non-fed cull beef and dairy cows and A-maturity, USDA Select grade steers. The results showed that the highest detection of sensory off-flavor was found in the non-fed beef cull cows, compared to all other cull cows and the A-maturity steers (Stelzleni & Johnson, 2010). Stelzleni & Johnson (2010) also reported that although fatty

acids are known to be precursors for off-flavors, the fatty acid profile was not a relevant factor for the off-flavors detected.

Two studies by Minchin *et al*. (2009, 2010) investigated the effects of different finishing diets on the meat quality of cull cow beef. In the first study (Minchin *et al*., 2009), different grass silage diets were compared with increasing inclusion of energy dense concentrates. The results showed no effect on carcass characteristics or meat tenderness, but there were improvements in color attributes and number of days to slaughter with the inclusion of a concentrate in the diet. The second study (Minchin *et al*., 2010) evaluated the effects of different finishing diets on the carcass and meat quality of cull cows. The treatments included combinations of cows that were either dried or milked during the over-wintering period, and diets were grass silage, grass silage with straw, or grass silage with concentrate. Results showed no difference in shear force values among the treatments, as well as increased number of days to slaughter in the treatments that included straw in the diet or milking over winter. Results from both studies suggest that adding a concentrate in the diet will get the cattle to slaughter quicker, while the benefits to tenderness may be limited. Consequently, focusing on improving tenderness with post-harvest tenderization methods could be more effective and feasible for industry application as compared to nutritional methods.

1.3.3 Improving Cull Cow Beef with Meat Processing

As previously discussed, post-harvest interventions to improve meat quality include aging, ES, alternative carcass suspension, mechanical tenderization, tumbling, and any other processes that are typically done to produce higher quality meat products. These methods may be more feasible because of the ability to be performed large scale in meat processing plants, rather than individual beef producers having to use expensive grain diets to improve quality attributes.

Aging is one of the most common methods that is used to improve the meat quality of cull cow beef. Even though aging may improve the tenderness of cull cow meat to a certain extent, in many cases the meat is still too tough to be acceptable for consumers (Boleman *et al*., 1996; Bhat *et al*., 2018b). However, this is considered to be dependent on the muscle (Alvarenga *et al*., 2021). Obuz *et al*. (2014) observed improvements in cull cow beef loin tenderness with postmortem aging and with the combination of aging and blade tenderization. Franco *et al*. (2009) found that shear force values of cull cow beef loin muscles were improved after being aged for 7 or 14 days, but

stated that a trained panel would be necessary to verify the increased tenderness levels. Vitale *et al*. (2014) performed a study that evaluated the effects of different aging periods in vacuum packaging on the shear force values of cull cow beef loins. Loins were aged for 0, 3, 6, 8, 14, or 21 days and then displayed in modified atmosphere packaging (MAP) for 0, 3, 6, or 9 days. For storage of 9 days, it was beneficial to use shorter aging times in vacuum packaging to improve beef loin tenderness. Improved tenderness was observed with the longer aging durations, but shelf life was decreased (Vitale *et al*., 2014).

Skeletal separation techniques also have the potential to improve the tenderness of cull cow meat. Mandell *et al*. (2006) observed an increase in tenderness of cull cow beef posterior rib and anterior loin, but not in the posterior loin, when the thoracic and lumbar vertebrae were severed in various locations. The *semimembranosus* had improved tenderness when skeletal separation techniques were used on the round muscle by severing the ischium and junction between the fourth and fifth sacral vertebrae (Mandell *et al*., 2006). A study by Streiter *et al*., (2012) evaluated the effects of different postmortem processing treatments and aging on the improvement of tenderness and eating quality for cull cow beef *longissimus lumborum, longissimus thoracis, semitendinosus,* and *semimembranosus* muscles. The treatments included: no postmortem processing, skeletal separation, enhancement with calcium ascorbate, and skeletal separation with enhancement. Results showed that skeletal separation decreased shear force for the loin muscles, and enhancement decreased the shear force for all of the muscles. Shear force values were decreased to a greater extent with the combination of skeletal separation and enhancement together. The sensory panel evaluation of *longissimus thoracis* steaks showed that the combination of postmortem processing treatments on cull cow beef was comparable to high quality steaks from young animals based on palatability traits (Streiter *et al*., 2012).

1.4   Implication - Tumbling Cull Cow Beef to Improve Tenderness

Taken together, tumbling has the potential to improve tenderness and other meat quality attributes of cull cow beef. Tumbling has been proven to improve the quality of meat products (Pietrasik & Shand, 2004; Dzudie & Okubanjo, 1999; Moon *et al*., 2007), especially for tenderness and water-holding capacity. Tenderness is increased when the muscle tissue is disrupted, which may cause the activation of calpains and release of cathepsins, increasing proteolytic activity with

aging (Troy & Tarrant, 1987; Jayasooriya *et al*., 2004; Canonico & Bird, 1970). This has been demonstrated in beef loins from A-maturity cattle (Tuell & Kim, 2021; Tuell *et al*., 2021), though no studies have evaluated if tumbling without a brine with additional aging would be beneficial to improve quality of cull cow beef.

Further, no studies have considered the most effective postmortem time point to apply a tumbling treatment to improve tenderness. It would be relevant to investigate the effect of fresh meat tumbling time on the tenderness and proteolytic activity of beef muscles. Tumbling fresh beef earlier postmortem could have the ability to improve aging potential. This may be especially true for beef loin muscles in which the majority of the tenderization occurs immediately after harvest (Nair *et al*., 2019). Also, beef loin muscles continue to tenderize during extended aging periods (Stolowski *et al*., 2006; Nair *et al*., 2019), therefore it could be advantageous to combine the tumbling application with postmortem aging. In Chapter 2, the postmortem time point of tumbling application on quality and palatability attributes will be investigated for A-maturity cattle. Chapter 3 will address the application of tumbling on cull cow beef loins as a potential strategy to improve tenderization and quality with aging.

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CHAPTER 2.   FRESH BEEF TUMBLING AT DIFFERENT POSTMORTEM TIMES TO IMPROVE TENDERNESS AND PROTEOLYTIC FEATURES OF *M. LONGISSIMUS LUMBORUM*

2.1   Abstract

The aim of this study was to determine the effects of fresh beef tumbling at different postmortem times on meat quality attributes and proteolytic features of loins (*M. longissimus lumborum*). At 1 day postmortem, loins were separated from one side of beef carcasses (n=12), cut into 4 sections, and assigned to no tumbling or tumbling for 90 minutes at 1, 6, or 11 days postmortem. Loin sections were evaluated immediately after tumbling or further aged to a total common postmortem time of 16 days. Purge loss from non-tumbled sections was similar to sections tumbled at any postmortem time ($P>0.05$), however tumbling at 1 day resulted in greater purge loss than 11 day ($P<0.05$). Thaw loss was not affected by tumbling ($P>0.05$), but cook loss increased when loins were tumbled at 6 or 11 days postmortem ($P<0.05$). Tumbling at 6 or 11 days postmortem increased CIE a^*, b^*, and chroma values ($P<0.05$), but L^* and hue angle were not affected ($P>0.05$). Beef sections tumbled at 1 or 6 days postmortem without additional aging had lower shear force values compared to the respective non-tumbled controls ($P<0.05$). Tumbling at 1 day postmortem with no further aging had similar shear force values compared to the 16 day aged non-tumbled controls ($P>0.05$). Western blot and myofibril fragmentation index results indicated that early postmortem tumbling did not have an immediate impact on protein degradation, but may have maximized calpain-1 activation. These results suggest that early postmortem tumbling coupled with aging can synergistically impact the improvements of beef loin tenderness and proteolysis, and shorten the necessary aging period.

Keywords: beef quality, calpain activation, meat tumbling, postmortem proteolysis, tenderness

2.2 Introduction

Beef tenderness is considered to be one of the most important palatability traits to consumers (Shackelford *et al.*, 2001; Miller, 2020; Warner *et al.*, 2021). Inconsistent and inadequate beef tenderness, as it varies greatly between and within different muscles (Belew *et al.*, 2003; Nair *et al.*, 2019), has been a major cause of consumer dissatisfaction (Warner *et al.*, 2021). Various post-harvest technologies have been developed and practiced in the meat industry, such as blade and needle tenderization with or without injection enhancement of non-meat ingredients (Yang *et al.*, 2021). Given the growing consumer demand for natural, safe, and minimally processed fresh meat products, some of the widely used mechanical tenderization methods may no longer be perceived as "consumer favorable" approaches (Yang *et al.*, 2021). Therefore, developing natural post-harvest processing systems that can be used to create consistently tender products would be beneficial for the beef industry.

Postmortem aging is the primary and most natural process used for beef tenderization. This process allows the endogenous proteolytic enzymes to continue to naturally degrade the muscle fibers, which results in improved tenderness (Kim *et al.*, 2018; Warner *et al.*, 2021). Although it is a natural and effective method to improve beef quality, it is a time-dependent process. According to the 2015 National Beef Tenderness Survey (Martinez *et al.*, 2017), beef steak aging durations at retail establishments averaged about 26 days, with the longest aging period being 102 days. Thus, it would be ideal to develop some post-harvest technology to shorten the aging period while maximizing the positive effects of aging on meat tenderness and overall quality.

For processed meat applications, tumbling is one of the most highly recognized and accepted techniques in the industry (Pietrasik & Shand, 2004). The tumbling process involves the physical disruption of meat structure while it rotates in a large metal drum (Cheng *et al.*, 2011; Addis & Schanus, 1979). As a result, tumbling improves brine incorporation, increases salt-soluble protein extraction, and improves meat tenderness and water-holding ability (Cassidy *et al.*, 1978; Krause *et al.*, 1978). Recently, however, it has been reported that tumbling without brine addition can be successful at improving instrumental tenderness and consumer liking of tenderness of fresh beef loins, likely through structural disintegration of muscle along with enhanced myofibrillar fragmentation (Tuell & Kim, 2021; Tuell *et al.*, 2021).

Physical disruptions of muscles, which occur during tumbling, may enhance activity of proteolytic enzymes (Koohmaraie, 1994; Jayasooriya *et al*., 2004; Kemp *et al*., 2010), which could influence more tenderizing effects. A recent study by Dang *et al*. (2022) found that the improved proteolysis that occurred in beef loins after being subjected to ultrasonication at 1d postmortem was associated with increased proteolytic enzyme activity. Ultrasonication and tumbling are both techniques that disrupt the ultrastructure of beef muscle tissue (Jayasooriya *et al*., 2004; Tuell *et al*., 2021). Increased availability of calcium ions at early postmortem could promote calpain protease activation, resulting in more proteolysis (Bhat *et al*., 2018; Cruzen *et al*., 1992). Given tumbling at early postmortem has the potential to induce more calpain activation possibly via the release of calcium ions in the muscle, it would be reasonable to postulate that applying tumbling early postmortem may influence an acceleration of cytoskeletal muscle protein degradation through increased availability of proteolytic enzymes. Therefore, the objective of this study was to determine the effects of tumbling at different postmortem times on the proteolytic features and meat quality attributes of fresh beef loins. Finding the most effective tumbling time would be critical to consistently provide consumers with high quality products, which may also have positive economic benefits through shortening the necessary duration of postmortem aging.

2.3   Materials and Methods

2.3.1   Raw Materials and Processing

Left-side beef loins (*M. longissimus lumborum*) from 12 different carcasses [USDA low Choice grade (USDA, 2017)] were used for this study. Eight loins were selected at one processing date and the remaining four selected at a second processing date. All loins were removed from carcasses at 1d postmortem. Each loin was cut into 4 sections and assigned to the four tumbling postmortem time (T) point treatments [non-tumbled (NT), tumbled at 1d (1d-T), 6d (6d-T), or 11d (11d-T)] with balanced allocation considering potential locational variation within each loin. Each section was weighed for initial weights and then vacuum packaged (CLARITY, Bunzl Processor Division, Riverside, MO, USA). A schematic diagram of the sample and treatment allocation is presented in **Figure 2.1**. The 1d-T loin sections were tumbled immediately, and the 6d-T and 11d-T sections were stored at 2°C until their assigned tumbling days (6d or 11d postmortem). The NT section was weighed immediately and had steaks cut from it at each tumbling date (1d, 6d, and

11d) and at the total aging period (16d). Each section was unpackaged and weighed before and after all aging and tumbling treatments. A Lance LT-30 500 lb capacity meat tumbler (Lance Industries, Hartford, WI, USA) set at 8.5 rpm for 90 minutes was used for all tumbling treatments by following the process of Tuell *et al*. (2021). After tumbling, beef sections were divided and assigned to either no further aging or aging at 2°C up to the common total aging time of 16d postmortem, such as 1d-T+15, 6d-T+10, and 11d-T+5. The 1d-T section had an additional steak cut from it after 7d of further aging. The purpose of this additional sample treatment being included in the study was to provide a sample that was tumbled early postmortem with a middle point of aging to compare against other tumbling and total aging treatment combinations. After all tumbling and aging treatments were completed, the samples were stored at -80°C until further analyses including meat quality and biochemical attribute measurements. The steak samples to be used for biochemical measurements were thawed and all excess fat and connective tissue were excluded. The remaining muscle was powdered using liquid nitrogen and a commercial blender (Waring Commercial, Stamford, CT, USA). The powdered samples were stored at -80°C until further use for biochemical analyses including pH, myofibril fragmentation index (MFI), and protein extraction for SDS-PAGE and western blotting.

2.3.2   pH Measurement

The pH values of the powdered steak samples were measured in the laboratory using a pH meter (Sartorius Basic Meter PB-11, Sartorius AG, Goettingen, Germany) that was standardized to pH 4.0 and pH 7.0 buffers before measuring the pH of the samples. In duplicate, powdered muscle tissue (3 g) was homogenized with DDI water (27 mL) for 10-15 s, then pH was measured and recorded for each individual sample.

2.3.3   Water-holding Ability

The measurements for the water-holding ability consisted of multiple measurements of purge loss including a total purge loss and each purge loss related to tumbling and aging treatments, thaw loss, and cook loss. Before weighing, samples were blotted with paper towel to remove the excess moisture from the meat surface. Purge loss from tumbling was expressed as the percent change in weight from immediately before and after tumbling. Purge loss before tumbling was expressed as the percent change in weight that occurred during the aging period before tumbling

was applied. Purge loss after tumbling was calculated as the percent change in weight that occurred between immediately after tumbling and at 16d postmortem. Total purge loss was the sum of all the purge loss measurements that occurred over the entire 16d from each loin section. Thaw loss was determined as the steak samples' percent change in weight from before frozen storage at -80°C to after the sample had been thawed prior to cooking. The samples were thawed for 16 hours in a 2°C cooler. Cooking loss was expressed as the steak samples' percent change in weight that occurred during the cooking process. Beef steak samples (2 cm thick) were cooked on an open-faced electric griddle (Model GR-150, Cuisinart, Stamford, CT, USA) that had the temperature set at 175°C. A type-T thermocouple (Omega Engineering, Stamford, CT, USA) connected to a data logger (Madge Tech, Inc., Warner, NH, USA) was inserted into the center of the steak samples to monitor the internal temperature. The steaks were cooked to an internal temperature of 41°C, flipped, and then cooked to 71°C. After cooking, the steaks were allowed 30 minutes of rest before blotting with paper towel and then weighing to determine the final cook weights of the samples.

