COMBINED TUMBLING AND POSTMORTEM AGING TO IMPROVE FRESH BEEF QUALITY, PALATABILITY, AND PROTEOLYSIS

by

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This work is dedicated to my parents and grandparents, who supported my passion for animals and food from a young age.

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ABSTRACT

Tenderness is a key sensory trait influencing beef palatability. Tumbling is a value-adding process that has been extensively applied and studied within the realm of processed meats. Various post-harvest strategies to ensure fresh beef reaches acceptable levels of tenderness have been employed, often with the aim of physically disrupting myofibrillar structure or enhancing the rate and extent of postmortem proteolysis. One such method would be the application of postmortem aging; however, the effectiveness of aging on tenderization is well-known to differ throughout individual muscles of the beef carcass. For inherently tough cuts, physical interventions such as mechanical tenderization are often used, although several detriments to quality attributes may be induced. Further, some modern consumers prefer meat products with no added non-meat ingredients. An alternative method of applying tumbling in the absence of a brine solution followed by additional postmortem aging could be a practical means to facilitate tenderization while potentially minimizing detriments to other eating quality attributes.

To evaluate the efficacy of tumbling without brine a method of beef tenderization, the process was first assessed in the *longissimus lumborum* muscle (n=9). In this study, muscles were allocated among 0, 60, and 90 minutes of tumbling, after which aging for 0, 7, and 14 days was conducted. Immediately after the application of the tumbling process, steaks from muscles that had been tumbled were considerably more tender (24.7 N and 21.6 N for 60 and 90 minutes, respectively) than non-tumbled controls (34.8 N). Steaks from the tumbled groups maintained greater instrumental tenderness throughout the course of the aging period. These results were supported by increases in myofibril fragmentation index, as well as increased troponin-T degradation during aging. However, cooking loss was increased in tumbled steaks, which could have implications for sensory juiciness. Considering this study demonstrated that tumbling without brine inclusion followed by postmortem aging resulted in profound changes to sensory traits, further study regarding its impacts on sensory attributes and proteolysis among different beef muscles was warranted.

The following study evaluated the combined tumbling and aging process on the quality, palatability, and proteolytic attributes of beef *longissimus lumborum* and *semitendinosus* muscles (n=16). Muscle sections were allocated among 0, 40, 80, and 120 minutes of tumbling, as well as

0 or 10 days of subsequent aging. Regardless of aging duration, tumbling for any duration increased instrumental tenderness of the *longissimus lumborum* but not *semitendinosus* muscle. Similar to the previous study, increased cooking loss was induced through tumbling. In both muscles, obvious fragmentation of the myofibrillar structure with tumbling was observed through increases in myofibril fragmentation index and transmission electron microscopy. Tumbling with aging favored the degradation of myofibrillar proteins including troponin-T and desmin; however, calpain-1 autolysis appeared mostly unchanged. Neither tumbling nor aging influenced the amount and properties of collagen, which may indicate why the process did not influence instrumental tenderness of the *semitendinosus* despite myofibrillar fragmentation and degradation. *Longissimus lumborum* muscles tumbled for any durations were rated by consumers (n=120) to be more tender with greater overall liking than control steaks. *Semitendinosus* steaks that were tumbled for 120 minutes and further aged had improved liking of tenderness with similar juiciness and flavor to control steaks at the same postmortem timepoint. These results indicated that tumbling without brine would result in myofibrillar fragmentation and favor the degradation of myofibrillar proteins during aging, while impacts on connective tissues would be minimal. Consequently, muscles without a high extent of background toughness would be effectively tenderized through tumbling, while the results would be more limited in inherently tough cuts.

Considering these results, the process was then applied to muscles of intermediate tenderness from the sirloin, specifically the *gluteus medius, biceps femoris,* and *tensor fasciae latae* muscles (n=16). Muscles were tumbled for 0 or 120 minutes, then aged for 0 or 10 additional days. Tumbling increased the instrumental tenderness of the *gluteus medius* and *tensor fasciae latae* but not the *biceps femoris*, regardless of aging time. Cooking loss was increased with tumbling in all muscles. Similarly, myofibrillar fragmentation was also increased in all muscles, and there was some evidence to suggest that tumbling with subsequent aging would aid in the degradation of troponin-T in the *biceps femoris*. To further understand how tumbling might affect specific descriptive sensory attributes, a trained panel (n=8) was conducted on aged samples. Tumbled *gluteus medius* steaks had greater myofibrillar tenderness than non-tumbled controls; however, tenderness scores of other muscles were not affected. There was some evidence that tumbling with aging could induce the generation of off-flavors in the *gluteus medius* and *tensor fasciae latae*, as well as decrease juiciness of the *biceps femoris*.

Taken together, these results support that tumbling without brine inclusion would be an effective strategy to improve beef tenderness and palatability, dependent on the traits of the individual cut. Improved tenderness would be primarily attributed to the fragmentation and degradation of myofibrillar structure. However, the results indicate that tenderization would be limited in cuts with a high extent of background toughness, which tumbling alone would be largely unable to disrupt. Future studies should focus on the effects of tumbling without brine inclusion with aging on oxidative stability and the potential introduction of hazards prior to industry application. Further elucidation of how the process could be optimized to maximize tenderization while minimizing potential negative impacts to flavor and juiciness would be beneficial to improving overall palatability.

CHAPTER 1. LITERATURE REVIEW

1.1 Overview of Beef Quality

The quality of meat is determined by the complex interaction of intrinsic and extrinsic traits that contribute to consumer satisfaction (Hocquette et al., 2012). Particularly, consumers' liking and acceptance of a meat product are heavily influenced by the sensory attributes including both eating quality (e.g., tenderness, juiciness, and flavor) and appearance quality (e.g., color and marbling) (Henchion et al., 2017). Often in research, objective measurements such as Warner-Bratzler shear force (WBSF) are used to approximate subjective measures such as consumer liking of tenderness (Warner et al., 2020). Accordingly, terms such as tenderness can often refer to both objective and subjective measures. However, it is also established that there is a disconnect between objective measures and the subjective experiences they aim to approximate. For instance, the various objective measures used to determine water-holding capacity (WHC) may not necessarily be highly correlated with sensory juiciness (Warner, 2017). Consequently, a holistic view of objective and subjective traits is often necessary to better understand how to provide consumers with consistently high-quality fresh beef products.

Historically, the tenderness of beef products has been considered as the primary sensory attribute which affects whether or not a product is deemed acceptable by the consumer (R. Miller, 2020). Accordingly, much research has been conducted on the topic of ensuring tenderization is achieved, and the National Beef Tenderness Surveys (NBTSs) have monitored this in the United States (Gonzalez & Phelps, 2018; Guelker et al., 2013; Martinez et al., 2017; Voges et al., 2007). While the most recent NBTSs conducted by Guelker et al. (2013) and Martinez et al. (2017) showed no substantial improvements in tenderness of fresh beef since previous surveys, most cuts from the rib and loin primals are considered as acceptably tender. Still, some inherently tough cuts from the round and chuck primals still exhibit unacceptable variation in tenderness (Guelker et al., 2013; Martinez et al., 2017), the reasons for which will be discussed in greater detail in the subsequent sections. As tenderness has become increasingly acceptable in recent decades, the focus of much research has shifted to better understanding the complex relationships between individual sensory traits and how they might affect overall palatability. For instance, the article by O'Quinn et al. (2018) demonstrated the importance in considering a full view of the sensory

attributes in ensuring consumers receive beef products that meet or exceed their expectations for eating quality. Most variation in overall palatability can be explained by the combination of both tenderness (43.4% of variance) and flavor (49.4% of variance) (O'Quinn et al., 2018). Accordingly, there is a high likelihood of a consumer finding a steak to be unacceptable for overall palatability if either tenderness (69% chance of unacceptability for overall palatability), flavor (77%), and juiciness (66%) individually are rated to be unacceptable (O'Quinn et al., 2018). The likelihood of an unacceptable eating experience increase further when two or more individual attributes are found to be unacceptable (O'Quinn et al., 2018). While tenderness of beef is the primary focus of this dissertation, consideration to the other meat and eating quality attributes must be given, taking into account the importance of all such factors in determining consumer liking and acceptance. The following sections will describe the objective and subjective measures that indicate beef quality, as well as the primary factors that may influence quality of fresh beef. Further, a review on how meat tumbling may influence these attributes in various beef muscles will be provided, with possible interactions with postmortem aging and protein degradation being of special interest.

1.2 Beef Quality Attributes

1.2.1 Tenderness

Objective tenderness of intact muscle foods is typically determined by the peak force via a force-deformation curve required to shear a sample of meat (Warner et al., 2020). Various techniques have been developed to measure objective tenderness, varying most often by the type of blade used to shear and the sample preparation. The most commonly applied method, WBSF, utilizes a V-shaped blade attachment matching the specifications set by the American Meat Science Association (AMSA, 2015). For WBSF, 1.27 cm cores would be obtained parallel to the direction of the myofibers, so that the blade will shear cores perpendicular to fiber direction. Another common method of objective tenderness would be slice shear force (SSF) (Shackelford et al., 1999a, 1999b). Unlike WBSF, SSF utilizes a straight-edged blade rather than V-shaped. Additionally, rather than 1.27 cm cores in WBSF, a slice measuring 1 cm in thickness and 5 cm in length is obtained (AMSA, 2015). In addition to the 1.27 cm diameter core for WBSF and 1 x 5 cm slice for SSF, some protocols have utilized a block measuring 1 square cm (Warner et al., 2020). For WBSF, it is recommended that at least six cores be obtained for measurement, and peak

shear force should for each core should be pooled prior to statistical analyses. It is generally recognized that SSF is slightly more correlated with sensory tenderness than WBSF (Battaglia et al., 2020; Shackelford et al., 1999b; Warner et al., 2020), although WBSF is superior in classifying samples as tender or not (Battaglia et al., 2020). For WBSF, it is recommended samples be chilled overnight at 2-5 °C prior to evaluation, while SSF may be performed on hot or chilled samples (AMSA, 2015). Evaluating shear force on chilled samples has been identified as advantageous for obtaining uniform cores and measuring all samples at a consistent temperature (AMSA, 2015). However, SSF measurement performed on hot samples may be more highly correlated with sensory tenderness scores than cold (Shackelford et al., 1999b), considering consumer and trained panelists would evaluate samples soon after the cooking process. Another consideration regarding conducting shear force measurements would be ensuring sample location is consistent due to the known locational differences in shear force within an individual muscle (Rhee et al., 2004).

When analyzing and interpreting WBSF data, it is generally recommended that muscles should not be compared (AMSA, 2015). The rationale for this is primarily due to shear force not adequately reflecting the contribution of connective tissues and sarcomere length to meat tenderness, as well as there being known discrepancies between objective and subjective measures (Rhee et al., 2004). Accordingly, shear force measurement is most appropriate to assess the contribution of proteolysis or disruption of muscle structure on tenderness within a single muscle, while trained or consumer sensory panels would be more appropriate to make comparisons between muscles. With that said, WBSF values between muscles are often compared in the literature to categorize muscles by tenderness levels (Sullivan & Calkins, 2011). Muscles with WBSF less than 3.9 kg (e.g., *psoas major, infraspinatus,* and *spinalis dorsi*) would be categorized as "tender," while those with WBSF greater than 4.6 kg (e.g., *biceps femoris, semitendinosus,* and *gluteus medius*) would be considered "tough." The threshold of 4.6 kg of WBSF as the dividing line between samples being considered as tough or not was established by Shackelford et al. (1991). The categorizations of tenderness can be broken down further into "very tender" (WBSF < 3.2 kg), "tender" (3.2 kg < WBSF < 3.9 kg), "intermediate" (3.9 kg < WBSF < 4.6 kg), and "tough" (WBSF > 4.6 kg) (Belew et al., 2003). These groupings have been used to determine the percentage of samples within a single muscle that would fall into each of these categories in the NBTSs (Guelker et al., 2013; Martinez et al., 2017; Voges et al., 2007). The most recent data suggest that although 95.9% of top loin steaks are categorized as very tender, there still exists a

small proportion (0.8%) that remains tough and would therefore likely fail to meet consumers' expectations for tenderness (Martinez et al., 2017). However, it appears progress has been made in this area, where in the 2010 survey, only 84.8% of top loins were very tender and 2.2% were tough (Guelker et al., 2013). For certain inherently tough muscles from the round primal, there still exists substantial variation in tenderness at the retail level. For instance, bottom round steaks would be categorized as 37.1% very tender, 31.4% tender, 17.1% intermediate, and 14.3% tough (Martinez et al., 2017). There may also exist variation within individual muscles between steaks found at the retail level and those used for foodservice. For example, 87.1% (2005), 91.1% (2010), and 86.1% (2015) of top sirloins were very tender at the retail level, while only 73.7% (2005), 58.1% (2010), 69.1% (2015) were very tender at the foodservice level (Guelker et al., 2013; Martinez et al., 2017; Voges et al., 2007). This observation could potentially be related to differences in the conditions and durations of postmortem aging used amongst steaks destined for retail or foodservice, as well as if any physical interventions (e.g., mechanical tenderization) were applied.

While it may intuitively seem that trained panel descriptive scores would be a subjective measurement, it is generally considered as an objective measure (AMSA, 2015). This would owe to the rigorous pre-screening, screening, and training conducted to form and maintain a trained panel. Furthermore, it is generally recommended that individual panelists be regularly evaluated to ensure performance is meeting or exceeding standards. Measures can be conducted using a 15-point scale such as that defined by Adhikari et al. (2011) or other scales (e.g., 100-point), with appropriately placed anchors. For example, to assess overall tenderness on a 15-point scale, anchors using shank (7.0), *longissimus lumborum* (9.0), and *psoas major* (14.0) muscles all cooked to the same internal temperature of 71 °C have been used (Foraker et al., 2020). Alternatively, the study by Ponce et al. (2019) employed a 100-point scale with anchors differing by both cut and degree of doneness. In the study, tenderness of the *semitendinosus* at 77 °C endpoint temperature was anchored at 30, *longissimus lumborum* at 71 °C at 55, and *psoas major* at 65 °C at 90. Trained descriptive panels have the ability to not only assess overall tenderness but also attributes such as initial tenderness and the amount of perceptible connective tissue that may provide additional insight into tenderness development (Jeremiah et al., 2003). Although these attributes can be assessed independently, they are often closely related to one another. For instance, the study by Jeremiah et al. (2003) employed a trained panel to evaluate the overall tenderness, initial

tenderness, and amount of perceptible connective tissue of 33 bovine muscles. Muscles such as the *psoas major*, *ilio-psoas*, *longissimus thoracis*, *infraspinatus*, and *spinalis dorsi* all consistently ranked high for initial tenderness and overall tenderness, as well as had a low amount of perceptible connective tissue. Conversely, the shank muscles, *deep pectoral*, and *superficial pectoral* were identified as tough by all of these same measures (Jeremiah et al., 2003). These results were corroborated by the review of Sullivan and Calkins (2011) who found the objective tenderness assessed by trained panel of the *psoas major* to be highest and the *biceps femoris* to be lowest. Between these extremes, *infraspinatus, longissimus lumborum, longissimus thoracis, rectus femoris, triceps brachii,* and *serratus ventralis* would be moderately tender, and *gluteus medius, semitendinosus,* and *semimembranosus* would be tough (Sullivan & Calkins, 2011). Measures of objective tenderness coupled with trained panel evaluations of juiciness and flavor can be used to determine which palatability attributes contribute to overall palatability. For example, the *semitendinosus* muscle typically ranks poorly for overall palatability primarily due to considerable deficiencies in juiciness and tenderness, as well as a slight deficiency in flavor (Jeremiah et al., 2003). Alternatively, the *rectus abdominis*, while having desirable juiciness and flavor, ranks below the mean for overall palatability due to an overriding deficiency in tenderness (Jeremiah et al., 2003). This type of information can be utilized to identify which individual palatability factors may need to be improved in order to have a net gain in overall palatability.