2.3.4   Instrumental Color

Immediately after each assigned tumbling and/or aging treatment, beef steaks were made to measure fresh meat surface color determination after a 60 minute bloom period. The CIE *L**, *a**, and *b** values were measured on 3 random locations of each steak using a colorimeter (Hunter MiniScan EZ, Reston, VA, USA). The hue angle and chroma values of the samples were calculated using the collected *a** and *b** values based on the American Meat Science Association (AMSA, 2012) color guidelines.

2.3.5   Warner-Bratzler Shear Force

After cooking, the steak samples were stored overnight at 4°C before performing the Warner-Bratzler shear force (WBSF) measurement. The American Meat Science Association guidelines (AMSA, 2015) were followed to determine the WBSF values of the beef steaks. From each steak sample, 8 cores (1.4 cm diameter) were cut parallel to the muscle fiber direction while avoiding large pieces of fat or connective tissue. The cooked meat tenderness measurement was performed using a calibrated TA-XT Plus Texture Analyser (Stable Micro System Ltd, Godalming, Surrey, UK) with a WBSF blade attachment. The blade cut through the cores, perpendicular to the

muscle fiber direction. With a test speed of 2 mm/sec, peak shear force (kg) was recorded and the mean values of all replicates were used for statistical analysis.

2.3.6   Whole Muscle Protein Extraction, SDS-PAGE, and Western Blotting

The overall process of whole muscle protein extraction, gel preparation, SDS-PAGE, and western blotting was performed aligning with the methods by Kim *et al*. (2013), with adjustments described by Setyabrata & Kim (2019). For whole muscle protein extraction, nano drop protein assay was performed to create samples with a consistent protein concentration of 4 mg/mL. SDS-PAGE was performed on all samples to check for consistent protein concentration. Troponin-T degradation, desmin degradation, and calpain-1 autolysis were analyzed by performing western blots. For electrophoresis, separating gels (15% for troponin-T, 12% for desmin, and 8% for calpain-1) loaded with 40 μg of protein ran for 3 hours at 130 V using a (Bio-Rad PowerPac Basis, Bio-Rad Laboratories, Hercules, CA, USA), and then gels were transferred onto polyvinylidene fluoride membranes for 90 minutes at 90 V in 1°C Tris-glycine buffer. Membranes were blocked in phosphate buffered saline-tween (PBST) solution diluted with 5% nonfat dry milk (w/v) for 1 hour and then incubated in primary antibody solution with 3% nonfat dry milk (w/v) in PBST with a 1:20,000 dilution of anti-troponin-T (T6277, Sigma Aldrich, St. Louis, MO, USA) and anti-desmin (D1022, Sigma Aldrich, St. Louis, MO, USA), and a 1:7,500 dilution of anti-calpain-1 (Thermofisher MA3-940) at 4°C overnight. After washing the membranes with PBST, secondary antibody incubation was applied for 1 hour in PBST with 3% nonfat dry milk (w/v) and with dilutions of 1:20,000, 1:15,000, and 1:10,000 of monoclonal goat anti-mouse IgG (H&L) horseradish peroxidase conjugate (170–6516, Bio-Rad Laboratories, Hercules, CA, USA) for troponin-T, desmin, and calpain-1, respectfully. Membranes were developed with enhanced chemiluminescent detection reagents (Thermo Fisher Scientific, Waltham, MA, USA) and imaged with a ChemiDoc-It[TS2] system (UVP GelDoc-It, Upland, CA, USA). Band intensities were quantified as a ratio comparing each band from each sample to the intact reference (1d-NT) band. For calpain-1 autolysis, the bands (80, 78, and 76 kDa) were quantified and expressed as a ratio of each individual band to the total intensity.

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2.3.7 Myofibril Fragmentation Index

Myofibril fragmentation index (MFI) was determined by following the method by Culler *et al*. (1978) with minor modifications. Powdered beef sample (2 g) was homogenized with 20 mL of cold MFI buffer [100 mM KCl, 20 mM potassium phosphate, 1 mM EGTA, 1 mM $MgCl_2$, and 1 mM NaN_3] for about 20 s, in duplicate. Samples were centrifuged two times at 1000 x g for 15 minutes at 4°C. Each time, the supernatant was removed and 20 mL (first time) or 5 mL (second time) of fresh cold MFI buffer was added to resuspend the pellet by vortex. The samples were strained using a no. 18 polyethylene strainer to remove any connective tissue. Protein quantification, also performed in duplicate, was done by comparing the samples (0.25 mL of suspension, 0.75 mL of cold MFI buffer, and 4 mL of biuret solution [1.5 g copper (II) sulphate pentahydrate, 6 g sodium potassium tartrate, 500 mL water, 300 mL 10% (w/v) NaOH, and 1 g KI]) to known BSA standards after reading absorbances at 540 nm using a microplate spectrophotometer (Epoch, BioTek Instruments, Inc., Winooski, VT, USA). After adjusting the protein concentration of the samples to 0.5 mg/mL by adding MFI buffer, MFI quantification was performed by reading the absorbance at 540 nm using a UV spectrophotometer (UV-1600PC, VWR International LLC, Radnor, PA, USA). The MFI values were calculated as $200 \times$ the sample absorbance at 540 nm.

2.3.8 Statistical Analysis

The one-way ANOVA statistical method was used to compare each of the total of 11 treatments [1d-NT, 6d-NT, 11d-NT, 16d-NT, 1d-T+0, 1d-T+7, 1d-T+15, 6d-T+0, 6d-T+10, 11d-T+0, and 11d-T+5] individually. All treatment levels were analyzed using the PROC MIXED procedure of SAS (version 9.4, SAS Institute, Inc., Cary, NC, USA) to construct the full model to find any differences between the treatment means. The Tukey pairwise comparison test was performed to evaluate the significant differences between the means of each treatment pair. Animal was used to serve as the random effect. By using a fixed effect model with PROC GLM to test all possible effects, it was confirmed that the residuals were normally distributed and the model assumptions were met. The data had the same goodness of fit as the full model, but suffered from overfitting when an additive model of PROC GENMOD was used, so it was recommended to use

the full model only. Least square means were separated with a statistical significance level set at *P*<0.05.

2.4   Results and Discussion

2.4.1   pH Measurement

For the pH of the beef loin sections, there were no particular tumbling treatment or aging effects found between the samples (*P*>0.05; **Table 2.1**). Tumbling did not affect the pH values of beef samples compared to the NT controls at each respective postmortem time point (*P*>0.05). Tuell & Kim (2021) found that tumbling fresh beef loins for 40 minutes increased the pH, but there were no pH differences found between the non-tumbled loins and the loins that were tumbled for 80 and 120 minutes. A significant increase in pH was found for the samples tumbled at 6d then further aged to 16d compared to the 6d-NT control. However, the changes in beef loin pH values due to tumbling and aging treatments were minimal in general.

2.4.2   Water-holing Ability

The impacts of tumbling at different postmortem times with further aging on water-holding ability of fresh beef loins were determined by sequential measurements of purge loss for loin sections, as well as thaw loss and cook loss of steak samples. Tumbling at 1d postmortem resulted in higher purge loss from the loin sections (*P*<0.05) compared to tumbling at 6d and 11d postmortem, when measured immediately after tumbling (**Table 2.2**). Moreover, after the allocated further aging, the 1d-T beef section maintained a higher purge loss compared to the other tumbling treatments (*P*<0.05). Greater total purge loss over 16d was found in sections tumbled at 1d postmortem (4.6%) compared to those tumbled at 11d (2.8%; *P*<0.05), while the NT and 6d-T treatments were intermediate (*P*>0.05). The purge loss results suggest that tumbling at early postmortem would result in higher losses relative to tumbling after an aging period. However, it is to be noted that when all purge losses were compiled together, there was no difference in total purge loss found between the NT control and the tumbling treatments (*P*>0.05), indicating aging is an overriding factor (than tumbling) inducing the loss of meat water as purge. This observation could suggest that although tumbling at early postmortem would result in more purge loss initially compared to the later postmortem time tumbling application, the total amount of purge loss at the

completion of aging would be equivalent regardless of tumbling treatments at different time postmortem. In fact, Tuell *et al*. (2021) also reported that there was no impact of tumbling on the purge loss of fresh beef loin sections when tumbled at 7d postmortem. However, they reported the significant aging impacts on purge loss of beef loin samples (Tuell *et al*., 2021)

Thaw loss of beef steaks from the 1d-NT section was higher compared to all other time points (*P*<0.05), except from the 1d-T+0 beef steak samples (**Table 2.1**). Tumbling alone did not affect thaw loss (*P*>0.05) compared to the NT controls at each respective time point. This observation is in agreement with Tuell *et al*. (2021), where they also reported no tumbling impacts on the thaw loss of beef loin steaks. However, when different aging time is considered, samples from 1d postmortem (1d-NT and 1d-T+0) without aging had more thaw loss (*P*<0.05), compared to samples at 11d. These results suggest that aging alone decreased the thaw loss and additional aging after tumbling also decreased the thaw loss at most of the time points.

For cook loss, tumbling resulted in an increase in amount of cook loss from beef steaks when tumbling was applied at 6d and 11d postmortem with no further aging (*P*<0.05) compared to the NT controls at each respective time point (**Table 2.1**). No considerable aging effects were found when comparing NT controls from each postmortem time point (P>0.05). Cook loss was also increased for previous studies (Tuell & Kim, 2021; Tuell *et al*., 2021) when tumbling was applied to beef loin sections at 5d or 7d postmortem. The increased cook loss could be due to the combined impact of thermal stress during cooking coupled with tumbling impacts, causing increased extra-myofibrillar space in the muscle, as physical stress such as ultrasonication, massaging, and tumbling techniques could impact cellular disruption of extra-myofibrillar structures (Sharedeh *et al*., 2015; Siró *et al*., 2009). While sensory traits were not evaluated for the current study, the study by Tuell & Kim (2021) found that a consumer sensory panel did not observe differences in juiciness of the tumbled samples compared to controls.

2.4.3 Instrumental Color

In general, tumbling without further aging affected *a** (redness), *b** (yellowness), and chroma (color intensity) of beef loin steaks (**Table 2.3**). Steak samples from the beef sections tumbled later postmortem (6d and 11d) had higher *a**, *b**, and chroma values compared to the NT controls at each respective time point (*P*<0.05). However, with further aging, the extent of

increases in redness, yellowness, and color intensity upon tumbling disappeared, indicating the immediate tumbling impacts on color attributes of beef surface were likely transient. This could be attributed to the fact that tumbling would affect initial blooming rate of fresh beef upon exposing the meat to aerobic conditions. No immediate tumbling impacts, however, were found on L^* (lightness) or hue angle (discoloration) compared to the NT controls at each time point ($P>0.05$).

2.4.4 Warner-Bratzler Shear Force

Tumbling had a significant impact on the WBSF values of beef samples (**Fig. 2.2**). The 1d-NT samples had the highest shear force values (4.14 kg) among treatments. Tumbling at 1d and 6d postmortem with no further aging, resulted in decreased shear force values compared to NT samples at the respective time points ($P<0.05$). As expected, when additional aging was included after tumbling, the shear force values were further decreased. Samples that were tumbled later (6d and 11d) and then aged, had the lowest WBSF values (below 2.05 kg). These results align with recent studies (Tuell & Kim, 2021; Tuell *et al*., 2021) in that tumbling positively impacted the immediate tenderness of beef loin muscles shown by decreased WBSF values. Importantly, tumbling at 1d postmortem, without further aging, achieved similar shear force values as the control samples that were aged to the full 16d without tumbling ($P>0.05$). These results suggest that tumbling (without brine incorporation) can increase the instrumental tenderness of beef loin samples and considerably shorten the required aging times. Tuell *et al*. (2021) also reported the immediate positive impacts of tumbling on instrumental tenderness values of beef loins, which had lower WBSF values ($P<0.05$) compared to non-tumbled beef loins aged for 2 weeks. Though the current study only measured instrumental tenderness, Tuell & Kim (2021) found that a consumer sensory panel was able to differentiate tenderness levels between tumbled samples compared to the controls.

2.4.5 Western Blot Analysis

For calpain-1 autolysis, although not significant, there was a strong trend ($P=0.055$) found in the 80 kDa intact band, where tumbling at 1d postmortem induced more autolysis of the 80 kDa band compared to the non-tumbled control (**Fig. 2.3. A. & Table 2.4**). Also, tumbling at 1d postmortem significantly decreased the intensity of the 78 kDa band of calpain-1 when compared to the NT counterpart. Further, there was a moderate trend ($P=0.075$) that tumbling at 1d

postmortem resulted in greater values of the 76 kDa band compared to the NT control. In fact, the intensity of the 76 kDa from the 1d-T+0 samples were equivalent to that of the NT samples at 16d postmortem ($P>0.05$), indicating that tumbling application at early postmortem (1d) accelerated autolysis of calpain-1 and likely its activity. The activation of calpain-1 mediated by calcium ion binding at micromolar concentration level, leads to the autolysis of the 80 kDa intact bands to the autolyzed 76 kDa form through a 78 kDa intermediate band (Lametsch *et al*., 2008). Thus, the rate and extent of autolysis of calpain-1 is often considered as an indirect indication of calpain-1 activation (Ma & Kim, 2020).

Conversely, tumbling at 6d postmortem had no impacts on the extent of calpain-1 autolysis when compared to the non-tumbled counterpart at the respective time point ($P<0.05$). However, it should be noted that the 6d-T+0 beef samples had equivalent extents of the 78 and 76 kDa bands to the 16d-NT samples ($P>0.05$), while the 6d-NT samples still had a greater extent of 78 kDa and lesser extent of 76 kDa bands compared to respective bands of samples from the 16d-NT treatment ($P<0.05$). No impacts of tumbling and further aging were found between the treatments when tumbling was applied at 11d postmortem ($P>0.05$). Taken together, it can be surmised that tumbling application at early postmortem times could result in early activation of calpain likely through the release of more calcium ions (Bhat *et al*., 2018; Cruzen *et al*., 2015) due to the physical disruption of muscle structure. However, tumbling impacts on the extent of calpain activation could be considerably diminished with the course of postmortem time periods for the application, indicating the importance of tumbling at early postmortem to maximize calpain activation.

Tumbling impacts on the level of myofibrillar structural protein degradation were determined by quantifying both intact and degradation products of troponin-T and desmin (**Fig. 2.3. B-C. & Table 2.4**). No significant tumbling impacts on troponin-T intact and degradation band 1 products were found. However, tumbling at 1d postmortem and further aging for 7d (1d-T+7) resulted in a similar extent of troponin-T degradation band 2 compared to the 16d-NT control ($P>0.05$). Furthermore, the 6d-T+0 samples had similar levels of troponin-T degradation band 2 to the 16d-NT control ($P>0.05$), while the 6d-NT samples had less extent of the degradation band compared to the 16d-NT control ($P<0.05$). These observations indicate the accelerated troponin-T degradation upon tumbling coupled with aging. Similar results were also found for desmin degradation, where a significant decrease in intact bands and increase in degradation products of

desmin were found in the 1d-T+7 samples compared to the 1d-NT ($P<0.05$), but those were comparable to respective bands from the 16d-NT control ($P>0.05$). It is of interest to note that tumbling at 11d postmortem with further aging for 5d resulted in a greater extent of desmin degradation products (band 2) compared to the 16d-NT ($P<0.05$), indicating a synergistic impact of tumbling coupled with aging on desmin degradation than aging alone. The results of the current study agree with the fresh beef loin tumbling study by Tuell *et al.* (2021) where troponin-T and desmin protein degradation was increased with combining tumbling and aging, but not with tumbling or aging alone.

2.4.6   Myofibril Fragmentation Index

The MFI results are in line with myofibrillar protein degradation, where tumbling at 1d postmortem with further aging for 7d (1d-T+7) resulted in a significant increase in MFI values compared to the 1d-NT and 1d-T+0 samples, but it had a similar extent of MFI values to the 16d-NT control ($P>0.05$; **Fig. 2.4**). Moreover, the 6d-T+0 had a similar level of MFI values to the 16d-NT control ($P>0.05$), while the 6d-NT had less extent of MFI values compared to the 16d-NT controls ($P<0.05$). These results support that tumbling coupled with aging results in an increase in extents of myofibrillar protein fragmentation of beef loins, which in turn positively affect meat tenderness. Interestingly, an immediate tumbling impact on MFI was found when tumbling was applied at 11d postmortem with no further aging, which had higher or equivalent MFI ($P<0.05$) compared to the 11d-NT or 16d-NT samples, respectively. Tuell *et al*. (2021) similarly found MFI values increased from both tumbling and aging treatments. These findings support that the tumbling effect on improving tenderness of beef loins is likely attributed to both the immediate physical disruption of muscle tissue and the enhancement of proteolysis.

2.5   Conclusion

The results of the present study confirm that tumbling application without the use of brine incorporation can improve instrumental tenderness of beef loins with minimal impacts on water-holding ability and surface meat color. Additionally, the present study found that while tumbling at various time postmortem would still exert its positive impacts on beef tenderness development, tumbling at early postmortem could result in greater impacts on meat tenderization by inducing accelerated calpain activation and subsequent increases in protein degradation. This observation

suggests that the beneficial impacts of tumbling can be further maximized through the identification of optimal time postmortem for the application. Moreover, the results of the current study support the idea that the postmortem aging time necessary to reach acceptable tenderness levels could be substantially shortened by an early postmortem tumbling application. These findings suggest that early postmortem fresh meat tumbling could have effects on the sensory attributes of beef muscles impacting the overall eating quality, though further study would be necessary to confirm this postulation.

2.6 Tables and Figures

Table 2.1 Water-holding and pH of beef loin steaks tumbled and aged at various postmortem time points (n=12).

Tumbling time	Treatment[1]	Thaw loss (%)	Cook loss (%)	pH
1d	1d-NT	3.1^a	19.6^c	5.47^{bc}
	1d-T+0	2.4^{ab}	20.5^{abc}	5.48^{bc}
	1d-T+7	1.6^c	19.8^c	5.51^{abc}
	1d-T+15	0.7^e	20.0^{bc}	5.53^{ab}
6d	6d-NT	1.9^{bc}	18.9^c	5.47^c
	6d-T+0	1.8^{bc}	23.8^{ab}	5.50^{abc}
	6d-T+10	0.8^{de}	18.9^c	5.54^a
11d	11d-NT	1.7^c	18.4^c	5.50^{abc}
	11d-T+0	1.4^{cd}	24.3^a	5.50^{abc}
	11d-T+5	0.8^{de}	20.9^{abc}	5.53^{ab}
16d	16d-NT	0.7^e	18.3^c	5.51^{abc}
SEM[2]		0.2	1.0	0.01
Significance of *P*-value		<0.001	<0.001	<0.001

[1]Tumbling and aging treatments
      NT: non-tumbled, T: tumbled, #d: tumbling time (d postmortem), +#: further aging (d)
[2]Standard error of the mean
[a-e]Means within a column with a common letter are not significantly different (*P*<0.05).

Table 2.2 Purge losses of beef loin sections tumbled and aged at various postmortem timepoints (n=12).

Treatment	Purge before tumbling (%)	Purge from tumbling (%)	Purge after tumbling (%)	Total purge after 16d (%)
NT[1]	-	-	-	4.0[ab]
1d-T[2]	-	0.81[a]	3.7[a]	4.6[a]
6d-T[3]	1.1	0.68[b]	1.7[b]	3.5[ab]
11d-T[4]	1.1	0.66[b]	1.1[b]	2.8[b]
SEM[5]	0.2	0.04	0.3	0.4
Significance of *P*-value	0.920	0.018	<0.001	0.007

[1]Non-tumbled
[2]Tumbled at 1d postmortem
[3]Tumbled at 6d postmortem
[4]Tumbled at 11d postmortem
[5]Standard error of the mean
[a,b]Means within a column with a common letter are not significantly different (*P*<0.05).

Table 2.3 Instrumental color measurements of beef loin steaks tumbled and aged at various postmortem time points (n=12).

Tumbling time	Treatment[1]	CIE *L**	CIE *a**	CIE *b**	Hue angle	Chroma
1d	1d-NT	42.0[c]	24.4[d]	17.9[e]	36.2[b]	30.2[e]
	1d-T+0	42.1[c]	25.4[cd]	18.7[de]	36.4[b]	31.6[de]
	1d-T+7	45.5[b]	26.3[bc]	20.1[bc]	37.4[a]	33.1[bcd]
	1d-T+15	46.8[ab]	26.5[bc]	20.4[bc]	37.6[a]	33.4[bc]
6d	6d-NT	46.6[ab]	25.5[cd]	19.8[cd]	37.8[a]	32.3[cd]
	6d-T+0	46.5[ab]	28.3[a]	21.7[a]	37.5[a]	35.6[a]
	6d-T+10	46.7[ab]	26.5[bc]	20.2[bc]	37.4[a]	33.3[bc]
11d	11d-NT	46.0[ab]	26.1[bc]	19.9[bcd]	37.4[a]	32.8[bcd]
	11d-T+0	46.9[ab]	28.4[a]	21.8[a]	37.5[a]	35.8[a]
	11d-T+5	48.1[a]	27.4[ab]	21.2[ab]	37.7[a]	34.6[ab]
16d	16d-NT	47.1[ab]	25.9[c]	20.0[bcd]	37.6[a]	32.7[bcd]
SEM[2]		0.6	0.4	0.3	0.2	0.5
Significance of *P*-value		<0.001	<0.001	<0.001	<0.001	<0.001

[1]Tumbling and aging treatments
    NT: non-tumbled, T: tumbled, #d: tumbling time (d postmortem), +#: further aging (d)
[2]Standard error of the mean
[a-e]Means within a column with a common letter are not significantly different (*P*<0.05).

Table 2.4 Western blot analysis of calpain-1 autolysis and troponin-T and desmin degradation of beef loin steaks tumbled and aged at various postmortem time points (n=12).

Tumbling time	Treatment[1]	Calpain-1 (%)			Troponin-T			Desmin		
		80 kDa	78 kDa	76 kDa	Intact	Deg. 1	Deg. 2	Intact	Deg. 1	Deg. 2
1d	1d-NT	0.20	0.32^a	0.48^d	1.04^a	0.09	0.54^d	1.19^a	0.56^c	0.78^{de}
	1d-T+0	0.15	0.29^b	0.57^{bcd}	1.03^{ab}	0.10	0.59^{cd}	1.14^a	0.57^c	0.75^e
	1d-T+7	0.14	0.21^{de}	0.65^{ab}	0.95^{ab}	0.10	0.76^{ab}	0.89^{bc}	0.76^b	0.98^{cd}
	1d-T+15	0.14	0.20^e	0.66^a	0.93^b	0.10	0.83^{ab}	0.81^c	0.83^{ab}	1.17^{bc}
6d	6d-NT	0.19	0.27^{bc}	0.54^{cd}	0.98^{ab}	0.11	0.71^{bc}	1.01^{ab}	0.72^b	0.92^{de}
	6d-T+0	0.15	0.24^{cd}	0.61^{abc}	1.03^{ab}	0.11	0.77^{ab}	0.92^{bc}	0.74^b	0.96^{cde}
	6d-T+10	0.15	0.20^e	0.66^a	0.94^{ab}	0.10	0.82^{ab}	0.75^c	0.85^{ab}	1.25^{ab}
11d	11d-NT	0.15	0.23^{de}	0.62^{abc}	0.97^{ab}	0.11	0.80^{ab}	0.89^{bc}	0.83^{ab}	1.19^b
	11d-T+0	0.15	0.21^{de}	0.64^{ab}	0.99^{ab}	0.11	0.89^a	0.81^{bc}	0.84^{ab}	1.30^{ab}
	11d-T+5	0.15	0.20^e	0.65^{ab}	0.97^{ab}	0.11	0.88^a	0.78^c	0.92^a	1.43^a
16d	16d-NT	0.15	0.21^{de}	0.64^{ab}	1.00^{ab}	0.11	0.87^a	0.87^{bc}	0.86^{ab}	1.20^b
SEM[2]		0.02	0.01	0.02	0.03	0.01	0.05	0.05	0.04	0.07
Significance of P-value		0.055	<0.001	<0.001	0.011	0.052	<0.001	<0.001	<0.001	<0.001

[1]Tumbling and aging treatments
     NT: non-tumbled, T: tumbled, #d: tumbling time (d postmortem), +#: further aging (d)
[2]Standard error of the mean
[a-e]Means within a column with a common letter are not significantly different (P<0.05).
Deg: degradation band
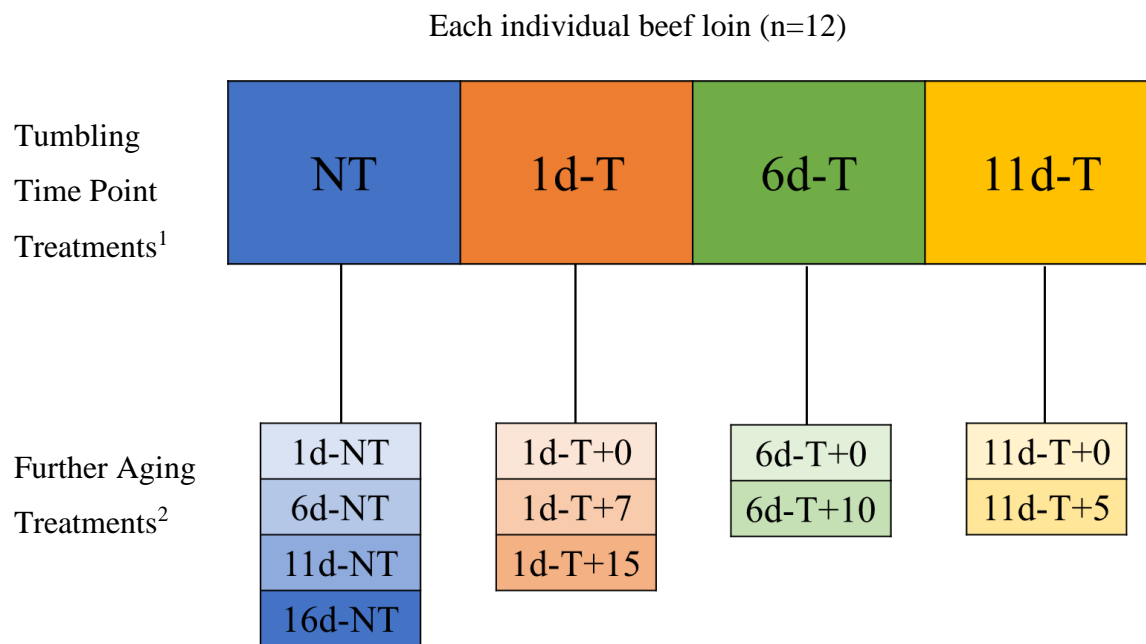
Figure 2.1 Schematic illustration of sample assignment to tumbling and aging treatments.

[1]Tumbling Time Point Treatments (random muscle positioning)
      NT: non-tumbled
      T: tumbled
      #d: postmortem day of tumbling
[2]Further Aging Treatments
      +d: days further aged after tumbling
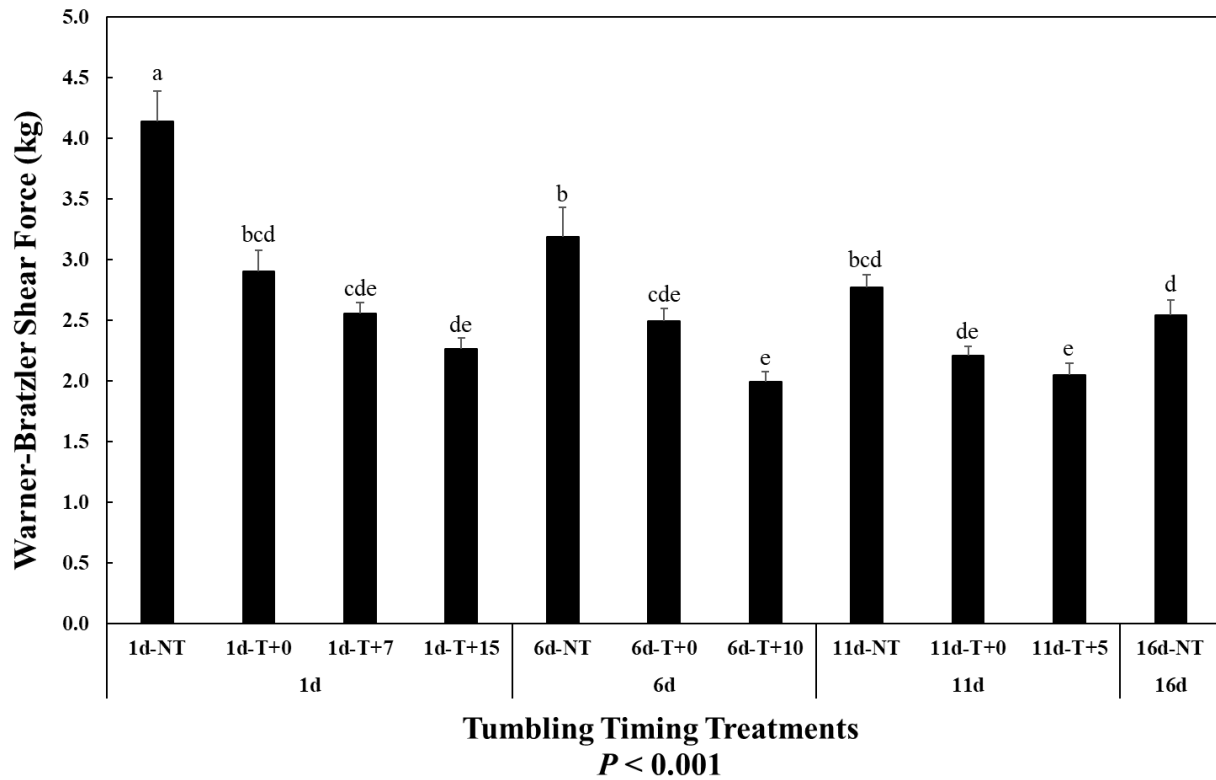
Figure 2.2 Effect of tumbling and aging treatments on Warner-Bratzler shear force (WBSF) values (kg) of beef loins (n=12).

Means lacking a common superscript (a-e) differ at (*P*<0.05).
Error bars indicate standard error of the mean.

A.

Calpain-1 autolysis

1d-NT 1d-T+0 1d-T+7 6d-NT 6d-T+0 11d-NT 11d-T+0 16d-NT

80 kDa →
78 kDa →
76 kDa →

B.

Troponin-T degradation

1d-NT 1d-T+0 1d-T+7 6d-NT 6d-T+0 11d-NT 11d-T+0 16d-NT

39 kDa →
30 kDa →

C.