The gold standard for determining subjective tenderness would be considered as quantitative consumer panel analysis (AMSA, 2015). To assess tenderness, consumers may be asked to rank a sample on a hedonic scale with at least five categories (e.g., extremely like, moderately like, somewhat like, neither like nor dislike, somewhat dislike, moderately dislike, extremely dislike), which can then be translated to numerical values. Alternatively, intensity scales may also be used (AMSA, 2015). While consumers can be asked to differentiate between "liking" and "level," often these results are nearly identical in practice (Guelker et al., 2013; Martinez et al., 2017). As previously discussed, subjective tenderness is a major determinant to the overall beef eating experience, accounting for 43.4% of variance in overall liking in the review by O'Quinn et al. (2018). Highlighting the importance of beef tenderness, in a scenario where tenderness is acceptable, the odds of a steak being rated as unacceptable by a consumer are only 1 in 10 (O'Quinn et al., 2018). Subjective tenderness is often shown to have moderate to high correlations with other measures related to tenderness including WBSF, amount of connective

tissue, and collagen content (Rhee et al., 2004). More detail on how these factors might influence tenderness will be provided in the subsequent sections.

1.2.2 Water-holding Capacity and Juiciness

As water makes up the largest component of fresh beef, the ability of meat to retain moisture is critical for product quality. This includes not only juiciness but also factors such as texture and appearance of the product alone and in the packaging (Aberle et al., 2012). Water within meat exists primarily in or around the myofibrils of the muscle cell, with 75% intramyofibrillar, 10% extramyofibrillar, and 15% extracellular (Warner, 2017). It is considered that most water (approximately 85%) in meat is considered immobilized or entrapped, with a smaller portion considered bound (Huff-Lonergan & Lonergan, 2005; Pearce et al., 2011). Water in the bound fraction is not truly bound but rather is tightly associated to charged groups of proteins (deMan et al., 2018). The remaining water is considered bulk or free water as it is readily expelled from meat upon the application of external forces (Aberle et al., 2012; Huff-Lonergan & Lonergan, 2005; Warner, 2017). Various factors are known to affecting the WHC of meat including net charge of muscle proteins, steric effects, extent of protein denaturation and functionality, and postmortem proteolysis (Aberle et al., 2012; Huff-Lonergan & Lonergan, 2005; Warner, 2017). Further, functional ingredients such as salts and phosphates can influence WHC (Warner, 2017), although they would be less relevant to the discussion of WHC of fresh meats. However, considering the widespread use of brine and marinade solutions with non-meat ingredients in relation to tumbled meats in the existing literature, some discussion regarding their impacts will be provided in the later sections. The primary methods of determining WHC can be separated based on whether external forces are applied or not (Warner, 2017). Methods that do not apply external forces would be gravimetric (e.g., drip loss) and purge losses during postmortem storage and freezing/thawing. Alternatively, methods that apply external forces would be compression WHC, centrifugation, filter paper method, and cooking loss. Correlations of measures of WHC to sensory juiciness are variable, and cooking loss is often considered as having the closest relationship (Warner, 2017).

While tenderness assessed by trained panel was considered an objective measure (AMSA, 2015), juiciness is often considered subjective regardless of whether panelists are trained or not

(Warner, 2017). In general, juiciness contributes less to the overall palatability of beef than tenderness and flavor (O'Quinn et al., 2018; Watson et al., 2008). Despite this, failing to meet consumer expectations for juiciness is still considered as a major determinant of overall acceptability. O'Quinn et al. (2018) reported that if juiciness was rated to be unacceptable by consumers, the likelihood of a beef product being rated as unacceptable overall would increase by a factor of 6.5. When evaluating juiciness, consumer panelists would be asked to assess liking or level of overall juiciness, similar to tenderness. However, trained panelists may be able to differentiate between initial and sustained juiciness, in addition to overall (Warner, 2017). Initial juiciness is considered to be related to moisture content, whereas sustained juiciness would be more dependent on the lubricating effect of intramuscular lipids (Warner, 2017; Winger & Hagyard, 1994). Juiciness is known to be correlated with multiple meat quality attributes such as cooking loss, WBSF, and sarcomere length (Rhee et al., 2004). However, the strength of these correlations may vary between individual beef muscles, where, for example, a significant correlation between juiciness and cooking loss was observed for *longissimus, gluteus medius,* and *biceps femoris,* but not *psoas major* and *semitendinosus* (Rhee et al., 2004). Overall, juiciness is less variable between beef muscles than tenderness (range of 2.52 versus 3.94 on a 9-point scale for juiciness and tenderness, respectively) (Jeremiah et al., 2003). In the study by Jeremiah et al. (2003), the *spinalis dorsi* was rated as the juiciest of 33 muscles by trained panel, while the *semitendinosus* was least juicy. The review of Sullivan and Calkins (2011) found the *infraspinatus*, *serratus ventralis,* and *longissimus lumborum* to be the juiciest, while the least juicy was the *semitendinosus*.

1.2.3 Flavor

As previously discussed, flavor is a primary driver of overall palatability of beef, accounting for more variance now than tenderness (O'Quinn et al., 2018). The development of flavor lexicons has allowed for standardized measures of flavor attributes and how they might contribute to the overall flavor of beef (Adhikari et al., 2011; Maughan et al., 2012). The lexicon by Maughan et al. (2012) included the basic tastes (bitter, salty, sour, sweet, umami) in addition to thirteen flavors characteristic of beef products. These included positive flavor attributes (e.g., those that contribute to increased overall liking) such as roast beef, browned, brothy, and others, as well

as flavors that would be considered as negative including barny, grassy, and gamey. Similarly, the lexicon developed by Adhikari et al. (2011) contained twenty-six flavor attributes that were defined and described in relation to a reference food. Certain specific flavor attributes may not be detectable in samples, rather samples are more frequently described by the basic tastes (sweet, sour, salty, bitter, umami) and certain beef-related attributes like beef identity, brown/roasted, metallic, and others (Adhikari et al., 2011). Considering the labor and resources necessary for trained sensory panel evaluation, other methods can be used to approximate objective flavor characteristics. One such method would be the use of an "electronic tongue" to detect specific flavor attributes including umami, salty, sour, bitter, and astringent (Ismail et al., 2020; Lee et al., 2019; X. Zhang et al., 2015).

Flavor, however, is also complex in that it encompasses both taste and odor. Accordingly, volatile compounds produced during the cooking of meat are often assessed to consider the odor component (Foraker et al., 2020; Legako et al., 2015).Through the use of a trained panel in tandem with volatile compound analysis, observations such as how certain flavor attributes and might change by treatments (e.g., over the course of postmortem aging) can be made. For instance, with extended postmortem aging, it has been observed that certain positive flavor attributes such as beef flavor, browned, and roasted in beef *longissimus* muscles might decrease, while negative attributes including oxidized, sour, and liver-like increase (Foraker et al., 2020). Considering the volatile compounds, Foraker et al. (2020) found several compounds to be negatively correlated to beef flavor and positively correlated to certain negative flavor attributes including oxidized and liver-like. As these compounds generally increase with extended postmortem aging, it is critical that potential detriments to flavor be minimized in efforts to improve tenderness. Specifically, as physical interventions might result in undesirable changes within the muscle such as the release of free radicals, special consideration to the possible generation of off-flavors must be given, as will be discussed in greater detail in the later sections.

While trained panelists could be used to identify specific flavor attributes (Adhikari et al., 2011; Foraker et al., 2020; Maughan et al., 2012), typically consumers would be asked to provide liking of flavor only (Legako et al., 2015; Ponce et al., 2019; Watson et al., 2008). Although it may be possible for untrained panelists to evaluate more flavor attributes than overall liking of flavor, this approach may not be recommended. Highlighting this, Watson et al. (2008) reported

that fatty taste, flavor, beef flavor, and liking of taste as assessed by consumer panelists would be highly redundant, thus liking of taste only would be recommended for this type of analysis.

1.3 Factors Influencing Beef Tenderness

It is well established that individual muscles throughout the beef carcass vary considerably in tenderness, and some may even exhibit locational differences in tenderness within the same muscle. For example, multiple studies have found muscles such as the *psoas major* and *diaphragm* to rank highly for tenderness, while those such as the *pectoralis profundus* and *flexor digitorum* are categorized as tough (Belew et al., 2003; Jeremiah et al., 2003; Torrescano et al., 2003). Concerning muscles that will be evaluated in the studies that comprise this dissertation, Sullivan and Calkins (2011) categorized the *longissimus lumborum* and *tensor fasciae latae* as intermediate tenderness and the *semitendinosus*, *gluteus medius*, and *biceps femoris* as tough. These discrepancies in tenderness between muscles can be attributed to major differences in the individual factors that determine tenderness, as well as the extent to which those factors drive overall texture. Three major factors are known to contribute to the tenderness of beef and will be discussed in detail. These would include the rate and extent of postmortem proteolysis, the amount and properties of connective tissues, and the length of sarcomeres within the myofibril (Warner et al., 2020). Understanding which of these factors would be most important to the tenderness of an individual beef muscle is critical to develop strategies aimed at improving eating quality and adding value to the beef carcass, especially considering the close relationship between tenderness and economic value (M. F. Miller et al., 2001; Shackelford et al., 2001).

1.3.1 Proteolysis

Following chilling of the carcass, beef is routinely stored under controlled, refrigerated conditions for a certain period of time in a process known as postmortem aging or conditioning (Aberle et al., 2012; Y. H. B. Kim et al., 2018; Warner et al., 2020). Typically, the aging process is conducted within anaerobic packaging to act as a protective barrier, referred to as wet aging. While the aging process influences multiple eating quality attributes including juiciness and flavor (Ponce et al., 2019; Warner, 2017), the primary purpose of postmortem aging is to improve tenderness through postmortem proteolysis (Y. H. B. Kim et al., 2018). Numerous factors are

known to contribute to the rate and extent of proteolysis in postmortem beef such as muscle type, aging conditions, sarcomere length, animal maturity, among others (Bratcher et al., 2005; Gruber et al., 2006; Weaver et al., 2008).

Prior to the examining the proteinase systems responsible for postmortem proteolysis, a discussion of changes occurring to muscle microstructure and ultrastructure during postmortem aging should be provided. Tenderization associated with postmortem proteolysis can in part be attributed to degradation of structures at the Z disk of the sarcomere (Huff Lonergan et al., 2010). It has also been suggested that tenderization may not be directly due to degradation of the Z disk but rather degeneration of the costameres and adjacent structures within and between sarcomeres (Taylor et al., 1995). One such protein associated with the Z disks and susceptible to postmortem proteolysis would be desmin, an intermediate filament protein. It is known desmin degrades during postmortem storage of bovine muscle, but it is debatable if this directly causes tenderization (Huff Lonergan et al., 2010). Another protein known to be degraded during postmortem storage of beef would be troponin-T (Lonergan et al., 2001; Penny & Dransfield, 1979). It has been suggested that troponin-T degradation is not only a marker of postmortem proteolysis but may also play a role in disruption of structural integrity at the ultrastructural level (Huff Lonergan et al., 2010). Other proteins of the myofibril that may be susceptible to postmortem proteolysis include titin and nebulin. It has also been suggested that myosin heavy chain and myosin light chain may be susceptible to postmortem proteolysis, which could have implications for tenderization through the disruption of actomyosin bonds (Lametsch et al., 2004).

The primary protease system considered to be responsible for the tenderization of beef would be the calpains (Aberle et al., 2012; Koohmaraie, 1992; Whipple & Koohmaraie, 1992). The calpain system consists of calpain-1 (also known as μ-calpain) and calpain-2 (also known as m-calpain) (Campbell & Davies, 2012; Goll et al., 2003). Both calpain forms are heterodimers that consist of 80 and 28 kDa subunits. Calpain enzymes have a cysteine protease core that is activated by the presence of calcium ions. Calpain-1 requires a lower concentration of calcium ions for half-maximal activation (approximately 30 μM) compared to calpain-2 (approximately 350 μM), hence why they were previously referred to as μ-calpain and m-calpain, respectively (Campbell & Davies, 2012). When incubated with calcium, calpain will autolyze, reducing the mass of the 80 kDa subunit to 76 kDa and 78 kDa for calpain-1 and calpain-2, respectively (Huff Lonergan et al., 2010). Accordingly, calpain autolysis shown by the relative ratios of each band following

immunoblotting is routinely used as a marker of calpain activity in postmortem muscle. It has been suggested by Boehm et al. (1998) that in bovine *semimembranosus* muscle, approximately 50% of calpain-1 remains tightly associated to the myofibrils during postmortem storage and exhibits no proteolytic activity. In addition to a portion of calpain that may remain inactive, it is well established that the presence of calpastatin inhibits proteolysis and has profound implications for tenderization (Cruzen et al., 2014; Lonergan et al., 2001; Whipple & Koohmaraie, 1992). Calpastatin is a polypeptide that is specific to calpain inhibition (Goll et al., 2003). It is considered that muscle function in the living animal influences the calpain system and subsequently postmortem proteolytic tenderization. Muscles used for locomotion (e.g. *semitendinosus, semimembranosus, biceps femoris*) may have a higher rate of protein turnover than those used for posture. It could also be considered that this would be attributed more to muscle type, as oxidative muscles typically exhibit less postmortem proteolysis with aging compared to glycolytic (Whipple & Koohmaraie, 1992). Stolowski et al. (2006) categorized seven bovine muscles in relation to their aging response, finding the *longissimus* and *gluteus medius* to exhibit aging potential up to 42 days, the *semitendinosus* to exhibit aging potential up to 28 days, and the *biceps femoris* to exhibit little to no aging response. Cruzen et al. (2014) assessed the proteolytic features of the bovine *longissimus, semimembranosus,* and *triceps brachii* of young and mature beef cattle. As expected, it was observed that less proteolysis indicated by lower caplain-1 activity and degradation of troponin-T occurred in mature animals, likely owing to an increased abundance of calpastatin. Regarding muscle type, abundances of calpain-1 were equivalent, though less calpain-2 and higher total calpastatin were found in *triceps brachii* compared to the *longissimus.* This led to less degradation of troponin-T and a higher abundance of titin during postmortem aging (Cruzen et al., 2014).

While calpains would be considered as the primary enzyme system responsible for tenderization, other proteinases have also been considered such as the cathepsins and caspases. Cathepsins B and L have been shown to have proteolytic activity on multiple myofibrillar and stromal proteins (Ertbjerg et al., 1999). Considering the cathepsins are located within the lysosomes, their relevancy to meat tenderization is debatable (Sentandreu et al., 2002). However, upon the application of external forces via physical interventions, it is reasonable to postulate cathepsins could be liberated from the lysosomes and thus degrade muscle proteins. For instance, the recent study by Wang et al. (2022) reported that damage from ultrasonication could increase

the activity of cathepsins B and L, leading to a increase in collagen solubility and disintegration of the perimysium. Also using ultrasonication but related to caspases, Dang et al. (2022) found that caspase-3 activity could be increased, which may have contributed to improved tenderization over the course of postmortem aging. However, it should also be noted that ultrasound damage also caused the release of calcium ions from the sarcoplasmic reticulum, leading to an increase in calpain-1 activity. Accordingly, it can be difficult to determine how individual proteases may be affected by physical interventions that cause multiple profound structural changes; however, enhanced proteolysis following a disruptive process is a well-studied phenomenon (Dang et al., 2022; H.-W. Kim et al., 2018; Setyabrata et al., 2019; Setyabrata & Kim, 2019; Wang et al., 2022).

Differences in the extent of proteolysis cannot explain all variation in tenderness among beef cuts (Anderson et al., 2012). Highlighting this, Rhee et al. (2004) found only a weak correlation between desmin degradation and subjective tenderness among eleven beef muscles, while no significant correlation between desmin degradation and WBSF was observed. However, this finding was observed to be muscle-specific, where a moderate correlation was observed in *longissimus, supraspinatus, infraspinatus,* and *triceps brachii* muscles. Similarly, the study by Stolowski et al. (2006) found calpastatin activity to be a poor indicator of objective tenderness and postmortem proteolysis in seven bovine muscles. In such instances, a lack of tenderization can often be attributed to a high extent of background toughness, which degradation of myofibrillar proteins would have little to no influence on. Highlighting this, Chun et al. (2020) reported that proteolysis as shown by troponin-T degradation would actually be greatest in the *tensor fasciae latae* and *gastrocnemius* muscles compared to the *longissimus lumborum*; however, the *longissimus lumborum* would have superior myofibrillar and overall tenderness scores. This observation would be mainly due to the *tensor fasciae latae* and *gastrocnemius* muscles having moderate and high levels of collagen, with the former exhibiting a high extent of collagen crosslinking (Chun et al., 2020). Accordingly, proteolysis alone would be a somewhat poor indicator of tenderness development, especially when considering cuts outside of the loin and rib primals that would be expected to have a large amount of intramuscular connective tissues.

1.3.2 Connective Tissues

Connective tissue comprises multiple organ and tissue systems. Relevant to the discussion of muscle structure and tenderness would be connective tissue proper (Aberle et al., 2012). Connective tissue is present in various forms throughout the muscle structure. Whole muscles are surrounded by epimysium, muscle bundles by perimysium, and muscle fibers by endomysium. Perimysium is the most abundant connective tissue in meat, comprising approximately 90% of total connective tissue (McCormick, 1999). Connective tissue proper primarily consists of collagen in a proteoglycan matrix, although other stromal proteins including elastin and reticulin would be important to beef texture as well (Lepetit, 2008). Collagen fibrils are comprised of tropocollagen molecules arranged in a triple helical structure. The types of tropocollagen molecules that make up the collagen fibril determine its type, which then determines the ability to form collagen fibers or other structures. Fibrous collagen (primarily Type I and III) is inelastic, poorly soluble, and is known to form cross-linked structures. It is generally recognized that collagen content is a major determinant of tenderness, especially in those muscles that were used for locomotive purposes in the living animal, especially with increasing animal maturity (Aberle et al., 2012). Specifically, the high degree of collagen cross-linking in mature beef animals (Alvarenga et al., 2021) and certain muscle types (Chun et al., 2020) is known to contribute to poor tenderness.

It has been shown that total collagen content and collagen solubility are highly correlated (r of 0.723 and 0.661, respectively) to shear force values of raw beef (Torrescano et al., 2003). For example, the *psoas major* has been shown to have the least shear force, total collagen content, and insoluble collagen content of twelve evaluated beef muscles (Torrescano et al., 2003). However, for cooked beef, the relationship between collagen content and solubility with instrumental tenderness is less apparent (Li et al., 2010). Chang et al. (2011) found collagen solubility of beef *semitendinosus* to fluctuate throughout varying endpoint temperatures and differ depending on cooking method. The 2010 NBTS included both dry- and moist-heat cookery to assess tenderness of beef round muscles; however, no substantial differences in instrumental tenderness values were found between cookery methods (Guelker et al., 2013). The study by Powell et al. (2000) applied a stepwise dry-heat cookery process to *semitendinosus* roasts, finding the process to decrease shear force and the amount of total collagen. Inconsistencies in collagen amount and solubility and the relationship to meat tenderness may be attributed to the thermal shortening of collagen induced during the cooking process (Lepetit, 2008) and differing thermal stability among collagen types

(Horgan et al., 1991). It has been shown that total collagen content may have a closer relationship with cooked beef tenderness than collagen solubility (Powell et al., 2000). The study by Jeremiah et al. (2003) found the amount of perceptible connective tissue to be high for round muscles like the *semitendinosus*, which was in general mirrored by scores of initial and overall tenderness.

Intramuscular connective tissue exhibits limited degradation relatively early postmortem but may be relevant to improved tenderness of extendedly aged beef (Nishimura et al., 1998). Nair et al. (2019) found bovine *semitendinosus* muscle to tenderize over the course of 14 days, although further tenderization was not observed past this. At or beyond 35 days of postmortem aging, however, tenderization of inherently tough round muscles has been shown (Juárez et al., 2010). As previously discussed, physical interventions may be a possible way to affect the properties of intramuscular connective tissues, either in a direct or indirect manner. For instance, it has been shown that damage produced from ultrasonic waves could result in some disruption to the ordered structure of the perimysium (Wang et al., 2022). With the application of further postmortem aging following ultrasonication, more extensive degradation of the perimysium could be observed, supported by increases in collagen solubility, greater activity of cathepsin B and L, and improved tenderization.

1.3.3 Sarcomere Length

Myofibers are composed of organelles known as myofibrils, which are then comprised of multiple myofilaments (Aberle et al., 2012). The thick myofilaments are predominantly myosin, whereas the thin filaments consist of actin. Regions of the myofilaments overlap in a repeating pattern over the length of the muscle fiber. Bands consisting of only the thin filaments are known as the isotropic (I) band, while those consisting of both thick and thin filaments are known as the anisotropic (A) band. Bisecting the I band would be the Z line, which is made up of ultra-thin filaments and serve as an attachment point for the thin filaments. Each region from Z line to adjacent Z line is known as a sarcomere. Sarcomeres are the contractile units of the muscle fiber (Ertbjerg & Puolanne, 2017). While sarcomeres are typically 2.5 µm in length in living muscle (Aberle et al., 2012), postmortem muscles typically have a sarcomere length of around 2.0 µm (Ertbjerg & Puolanne, 2017). Further, substantial intermuscular variation has been observed for sarcomere length (Torrescano et al., 2003). This may have implications for postmortem proteolysis

(Weaver et al., 2008); however, the role of sarcomere length on tenderness can vary between muscles.

In a survey of 14 raw bovine muscles, Torrescano et al. (2003) found sarcomere length to vary from 1.57 to 3.42 µm. For some muscles like the *psoas major*, the results were intuitive where the longest sarcomere length contributed to the least shear force values. However, the shortest sarcomere lengths were observed in the *longissimus lumborum* and *gluteus medius* which were observed to be the fourth and fifth most tender muscles, respectively. Accordingly, the correlation between sarcomere length and instrumental tenderness has been reported to be weak (Rhee et al., 2004; Torrescano et al., 2003). It has been suggested that sarcomere length is relevant to tenderness only when postmortem glycolysis is slow or delayed, whereas no relationship between sarcomere length and tenderness may be apparent when rapid postmortem glycolysis occurs (Smulders et al., 1990). The relationship between sarcomere length and tenderness in raw beef is typically weak (Torrescano et al., 2003), yet there is more evidence to suggest sarcomere length is more related to tenderness of cooked beef (Ertbjerg & Puolanne, 2017; Wheeler et al., 2002). This could be related to heat causing further contraction and coagulation of muscle proteins (Ertbjerg & Puolanne, 2017). Further, it has been hypothesized that the discrepancies in tenderness and sarcomere length for certain muscles (e.g., the *longissimus lumborum* has short sarcomeres but is generally tender) could be attributed to increased fracturing near the Z line being related to short sarcomeres (Ertbjerg & Puolanne, 2017; Torrescano et al., 2003). Weaver et al. (2008) found that stretching of bovine *semitendinosus* would not only decrease shear force attributable to increased sarcomere length but would also enhance proteolysis during aging shown by the degradation of troponin-T. While sarcomere length is important to consider when comparing tenderness between muscles, improving the tenderness of individual muscles would likely be more dependent on disrupting the structural integrity of the muscle through the degeneration of myofibrillar and stromal proteins. However, it is reasonable to expect that some interaction of sarcomere length with postmortem proteolysis may occur.

1.4 Tumbling

Tumbling has been widely applied in the industry for processed meat applications. However, tumbling without the inclusion of a brine or marinade solution has typically not been considered

as a method of improving the meat and eating quality attributes of fresh beef products. Generally, tumbling is utilized as a method to distribute cure ingredients throughout the meat structure via mechanical forces (Aberle et al., 2012). The application of tumbling has been shown to result in several desirable quality improvements such as improved product yield, WHC and juiciness, and tenderness. Often, products are injected with functional ingredients prior to treatment, or liquid media is added to the drum interior which aid in flavoring and protein extraction (C.-S. Cheng & Parrish, 1978; Hultin et al., 1995; Pietrasik & Shand, 2004). Product enhancement can also be applied after tumbling in a process known as pre-tumbling (Boles & Shand, 2002). The tumbling process is similar to massaging in that agitation is used, although massaging aims primarily to create friction on the meat surface to improve binding. Regarding tumbling, Daudin et al. (2016) categorized the mechanical forces that would be induced into two phases: a brief deformation resulting from the drop and the compressive forces resulting from contact with other pieces. The deformation phase is considered as the primary action that influences product quality (Daudin et al., 2016). Though the current literature regarding application of meat tumbling to fresh meat products is limited, the following sections will summarize the principal effects on meat quality attributes, as well as what factors may contribute.

1.4.1 Effects on Quality Attributes

Tenderness

While tumbling would affect other meat and eating quality traits, as will be later discussed, the primary rationale for applying the process would be tenderization. Numerous studies have found tumbling to improve the quality of muscle foods, even for inherently tough cuts such as the *rectus abdominis*, *biceps femoris*, *semitendinosus*, and others (J. Cheng & Ockerman, 2003; Chow et al., 1986; Dzudie & Okubanjo, 1999; Gao et al., 2014; Garmyn et al., 2020; Krause et al., 1978; Molina et al., 2005; Morrow et al., 2019). Intermuscular differences would be expected to have a large role in the effectiveness of the tumbling process on tenderization. Molina et al. (2005) reported that of the eight evaluated muscles from the beef chuck, only the *subscapularis* would have decreased WBSF compared to the non-tumbled control group. Other muscles with WBSF values unaffected by tumbling included the *complexus, latissimus dorsi, rhomboideus, splenius, serratus ventralis, supraspinatus,* and *triceps brachii* (Molina et al., 2005). Interestingly, while it

may be expected that the tenderness of the *subscapularis* was improved through tumbling as it is already a tender muscle (Sullivan & Calkins, 2011), the *serratus ventralis* would also be considered as inherently tender and was not tenderized. Further, significant tenderization was achieved through needle pumping in the *complexus* and *triceps brachii* (Molina et al., 2005), both muscles of intermediate tenderness (Sullivan & Calkins, 2011). This would likely be related to the interrelationships between tenderness and water-holding (Rhee et al., 2004; Warner, 2017; Warner et al., 2020), as well as differences in the incorporation of functional ingredients by enhancement method (Gao et al., 2014; Molina et al., 2005; Morrow et al., 2019). This phenomenon will be discussed in greater detail in the subsequent section. While there may be some inconsistencies in the tenderization achieved through tumbling by muscle, it could be surmised that the process would be most effective on muscles that would already be considered inherently tender without a large contribution of background toughness. For instance, the study by Gao et al. (2014) found that continuous and intermittent tumbling for a total time of 8 hours would decrease shear force of porcine *longissimus thoracis et lumborum* muscles from 27.75 N to 12.41 and 14.93 N, respectively. This would be equivalent to 76.4% and 60.1% decreases in shear force for continuous and intermittent tumbling, respectively (Gao et al., 2014). While significant decreases in shear force have been reported by numerous studies as previously discussed, the amount of tenderization achievable would undoubtedly be limited by the individual muscle. For example, J. Cheng and Ockerman (2003) reported the difference in shear force of beef bottom round roasts tumbled intermittently for 18 hours at 10 minutes per hour would be only 17.3%. Similarly, Chow et al. (1986) applied an intermittent tumbling treatment (15 minutes per hour) for 9 or 18 hours, decreasing the Instron tenderness of porcine shoulder muscles by 28.0% and 37.5%, respectively (specific shoulder muscles used was not specified; however, it would be expected the muscles used would be inherently tough in general). In relation to beef muscles tumbled in the absence of a brine solution, the present literature is somewhat limited. Morrow et al. (2019) reported that beef *rectus abdominis* muscles tumbled without a marinade would have no significant decrease in SSF, although considerable improvements would be found through tumbling with enhancement. Conversely, N'Gatta et al. (2021) found that textural properties of the beef *semitendinosus* could be influenced by the application of physical forces via a tumbling simulator; however, drastic differences in tumbling treatments were used between studies. Specifically, Morrow et al. (2019) tumbled for approximately 200 cumulative rotations, whereas N'Gatta et al. (2021) applied 2,500

to 13,000 consecutive compression cycles. As such, the current literature suggests that the instrumental tenderness of inherently tough cuts could be improved through tumbling, although the inclusion of a brine or an extended duration would likely be necessary.

In addition to instrumental tenderness, consumer liking of tenderness has been shown to be affected by tumbling as well. The study by Garmyn et al. (2020) reported that tumbling with a brine of sodium chloride and either sodium tripolyphosphate or sodium bicarbonate would improve consumer liking of tenderness of beef *diaphragm, transversus abdominis, gracilis, obliquus abdominus internus,* and *rectus abdominis* muscles compared to non-tumbled controls. Intermuscular differences in tenderness liking were also reported, where the *obliquus abdominus internus* and *diaphragm* were more tender than the other muscles, though this was not dependent on tumbling treatment. While other quality traits were also improved with tumbling, it could be expected that the improvements in tenderness would act as a major driver in the improved overall liking of tumbled muscles (Garmyn et al., 2020). Similarly, Morrow et al. (2019) found consumer tenderness of *rectus abdominis* muscles tumbled with the inclusion of a marinade would be significantly improved compared to the non-tumbled control group. However, the muscles tumbled with a marinade had inferior tenderness compared to those muscles that were either injected with a marinade solution or injected with a marinade and subsequently tumbled. Further, the muscles that were tumbled without a marinade had comparable subjective tenderness to the non-tumbled control group (Morrow et al., 2019).