Desmin degradation

1d-NT 1d-T+0 1d-T+7 6d-NT 6d-T+0 11d-NT 11d-T+0 16d-NT

55 kDa →
38 kDa →
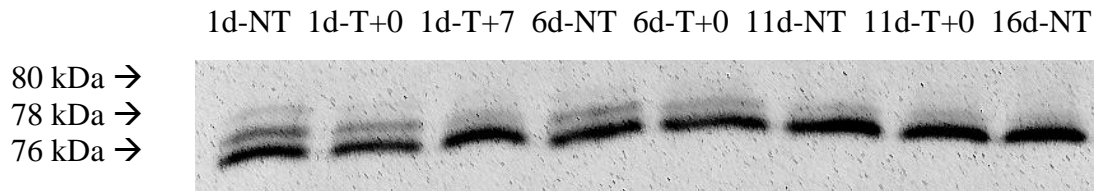
Figure 2.3 Representative western blot images from beef loins (n=12) for calpain-1 autolysis (A), troponin-T degradation (B), and desmin degradation (C).

Figure 2.4 Effect of tumbling and aging on myofibril fragmentation index (MFI) values of beef loins (n=12).

Means lacking a common superscript (a-f) differ at ($P<0.05$).

Error bars indicate standard error of the mean.
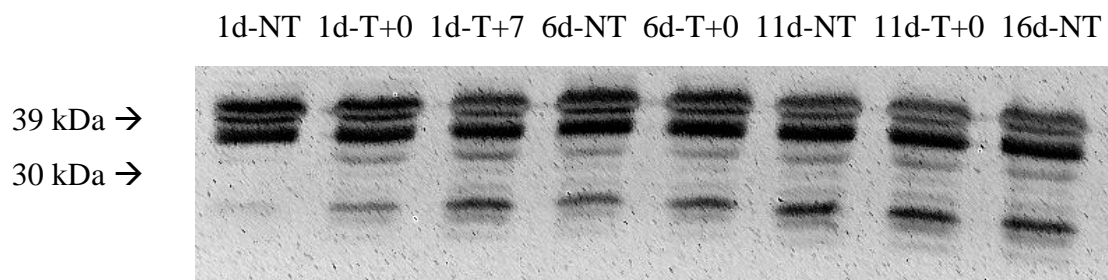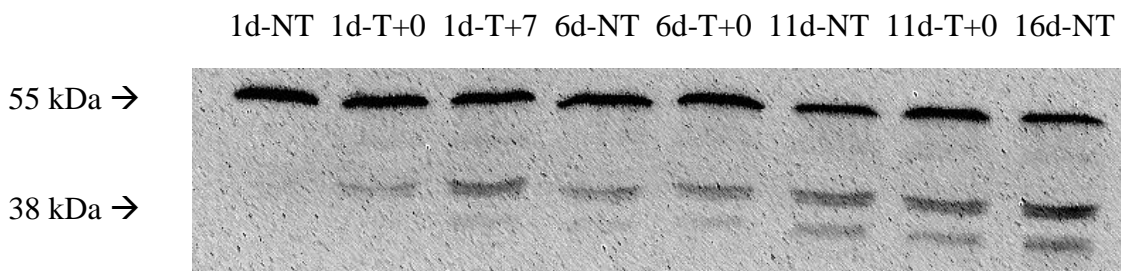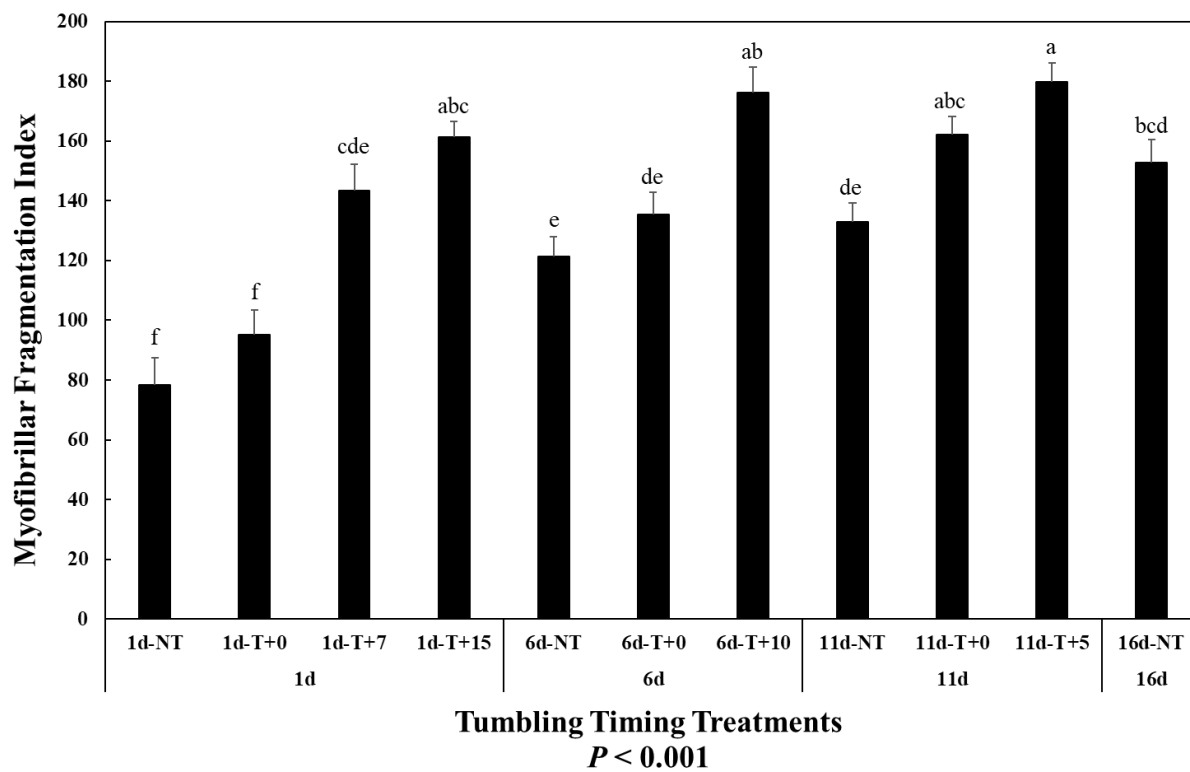
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CHAPTER 3.   APPLICATION OF FRESH BEEF TUMBLING TO ENHANCE TENDERNESS AND PROTEOLYSIS OF CULL COW LOINS (*M. LONGISSIMUS LUMBORUM*)

3.1   Abstract

This study investigated the effect of fresh beef tumbling and postmortem aging on the quality and proteolysis of loins (*M. longissimus lumborum*) from cull cows. Loins from culled cows (Holstein breed, fat utility, >30 months) were obtained at 5d postmortem, sectioned, and assigned to tumbling treatments [NT (non-tumbled), T (tumbled), and TS (tumbled with spike mat)]. Sub-sections were made and assigned to either 0d or 14d of additional aging after tumbling. The results suggest that, in general, postmortem aging but not tumbling would affect instrumental tenderness and proteolysis of cull cow beef loins. However, without additional aging both tumbling methods improved consumer liking of tenderness of beef loins ($P<0.05$), although differences were negligible among aged muscles ($P>0.05$). Tumbling with the spike mat induced higher moisture losses compared to the non-tumbled controls shown by purge, drip, and cook losses ($P<0.05$), but juiciness was comparable among treatments ($P>0.05$). These results indicate that aging would be effective at improving the quality and palatability of cull cow beef loins, although tumbling could improve consumer liking of tenderness at earlier postmortem times.

Keywords: beef quality, cull cow, meat tumbling, tenderness

3.2 Introduction

According to the US Department of Agriculture 2020 livestock slaughter summary (USDA National Agriculture Statistics Service, 2021), culled beef and dairy cows accounted for 19.7% of total beef slaughtered, estimated at 6.4 million carcasses annually. Despite making up such a large percentage of the total beef slaughtered, beef from culled animals is considered underutilized and is often not sold as premium cuts. Cull cow beef is typically further processed owing to inferior meat quality, specifically poor tenderness (Alvarenga *et al*., 2021; Streiter *et al*., 2012; Lucero-Borja *et al*., 2014; Stelzleni *et al*., 2007; Gredell *et al*., 2008).

To improve inferior quality attributes (particularly toughness) of meat, various post-harvest meat processing practices have been developed. Among those practices, blade and needle tenderization is one of the most common methods used by the meat/food industry (Yang *et al*., 2021). This process mechanically tenderizes meat by deep penetration of tiny blades or needles, either through a repetitive up-and-down motion applied on a conveyor or as a manual process (Pietrasik and Shand, 2004). The process disrupts the muscle fibers and connective tissues, resulting in improved meat tenderness (Yang *et al*., 2021). However, a cross-contamination risk is an inevitable consequence of blade/needle penetration process, and increased potential for incorporating and distributing foodborne pathogens within and between cuts (Yang *et al*., 2021; Luchansky *et al*., 2008). Hence, there exists a need for developing natural post-harvest manufacturing systems that can be used to improve eating quality attributes and marketability of fresh beef subprimals that do not pose a food safety risk.

Postmortem aging, as a natural value-adding process, plays a pivotal role in determining beef palatability and thus has been extensively practiced by the industry for years (Warner *et al*., 2021). However, several studies have suggested that postmortem aging would not be sufficient to achieve tenderization in cull cow muscles (Boleman *et al*., 1996; Bhat *et al*., 2018). Considering the tenderness of cull cow beef often owes to the amount and properties of intramuscular connective tissues (Purslow, 2014; Purslow, 2018; Obuz *et al*., 2014; Miller *et al*., 1987) which are known to vary between muscles (Rhee *et al*., 2004), an additional processing system may be required to accelerate and maximize the beneficial impacts of aging. For certain beef muscles,

applying a combination of mechanical tenderization with additional postmortem aging may be an effective strategy to overcome deficiencies in tenderness (Obuz *et al*., 2014).

Specifically, meat tumbling may be effective at achieving this aim through disrupting myofibrillar structure with minimal detriments to other quality attributes (Pietrasik & Shand, 2004; Dzudie & Okubanjo, 1999; Moon *et al*., 2007). In fact, several recent studies have considered the application of tumbling without brine as a method to improve fresh beef quality (Tuell & Kim, 2021; Tuell *et al*., 2021). A recent study from Tuell & Kim (2021) reported that tumbling of vacuum packaged beef loins resulted in an immediate positive impact on tenderness (almost a 40% improvement) without any further aging. Furthermore, Nondorf *et al*. (2021) found that early postmortem tumbling application coupled with aging synergistically improved beef loin tenderness and proteolysis through the early activation of calpain coupled with physical disruption. These observations suggest that fresh beef tumbling could be used as a simple, natural, energy-efficient, and readily applicable processing method, considering the increasing demand for natural meat processing (without addition of non-meat ingredients).

Taken together, the objective of the current study was to determine the effect of fresh beef tumbling on meat quality, myofibrillar protein degradation, and consumer sensory attributes of cull cow loins. In particular, given cull beef has a higher amount of intramuscular connective tissue compared to fed cattle (Purslow, 2014; Purslow, 2018), the impact of using a spiked liner within the tumbler was also determined as a possible means to improve tenderness of cull cow beef loins. The results of the present study could provide insights and practical implications to the beef industry regarding how to ensure quality of certain cuts from cull beef and thus being marketed as tenderness guaranteed cuts that meet or exceed consumer expectations.

3.3   Materials and Methods

3.3.1   Raw Materials and Processing

Beef loin (*longissimus lumborum*) muscles (n=12) from culled Holstein dairy cow carcasses [fat utility cow, over 30 months of age (USDA, 2017)] were received at 5d postmortem from a commercial processor. Each muscle was cut into 3 sections and assigned to each tumbling treatment [NT (non-tumbled), T (tumbled), and TS (tumbled with a spike mat)] while balancing

for muscle position. Sections were halved, and each half was randomly assigned to aging treatments of either 0d or 14d of additional aging after tumbling treatment. Each section was then weighed and vacuum packaged into 4 mil bags (CLARITY, Bunzl Processor Division, Riverside, MO, USA). The non-tumbled sections were either additionally aged for 14d in the packaging or they were packaged for 90 min and then reweighed to measure the purge loss induced through packaging only. The tumbled sections were tumbled for 90 min at 8.5 rpm using a Lance LT-30 500 lb capacity meat tumbler (Lance Industries, Hartford, WI, USA) with or without the addition of a spiked mat (26" x 6', Multi Grip Ribbed Clear Runner Rug Carpet Protector Mat, Sweet Home Stores, Carlstadt, NJ, USA) adhered to the interior of the drum with 2-inch double-sided carpet tape (YYXLIFE, Shenzhen Youyixiang Technology Co., China). Images of the tumbler with the spike mat inserted are provided in **Figure 3.1**. After tumbling treatments, sections were further aged for 0d or 14d at 2°C with additional vacuum packaging over the existing ones to ensure anaerobic conditions were maintained. After completion of the treatments, sections were reweighed to measure purge loss, and steaks were made. Purge was collected from the bags and stored at -80°C for detection of plasticizers. One steak each (2 cm thickness) was collected for Warner-Bratzler shear force (WBSF) measurement and consumer panel evaluation. The remaining sample was collected for biochemical measurements and was frozen at -80°C until analysis. Prior to biochemical analyses, the thawed muscle was trimmed of excess fat, snap-frozen in liquid nitrogen, and finely powdered. The WBSF and consumer panel steaks were frozen at -40°C.

3.3.2 pH Measurement

The pH measurements were determined on the sample collected for biochemical analyses, prior to powdering. A meat pH probe (HI 99163, Hanna Instrument, Inc., Warner, NH, USA) was standardized to pH 4.0 and 7.0 buffers, and then used to measure the pH of the samples in duplicate by inserting the probe into two random locations.

3.3.3 Water-holding Ability

Water-holding ability was assessed using purge loss, drip loss, thaw loss, and cook loss. Before each measurement, samples were blotted gently with paper towel to remove excess purge from the meat surface. The purge loss measurement was calculated as the percent change in weight loss that occurred due to the tumbling and/or additional aging processes. Drip loss measurement

was done according to the Honikel method (Honikel, 1998), prior to freezing. Samples used for drip loss were cut into cubic samples (15-35 g) with fat and connective tissue removed. Cubes were hung using nylon netting inside airtight plastic containers at 2°C. After 48 h, samples were reweighed to determine the percent drip loss. Thaw loss was determined as the percent weight loss of the steaks collected for WBSF prior to and after freezing and subsequent thawing for 24 h at 2°C. These steaks were then cooked to determine cook loss prior to WBSF measurement. A thermocouple (Omega Engineering, Stamford, CT, USA) with a data logger (Madge Tech, Inc., Warner, NH, USA) was inserted into the center of each steak to monitor the increasing temperature. Steaks were cooked to an internal temperature of 71°C on an electric griddle (Model GR-150, Cuisinart, Stamford, CT, USA) set at 175°C. The steak was flipped once at 41°C. Each steak rested for 30 min after cooking. Cook loss was determined as the percent weight loss prior to and after the cooking process. These steaks were then wrapped individually in foil and stored at 4°C for approximately 16 h prior to WBSF measurements.

3.3.4 Instrumental Color

Instrumental color was assessed by using a colorimeter (Hunter MiniScan EZ, Reston, VA, USA) to measure CIE *L**, *a**, and *b** values at 3 randomly determined locations on the surface of each sample, avoiding excess fat and connective tissue. Color was measured on the frozen/thawed steak prior to cooking as previously described, following an oxygenation period of 60 min at 2°C. Hue angle and chroma values were calculated using the *a** and *b** values based on the color guidelines set by the American Meat Science Association (AMSA, 2012).

3.3.5 Warner-Bratzler Shear Force

The cooked steaks were used to measure WBSF by following the American Meat Science Association guidelines (AMSA, 2015). Six cores (1.4 cm diameter) were cut from each sample by cutting parallel to myofiber direction. Cores were measured using a TA-XT Plus Texture Analyser (Stable Micro Systems Ltd, Godalming, Surrey, UK) with a Warner-Bratzler blade attached. The test speed was set at 2 mm/sec, per manufacturer recommendation. The peak shear force (kg) of the blade cutting perpendicularly through the muscle fibers was recorded, and averages were determined from each steak sample. If a core exceeded the maximum capacity of the 5 kg load cell

(7 kg maximum), the data was discarded and an additional core was measured to ensure a total of six cores per sample.

3.3.6   Myofibril Fragmentation Index

The measurement for MFI was completed by following the protocol set by Culler *et al.* (1978), with some adjustments. Powdered beef loin (2 g) was homogenized in duplicate with cold MFI buffer (20 mL [100 mM potassium chloride, 20 mM potassium phosphate, 1 mM egtazic acid, 1 mM magnesium chloride, and 1 mM sodium azide]). The homogenates were centrifuged twice at 1000 x g at 4°C for 15 min. After each centrifugation, supernatants were removed and more MFI buffer was added to the tubes to resuspend the pellet, then each sample was strained to remove any existing connective tissue. Protein quantification was performed in duplicate by using a microplate spectrophotometer (Epoch, BioTek Instruments Inc., Winooski, VT, USA) to compare the absorbances (540 nm) of suspension samples to BSA standards, and then by diluting each suspension sample with MFI buffer to a common protein concentration of 0.5 mg/mL. The MFI quantification was performed by duplicating the suspension samples diluted with MFI buffer to the appropriate protein concentration and reading the absorbances (540 nm) of each sample using a UV spectrophotometer (UV-1600PC, VWR International LLC, Radnor, PA, USA). The MFI values were then calculated by multiplying 200 to the absorbances read.

3.3.7   Whole Muscle Protein Extraction, SDS-PAGE, and Western Blotting

Protein extraction, SDS-PAGE, and western blotting procedures were carried out following the methods described by Kim *et al.* (2013), along with the modifications made by Setyabrata & Kim (2019). Whole muscle protein extraction was completed by performing nano drop protein assay to make samples with similar protein concentration (4 mg/mL). To check for consistent protein concentration, SDS-PAGE was performed. For western blotting, troponin-T degradation, desmin degradation, and calpain-1 autolysis were analyzed by loading 40 µg of protein on to 15%, 12%, or 8% separating gels, respectfully. Using a Bio-Rad PowerPac Basic system (Bio-Rad Laboratories, Hercules, CA, USA), electrophoresis ran at 130 V for 3 h and then proteins were transferred from gels onto polyvinylidene fluoride membranes at 90 V for 90 min in 1°C Tris-glycine buffer. Membranes were blocked with 5% (w/v) nonfat dry milk in phosphate buffer saline-tween (PBST) for 1 h. In 3% (w/v) nonfat dry milk and PBST with a 1:20,000 concentration

of troponin-T (T6277, Sigma Aldrich, St. Louis, MO, USA) and desmin (D1022, Sigma Aldrich, St. Louis, MO, USA), and a 1:7,500 concentration of calpain-1 (Thermofisher MA3-940), membranes were incubated in the primary antibody solution overnight at 4°C. For the secondary antibody solution, membranes were covered for 1 h with 3% (w/v) nonfat dry milk in PBST with concentrations of 1:20,000, 1:15,000, and 1:10,000 of monoclonal goat anti-mouse IgG (H&L) horseradish peroxidase conjugate (170–6516, Bio-Rad Laboratories, Hercules, CA, USA) for troponin-T, desmin, and calpain-1, respectfully. Western blot imaging was performed using a ChemiDoc-ItTS2 imaging system (UVP GelDoc-It, Upland, CA, USA) and enhanced chemiluminescent reagents (Thermo Fisher Scientific, Waltham, MA). To calculate the degradation of desmin and troponin-T, band densities were quantified using the Visionworks LS Analysis Software (UVP, Upland, CA, USA). Each band was compared to the intact reference band (NT-A) used on each gel. To analyze calpain-1 autolysis, the densities of the bands (80, 78, and 76 kDa) were quantified and expressed as a ratio out of 100% for the total intensity.

3.3.8 Transmission Electron Microscopy Imaging

Transmission electron microscopy (TEM) was performed on the samples to analyze their muscle ultrastructure. After the tumbling and aging treatments were applied, 3 replicates from each treatment from 3 random loins were used as representative samples. Samples were collected by cutting parallel to muscle fiber direction from the inside of the muscle. These samples were stored at 4°C in 2.5% glutaraldehyde buffer and then fixated with 1% osmium tetroxide with 0.8% ferricyanide. After the fixation process, samples were dehydrated with ethanol dilutions and then embedded into a resin. From these resin samples, semi-thin sections were created and viewed under a microscope to see if there was proper fiber direction before embedding the samples onto tiny copper grids. A Tecnai T12 microscope (FEI Company, Hillsboro, OR, USA) was used for the TEM imaging at both 2,550 × and 11,500 × magnification. The Gatan DigitalMicrograph software (v.3.31.2360.0, Gatan, Inc., Pleasanton, CA, USA) was used to analyze TEM images of the representative samples.

3.3.9 Consumer Sensory Panel

The consumer sensory panel evaluation protocol was approved by the Purdue Institutional Review Board (IRB 2019-16). The sensory panel (n=72) was conducted over a total of 12 sessions

with 6 panelists per session, following the American Meat Science Association (AMSA, 2015) sensory guidelines. Steaks from each treatment combination were randomly selected, and each treatment was designated with a randomly determined 4-digit code. The evaluation form asked panelists to anonymously provide demographic information, beef consumption habits, and evaluation of the beef loin samples (**Tables 3.4 & 3.5**). Panelists were compensated with $5 USD gift cards after submitting the completed evaluation form.

Steaks for consumer panel evaluation were cooked in the same manner as described for WBSF determination. After cooking, steaks were kept in foil containers for no more than 5 min inside an oven set at 50°C. Steaks were then cut into uniform cubes (1.27 × 1.27 cm). Panelists received 2 cubes for each sample, in a random, predetermined order. Panelists were provided distilled water, unsalted crackers, an expectorant cup, toothpicks, and napkins. The consumer panel evaluation took place in a sensory room with red incandescent lighting to mask color differences. The evaluation of samples was performed in accordance with Berger *et al*. (2018). Each panelist received an untreated, low Choice (USDA, 2017) beef loin sample at 5d postmortem prior to receiving the treated samples. Consumers were asked to evaluate the samples on a continuous line scale ranging from 0 (extreme dislike) to 100 (extreme liking) of tenderness, juiciness, flavor, and overall liking. The acceptability of each attribute was measured as panelists declared each sample as acceptable or unacceptable, and provided the perceived quality level (unsatisfactory, everyday quality, better than everyday quality, premium quality) of each sample. All responses were recorded on iPad devices (Apple Inc., Cupertino, CA, USA) using the Qualtrics software (Provo, UT, USA).

3.3.10 Plasticizer Detection

Any leakage of microplastic materials from the meat-packaging interface upon tumbling was determined by using a vibrational spectroscopic detection method (Nunes *et al*., 2020). Meat exudates from specimens before and after tumbling were collected and measured using Fourier Transform Infrared (FTIR) with Thermo Nicolet Nexus FTIR (Thermo Fisher, US). Qualitative analysis of each spectra data was conducted by comparing individual spectra peaks and wavenumbers between samples.

3.3.11 Statistical Analysis

The experimental design for this study was a balanced complete block design with a factorial arrangement of 3 tumbling methods (NT, T, and TS) across 2 additional aging treatments (0d and 14d). All data was analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC, USA). Least square means were separated by a statistical significance of $P<0.05$.

The consumer sensory panel data was analyzed as a split-split-plot. The whole plot was represented by animal, sub-plot was represented by tumbling method, and sub-sub-plot was represented by the additional aging treatment. Animal and panel session were included as random effects. Least square means were separated by using a binomial error distribution model that assessed the consumer acceptability and perceived quality of samples using the ILINK and PROC GLIMMIX procedure in SAS.

3.4   Results and Discussion

3.4.1   pH Measurement

There was a significant tumbling impact on pH of beef samples (**Table 3.1**). Tumbling with the spike mat (TS) had a slightly lower pH than the non-tumbled (NT) control ($P<0.05$), but had similar pH compared to samples tumbled without the spike mat (T; $P>0.05$). The pH of the NT and T samples were similar ($P>0.05$). The finding of tumbling not influencing pH is similar to results reported by Morrow *et al*. (2019), who found no differences in pH when beef *rectus abdominus* samples were tumbled without brine. No aging effect on the pH was observed ($P>0.05$), nor was there a significant interaction.

3.4.2   Water-holding Ability

The impacts of tumbling application on water-holding ability of beef samples were determined by performing various percent weight loss measurements including purge loss, drip loss, thaw loss, and cook loss (Setyabrata & Kim, 2019). For purge loss, there was a significant interaction between the tumbling and additional aging treatments. Initially, there was no tumbling-induced purge loss when measured immediately after tumbling application ($P>0.05$; **Fig. 3.2**).

However, after 2 weeks of additional aging, the TS samples had a higher purge loss compared to the NT and T samples (P=0.01), while there was no difference between NT and T (P>0.05). These results, partially agree with Tuell et al. (2021), where they reported no significant tumbling impacts on purge loss of samples when assigned to fresh beef tumbling for either 60 min or 90 min at 8.5 rpm. In the present study, tumbling with the spike mat might result in more structural alteration of beef muscle and thus release more myowater upon additional postmortem aging. For drip loss and thaw loss, there was no difference between treatments (P>0.05; **Table 3.1**). Additional aging, however, decreased the amount of drip loss and thaw loss of beef samples (P<0.01), regardless of tumbling application. Tumbling affected the amount of cook loss of beef samples, where TS samples had a greater cook loss compared to NT (P<0.05), but was similar to T samples (P>0.05; **Table 3.1**). No additional aging impact on cook loss was found in the beef samples (P>0.05). Recent studies also found that fresh beef tumbling increased cook loss in fresh beef loins (Tuell & Kim, 2021; Tuell *et al*., 2021). The increased cook loss from using the spike mat while tumbling might be due to the tumbling treatment causing greater myofibrillar space that has been observed with ultrasonication, massaging, and tumbling methods (Sharedeh *et al*., 2015; Siró *et al*., 2009).

3.4.3   Instrumental Color

Tumbling treatments did not significantly affect *L**, *a**, *b**, hue angle, or chroma values of the cull cow beef loin steak samples (**Table 3.2**). The additional aging treatment of 14d increased *L**, *a**, *b**, and chroma values compared to beef samples that were not additionally aged after tumbling treatment (P<0.01). These results are similar to the findings of Nondorf *et al*. (2021), where they found no fresh beef tumbling impacts on most instrumental color attributes, but resulted in a slight increase in *a** and chroma values. The only instrumental color measurement that had an interaction between tumbling and additional aging treatments was hue angle (P<0.05). The beef samples tumbled with the spike mat with no additional aging had lower hue angle values than any other treatment combination (P<0.05). The results in the current study indicate that tumbling does not have a large impact on surface color of cull cow beef loin steaks.

3.4.4   Warner-Bratzler Shear Force

Tumbling had no significant impacts on the WBSF values of beef samples, regardless of additional aging (**Fig. 3.3. A**). Although beef steak samples from tumbled (T and TS) samples had

numerically lower WBSF values compared to the non-tumbled control, the difference was not statistically significant (P=0.176). Our results are in disagreement with the findings from several other similar studies, where fresh beef tumbling resulted in an immediate tenderness improvement (assessed by WBSF) of beef loins (Nondorf *et al*., 2021; Tuell *et al*., 2021; Tuell & Kim, 2021). The contradictory result of the current study could be likely attributed to background toughness of loins from cull cow beef. As an animal matures, less collagen synthesis and turnover occur, and inter and intramolecular crosslinks develop between collagen fibers (Purslow, 2005; Cross *et al*., 1984). Molecular cross-links stabilize collagen against heat denaturation (thus less soluble and more heat stable), resulting in relatively tougher meat or a possible decrease in aging response of meat (Purslow, 2005; Cross *et al*., 1984). In fact, in the present study, although postmortem aging significantly decreased the WBSF values of beef samples (**Fig. 3.3. B**), only a marginal decrease (0.3 kg) was found in beef samples after 14d of additional aging. In contrast, in those similar studies, where beef loins from carcasses with A maturity (less than 30 months of age) were used, a considerable decrease in WBSF (about 1 kg or more) was reported for non-tumbled beef samples during the similar aging period (Nondorf *et al*., 2021; Tuell *et al*., 2021; Tuell & Kim, 2021). Furthermore, Tuell *et al*. (2021) reported muscle-specific impacts of tumbling on instrumental tenderness of beef samples, where fresh beef tumbling significantly decreased WBSF values of loin muscles, but not *semitendinosus* muscles (P>0.05). They also reported *semitendinosus* muscles having a greater amount of total collagen contents (7.78 mg/g vs. 4.81 mg/g) and a lower value of collagen solubility (7% vs. 12%) compared to loin muscles, indicating a potential hinderance of background toughness on tumbling impacts for WBSF (Tuell *et al*., 2021). Future studies should evaluate the effects of fresh beef tumbling methods and aging periods on other, tougher beef muscles to see if tenderness is improved.

3.4.5 Myofibril Fragmentation Index

Tumbling had no significant impacts on the MFI values of beef samples, regardless of additional aging time (**Fig. 3.4. A**). Although tumbled (T and TS) beef steak samples had numerically higher MFI values compared to the non-tumbled control, the difference was not statistically significant (P=0.148), similar to WBSF results. However, as expected, additional aging increased MFI values (P<0.0001; **Fig. 3.4. B**), indicating a gradual increase in the extent of myofibrillar protein degradation upon aging. In terms of tumbling impacts on MFI, there are

several inconsistent results reported in recent literature. Tuell *et al.* (2021) reported increased MFI of beef loins upon tumbling treatment (both 60 min and 90 min at 8.5 rpm), regardless of aging times. Nondorf *et al.* (2021) found immediate tumbling impacts on MFI values were not observed when tumbling was applied at either 1d or 6d postmortem. However, they found an immediate increase in MFI as well as additional increases with further aging ($P<0.05$), when tumbling was applied to beef loin sections at 11d postmortem. While it remains unclear, the extent of muscle structure integrity (along with inter and intramuscular cross-links of collagen) could be attributed to the different responses in myofibrillar protein fragmentation upon tumbling. Further research would be beneficial to determine this postulation.

3.4.6   Western Blot Analysis

Tumbling treatment did not significantly affect any of the intact or degradation bands for troponin-T, desmin, or calpain-1 (**Table 3.3. & Fig. 3.5. A-C**). Additional aging after tumbling affected the abundance of the degraded products of those proteins ($P<0.05$), where a significant decrease in troponin-T intact band and calpain-1 band 2 (78 kDa) was found with aging. As expected, the additional aging also increased the abundance of troponin-T and desmin degradation products, and the fully autolyzed form of calpain-1 (76 kDa; $P<0.0001$). However, intact desmin and calpain-1 band 1 (80 kDa) were not significantly affected by aging. No interactions were found between tumbling and additional aging treatments for any of the troponin-T, desmin, or calpain-1 intact and degradation bands ($P>0.05$). The western blotting results are in line with the MFI results indicating no distinct tumbling impacts on calpain-1 activation and subsequent changes in myofibrillar structural proteins. Nondorf *et al.* (2021) determined that tumbling application at 1d postmortem of beef loins resulted in a greater extent of calpain-1 autolysis compared to non-tumbled controls ($P<0.05$). However, they also found no tumbling impacts on calpain autolysis when tumbling applied at either 6d or 11d postmortem ($P>0.05$), possibly indicating the time-sensitive response of tumbling on calpain-1 activation. Tuell *et al.* (2021) also found no differences in the extent of calpain-1 autolysis of beef samples, when tumbling was applied at 5d postmortem, although they still observed some interactive effects of tumbling coupled with aging on desmin and troponin-T degradation.