Understanding the structural changes that occur during the tumbling process is critical to elucidating the ways in which tenderization would be achieved. As previously discussed, tenderness is highly dependent on the integrity of the myofibrils (Huff Lonergan et al., 2010; Y. H. B. Kim et al., 2018; Taylor et al., 1995; Warner et al., 2020). Previously, Tyszkiewicz et al. (1997) evaluated the ultrastructure of mechanically tenderized porcine *biceps femoris* muscles. At 3 hours postmortem, integrity of the myofibrils of the control and blade tenderized muscles was preserved, though myofibrillar damage was apparent in those muscles subjected to grinding and a meat "activator" (defined as a tubular device that exposed the meat to pressure and other mechanical forces). While grinding resulted in longitudinal fracturing at the Z line, the activator treated muscles had a near complete loss of contractile structure. Interestingly, at 48 hours postmortem, the activator treated muscles were categorized by extended sarcomeres and fracturing between the A and I bands, rather than at the Z line. Similar findings were reported by Katsaras

and Budras (1993) in tumbled porcine *semimembranosus, adductor, biceps femoris,* and *semitendinosus* muscles. Tumbling resulted in the disintegration of muscle cells near the external surface exposed to the most kinetic force, although ultrastructural integrity could be preserved in the internal portions of the product. Other relevant structural changes would include the swelling of myofibrils induced from brine inclusion and subsequent release of granular protein into the intermyofibrillar space (Katsaras & Budras, 1993). Considering Morrow et al. (2019) reported that tumbling without enhancement would not affect textural properties, it could be argued that the swelling of myofibrils associated with brine inclusion would be more relevant to tenderness than the disruption of myofibrillar structure. This postulation would be partially supported by Molina et al. (2005) who found several muscles from the beef chuck could be tenderized through needle pumping but not tumbling. However, Gao et al. (2014) reported that tumbling with a brine would result in lower shear force than static marination alone, which may suggest that the physical disruptions to myofibrillar structure would be a major factor influencing tenderness. With that said, differences in diffusion of functional ingredients between tumbling and static marination could also account for this observation (Sharedeh et al., 2015). As previously discussed, physical disruptions alone may be able to affect textural properties. N'Gatta et al. (2021) found that extended tumbling (2,500 to 13,000 consecutive compression cycles) would contribute to the formation of amorphous zones in *semitendinosus* muscles via compaction of the myofibers. Likely the means through which tumbling with a brine improves tenderness would be through a combination of physical disruptions and myofibrillar swelling. Without brine inclusion, it may be possible that physical disruptions alone could influence tenderness, although more extensive application would be required.

While the effects of tumbling on postmortem proteolysis are not well characterized, some insight can be provided through previous research regarding ultrasonication. Ultrasonication in muscle foods has been used to generate physical forces that can aid in brine penetration, reduce microbial loads, and improve tenderization (Alarcon-Rojo et al., 2019; Bekhit et al., 2014; Peña-Gonzalez et al., 2019). Recently, Dang et al. (2022) found that ultrasound damage in beef *longissimus lumborum et thoracis* muscles would not only result in an immediate decrease in WBSF relative to the control group but also accelerate postmortem proteolysis of myofibrillar proteins including titin, desmin, and troponin-T. Specifically, the treatment group was shown to immediately decrease relative abundance of titin, as well as increase the degradation of troponin-

T and desmin with the application of additional postmortem aging. This finding was attributed to accelerated autolysis of calpain-1 through an increase in free calcium in the cytosol (Dang et al., 2022). Similar observations have been made regarding the effects on beef *semimembranosus* muscles with aging. Stadnik et al. (2008) reported that ultrasound damage would result in an initial swelling of the myofibrils followed by increased fragmentation of the sarcomeres at the Z line with aging. Further, it may also be possible that physical disruptions could affect not only myofibrillar but also stromal proteins. For instance, Wang et al. (2022) found that ultrasound damage would disrupt the perimysium of beef *semitendinosus* muscles, resulting in an increase in collagen solubility. Consequently, tenderness was improved both immediately and with aging, likely through a combination of physical disruptions alone combined with enhanced proteolysis through the release of cathepsin B and L (Wang et al., 2022). Taken together, it is reasonable to postulate that physical disruptions induced through tumbling would result in similar changes to proteolysis with aging as has been reported with ultrasonication.

Water-holding Capacity and Juiciness

As most tumbling studies have utilized a brine or marinade solution, the direct effects on water-holding capacity and juiciness would be difficult to categorize. Most studies have observed a positive effect of tumbling with a brine on WHC of both raw and cooked product (J. Cheng & Ockerman, 2003; Chow et al., 1986; Dzudie & Okubanjo, 1999; Gao et al., 2014; Garmyn et al., 2020; Moon et al., 2007; Morrow et al., 2019). Moon et al. (2007) reported that moisture content of restructured beef *pectoralis profundus* muscles would increase with tumbling duration. However, increasing tumbling duration resulted in higher drip loss but lower cooking loss (Moon et al., 2007). Similarly, Dzudie and Okubanjo (1999) reported higher moisture content in both raw and cooked goat hams as tumbling duration increased from 2 to 6 hours, supported by improved WHC and decreased cooking loss. Decreased cooking loss was also reported by Gao et al. (2014) in tumbled porcine *longissimus lumborum et thoracis* muscles. Morrow et al. (2019) found that tumbling with a marinade would increase both raw and cooked moisture content of *rectus abdominis* muscles, which would be maintained through cooking. Accordingly, it can be concluded that tumbling with a brine would result in increased moisture content in raw product. Improved moisture content is typically maintained upon the application of external forces

including pressing and heating, likely through improvements in the functionality of myofibrillar proteins.

As previously discussed, tumbling with a brine would result in swelling of the myofibrils (Katsaras & Budras, 1993), which would likely have profound effects on water-holding (Huff-Lonergan & Lonergan, 2005; Hughes et al., 2014; Warner, 2017). Further, tumbling of muscle foods with a brine would increase the amount of sodium chloride in the product (Bombrun et al., 2014; Dzudie & Okubanjo, 1999; Sharedeh et al., 2015), which would be relevant to factors such as ionic strength and the functionality of myofibrillar proteins (Chen et al., 2017; Huff-Lonergan & Lonergan, 2005; Puolanne & Peltonen, 2013; Warner, 2017). Salt content has been shown to influence the number of hydrophobic sites and disulfide bridge formation in porcine *semimembranosus* muscles, which would be relevant to protein functionality and gel formation (Bombrun et al., 2014). However, Bombrun et al. (2014) reported that extractability of myosin would not be significantly affected by brine salt content, while extractability of actin would actually decrease. In general, though, it is accepted that myofibrillar protein functionality increases at high ionic strengths (Chen et al., 2017). Tyszkiewicz et al. (1997) reported that the application of mechanical forces in the absence of a brine would increase the amount of extractable water and salt soluble proteins in porcine *biceps femoris* muscles. Previously, Farouk et al. (2012) described a "sponge effect" to elucidate why WHC may improve with postmortem aging. It has been proposed that fragmentation of myofibrillar structure would inhibit moisture from easily leaving the muscle structure through drip channels. The disruption of drip channels with postmortem aging has been shown with scanning electron microscopy, accompanied by an increase in protein extractability with aging duration (Farouk et al., 2012). This phenomenon could help explain, in part, why improved WHC of tumbled muscles is generally maintained when external forces are applied. While information regarding WHC of muscles tumbled without a brine is limited, Morrow et al. (2019) found comparable moisture content in raw and cooked beef *rectus abdominis* muscles tumbled without a brine compared to the control group. Similarly, Dang et al. (2022) reported comparable cooking loss at all postmortem times in ultrasonicated beef *longissimus thoracis et lumborum* muscles compared to controls. Accordingly, it may be that physical forces generated by tumbling would result in myofibrillar fragmentation or increased protein functionality, minimizing possible detriments to WHC. However, several studies have also observed water-holding to decrease with postmortem aging (Dang et al., 2022; Purslow et al., 2016; Warner, 2017),

contesting this supposition. Further complicating this relationship, the effects of tumbling on WHC have also been demonstrated to be muscle specific. Molina et al. (2005) found tumbling to decrease cooking loss of *complexus, latissimus dorsi, splenius,* and *subscapularis* muscles compared to non-tumbled controls, while no differences were found for *rhomboideus, serratus ventralis, supraspinatus,* and *triceps brachii*. These differences may be related to inconsistencies in whether tumbling results in an increase or decrease in extracellular space, which may be dependent on muscle type and brine inclusion (N'Gatta et al., 2021; Sharedeh et al., 2015; Siró et al., 2009).

The relationship of WHC to meat color has been well described (Hughes et al., 2014). Gao et al. (2014) found tumbling to increase the CIE *L** (lightness) and CIE *a** (redness) values of porcine *longissimus thoracis et lumborum* muscles. This finding would likely be attributable to an increase in extracellular space generated through tumbling with brine inclusion. Supporting this, an increase in extracellular space has been reported with massaging, tumbling, and ultrasonication (Sharedeh et al., 2015; Siró et al., 2009), although the inverse relationship has also been observed (N'Gatta et al., 2021). Effects of tumbling on color may also be related to the increase in pH typically found through the inclusion of phosphates (J. Cheng & Ockerman, 2003; Dzudie & Okubanjo, 1999; Gao et al., 2014; Garmyn et al., 2020). Regarding the impacts of tumbling only, J. Cheng and Ockerman (2003) reported that while pH would increase with the concentration of phosphate, the pH of tumbled (5.89) and non-tumbled (5.86) beef bottom rounds would be practically equal. Supporting this, most studies support that ultrasonication would have minimal effects on influencing postmortem pH (Alarcon-Rojo et al., 2019; Dang et al., 2022; Stadnik et al., 2008). Taken together, it would be expected that tumbling without the inclusion of a brine with phosphates would have negligible impacts on pH. Regarding color, the present literature suggests that tumbling with brine inclusion would increase lightness, likely through an increase in extracellular space. Without brine inclusion, it may be that color would be affected in an inverse manner through a decrease in extracellular space (N'Gatta et al., 2021); however, no studies have reported the color attributes of beef tumbled without brine inclusion.

Although the relationship between WHC and sensory juiciness often varies (Rhee et al., 2004; Warner, 2017), tumbling has generally been shown to improve both in tandem. Gao et al. (2014) reported that juiciness of porcine *longissimus thoracis et lumborum* muscles would improve with both static marination and continuous and intermittent tumbling relative to non-enhanced controls. Dzudie and Okubanjo (1999) found that juiciness of tumbled goat hams would increase

with tumbling time, regardless of whether the muscle was in a pre- or post-rigor state. This is in contrast with Chow et al. (1986) who reported that tumbling would not affect juiciness but that the attribute would be affected by rigor state. Likely, juiciness would be closely related to brine penetration, as several studies have observed differences in juiciness by enhancement method (Gao et al., 2014; Molina et al., 2005; Morrow et al., 2019). Supporting this, Gao et al. (2014) found juiciness to improve regardless of enhancement method, and continuous and intermittent tumbling were more effective than static marination at improving juiciness. Conversely, Morrow et al. (2019) reported that injection with marinade was more beneficial to juiciness than tumbling with marinade for beef *rectus abdominis* muscles. These discrepancies can likely be explained by the differences in tumbling and brine penetration between muscles. Molina et al. (2005) found that tumbling would improve juiciness of beef *latissimus dorsi, subscapularis, serratus ventralis, supraspinatus,* and *triceps brachii* muscles, but not for *complexus, rhomboideus,* and *splenius* muscles. For tumbling without a brine, the current literature suggests impacts on juiciness would be largely minimal. Morrow et al. (2019) reported juiciness of beef *rectus abdominis* muscles tumbled without a marinade would be comparable to non-tumbled control muscles.

Flavor and Oxidative Stability

The literature regarding the effects of tumbling on flavor are more mixed than tenderness, WHC, and juiciness. Several studies have reported that tumbling with enhancement would improve liking of flavor compared to non-enhanced controls (Gao et al., 2014; Garmyn et al., 2020; Krause et al., 1978; Morrow et al., 2019). This finding would likely be attributable to penetration of the brine solution into the meat aiding in flavoring. However, other studies have observed no beneficial effect of tumbling on flavor (Chow et al., 1986; Dzudie & Okubanjo, 1999). Chow et al. (1986) found that tumbled porcine shoulder muscles would have comparable juiciness to non-tumbled controls, although flavor would be superior in pre-rigor compared to post-rigor muscles. In partial disagreement, Dzudie and Okubanjo (1999) found that neither flavor nor saltiness of goat hams would be affected by tumbling, regardless of rigor state. Molina et al. (2005) reported that tumbling would increase beef flavor intensity, dependent on the individual beef chuck muscle. The study by Garmyn et al. (2020) observed that tumbling with enhancement would increase flavor liking, regardless of which muscle was evaluated. For tumbling without a brine, it would be

expected that liking of flavor and saltiness would be generally unaffected. The study by Morrow et al. (2019) found that tumbling without a brine would not affect flavor liking or saltiness of beef *rectus abdominis* muscles relative to the control group.

With that said, it is reasonable to postulate that tumbling without brine inclusion could influence flavor attributes with the application of subsequent postmortem aging. The study by Morrow et al. (2019) conducted the trained panel on beef steaks that had been immediately frozen following processing. Accordingly, it would be expected that the effects tumbling could have on oxidative stability would be somewhat limited. It has been postulated that tumbling would disrupt the phospholipid bilayers of the muscle fibers, as well as release free radicals into the system (J. Cheng & Ockerman, 2003). Similar observations regarding oxidative stability through ultrasound damage have been made. For instance, Peña-Gonzalez et al. (2019) found that ultrasound damage would negatively impact color and flavor, specifically causing discoloration and an increase in metallic and oily off-flavors. With the application of subsequent postmortem aging, the release of reactive oxygen species (ROS) has been shown, likely related to mitochondrial disfunction (Dang et al., 2022). Tumbling has also been shown to increase the heme iron content of cooked beef bottom rounds initially during storage, although the differences would be negligible as storage progressed (J. Cheng & Ockerman, 2003). Regarding lipid oxidation, muscles that were tumbled with no phosphate inclusion had significant increases in 2-thiobarbituric acid reactive substances (TBARS) values (J. Cheng & Ockerman, 2003). For protein oxidation, Bombrun et al. (2014) reported that the content of carbonyl groups would be unaffected by tumbling, regardless of brine salinity; however, carbonyl groups would form upon heating. For thiol groups, tumbling alone resulted in less oxidation compared to muscles tumbled with a brine (Bombrun et al., 2014), which may be related to the pro-oxidative effects of salt. The addition of antioxidants has been used to prevent the deterioration in oxidative stability associated with tumbling (J.-H. Cheng et al., 2011). Enhancement with a brine solution with ascorbic acid or α-tocopherol has been shown to prevent lipid oxidation during storage, with no differences between muscles that were injected but not tumbled and those that were tumbled. This may also be related to the chelating ability of phosphates (J. Cheng & Ockerman, 2003).

While no studies have evaluated the effects of tumbling without a brine on oxidative stability, it would be expected that shelf-life would be compromised compared to muscles tumbled with brines containing phosphates and antioxidants. But, as Bombrun et al. (2014) found that

tumbling without salt would not negatively influence protein oxidation, at least initially, it may be that oxidative stability of muscles tumbled without a brine would be comparable to muscles without tumbling and enhancement. Further research would be necessary to determine how oxidation and flavor would be affected in muscles tumbled without a brine.