3.4.7 Transmission Electron Microscopy Imaging

The TEM images support the other measured data in the current study, showing that there was an evident effect of additional aging on the disruption of muscle structure (**Fig. 3.6. A-B**). While it was not as obvious, there was still a slight tumbling effect seen in the tumbled (T and TS) samples compared to the non-tumbled controls, where the tumbled samples showed more fragmented muscle structures. Overall, the visual evaluation of the TEM images agreed with the WBSF, MFI, and western blot results that suggest additional aging, and not tumbling treatments, caused the greatest differences in tenderness and proteolysis of the fresh cull cow beef loin muscles.

3.4.8 Consumer Sensory Panel

Demographic data showed that the majority of consumer panelists consumed beef frequently (57% for 1-3 times/week and 32% for 4-6 times/week; **Table 3.4**). When the consumer panelists were asked which beef palatability trait was the most important to them, 47.2% responded with "flavor", while 37.5% said "tenderness", and 15.3% said "juiciness". O'Quinn *et al*. (2018) similarly found that a large number of consumers ranked flavor as the most important palatability trait, followed by tenderness.

A significant interaction between tumbling and aging was found in tenderness liking, where the consumer panel determined that steak samples from tumbled beef sections (both T and TS) were more tender than the samples from the non-tumbled control ($P<0.05$), when no additional aging was applied (**Fig. 3.7. A**). After the additional 2 weeks of aging, however, there was no difference in tenderness liking found between treatments ($P>0.05$). However, it is of interest to note that samples from tumbled beef sections (T and TS) with no additional aging had equivalent tenderness levels as the non-tumbled and further aged beef samples ($P>0.05$), indicating tumbling considerably improved perceived tenderness of beef samples without additional aging. This result is supported by a similar study by Tuell *et al*. (2021), where the consumer panel (n=120) determined higher tenderness scores of tumbled beef samples compared to non-tumbled counterparts ($P<0.05$). No significant tumbling or additional aging effects were found on the consumer liking of flavor and juiciness (**Table 3.5**). Along with tenderness, there was a significant interaction between tumbling and additional aging on the overall liking of the beef samples (**Fig. 3.7. B**). The treatment combinations ranked similarly in both tenderness and overall liking of beef

samples, where both tumbling and additional aging improved the consumer liking of samples compared to the non-tumbled controls that were not additionally aged. The results of the current study indicate that tumbling may improve consumer liking of tenderness at shorter durations of postmortem aging, although the differences would be negligible as the additional aging duration increases.

While the consumer panel found tumbling treatments to improve the tenderness and overall liking of beef samples, the WBSF results of the current study did not show significant improvement of instrumental tenderness with tumbling. However, it has been observed in several other studies that WBSF values do not always properly reflect the sensory analysis of tenderness of various beef muscles, including the loin (Strydom *et al*., 2015; Rhee *et al*., 2004; AMSA, 2015). Discrepancies between WBSF values and sensory analysis could potentially be related to the connective tissues and myofibrillar properties of the muscles (Strydom *et al*., 2015), which may be especially true with the analysis of cull cow beef tenderness.

When consumers declared each sample as "unacceptable" or "acceptable" in each category, there were no significant tumbling or additional aging effects found for flavor, juiciness, or overall acceptability (**Table 3.5**). Additional aging improved the tenderness acceptability of beef samples ($P<0.05$), but there were no significant differences found between the different tumbling methods. Similar to other tenderness and proteolysis measurements, the additional aging had a stronger impact on tenderization of the beef loins compared to tumbling methods, though numerically the tumbled sections (both T and TS) had improved tenderness and overall acceptability compared to the non-tumbled controls ($P>0.05$). For the perceived quality, consumer panelists could declare each sample's overall quality as "unsatisfactory," "everyday," "better than everyday," or "premium." There were no tumbling or additional aging differences found between samples labeled by consumers as unsatisfactory, everyday, or premium quality ($P>0.05$). There was a tumbling difference found between samples, where T samples were more frequently declared as "better than everyday quality" compared to the non-tumbled samples ($P<0.05$), but no significant differences were found between the tumbled sections (T and TS).

3.4.9 Plasticizer Detection

The impact of tumbling on any potential release of plasticizer from the meat-packages interface was investigated. Meat exudate was collected from tumbled and non-tumbled meat samples to determine the presence of microplastics using FTIR, a vibrational spectroscopy method. The FTIR method is an effective and widely used tool for detecting microplastics and identifying unknown chemical materials/contaminants in food. Nunes *et al*. (2020) also used FTIR-ATR methods on bovine meat purges to identify adulterants. The spectra data observed from the current study showed the meat purges indicated almost identical spectra peaks and wavenumbers between tumbled and non-tumbled control samples (**Fig. 3.8**). These results confirm the absence of microplastic materials from the interface of meat vacuum packages, ensuring the safety around packaging materials subjected under long-time duration with slow tumbling speed application (90 min at 8.5 rpm).

3.5 Conclusion

The results of the current study indicate that tumbling improved the tenderness and overall liking of cull cow beef loins without additional aging. However, WBSF and MFI values were not affected by tumbling application. Connective tissues and other myofibrillar properties of the cull cow beef may play a role in explaining the discrepancies between instrumental tenderness and the consumer liking of tenderness of the beef loins. As expected, aging was favorable for the improvement of tenderness and proteolysis of cull cow beef loins. Further research should be conducted to investigate the effect of different tumbling methods on the tenderization and eating quality of tougher cull cow beef muscles.

3.6 Tables and Figures

Table 3.1 Effect of fresh beef tumbling method and additional aging on water-holding ability and pH of cull cow beef loins (n=12).

	Purge loss (%)	Drip loss (%)	Thaw loss (%)	Cook loss (%)	pH value
Tumbling					
Non-tumbled	1.357	1.016	2.001	21.17[b]	5.80[a]
Tumbled	1.576	1.176	2.030	22.62[ab]	5.78[ab]
Tumble+spike	2.135	1.207	2.063	23.18[a]	5.73[b]
SEM	0.002	0.001	0.002	0.01	0.10
Additional aging					
0d	0.619	1.272[x]	2.303[x]	22.81	5.78
14d	2.759	0.994[y]	1.760[y]	21.84	5.76
SEM	0.002	0.001	0.002	0.01	0.10
Significance of *P*-value					
Tumbling	0.0054	0.0978	0.8994	0.0437	0.0268
Additional aging	<0.0001	0.0006	<0.0001	0.1455	0.3001
Tumbling ×	0.0117	0.4012	0.8781	0.0792	0.9429
Additional aging					

[a,b]Means lacking a common superscript within a column differ due to fresh beef tumbling method (*P*<0.05).

[x,y]Means lacking a common superscript within a column differ due to further aging (*P*<0.05).

Table 3.2 Effect of fresh beef tumbling method and additional aging on instrumental color attributes of cull cow beef loins (n=12).

	CIE L*	CIE a*	CIE b*	Hue angle	Chroma
Tumbling					
Non-tumbled	37.1	25.1	18.3	35.8	31.1
Tumbled	38.4	25.3	18.6	36.0	31.4
Tumble+spike	37.3	24.5	17.7	35.5	30.2
SEM	1.1	0.9	0.9	0.6	1.2
Additional Aging					
0d	36.2^y	24.3^y	17.4^y	35.3	30.0^y
14d	39.0^x	25.6^x	18.9^x	36.2	31.8^x
SEM	1.1	0.9	0.9	0.6	1.2
Significance of *P*-value					
Tumbling	0.1923	0.1171	0.1481	0.5260	0.1047
Additional aging	<0.0001	0.0008	0.0003	0.0090	0.0003
Tumbling × *Additional aging*	0.7604	0.6398	0.2855	0.0251	0.6211

^{x,y}Means lacking a common superscript within a column differ due to further aging ($P<0.05$).

Table 3.3 Effect of fresh beef tumbling methods and additional aging on quantitative abundance of desmin (intact and degraded), troponin-T (intact and degraded), and calpain-1 autolysis of beef loins (n=12).

	Troponin-T			Desmin			Calpain-1 (%)		
	Intact	Deg 1	Deg 2	Intact	Deg 1	Deg 2	Band 1	Band 2	Band 3
Tumbling									
Non-tumbled	1.35	0.11	0.72	1.01	0.28	0.29	17.04	26.07	56.89
Tumbled	1.29	0.11	0.72	1.00	0.28	0.27	17.02	25.21	57.77
Tumble+spike	1.32	0.11	0.73	1.01	0.29	0.28	17.00	26.32	56.68
SEM	0.05	0.01	0.04	0.04	0.03	0.04	0.01	0.01	0.01
Additional aging									
0d	1.36^x	0.093^y	0.56^y	1.04	0.23^y	0.17^y	17.45	30.64^x	51.91^y
14d	1.28^y	0.124^x	0.89^x	0.97	0.34^x	0.39^x	16.56	21.09^y	62.31^x
SEM	0.05	0.005	0.04	0.03	0.03	0.04	0.01	0.01	0.01
Significance of *P*-value									
Tumbling	0.3191	0.7637	0.9646	0.9498	0.8428	0.8942	0.9988	0.5078	0.7103
Additional aging	0.0135	<0.0001	<0.0001	0.0502	<0.0001	<0.0001	0.1203	<0.0001	<0.0001
Tumbling × Additional aging	0.7208	0.7424	0.5469	0.9525	0.3410	0.5800	0.7116	0.2958	0.3615

[x,y]Means lacking a common superscript within a column differ due to further aging (*P*<0.05).
Deg: degradation products

Table 3.4 Demographic characteristics of consumers (n=72) that participated in the sensory panel.

Demographic Question	Response Option	Frequency (%)
Gender	Male	41.7
	Female	58.3
Household Size	1	38.9
	2	30.5
	3	8.3
	4	15.3
	5	4.2
	>5	2.8
Marital Status	Single	61.1
	Married	38.9
Age	<20	1.4
	20-29	48.6
	30-39	31.9
	40-49	8.3
	50-59	4.2
	>60	5.6
Ethnic Origin	African-American	6.9
	Asian	25.0
	Caucasian/White	48.6
	Hispanic	11.1
	Mixed Race	1.4
	Other	7.0
Annual Household Income	<$25,000	36.1
	$25,000-$34,999	11.1
	$35,000-$49,999	9.7
	$50,000-$74,999	11.1
	$75,000-$99,999	8.3
	$100,000-$149,999	12.5
	$150,000-$199,999	2.8
	>$199,999	1.4
	Prefer not to disclose	7.0
When eating beef, what palatability trait is most important to you?	Flavor	47.2
	Juiciness	15.3
	Tenderness	37.5
When eating beef, what degree of doneness do you prefer?	Rare	0.0
	Medium-Rare	34.7
	Medium	30.6
	Medium-Well	25.0
	Well Done	9.7
How many times per week do you consume beef?	1-3 (Times/Week)	57.0
	4-6 (Times/Week)	31.9
	>7 (Times/Week)	11.1

Table 3.5 Effect of fresh beef tumbling methods and additional aging on consumer sensory panel (n=72) liking, acceptability, and perceived quality of cull cow beef loin steak samples.

	Liking				Acceptability (%)				Perceived quality (%)			
	F	J	T	O	F	J	T	O	US	E	BE	P
Tumbling												
Non-tumbled	62.26	65.28	62.04	60.32	83.33	84.03	75.69	75.00	25.35	48.59	17.61^b	8.45
Tumbled	63.42	64.19	66.97	64.38	87.50	80.56	84.03	80.56	19.72	38.03	33.80^a	8.45
Tumble+spike	67.14	64.47	67.90	66.32	88.89	86.11	84.72	85.42	15.28	46.53	27.08^{ab}	11.11
SEM	1.96	2.87	2.81	2.40	3.32	3.97	4.57	4.02	3.74	4.30	3.96	2.62
Additional aging												
0d	64.46	64.04	62.43	62.44	89.81	82.41	77.31^y	79.63	20.09	43.92	26.64	9.35
14d	64.08	65.25	68.84	64.90	83.33	84.72	85.65^x	81.02	20.09	44.86	25.70	9.35
SEM	1.68	2.64	2.56	2.15	2.81	3.31	3.92	3.13	2.86	3.55	3.05	1.99
Significance of *P*-value												
Tumbling	0.1352	0.9195	0.0967	0.0738	0.3239	0.4197	0.1389	0.1128	0.1444	0.1627	0.0144	0.6067
Additional aging	0.8543	0.5911	0.0083	0.2533	0.0519	0.4741	0.0427	0.8571	0.8004	0.8250	0.8795	0.9197
Tumbling × *Additional aging*	0.3970	0.0905	0.0499	0.0270	0.5301	0.3179	0.0712	0.2292	0.1515	0.1464	0.1675	0.3672

[a,b]Means lacking a common superscript within a column differ due to fresh beef tumbling method (*P*<0.05).

[x,y]Means lacking a common superscript within a column differ due to further aging (*P*<0.05).

T: tenderness, J: juiciness, F: flavor, O: overall, US: unsatisfactory, E: everyday, BE: better than everyday, P: premium
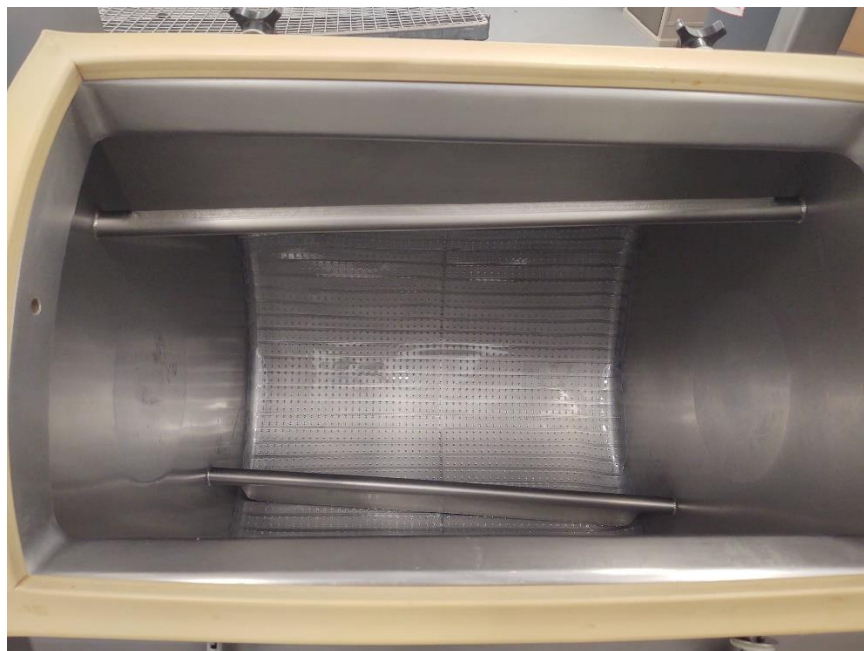
A.

B.

Figure 3.1 Images of tumbling treatments, including the exterior (A) and interior (B) of the meat tumbler with the spike mat inserted.
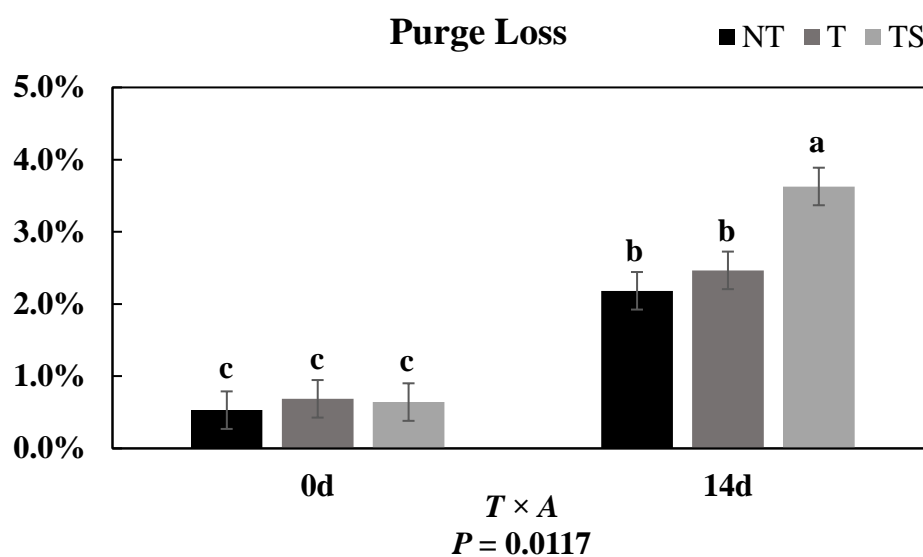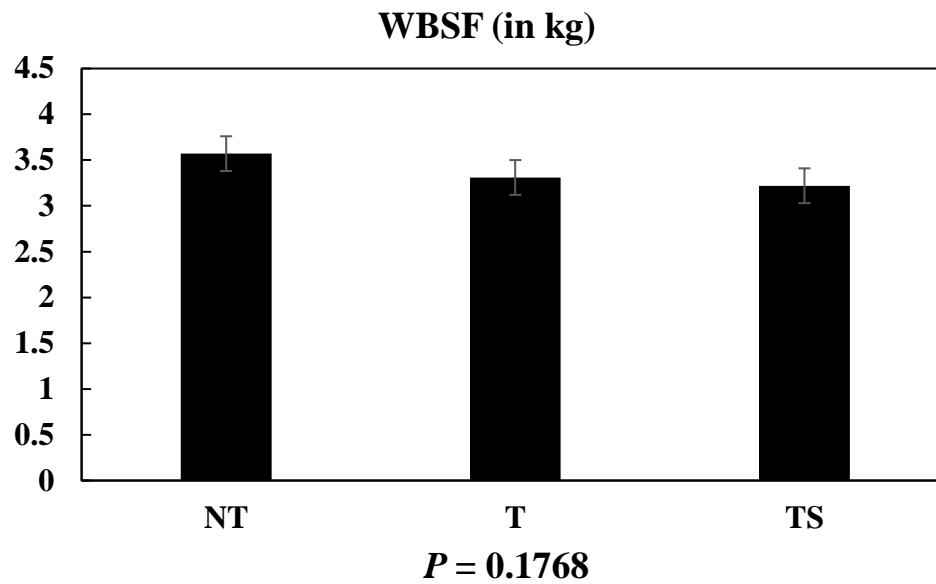
Figure 3.2 Effect of tumbling and additional aging treatments on the purge loss of cull cow beef loins.

Means lacking a common superscript (a-c) differ at ($P<0.05$).
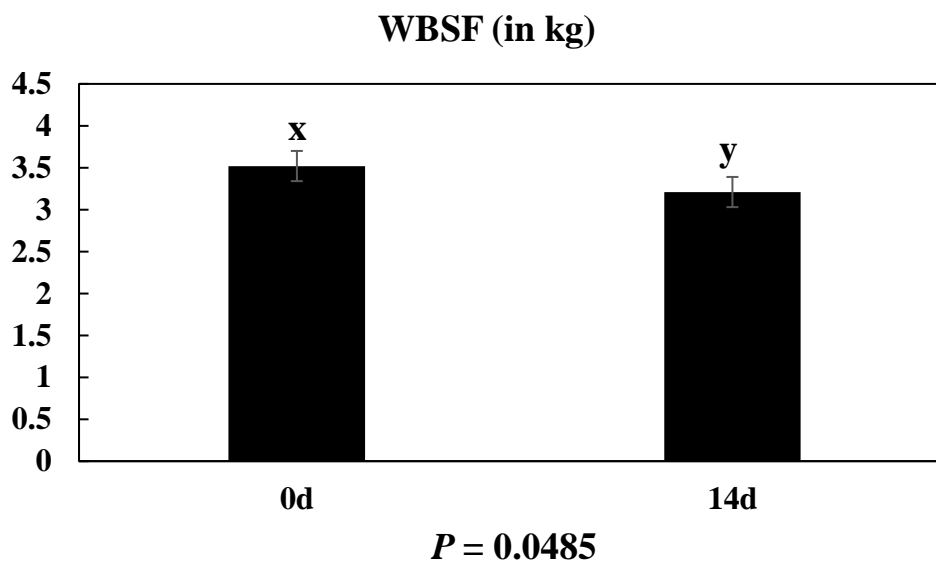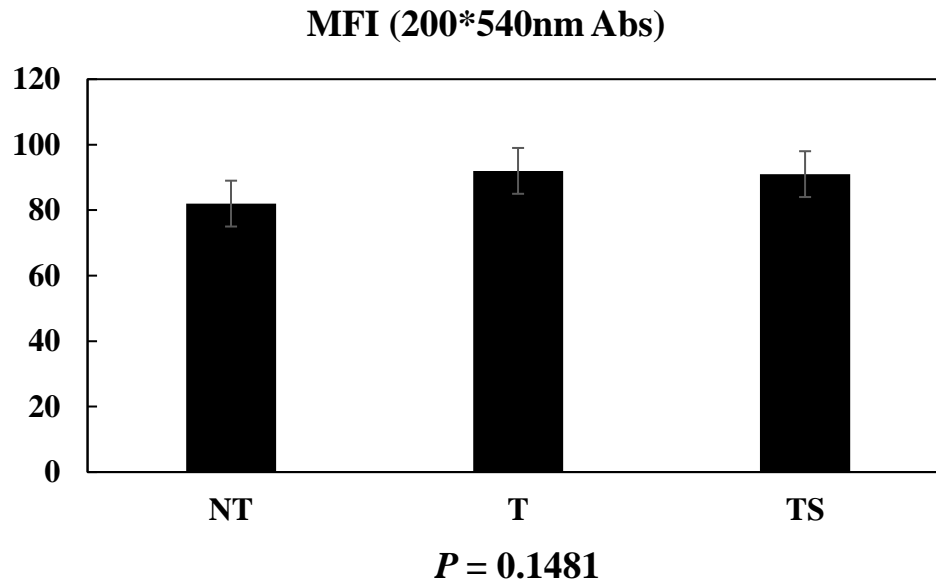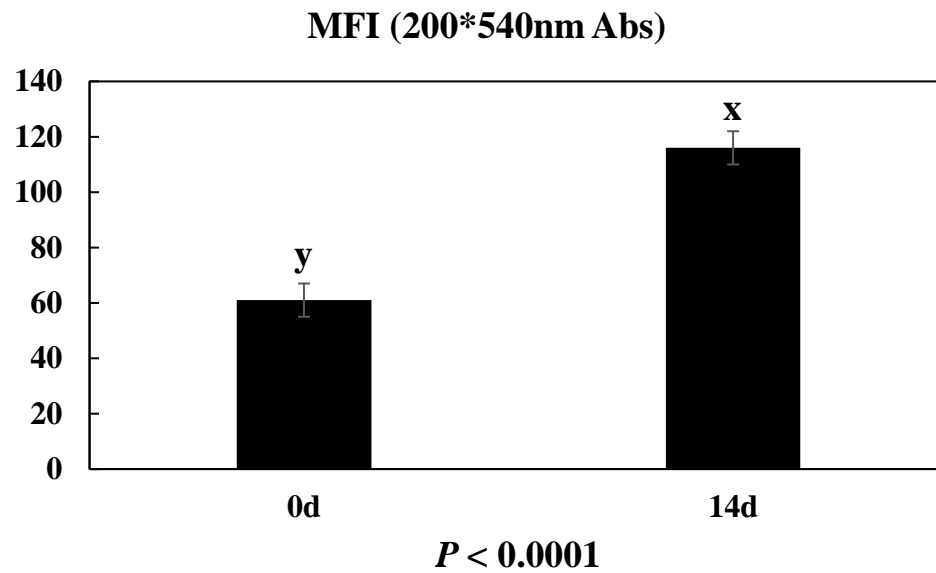Error bars indicate standard error of the mean.

A.

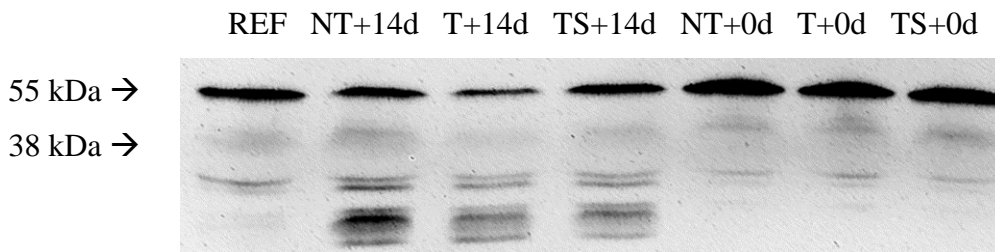**WBSF (in kg)**

P = 0.1768

B.

WBSF (in kg)

P = 0.0485

Figure 3.3 Effect of tumbling (A) and additional aging (B) treatments on Warner-Bratzler shear force (WBSF) values (kg) of cull cow beef loins (n=12).
Means lacking a common superscript (x,y) differ at (*P*<0.05).
Error bars indicate standard error of the mean.

A.

MFI (200*540nm Abs)

$P = 0.1481$

B.

MFI (200*540nm Abs)

$P < 0.0001$

Figure 3.4 Effect of tumbling (A) and additional aging (B) treatments on myofibril fragmentation index (MFI) values of cull cow beef loins (n=12).
Means lacking a common superscript (x,y) differ at ($P<0.05$).
Error bars indicate standard error of the mean.

A.

Troponin-T degradation

REF NT+14d T+14d TS+14d NT+0d T+0d TS+0d

39 kDa →

30 kDa →

B.

Desmin degradation

REF NT+14d T+14d TS+14d NT+0d T+0d TS+0d

55 kDa →

38 kDa →

C.

Calpain-1 autolysis

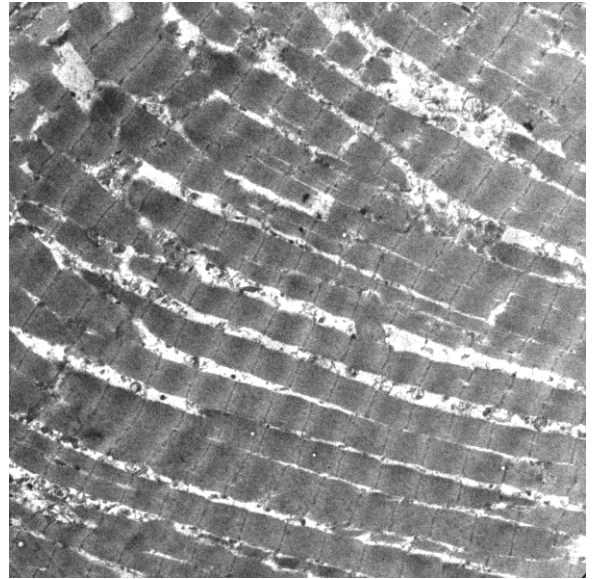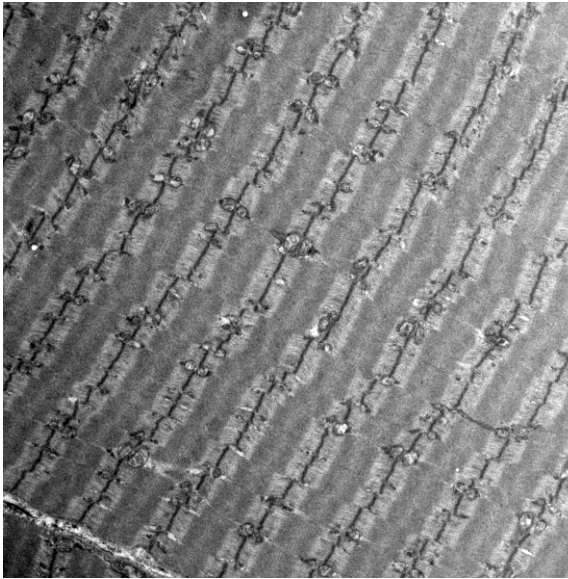REF NT+14d T+14d TS+14d NT+0d T+0d TS+0d