1.4.2 Tumbling Factors

Duration

Of all the factors related to tumbling that may influence final product quality, duration is undoubtedly the most studied. The duration of tumbling has been shown to affect multiple attributes including yield, proximate composition, appearance quality, and eating quality (Chow et al., 1986; Dzudie & Okubanjo, 1999; Krause et al., 1978; Moon et al., 2007). Increased yield with tumbling duration was reported in multiple instances including pork hams (Krause et al., 1978) and shoulders (Chow et al., 1986). As yield would refer to the ratio of cooked to raw weight, it can also be assumed that yield was increased with tumbling duration in goat ham (Dzudie & Okubanjo, 1999) and beef *pectoralis profundus* muscles (Moon et al., 2007), although it was not implicitly stated. This assumption would be based on an increase in proximate moisture content with tumbling duration, coupled with lower cooking losses (Dzudie & Okubanjo, 1999; Moon et al., 2007). The studies by Dzudie and Okubanjo (1999) and Moon et al. (2007) both reported a decrease in percent crude protein with tumbling duration, likely driven by brine pickup increasing the relative moisture content. Dzudie and Okubanjo (1999) reported that ash content would also increase with tumbling duration in both raw and cooked goat hams due to the incorporation salt; however, Moon et al. (2007) found comparable ash content with tumbling. This may be due to differences in the type of product or relative durations of tumbling [Dzudie and Okubanjo (1999) tumbled for 2, 4, or 6 hours, while Moon et al. (2007) tumbled for 5, 15, or 25 minutes]. It would be expected that these discrepancies would owe to differences in meat deformation and subsequent salt migration associated with tumbling duration (Daudin et al., 2016; Sharedeh et al., 2015; Siró et al., 2009). Regarding proximate composition of meat tumbled without a brine, it would be expected that ash content would be largely unaffected as no non-meat ingredients would be added. As comparable moisture contents between beef *rectus abdominis* muscles that were not tumbled

or tumbled without a brine were previously reported (Morrow et al., 2019), it may be that negligible impacts on proximate protein and lipid would be induced, at least on a percentage basis.

Regarding WHC, increasing tumbling duration has generally been shown to be beneficial. Both Dzudie and Okubanjo (1999) and Moon et al. (2007) reported decreased cooking loss with increasing tumbling duration. Further, Dzudie and Okubanjo (1999) reported an increase in WHC with tumbling duration, assessed by the centrifugation method. This is in some disagreement with Chow et al. (1986) who found that tumbling would increase WHC compared to non-tumbled, but no differences would be found between 9 and 18 hours. Likely related to this, no differences in juiciness were observed across tumbling durations (Chow et al., 1986). While Moon et al. (2007) reported cooking loss would decrease with tumbling duration (from 5 to 25 minutes), drip loss was shown to increase slightly. Despite this, juiciness increased with longer durations of tumbling (Moon et al., 2007), highlighting the importance of holistically evaluating meat and eating quality attributes. Increased juiciness with tumbling duration (from 2 to 6 hours) was corroborated by Dzudie and Okubanjo (1999). As previously discussed, short-term tumbling (20 minutes at 10 rpm) did not negatively impact water-holding and juiciness of beef *rectus abdominis* muscles (Morrow et al., 2019). However, it is presently poorly understood if longer durations of tumbling that would likely be necessary to improve tenderness would negatively impact WHC and juiciness. It would be expected that at a certain threshold these qualities would be compromised, although it may be that improved tenderness would mitigate potential detriments to juiciness to an extent.

Pattern

Tumbling processes can be applied either continuously or intermittently, although the relative advantages of either are inconsistent in the literature. Cassidy et al. (1978) found that tumbling intermittently (18 hours at 10 minutes per hour, resulting in 180 minutes of actual tumbling) would cause greater disruptions to myofibrillar structure than tumbling continuously for the same duration. Myofibers near the surface of the porcine leg muscles would have comparable clarity of striations and disruptions of the cell membrane between continuous and intermittent treatments, while intermittent tumbling would result in more disorganization of the cell nuclei. For myofibers deeper within the product, intermittent tumbling would result in a similar amount of disruption to the cell membranes compared to continuous, while greater disorganization of nuclei

and less clarity of striation would be observed (Cassidy et al., 1978). Krause et al. (1978) reported that intermittent tumbling would increase product yield of cured hams compared to a similar duration of continuous tumbling, although trained panelists evaluated them to be similar. Conversely, Gao et al. (2014) found that a similar duration of continuous tumbling would be advantageous over intermittent tumbling for porcine *longissimus thoracis et lumborum* muscles. Compared to intermittent, continuous tumbling resulted in greater product yield, higher pH, lower CIE L^* and a^* values, higher CIE b^* values, lower cooking loss, and lower shear force. The WHC as assessed by pressing loss was comparable between tumbling patterns. These findings were corroborated by trained panel, as continuous tumbling resulted in superior tenderness, flavor, juiciness, and overall acceptability (Gao et al., 2014). It is unclear why these discrepancies in product quality by tumbling method would be observed, although it is reasonable that it could result in part from intermuscular differences. While not assessing differences between tumbling patterns, Lachowicz et al. (2003) found that textural properties of individual pork leg muscles would respond differently to varying durations of massaging. It was concluded that the more inherently tender a muscle was, the shorter duration that would be required to achieve the desired outcome for tenderness (Lachowicz et al., 2003). Consequently, it may be that intermittent tumbling would be more beneficial to inherently tough muscles via greater disruptions to the product interior, although the existing literature regarding tumbling pattern, ultrastructural features, and tenderness is limited.

Rigor State

The effects of rigor state on the quality attributes of tumbled products have also had conflicting results between studies. For pork shoulder, it has been shown that yield would increase with tumbling duration, regardless of rigor state (Chow et al., 1986). Similarly, WHC, tenderness, salt and nitrite contents, and textural properties would be similar between pre- and post-rigor muscles. However, tumbled post-rigor muscle was shown to be rated poorer for flavor and juiciness compared to pre-rigor (Chow et al., 1986). Mostly in agreement, Dzudie and Okubanjo (1999) found that rigor state would not affect raw or cooked proximate composition, pH, and shear force of tumbled goat hams. However, pre-rigor muscle did exhibit greater WHC but lower cooking loss. As improved WHC but worse cooking loss would be expected to cancel one another

out to a certain extent, juiciness was unaffected by rigor state (Dzudie & Okubanjo, 1999). No previous studies have applied tumbling to pre-rigor muscle in the absence of a brine solution. However, it may be expected that without the addition of salt and other functional ingredients, the benefits of utilizing pre-rigor muscle would not be found. For instance, the addition of salt to pre-rigor beef *semimembranosus* muscles has been shown to reduce the extent of glycolysis, resulting in greater ultimate pH and protein functionality (Claus & Sørheim, 2006). However, in pre-rigor beef *semitendinosus* muscles without the addition of salt, poorer protein functionality has been reported (Farouk & Swan, 1998). Consequently, it may not be recommended to utilize pre-rigor muscle for tumbling without the incorporation of a brine solution, although this postulation could warrant further research. Rather, the time at which tumbling is applied to post-rigor muscle would likely be more influential on tenderization and other quality attributes, considering the profound changes in proteolytic enzyme activity with aging (Boehm et al., 1998; Y. H. B. Kim et al., 2018; Koohmaraie & Geesink, 2006; Stolowski et al., 2006; W. G. Zhang et al., 2006). However, this too has not been previously studied.

Ingredients

Numerous studies have been conducted regarding the ingredients used in the brine or marinade of tumbled product (Bombrun et al., 2014; Cassidy et al., 1978; J. Cheng & Ockerman, 2003; J.-H. Cheng et al., 2011; Garmyn et al., 2020; Krause et al., 1978). Krause et al. (1978) found that brine including sodium tripolyphosphate would improve external appearance and color, sliceability, taste, aroma, and yield of cured pork shoulders. Both sodium tripolyphosphate and sodium bicarbonate have been shown to influence quality parameters of tumbled beef muscles (Garmyn et al., 2020). Relative to non-enhanced muscles, pH would be increased, while brines including sodium tripolyphosphate would be more advantageous at reducing cooking loss than sodium bicarbonate. Despite this, sodium bicarbonate enhancement was more effective at improving consumer liking of tenderness and juiciness compared to sodium tripolyphosphate, though flavor, overall liking, and consumer satisfaction would be comparable (Garmyn et al., 2020). As previously discussed, sodium tripolyphosphate may also be beneficial at reducing lipid oxidation during storage of precooked beef roasts (J. Cheng & Ockerman, 2003). Similarly, brines may incorporate antioxidative compounds such as ascorbic acid and α-tocopherol as a strategy to

inhibit oxidation induced through tumbling (J.-H. Cheng et al., 2011). It would also be expected that the effectiveness of functional ingredients in influencing product quality would differ by incorporation method. For example, Molina et al. (2005) found differences in product quality of beef chuck muscles by brine incorporation method (vacuum-tumbling, needle-pumping, and static marination) and muscle type, despite identical brine compositions. As discussed previously, this would likely owe to differences in how individual muscles respond to physically disruptive treatments (Lachowicz et al., 2003), as well as diffusion of functional ingredients into the product (Bombrun et al., 2014; Sharedeh et al., 2015; Siró et al., 2009). Considering tumbling without a brine would not incorporate any added functional ingredients, it may be expected that some quality and oxidative stability attribute would be negatively affected to an extent. However, considering oxidative stability may be extended in vacuum packaging (Y. H. B. Kim et al., 2018; Lorenzo & Gómez, 2012; Ramanathan et al., 2011), it is currently unclear to what extent oxidative stability may be compromised in products tumbled in anaerobic packaging.

Others

Other factors that may be relevant to the quality of tumbled product would be the presence of a vacuum within the drum (Arboix, 2004; Ghavimi et al., 1986; Solomon et al., 1980), tumbling speed (Lin et al., 1990), drop distance, and tumbler fill. The presence of a vacuum in the tumbler has been shown to improve the incorporation of brine through negative vacuum pressure, as well as prevent the formation of air bubbles on the meat surface (Arboix, 2004; Bosse (née Danz) et al., 2018; Solomon et al., 1980). Tumbling in a vacuum bag, however, has not been previously studied. However, it may be expected that the use of a vacuum bag as a protective barrier would prevent the formation of exudate on the muscle surface. While the literature regarding tumbler speed is limited, Lin et al. (1990) found that speed would affect textural attributes of pork hams. This finding was attributed to higher speeds inducing greater disruptions to myofibrillar structure. While it may be expected that the extent of physical disruptions would differ between tumblers by altering drop distance and thus product deformation, this factor, along with tumbler fill, has received little attention. Recently, Daudin et al. (2016) explored the use of a tumbling simulator for laboratory application, which could replicate the mechanical forces that would differ by varying drop distances and tumbler fill.

1.5 Objectives

This literature review has highlighted that while tumbling is well-studied process for processed meat applications, there is presently limited information regarding the potential applications in fresh beef without brine inclusion. Improvements to product tenderness through tumbling would be through the combined impacts of physical disruptions and myofibrillar swelling. For tumbling without brine inclusion, it may be expected that similar disruptions to the micro- and ultrastructure of the meat would be induced; however, limited myofibrillar swelling would be induced. Accordingly, how tenderness may be affected is not well-understood. The driving factor which influences the quality of tumbled product is generally considered as the duration (Chow et al., 1986; Dzudie & Okubanjo, 1999; Krause et al., 1978; Moon et al., 2007), thus differences owing to this factor will be examined in the following chapters. Considering the discrepancies in the effectiveness of tumbling by individual beef muscle (Garmyn et al., 2020; Lachowicz et al., 2003; Molina et al., 2005), as well as the known intermuscular differences in aging potential (Anderson et al., 2012; Juárez et al., 2010; Nair et al., 2019; Stolowski et al., 2006), the process will be evaluated in various beef cuts. While tumbling without brine inclusion may not result in myofibrillar swelling, it is reasonable to postulate that the physical disruptions caused could enhance the activity of proteolytic enzymes relevant to the tenderization process (Dang et al., 2022). The objective of this dissertation is to examine the application of tumbling without brine inclusion followed by further postmortem aging as a method to improve quality, palatability, and proteolytic attributes of beef *longissimus lumborum*, *semitendinosus*, *gluteus medius*, *biceps femoris*, and *tensor fasciae latae* muscles.

1.6 References

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2012). *Principles of Meat Science* (5th ed.). Kendall Hunt Publishing Company.

Adhikari, K., Iv, E. C., Miller, R., Vázquez-Araújo, L., Bhumiratana, N., & Philip, C. (2011). Development of a Lexicon for Beef Flavor in Intact Muscle. *Journal of Sensory Studies*, *26*(6), 413–420. https://doi.org/10.1111/j.1745-459X.2011.00356.x

Alarcon-Rojo, A. D., Carrillo-Lopez, L. M., Reyes-Villagrana, R., Huerta-Jiménez, M., & Garcia-Galicia, I. A. (2019). Ultrasound and meat quality: A review. *Ultrasonics Sonochemistry*, *55*, 369–382. https://doi.org/10.1016/j.ultsonch.2018.09.016

Alvarenga, T. I. R. C., Palendeng, M., Thennadil, S., McGilchrist, P., Cafe, L. M., Almeida, A. K., & Hopkins, D. L. (2021). Is meat from cull cows tougher? *Meat Science*, *177*, 108498. https://doi.org/10.1016/j.meatsci.2021.108498

AMSA. (2015). *Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat*. American Meat Science Association.

Anderson, M. J., Lonergan, S. M., Fedler, C. A., Prusa, K. J., Binning, J. M., & Huff-Lonergan, E. (2012). Profile of biochemical traits influencing tenderness of muscles from the beef round. *Meat Science*, *91*(3), 247–254. https://doi.org/10.1016/j.meatsci.2012.01.022

Arboix, J. A. (2004). Ham production/cooked ham. In *Encyclopedia of Meat Sciences* (W.K. Jensen (Ed.), pp. 262–267). Oxford: Academic Press.

Battaglia, C., Vilella, G. F., Bernardo, A. P. S., Gomes, C. L., Biase, A. G., Albertini, T. Z., & Pflanzer, S. B. (2020). Comparison of methods for measuring shear force and sarcomere length and their relationship with sensorial tenderness of longissimus muscle in beef. *Journal of Texture Studies*, *51*(2), 252–262. https://doi.org/10.1111/jtxs.12473

Bekhit, A. E.-D. A., Carne, A., Ha, M., & Franks, P. (2014). Physical Interventions to Manipulate Texture and Tenderness of Fresh Meat: A Review. *International Journal of Food Properties*, *17*(2), 433–453. https://doi.org/10.1080/10942912.2011.642442

Belew, J. B., Brooks, J. C., McKenna, D. R., & Savell, J. W. (2003). Warner–Bratzler shear evaluations of 40 bovine muscles. *Meat Science*, *64*(4), 507–512. https://doi.org/10.1016/S0309-1740(02)00242-5

Boehm, M. L., Kendall, T. L., Thompson, V. F., & Goll, D. E. (1998). Changes in the calpains and calpastatin during postmortem storage of bovine muscle. *Journal of Animal Science*, *76*(9), 2415. https://doi.org/10.2527/1998.7692415x

Boles, J. A., & Shand, P. J. (2002). Tumbling regime effects on the processing characteristics and tenderness of cooked roast beef. *Journal of Muscle Foods*, *13*(1), 25–35. https://doi.org/10.1111/j.1745-4573.2002.tb00318.x

Bombrun, L., Gatellier, P., Carlier, M., & Kondjoyan, A. (2014). The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking. *Meat Science*, *96*(1), 5–13. https://doi.org/10.1016/j.meatsci.2013.06.029

Bosse (née Danz), R., Müller, A., Gibis, M., Weiss, A., Schmidt, H., & Weiss, J. (2018). Recent advances in cured raw ham manufacture. *Critical Reviews in Food Science and Nutrition*, *58*(4), 610–630. https://doi.org/10.1080/10408398.2016.1208634

Bratcher, C. L., Johnson, D. D., Littell, R. C., & Gwartney, B. L. (2005). The effects of quality grade, aging, and location within muscle on Warner–Bratzler shear force in beef muscles of locomotion. *Meat Science*, *70*(2), 279–284. https://doi.org/10.1016/j.meatsci.2005.01.013

Campbell, R. L., & Davies, P. L. (2012). Structure-function relationships in calpains. *Biochemical Journal*, *447*(3), 335–351. Scopus. https://doi.org/10.1042/BJ20120921

Cassidy, R. O., Ockerman, H. W., Krol, B., Roon, P. S., Plimpton, R. F., & Cahill, V. R. (1978). Effect of Tumbling Method, Phosphate Level and Final Cook Temperature on Histological Characteristics of Tumbled Porcine Muscle Tissue. *Journal of Food Science*, *43*(5), 1514–1518. https://doi.org/10.1111/j.1365-2621.1978.tb02532.x

Chang, H., Wang, Q., Xu, X., Li, C., Huang, M., Zhou, G., & Dai, Y. (2011). Effect of Heat-Induced Changes of Connective Tissue and Collagen on Meat Texture Properties of Beef Semitendinosus Muscle. *International Journal of Food Properties*, *14*(2), 381–396. https://doi.org/10.1080/10942910903207728

Chen, X., Tume, R. K., Xu, X., & Zhou, G. (2017). Solubilization of myofibrillar proteins in water or low ionic strength media: Classical techniques, basic principles, and novel functionalities. *Critical Reviews in Food Science and Nutrition*, *57*(15), 3260–3280. https://doi.org/10.1080/10408398.2015.1110111

Cheng, C.-S., & Parrish, F. C. (1978). Molecular Changes in the Salt-Soluble Myofibrillar Proteins of Bovine Muscle. *Journal of Food Science*, *43*(2), 461–463. https://doi.org/10.1111/j.1365-2621.1978.tb02330.x

Cheng, J., & Ockerman, H. W. (2003). Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. *Meat Science*, *65*(4), 1353–1359. https://doi.org/10.1016/S0309-1740(03)00057-3

Cheng, J.-H., Wang, S.-T., Sun, Y.-M., & Ockerman, H. W. (2011). Effect of phosphate, ascorbic acid and α-tocopherol injected at one-location with tumbling on quality of roast beef. *Meat Science*, *87*(3), 223–228. https://doi.org/10.1016/j.meatsci.2010.10.013

Chow, H.-M., Ockerman, H. W., Cahill, V. R., & Parrett, N. A. (1986). Evaluation of Cured, Canned Pork Shoulder Tissue Produced by Electrical Stimulation, Hot Processing and Tumbling. *Journal of Food Science*, *51*(2), 288–291. https://doi.org/10.1111/j.1365-2621.1986.tb11111.x

Chun, C. K. Y., Wu, W., Welter, A. A., O'Quinn, T. G., Magnin-Bissel, G., Boyle, D. L., & Chao, M. D. (2020). A preliminary investigation of the contribution of different tenderness factors to beef loin, tri-tip and heel tenderness. *Meat Science*, *170*, 108247. https://doi.org/10.1016/j.meatsci.2020.108247

Claus, J. R., & Sørheim, O. (2006). Preserving pre-rigor meat functionality for beef patty production. *Meat Science*, *73*(2), 287–294. https://doi.org/10.1016/j.meatsci.2005.12.004

Cruzen, S. M., Paulino, P. V. R., Lonergan, S. M., & Huff-Lonergan, E. (2014). Postmortem proteolysis in three muscles from growing and mature beef cattle. *Meat Science*, *96*(2, Part A), 854–861. https://doi.org/10.1016/j.meatsci.2013.09.021

Dang, D. S., Stafford, C. D., Taylor, M. J., Buhler, J. F., Thornton, K. J., & Matarneh, S. K. (2022). Ultrasonication of beef improves calpain-1 autolysis and caspase-3 activity by elevating cytosolic calcium and inducing mitochondrial dysfunction. *Meat Science*, *183*, 108646. https://doi.org/10.1016/j.meatsci.2021.108646

Daudin, J.-D., Sharedeh, D., Favier, R., Portanguen, S., Auberger, J.-M., & Kondjoyan, A. (2016). Design of a new laboratory tumbling simulator for chunked meat: Analysis, reproduction and measurement of mechanical treatment. *Journal of Food Engineering*, *170*, 83–91. https://doi.org/10.1016/j.jfoodeng.2015.09.020

deMan, J. M., Finley, J. W., Hurst, W. J., & Lee, C. Y. (2018). *Principles of Food Chemistry*. Springer International Publishing. https://doi.org/10.1007/978-3-319-63607-8

Dzudie, T., & Okubanjo, A. (1999). Effects of rigor state and tumbling time on quality of goat hams. *Journal of Food Engineering*, *42*(2), 103–107. https://doi.org/10.1016/S0260-8774(99)00097-7

Ertbjerg, P., Larsen, L. M., & Moller, A. J. (1999). Effect of prerigor lactic acid treatment on lysosomal enzyme release in bovine muscle. *Journal of the Science of Food and Agriculture*. https://scholar.google.com/scholar_lookup?title=Effect+of+prerigor+lactic+acid+treatment+on+lysosomal+enzyme+release+in+bovine+muscle&author=Ertbjerg%2C+P.&publication_year=1999

Ertbjerg, P., & Puolanne, E. (2017). Muscle structure, sarcomere length and influences on meat quality: A review. *Meat Science*, *132*, 139–152. https://doi.org/10.1016/j.meatsci.2017.04.261

Farouk, M. M., Mustafa, N. Md., Wu, G., & Krsinic, G. (2012). The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with ageing. *Meat Science*, *90*(3), 670–677. https://doi.org/10.1016/j.meatsci.2011.10.012

Farouk, M. M., & Swan, J. E. (1998). Effect of rigor temperature and frozen storage on functional properties of hot-boned manufacturing beef. *Meat Science*, *49*(2), 233–247. https://doi.org/10.1016/S0309-1740(97)00134-4

Foraker, B. A., Gredell, D. A., Legako, J. F., Stevens, R. D., Tatum, J. D., Belk, K. E., & Woerner, D. R. (2020). Flavor, Tenderness, and Related Chemical Changes of Aged Beef Strip Loins. *Meat and Muscle Biology*, *4*(1). https://doi.org/10.22175/mmb.11115

Gao, T., Li, J., Zhang, L., Jiang, Y., Ma, R., Song, L., Gao, F., & Zhou, G. (2014). Effect of Different Tumbling Marination Treatments on the Quality Characteristics of Prepared Pork Chops. *Asian-Australasian Journal of Animal Sciences*, *28*(2), 260–267. https://doi.org/10.5713/ajas.14.0511

Garmyn, A., Hardcastle, N., Bendele, C., Polkinghorne, R., & Miller, M. (2020). Exploring Consumer Palatability of Australian Beef Fajita Meat Enhanced with Phosphate or Sodium Bicarbonate. *Foods*, *9*(2), 177. https://doi.org/10.3390/foods9020177

Ghavimi, B., Rogers, R. W., Althen, T. G., & Ammerman, G. R. (1986). Effects of Nonvacuum, Vacuum and Nitrogen Back-Flush Tumbling on Various Characteristics of Restructured Cured Beef. *Journal of Food Science*, *51*(5), 1166–1168. https://doi.org/10.1111/j.1365-2621.1986.tb13074.x

Goll, D. E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The Calpain System. *Physiological Reviews*, *83*(3), 731–801. https://doi.org/10.1152/physrev.00029.2002

Gonzalez, J. M., & Phelps, K. J. (2018). United States beef quality as chronicled by the National Beef Quality Audits, Beef Consumer Satisfaction Projects, and National Beef Tenderness Surveys—A review. *Asian-Australasian Journal of Animal Sciences*, *31*(7), 1036–1042. https://doi.org/10.5713/ajas.18.0199

Gruber, S. L., Tatum, J. D., Scanga, J. A., Chapman, P. L., Smith, G. C., & Belk, K. E. (2006). Effects of postmortem aging and USDA quality grade on Warner-Bratzler shear force values of seventeen individual beef muscles1. *Journal of Animal Science*, *84*(12), 3387–3396. https://doi.org/10.2527/jas.2006-194

Guelker, M. R., Haneklaus, A. N., Brooks, J. C., Carr, C. C., Delmore, R. J., Griffin, D. B., Hale, D. S., Harris, K. B., Mafi, G. G., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Martin, J. N., Miller, R. K., Raines, C. R., VanOverbeke, D. L., Vedral, L. L., Wasser, B. E., & Savell, J. W. (2013). National Beef Tenderness Survey–2010: Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. *Journal of Animal Science*, *91*(2), 1005–1014. https://doi.org/10.2527/jas.2012-5785

Henchion, M. M., McCarthy, M., & Resconi, V. C. (2017). Beef quality attributes: A systematic review of consumer perspectives. *Meat Science*, *128*, 1–7. https://doi.org/10.1016/j.meatsci.2017.01.006

Hocquette, J.-F., Botreau, R., Picard, B., Jacquet, A., Pethick, D. W., & Scollan, N. D. (2012). Opportunities for predicting and manipulating beef quality. *Meat Science*, *92*(3), 197–209. https://doi.org/10.1016/j.meatsci.2012.04.007

Horgan, D. J., Jones, P. N., King, N. L., Kurth, L. B., & Kuypers, R. (1991). The relationship between animal age and the thermal stability and cross-link content of collagen from five goat muscles. *Meat Science*, *29*(3), 251–262. https://doi.org/10.1016/0309-1740(91)90054-T

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle—Lessons on mechanisms of meat tenderization. *Meat Science*, *86*(1), 184–195. https://doi.org/10.1016/j.meatsci.2010.05.004

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, *71*(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, *98*(3), 520–532. https://doi.org/10.1016/j.meatsci.2014.05.022

Hultin, H. O., Feng, Y., & Stanley, D. W. (1995). A Re-Examination of Muscle Protein Solubility. *Journal of Muscle Foods*, *6*(2), 91–107. https://doi.org/10.1111/j.1745-4573.1995.tb00560.x

Ismail, I., Hwang, Y.-H., & Joo, S.-T. (2020). Low-temperature and long-time heating regimes on non-volatile compound and taste traits of beef assessed by the electronic tongue system. *Food Chemistry*, *320*, 126656. https://doi.org/10.1016/j.foodchem.2020.126656

Jeremiah, L. E., Gibson, L. L., Aalhus, J. L., & Dugan, M. E. R. (2003). Assessment of palatability attributes of the major beef muscles. *Meat Science*, *65*(3), 949–958. https://doi.org/10.1016/S0309-1740(02)00307-8

Juárez, M., Larsen, I. L., Gibson, L. L., Robertson, W. M., Dugan, M. E. R., Aldai, N., & Aalhus, J. L. (2010). Extended ageing time and temperature effects on quality of sub-primal cuts of boxed beef. *Canadian Journal of Animal Science*, *90*(3), 361–370. https://doi.org/10.4141/CJAS09079

Katsaras, K., & Budras, K.-D. (1993). The Relationship of the Microstructure of Cooked Ham to its Properties and Quality. *LWT - Food Science and Technology*, *26*(3), 229–234. https://doi.org/10.1006/fstl.1993.1050

Kim, H.-W., Kim, J.-H., Seo, J.-K., Setyabrata, D., & Kim, Y. H. B. (2018). Effects of aging/freezing sequence and freezing rate on meat quality and oxidative stability of pork loins. *Meat Science*, *139*, 162–170. https://doi.org/10.1016/j.meatsci.2018.01.024

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. *Meat Science*, *144*, 74–90. https://doi.org/10.1016/j.meatsci.2018.04.031

Koohmaraie, M. (1992). The role of Ca2+-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. *Biochimie*, *74*(3), 239–245. https://doi.org/10.1016/0300-9084(92)90122-U

Koohmaraie, M., & Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, *74*(1), 34–43. https://doi.org/10.1016/j.meatsci.2006.04.025

Krause, R. J., Ockerman, H. W., Krol, B., Moerman, P. C., & Plimpton, R. F. (1978). Influence of Tumbling, Tumbling Time, Trim and Sodium Tripolyphosphate on Quality and Yield of Cured Hams. *Journal of Food Science*, *43*(3), 853–855. https://doi.org/10.1111/j.1365-2621.1978.tb02438.x

Lachowicz, K., Sobczak, M., Gajowiecki, L., & Żych, A. (2003). Effects of massaging time on texture, rheological properties, and structure of three pork ham muscles. *Meat Science*, *63*(2), 225–233. https://doi.org/10.1016/s0309-1740(02)00073-6

Lametsch, R., Roepstorff, P., Møller, H. S., & Bendixen, E. (2004). Identification of myofibrillar substrates for μ-calpain. *Meat Science*, *68*(4), 515–521. https://doi.org/10.1016/j.meatsci.2004.03.018

Lee, H. J., Choe, J., Kim, M., Kim, H. C., Yoon, J. W., Oh, S. W., & Jo, C. (2019). Role of moisture evaporation in the taste attributes of dry- and wet-aged beef determined by chemical and electronic tongue analyses. *Meat Science*, *151*, 82–88. https://doi.org/10.1016/j.meatsci.2019.02.001

Legako, J. F., Brooks, J. C., O'Quinn, T. G., Hagan, T. D. J., Polkinghorne, R., Farmer, L. J., & Miller, M. F. (2015). Consumer palatability scores and volatile beef flavor compounds of five USDA quality grades and four muscles. *Meat Science*, *100*, 291–300. https://doi.org/10.1016/j.meatsci.2014.10.026

Lepetit, J. (2008). Collagen contribution to meat toughness: Theoretical aspects. *Meat Science*, *80*(4), 960–967. https://doi.org/10.1016/j.meatsci.2008.06.016

Li, C. B., Zhou, G. H., & Xu, X. L. (2010). Dynamical Changes of Beef Intramuscular Connective Tissue and Muscle Fiber during Heating and their Effects on Beef Shear Force. *Food and Bioprocess Technology*, *3*(4), 521–527. https://doi.org/10.1007/s11947-008-0117-3

Lin, G. C., Mittal, G. S., & Barbut, S. (1990). Effects of tumbling speed and cumulative revolutions on restructured hams' quality. *Journal of Food Processing and Preservation*, *14*(6), 467–479. https://doi.org/10.1111/j.1745-4549.1990.tb00147.x

Lonergan, S. M., Huff-Lonergan, E., Wiegand, B. R., & Kriese-Anderson, L. A. (2001). Postmortem Proteolysis and Tenderization of Top Loin Steaks from Brangus Cattle1. *Journal of Muscle Foods*, *12*(2), 121–136. https://doi.org/10.1111/j.1745-4573.2001.tb00304.x

Lorenzo, J. M., & Gómez, M. (2012). Shelf life of fresh foal meat under MAP, overwrap and vacuum packaging conditions. *Meat Science*, *92*(4), 610–618. https://doi.org/10.1016/j.meatsci.2012.06.008

Martinez, H. A., Arnold, A. N., Brooks, J. C., Carr, C. C., Gehring, K. B., Griffin, D. B., Hale, D. S., Mafi, G. G., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Miller, R. K., VanOverbeke, D. L., Wasser, B. E., & Savell, J. W. (2017). National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. *Meat and Muscle Biology*, *1*(1), 138. https://doi.org/10.22175/mmb2017.05.0028