80 kDa →
78 kDa →
76 kDa →
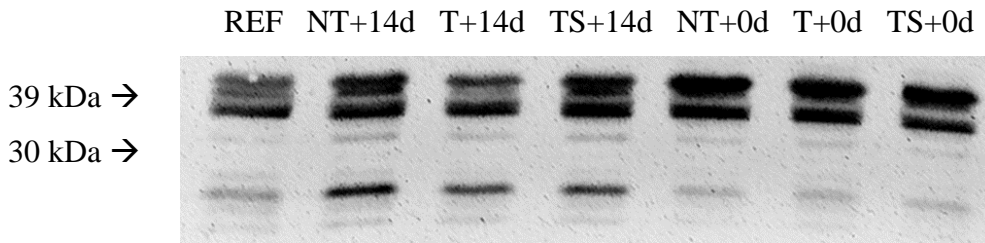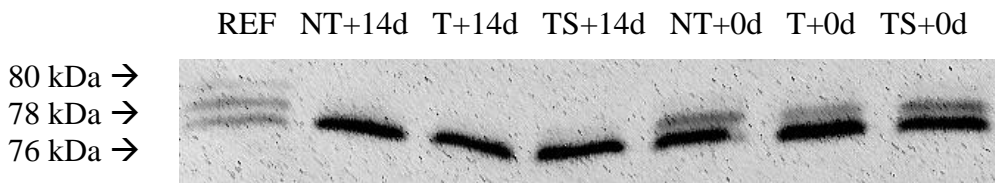
Figure 3.5 Representative western blot images for cull cow beef loins (n=12) for troponin-t degradation (A), desmin degradation (B), and calpain-1 autolysis (C).
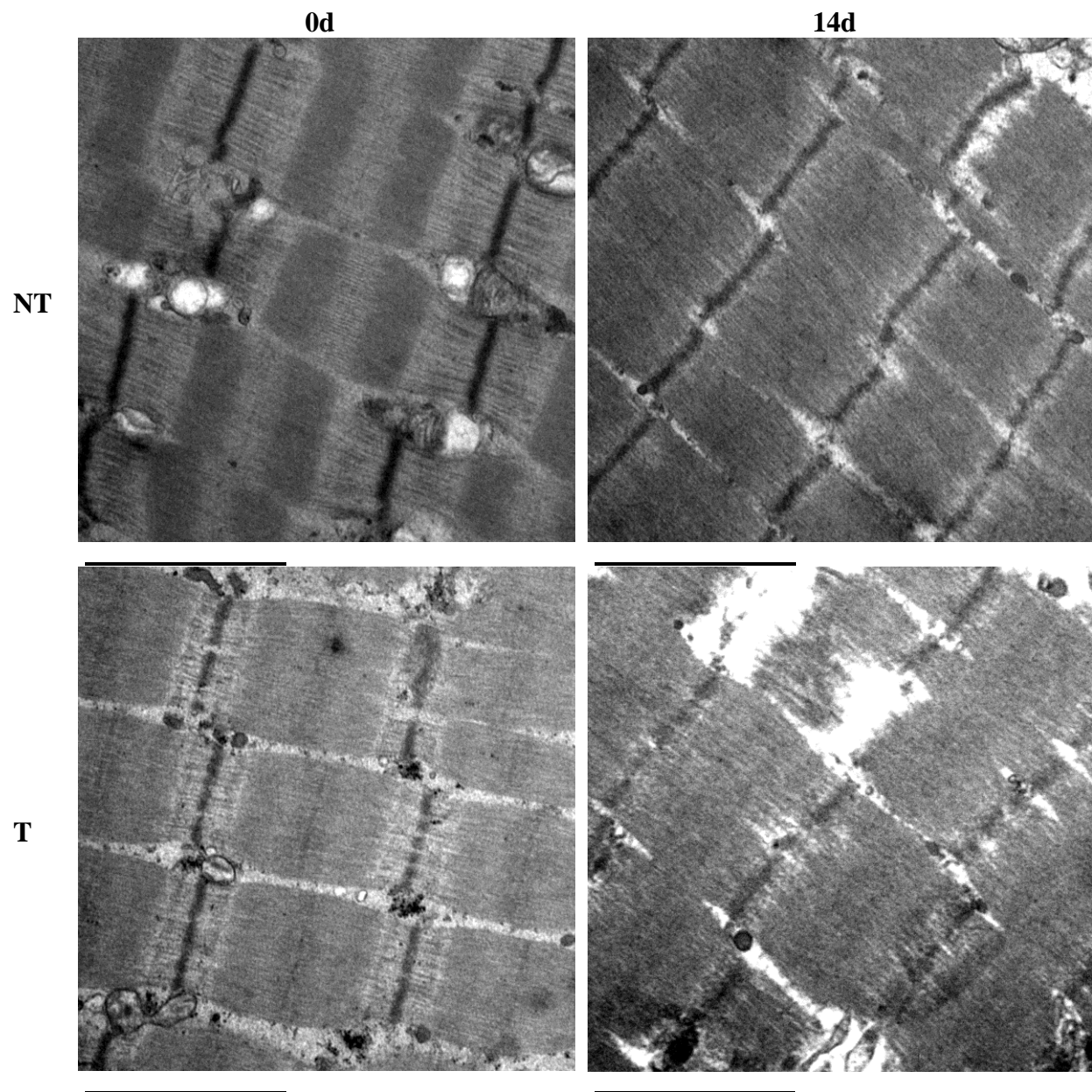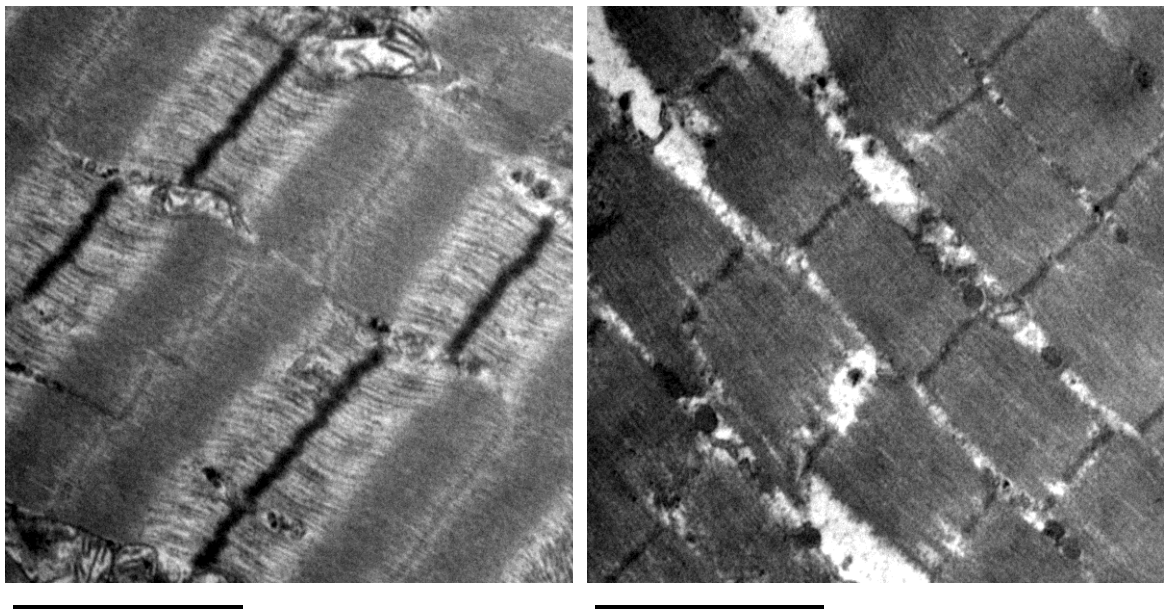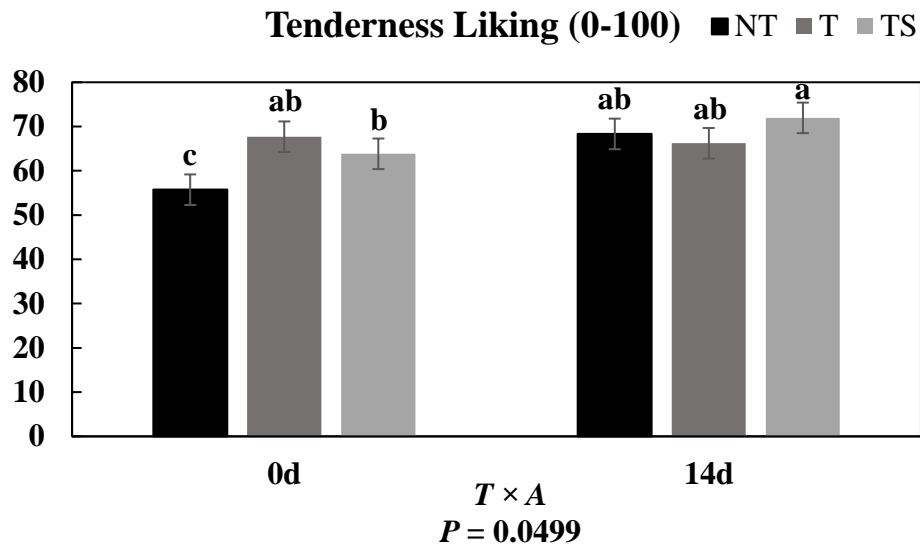
A.

0d   **14d**

NT

T

TS

B.

0d   14d

NT

T
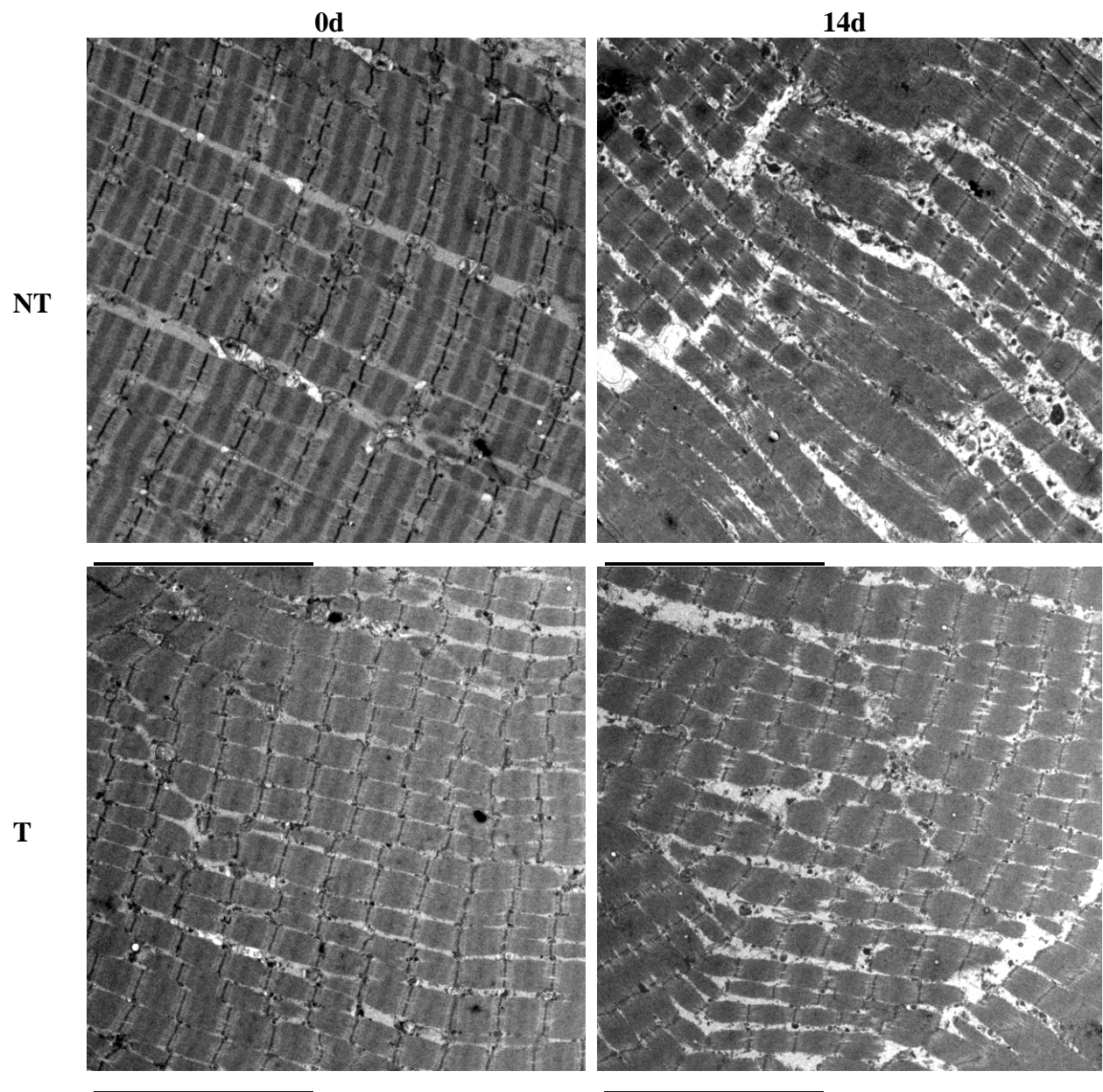
TS

Figure 3.6 Representative transmission electron microscopy images of cull cow beef loins at 2,550 × (A) and 11,500 × (B) magnification.

A.

Tenderness Liking (0-100) ■NT ■T ■TS

$T \times A$
$P = 0.0499$

B.

Overall Liking (0-100) ■NT ■T ■TS
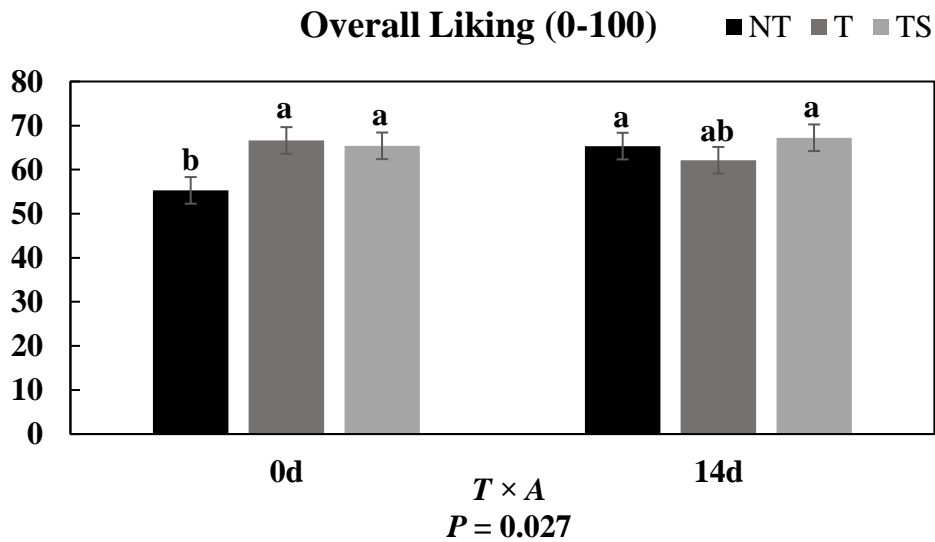

$T \times A$
$P = 0.027$

Figure 3.7 Effect of tumbling and additional aging treatments on the consumer panel tenderness liking (A) and overall liking (B) of cull cow beef loins.

Means lacking a common superscript (a-c) differ at ($P<0.05$).
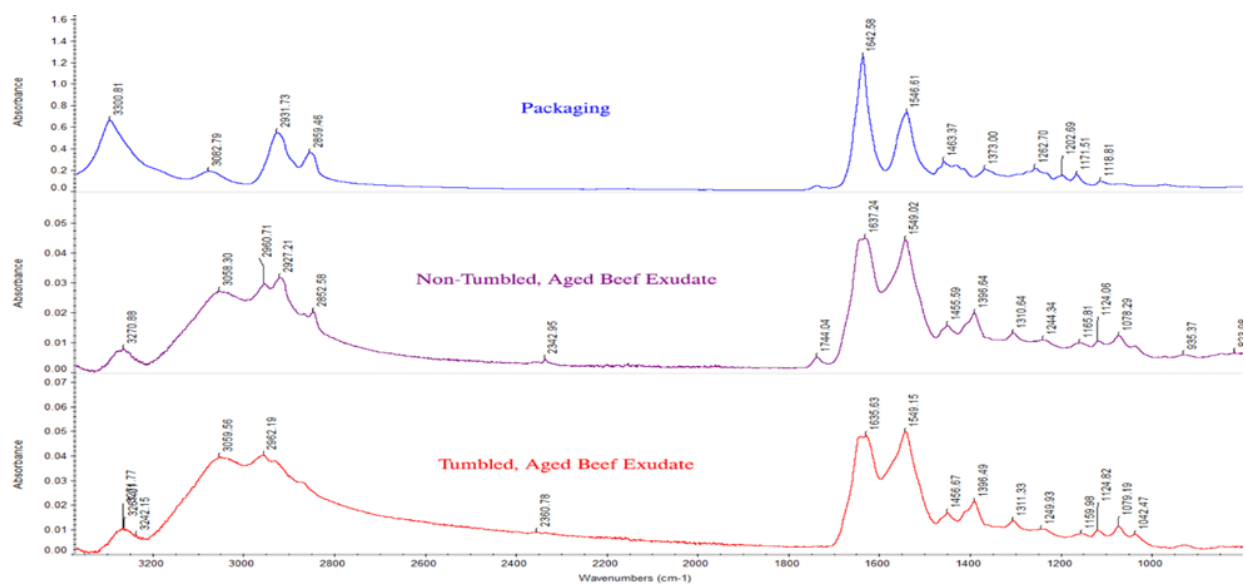Error bars indicate standard error of the mean.

Figure 3.8 Fourier Transform Infrared (FTIR) spectra of meat exudate from tumbled and non-tumbled cull cow beef loins.

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