Maughan, C., Tansawat, R., Cornforth, D., Ward, R., & Martini, S. (2012). Development of a beef flavor lexicon and its application to compare the flavor profile and consumer acceptance of rib steaks from grass- or grain-fed cattle. *Meat Science*, *90*(1), 116–121. https://doi.org/10.1016/j.meatsci.2011.06.006

McCormick, R. (1999). Extracellular modifications to muscle collagen: Implications for meat quality. *Poultry Science*, *78*(5), 785–791. https://doi.org/10.1093/ps/78.5.785

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, *79*(12), 3062. https://doi.org/10.2527/2001.79123062x

Miller, R. (2020). Drivers of Consumer Liking for Beef, Pork, and Lamb: A Review. *Foods*, *9*(4). https://doi.org/10.3390/foods9040428

Molina, M. E., Johnson, D. D., West, R. L., & Gwartney, B. L. (2005). Enhancing palatability traits in beef chuck muscles. *Meat Science*, *71*(1), 52–61. https://doi.org/10.1016/j.meatsci.2005.04.004

Moon, S. S., Yang, H. S., Park, G. B., & Joo, S. T. (2007). The Effect of Tumbling Time on the Quality and Binding Ability of Restructured Beef M. Pectoralis profundus with Alginate Binder. *Asian-Australasian Journal of Animal Sciences*, *20*(3), 418–423. https://doi.org/10.5713/ajas.2007.418

Morrow, S. J., Garmyn, A. J., Hardcastle, N. C., Brooks, J. C., & Miller, M. F. (2019). The Effects of Enhancement Strategies of Beef Flanks on Composition and Consumer Palatability Characteristics. *Meat and Muscle Biology*, *3*(1), 457. https://doi.org/10.22175/mmb2019.07.0030

Nair, M. N., Canto, A. C. V. C. S., Rentfrow, G., & Suman, S. P. (2019). Muscle-specific effect of aging on beef tenderness. *LWT*, *100*, 250–252. https://doi.org/10.1016/j.lwt.2018.10.038

N'Gatta, K. C. A., Kondjoyan, A., Favier, R., Rouel, J., Vénien, A., Astruc, T., Gruffat, D., & Mirade, P.-S. (2021). Impact of Tumbling Process on the Toughness and Structure of Raw Beef Meat Pieces. *Foods*, *10*(11), 2802. https://doi.org/10.3390/foods10112802

Nishimura, T., Liu, A., Hattori, A., & Takahashi, K. (1998). Changes in mechanical strength of intramuscular connective tissue during postmortem aging of beef. *Journal of Animal Science*, *76*(2), 528. https://doi.org/10.2527/1998.762528x

O'Quinn, T. G., Legako, J. F., Brooks, J. C., & Miller, M. F. (2018). Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience1. *Translational Animal Science*, *2*(1), 26–36. https://doi.org/10.1093/tas/txx008

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes—A review. *Meat Science*, *89*(2), 111–124. https://doi.org/10.1016/j.meatsci.2011.04.007

Peña-Gonzalez, E., Alarcon-Rojo, A. D., Garcia-Galicia, I., Carrillo-Lopez, L., & Huerta-Jimenez, M. (2019). Ultrasound as a potential process to tenderize beef: Sensory and technological parameters. *Ultrasonics Sonochemistry*, *53*, 134–141. https://doi.org/10.1016/j.ultsonch.2018.12.045

Penny, I. F., & Dransfield, E. (1979). Relationship between toughness and troponin T in conditioned beef. *Meat Science*, *3*(2), 135–141. https://doi.org/10.1016/0309-1740(79)90015-9

Pietrasik, Z., & Shand, P. J. (2004). Effect of blade tenderization and tumbling time on the processing characteristics and tenderness of injected cooked roast beef. *Meat Science*, *66*(4), 871–879. https://doi.org/10.1016/j.meatsci.2003.08.009

Ponce, J., Brooks, J. C., & Legako, J. F. (2019). Consumer Liking and Descriptive Flavor Attributes of M. Longissimus Lumborum and M. Gluteus Medius Beef Steaks Held in Varied Packaging Systems. *Meat and Muscle Biology*, *3*(1). https://doi.org/10.22175/mmb2018.12.0041

Powell, T. H., Dikeman, M. E., & Hunt, M. C. (2000). Tenderness and collagen composition of beef semitendinosus roasts cooked by conventional convective cooking and modeled, multi-stage, convective cooking. *Meat Science*, *55*(4), 421–425. https://doi.org/10.1016/S0309-1740(99)00171-0

Puolanne, E., & Peltonen, J. (2013). The effects of high salt and low pH on the water-holding of meat. *Meat Science*, *93*(2), 167–170. https://doi.org/10.1016/j.meatsci.2012.08.015

Purslow, P. P., Oiseth, S., Hughes, J., & Warner, R. D. (2016). The structural basis of cooking loss in beef: Variations with temperature and ageing. *Food Research International*, *89*, 739–748. https://doi.org/10.1016/j.foodres.2016.09.010

Ramanathan, R., Mancini, R. A., & Dady, G. A. (2011). Effects of pyruvate, succinate, and lactate enhancement on beef longissimus raw color. *Meat Science*, *88*(3), 424–428. https://doi.org/10.1016/j.meatsci.2011.01.021

Rhee, M. S., Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2004). Variation in palatability and biochemical traits within and among eleven beef muscles. *Journal of Animal Science*, *82*(2), 534–550. https://doi.org/10.2527/2004.822534x

Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science & Technology*, *13*(12), 400–421. https://doi.org/10.1016/S0924-2244(02)00188-7

Setyabrata, D., & Kim, Y. H. B. (2019). Impacts of aging/freezing sequence on microstructure, protein degradation and physico-chemical properties of beef muscles. *Meat Science*, *151*, 64–74. https://doi.org/10.1016/j.meatsci.2019.01.007

Setyabrata, D., Tuell, J. R., & Kim, Y. H. B. (2019). The Effect of Aging/Freezing Sequence and Freezing Rate on Quality Attributes of Beef Loins. *Meat and Muscle Biology*, *3*(1), 488. https://doi.org/10.22175/mmb2019.08.0034

Shackelford, S. D., Morgan, J. B., Cross, H. R., & Savell, J. W. (1991). Identification of Threshold Levels for Warner-Bratzler Shear Force in Beef Top Loin Steaks. *Journal of Muscle Foods*, *2*(4), 289–296. https://doi.org/10.1111/j.1745-4573.1991.tb00461.x

Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1999a). Tenderness classification of beef: II. Design and analysis of a system to measure beef longissimus shear force under commercial processing conditions. *Journal of Animal Science*, *77*(6), 1474–1481. https://doi.org/10.2527/1999.7761474x

Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1999b). Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. *Journal of Animal Science*, *77*(10), 2693–2699. https://doi.org/10.2527/1999.77102693x

Shackelford, S. D., Wheeler, T. L., Meade, M. K., Reagan, J. O., Byrnes, B. L., & Koohmaraie, M. (2001). Consumer impressions of Tender Select beef. *Journal of Animal Science*, *79*(10), 2605. https://doi.org/10.2527/2001.79102605x

Sharedeh, D., Mirade, P.-S., Venien, A., & Daudin, J.-D. (2015). Analysis of salt penetration enhancement in meat tissue by mechanical treatment using a tumbling simulator. *Journal of Food Engineering*, *166*, 377–383. https://doi.org/10.1016/j.jfoodeng.2015.06.023

Siró, I., Vén, Cs., Balla, Cs., Jónás, G., Zeke, I., & Friedrich, L. (2009). Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. *Journal of Food Engineering*, *91*(2), 353–362. https://doi.org/10.1016/j.jfoodeng.2008.09.015

Smulders, F. J. M., Marsh, B. B., Swartz, D. R., Russell, R. L., & Hoenecke, M. E. (1990). Beef tenderness and sarcomere length. *Meat Science*, *28*(4), 349–363. https://doi.org/10.1016/0309-1740(90)90048-B

Solomon, L. W., Norton, H. W., & Schmidt, G. R. (1980). Effect of Vacuum and Rigor Condition on Cure Absorption in Tumbled Porcine Muscles. *Journal of Food Science*, *45*(3), 438–440. https://doi.org/10.1111/j.1365-2621.1980.tb04069.x

Stadnik, J., Dolatowski, Z. J., & Baranowska, H. M. (2008). Effect of ultrasound treatment on water holding properties and microstructure of beef (m. Semimembranosus) during ageing. *LWT - Food Science and Technology*, *41*(10), 2151–2158. https://doi.org/10.1016/j.lwt.2007.12.003

Stolowski, G. D., Baird, B. E., Miller, R. K., Savell, J. W., Sams, A. R., Taylor, J. F., Sanders, J. O., & Smith, S. B. (2006). Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. *Meat Science*, *73*(3), 475–483. https://doi.org/10.1016/j.meatsci.2006.01.006

Sullivan, G. A., & Calkins, C. R. (2011). Ranking Beef Muscles for Warner–Bratzler Shear Force and Trained Sensory Panel Ratings from Published Literature. *Journal of Food Quality*, *34*(3), 195–203. https://doi.org/10.1111/j.1745-4557.2011.00386.x

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science*, *73*(5), 1351–1367. https://doi.org/10.2527/1995.7351351x

Torrescano, G., Sánchez-Escalante, A., Giménez, B., Roncalés, P., & Beltrán, J. A. (2003). Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics. *Meat Science*, *64*(1), 85–91. https://doi.org/10.1016/S0309-1740(02)00165-1

Tyszkiewicz, I., Kłossowska, B. M., Wieczorek, U., & Jakubiec-Puka, A. (1997). Mechanical Tenderisation of Pork Meat: Protein and Water Release due to Tissue Damage. *Journal of the Science of Food and Agriculture*, *73*(2), 179–185. https://doi.org/10.1002/(SICI)1097-0010(199702)73:2<179::AID-JSFA699>3.0.CO;2-#

Voges, K. L., Mason, C. L., Brooks, J. C., Delmore, R. J., Griffin, D. B., Hale, D. S., Henning, W. R., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Miller, R. K., Morgan, J. B., Baird, B. E., Gwartney, B. L., & Savell, J. W. (2007). National beef tenderness survey – 2006: Assessment of Warner–Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. *Meat Science*, *77*(3), 357–364. https://doi.org/10.1016/j.meatsci.2007.03.024

Wang, L., Li, J., Teng, S., Zhang, W., Purslow, P. P., & Zhang, R. (2022). Changes in collagen properties and cathepsin activity of beef M. semitendinosus by the application of ultrasound during post-mortem aging. *Meat Science*, *185*, 108718. https://doi.org/10.1016/j.meatsci.2021.108718

Warner, R. (2017). Chapter 14—The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. In F. Toldra´ (Ed.), *Lawrie´s Meat Science (Eighth Edition)* (pp. 419–459). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100694-8.00014-5

Warner, R., Miller, R., Ha, M., Wheeler, T. L., Dunshea, F., Li, X., Vaskoska, R., & Purslow, P. (2020). Meat Tenderness: Underlying Mechanisms, Instrumental Measurement, and Sensory Assessment. *Meat and Muscle Biology*, *4*(2). https://doi.org/10.22175/mmb.10489

Watson, R., Gee, A., Polkinghorne, R., & Porter, M. (2008). Consumer assessment of eating quality—Development of protocols for Meat Standards Australia (MSA) testing. *Australian Journal of Experimental Agriculture*, *48*(11), 1360. https://doi.org/10.1071/EA07176

Weaver, A. D., Bowker, B. C., & Gerrard, D. E. (2008). Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. *Journal of Animal Science*, *86*(8), 1925–1932. https://doi.org/10.2527/jas.2007-0741

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2002). Technical note: Sampling methodology for relating sarcomere length, collagen concentration, and the extent of postmortem proteolysis to beef and pork longissimus tenderness. *Journal of Animal Science*, *80*(4), 982–987. https://doi.org/10.2527/2002.804982x

Whipple, G., & Koohmaraie, M. (1992). Effects of lamb age, muscle type, and 24-hour activity of endogenous proteinases on postmortem proteolysis. *Journal of Animal Science*, *70*(3), 798–804. https://doi.org/10.2527/1992.703798x

Winger, R. J., & Hagyard, C. J. (1994). Juiciness—Its importance and some contributing factors. In A. M. Pearson & T. R. Dutson (Eds.), *Quality Attributes and their Measurement in Meat, Poultry and Fish Products* (pp. 94–124). Springer US. https://doi.org/10.1007/978-1-4615-2167-9_4

Zhang, W. G., Lonergan, S. M., Gardner, M. A., & Huff-Lonergan, E. (2006). Contribution of postmortem changes of integrin, desmin and μ-calpain to variation in water holding capacity of pork. *Meat Science*, *74*(3), 578–585. https://doi.org/10.1016/j.meatsci.2006.05.008

Zhang, X., Zhang, Y., Meng, Q., Li, N., & Ren, L. (2015). Evaluation of Beef by Electronic Tongue System TS-5000Z: Flavor Assessment, Recognition and Chemical Compositions According to Its Correlation with Flavor. *PLOS ONE*, *10*(9), e0137807. https://doi.org/10.1371/journal.pone.0137807

CHAPTER 2. CAN TUMBLING WITHOUT BRINE IMPROVE TENDERNESS AND PROTEOLYSIS OF BEEF LOIN MUSCLES?

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2.1 Abstract

Tumbling of intact muscle foods has been widely applied to processed meats using brine solution. However, the use of tumbling without brine on fresh beef muscles has not been fully examined. Therefore, this study aimed to evaluate fresh beef tumbling on meat quality and proteolytic features of loin (*longissimus lumborum*) muscles. Moreover, interactions with the duration of postmortem aging were investigated. Loins (n=9) at 7d postmortem were sectioned and allocated among two tumbling (T) treatment groups at 60 (T60) or 90 (T90) minutes, as well as a non-tumbled control (T0) group. After treatment, sub-sections were made and divided among 0d, 7d, or 14d of further aging. Meat quality was assessed by shear force values, water-holding ability, and color attributes. The extent of proteolysis was determined by quantification of desmin and troponin-T, myofibril fragmentation index (MFI), and transmission electron microscopy. An interaction between fresh beef tumbling and aging duration was observed in shear force values (P=0.032). At 0d, muscles from T90 exhibited lower shear force (21.6 N) compared to T0 (34.8 N) and T60 (24.7 N) groups. Muscles from T60 and T90 groups maintained lower shear force than T0 controls at each respective aging duration. Higher cooking loss (P=0.011) but not purge loss (P=0.412) was observed in the T60 and T90 groups compared to T0. Shear force results were supported by higher MFI in T60 and T90 groups than T0 controls (P<0.001), as well as the disappearance of intact troponin-T with further aging (P=0.009). Transmission electron microscopy supported increased initial tenderness was primarily caused by physical disruptions to myofibrillar structure, though fresh beef tumbling may facilitate proteolysis with further aging.

Keywords: instrumental tenderness, meat tumbling, muscle ultrastructure, postmortem proteolysis, transmission electron microscopy

2.2 Introduction

Tenderness is one of the primary sensory attributes which dictates the overall palatability of fresh beef (O'Quinn et al., 2018; Miller, 2020; Warner et al., 2020). It has been assessed that if tenderness is rated to be unacceptable, there would be a 69% chance that a consumer will deem overall palatability to be unacceptable as well (O'Quinn et al., 2018). Several studies have supported that consumers will pay a premium for beef with known tenderness (Boleman et al., 1997; Miller et al., 2001; Shackelford et al., 2001). Accordingly, understanding the mechanisms by which tenderness can be affected is critical to ensuring consistent product quality. Tenderness is primarily influenced by the integrity of the myofibrils, sarcomere length, and connective tissues (Warner et al., 2020), though the contribution of each can differ by individual muscle (Rhee et al., 2004). For the beef *longissimus thoracis et lumborum* muscle, Warner-Bratzler shear force (WBSF) values are moderately correlated with the amount of connective tissue and degradation of desmin (a marker of proteolysis) and weakly correlated with sarcomere length (Rhee et al., 2004). While the US beef industry has shown marked improvements in fresh beef quality and consistency in recent decades (Voges et al., 2007; Guelker et al., 2013; Martinez et al., 2017; Gonzalez and Phelps, 2018), meeting consumer expectations for eating quality, especially tenderness, remains critical.

Several postharvest strategies have been used to improve tenderness of fresh beef, though the ability to accomplish this without negatively impacting other quality attributes or sensory traits is often limited. For example, mechanical tenderization is commonly applied to improve tenderness of beef by physically disrupting muscle structure through penetration with blades or needles. While mechanical tenderization can be effective at improving tenderness, this often comes at the expense of other eating quality traits including juiciness and flavor (Glover et al., 1977; Jeremiah et al., 1999), which are also crucial to the overall palatability of beef (O'Quinn et al., 2018). Tenderization methods that penetrate the meat may introduce foodborne pathogens (Luchansky et al., 2008), leading consumers to express concerns regarding the safety of such products (Yang et al., 2021). Consequently, this may lead consumers to cook mechanically tenderized beef products to a higher degree of doneness (Yang et al., 2021), which would be expected to negatively impact palatability to an extent (McKillip et al., 2017).

Meat tumbling has been widely implemented for processed meat applications to improve eating quality and enhance the pickup of brine solutions. During the tumbling process, physical

disruptions to the muscle structure occur through contact with the rotating drum and other pieces of meat (Martin, 2001). In general, the tenderness of tumbled muscle foods increases with the duration of tumbling (Dzudie and Okubanjo, 1999; Pietrasik and Shand, 2004; Moon et al., 2007). However, it is poorly understood how quality of fresh beef would be affected through the application of tumbling without the use of a brine solution. Recently, Morrow et al. (2019) observed no positive effect of tumbling without brine on tenderization of beef *rectus abdominis* muscles. However, beef tenderization is known to be muscle-specific (Juárez et al., 2010; Y.H.B. Kim et al., 2018; Nair et al., 2019), and the flank muscle is often described as tougher than the loin owing to larger muscle fibers and higher amount of connective tissue (Jeremiah et al., 2003; Couvreur et al., 2019). As physical disruptions to muscle structure could enhance the activity of proteolytic enzymes (H.-W. Kim et al., 2018; Y.H.B. Kim et al., 2018; Setyabrata et al., 2019; Setyabrata and Kim, 2019), it stands to reason that tumbling with further aging could be an effective strategy of tenderization in certain muscles. We hypothesized that tumbling fresh beef loin muscles without brine would improve tenderization through physical disruptions to muscle structure, enhanced proteolysis with further aging, or some interaction between those factors.

2.3 Materials and Methods

2.3.1 Raw Materials and Processing

Beef loins (*longissimus lumborum;* n=9; USDA Select grade) were obtained from a commercial processor with 3 loins at one processing date and the remaining 6 at a later date, each from different carcasses. The loins were stored in vacuum packaging at 2 °C until 7d postmortem. At 7d, loins were divided into three equal length sections averaging 1.75 ± 0.05 (standard error) kg per section and assigned to tumbling treatments at 60 (T60) or 90 (T90) min, or non-tumbled control (T0). Treatments were applied in a balanced complete block design for equal distribution among muscle position to account for locational differences throughout the loin muscle (Rhee et al., 2004). Sections were vacuum sealed in two layers of 3-mil thickness vacuum packaging (CLARITY, Bunzl Processor Division, Riverside, MO), and weights were recorded prior to packaging. Tumbling for each respective duration was performed within a Lance LT-30 500 lb capacity meat tumbler (Lance Industries, Hartford, WI) at 8.5 rpm. Following tumbling, samples were removed from the packaging, gently blotted with a paper towel, and reweighed. Sections

from the T0 group were packaged for a similar duration as the treatment groups to account for purge loss induced from vacuum packaging. Each section was then further cut into three equal length sub-sections, repackaged, and randomly allocated among aging durations [0d (no further aging), 7d, 14d] at 2 °C. One additional steak (2.54 cm thickness) was collected from each sub-section from the 0d further aging group for instrumental color measurement over 7d of simulated retail display as later described. At the completion of each respective aging duration, steaks (2.54 cm thickness) were made to determine cooking loss and shear force, while remaining muscle samples for biochemical analyses were frozen and stored at -80 °C.

2.3.2 Water-holding Ability

Water-holding ability of beef muscles was indirectly assessed by purge, display, and cooking losses (Setyabrata and Kim, 2019). Purge loss was assessed as the percentage weight loss before and after tumbling and further aging. Display loss was determined on 0d further aged samples only as the weight loss before and after 7d of aerobic display storage. Cooking loss was assessed by cooking the samples on an open-faced electric griddle (Model GR-150, Cuisinart, Stamford, CT) set at 135 °C. A type-T thermocouple (Omega Engineering, Stamford, CT) was used to monitor internal temperature, and steaks were flipped at 41 °C and cooked until 71 °C was reached. After 30 min of resting at room temperature, the steaks were gently blotted and reweighed. The percent difference between raw and cooked weights was calculated as cooking loss.

2.3.3 Instrumental Display Color

Steaks (2.54 cm thick) from the 0d further aging group were displayed for 7d at 2 °C on polystyrene foam trays with polyvinylchloride overwrap film (23,000 cm^3 O^2/m^2/24 h at 23 °C). Lighting was provided by fluorescent bulb at approximately 1,450 lx (OCTRON T8 Lamps, Wilmington, MA). A colorimeter (Hunter MiniScan EZ, Reston, VA) was used to measure CIE *L** (lightness), CIE *a** (redness), and CIE *b** (yellowness) values at 1d, 4d, and 7d of aerobic display. Three readings were taken per steak at each time point and pooled prior to statistical analysis. The colorimeter was equipped with a 25 mm diameter measuring device, illuminant A, and degree observer of 10°. The degree of discoloration was determined by hue angle, and color

saturation (chroma) values were calculated from CIE *a** and CIE *b** in accordance to American Meat Science Association guidelines (AMSA, 2012).

2.3.4 Warner-Bratzler Shear Force

Steaks used for cooking loss measurement were individually wrapped in foil and stored at 4 °C for 16 h. For Warner-Bratzler shear force (WBSF) measurement was conducted according to American Meat Science Association guidelines (AMSA, 2015). Cores (1.27 cm diameter; at least 6 per steak) were collected by cutting parallel to fiber direction, avoiding visible connective tissue and fat. Shear force was measured using a texture analyzer (TA-XT Plus Texture Analyser, Stable Micro System Ltd, Godalming, Surrey, UK) with WBSF V-shaped blade attachment. Cores were sheared perpendicular to fiber direction, and peak shear force (N) was recorded. Prior to statistical analysis, the mean shear force per sample was determined.

2.3.5 Myofibril Fragmentation Index

Fragmentation of myofibrils was assessed in duplicate using the myofibril fragmentation index (MFI) protocol of Culler et al. (1978) with some modification. Approximately 4 g of scissor minced sample was homogenized in a 1:10 (w/v) ratio with MFI buffer (100 mM potassium chloride, 20 mM potassium phosphate, 1 mM egtazic acid, 1 mM magnesium chloride, and 1 mM sodium azide pH 7.0) at 2 °C for 45 sec. The homogenate was centrifuged at 1000 ×g for 15 min, after which the supernatant was discarded. The pellet was resuspended in 40 mL of MFI buffer. The centrifugation and removal of supernatant was repeated. After resuspending the pellet in 10 mL of MFI buffer, the sample was strained to remove connective tissue. An additional 10 mL of MFI buffer was used to pass myofibrils through the strainer. Afterwards, protein concentration of the filtrate was quantified by comparison to known bovine serum albumin standards to dilute the samples to a concentration of 0.5 mg/mL. A UV spectrophotometer (VWR UV-1600 PC, VWR International, San Francisco, CA) was used to measure absorbance at 540 nm, and MFI was determined by multiplying the absorbance value by 200.

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

Extraction of muscle proteins was conducted using the protocol of Y.H.B. Kim et al. (2010) with modifications described by Setyabrata et al. (2019). SDS-PAGE load checks were conducted to ensure consistent protein concentration. Western blotting procedures were performed in accordance to Setyabrata et al. (2019). Briefly, 15% bis-acrylamide (100:1) separating gel with 5% stacking gel was loaded with 40 µg of protein, and electrophoresis was conducted for 3 h at 130 V (Bio-Rad PowerPac Basis, Bio-Rad Laboratories, Hercules, CA). The transfer of proteins to transfer membranes (IPVH00010, Millipore, Billerica, MA) was conducted for 90 min at 90 V in Tris-glycine buffer. Blocking was conducted for 1 h at room temperatures with 5% (w/v) nonfat dry milk in phosphate buffer saline-tween (PBST). Incubation for 16 h at 4 °C with a 1:20,000 dilution of monoclonal mouse anti-desmin (D1022, Sigma Aldrich, St. Louis, MO, USA) or a 1:40,000 dilution of anti-troponin-T (T6277, Sigma Aldrich, St. Louis, MO, USA) in 3% (w/v) nonfat dry milk in PBST was applied. After three washes in PBST, a 1:20,000 (desmin) or 1:15,000 dilution (troponin-T) of monoclonal goat anti-mouse IgG (H&L) horseradish peroxidase conjugate (170–6516, Bio-Rad Laboratories, Hercules, CA) in 3% (w/v) nonfat dry milk in PBST was applied for 1 h at room temperature. The washing steps were repeated prior to imaging. Imaging was conducted by developing membranes with enhanced chemiluminescence reagents (Thermo Fisher Scientific, Waltham, MA) and visualizing with a ChemiDoc-It^{TS2} imager (UVP, Upland, CA). Intact and degradation bands were quantified by comparison of band intensity to the intact band of an internal reference sample (each same T0 control at 0d further aging).

2.3.7 Muscle Ultrastructure

Muscle ultrastructure was assessed by transmission electron microscopy (TEM). Representative samples per tumbling and aging treatment combination were collected parallel to fiber direction, approximately 2 cm from the exterior of the section. Samples were stored in 2.5% glutaraldehyde buffer at 4 °C until fixation. Samples were rinsed 3 times in 0.1 M cacodylate buffer prior to fixation with 1% osmium tetroxide with 0.8% ferricyanide. Dehydration was conducted through a series of ethanol dilutions (50%, 75%, 95%, 100% × 3), after which samples were immersed in acetonitrile and acetonitrile with Spurr's resin [2:1 (v/v) followed by 1:2 (v/v)]. Samples were embedded in 100% Spurr's resin for 2 h at 50 °C. Microtomy was performed to

ensure samples were parallel to muscle fiber direction. Samples were embedded on copper TEM grids, and imaging was performed (Tecnai T12, FEI Company, Hillsboro, OR) at 11,500× magnification. Images were viewed with Gatan DigitalMicrograph software (v.3.31.2360.0, Gatan, Inc., Pleasanton, CA).

2.3.8 Statistical Analysis

The design of this experiment was a balanced complete block design with a factorial arrangement of 3 tumbling durations (T0, T60, T90) by 3 further aging durations (0d, 7d, 14d). Data were analyzed with the PROC GLIMMIX procedure of SAS (9.4; SAS Institute, Cary, NC). Fresh beef tumbling and aging durations were considered as fixed effects with each carcass serving as a block. Color data were analyzed as a split-plot with tumbling duration as whole plot and display day as sub-plot. Means were separated by least significant differences with statistical significance set at $P<0.05$. Pearson correlation coefficients were determined using the PROC CORR procedure. No effect of processing date was found for any attribute ($P>0.05$) and thus was omitted from the final statistical model.

2.4 Results and Discussion

2.4.1 Water-holding Ability

Multiple weight loss measurements including the purge loss of beef sections and cooking and display weight losses of beef steaks were used to assess water-holding ability. No significant tumbling impact on purge loss of beef sections was found ($P=0.412$; Table 2.1). Further aging increased purge losses, where an additional 14d aging induced the highest purge loss from beef samples followed by 7d and 0d aging ($P<0.001$). For cooking loss of steaks, fresh beef tumbling resulted in more weight loss during cooking at 24.9% and 26.0% for T60 and T90, respectively, compared to 22.9% for the T0 controls ($P=0.011$). Aging had no impact on cooking loss ($P=0.885$), nor was there any interaction between tumbling and aging observed for both cooking and purge losses ($P=0.710$). Display weight loss over 7d of simulated retail display was assessed on steaks from 0d further aged beef sections only, where no effect of tumbling was found ($P=0.362$).

As most published studies regarding tumbling are associated with the addition of brine solution, information regarding how tumbling without brine would influence water-holding of fresh meat products is less available. Several tumbling studies utilizing a brine solution have found positive tumbling impacts on water-holding attributes of muscle foods (Boles and Shand, 2002; Pietrasik and Shand, 2004; Moon et al., 2007). These effects are known to be dependent upon factors including tumbling duration and speed (Lin et al., 1990; Dzudie and Okubanjo, 1999; Pietrasik and Shand, 2004; Moon et al., 2007). Ockerman et al. (1978) reported no changes in final yield and moisture loss through short-term tumbling (30 min), while improvements in tenderness and cohesiveness of cured cooked hams were found. Conversely, Pietrasik and Shand (2004) found a decrease in purge loss of cooked beef roast when extended tumbling (16 h) with brine enhancement was applied. The results of the present study indicate that although no brine solution is included, tumbling of beef loins would have minimal effects on immediate purge loss after tumbling, nor would further purge loss during aging be induced. Further, no adverse tumbling impact on display weight loss of fresh beef steaks was found, though this attribute was assessed in the 0d further aged group only. As water-holding is highly dependent on the extramyofibrillar fraction of water (Pearce et al., 2011; Warner, 2017), it may be that the tumbling conditions used in the present study would have a negligible effect on mobilizing this fraction. However, an increase in cooking loss of steaks from tumbled beef sections was observed. As most previous tumbling research has added brine solution which would be expected to affect the solubility and functionality of muscle proteins, the effects of tumbling only are presently poorly categorized. Various factors are known to contribute water loss during cooking including shrinkage of the protein network and denaturation of muscle proteins (Warner, 2017). It could be postulated that tumbling would result in larger gaps between cooked muscle fibers, resulting in more cooking loss (Pearce et al., 2011). Morrow et al. (2019) found cooking loss of beef *rectus abdominis* muscles tumbled without brine to be higher relative to the non-tumbled control, in agreement with the findings in the present study. However, despite increased cooking loss, moisture content of both the raw and cooked products were equivalent, and consumer juiciness scores between tumbled without brine and control muscles were comparable (Morrow et al., 2019). As the correlation between cooking loss and sensory juiciness of the *longissimus lumborum et thoracis* is weak (Rhee et al., 2004), further understanding of how tumbling would affect juiciness of beef loin steaks would be warranted.

2.4.2 Instrumental Display Color

No interactions between tumbling treatments and display day were observed for any color attribute (P>0.05; Table 2.2). Fresh beef tumbling resulted in lower CIE a^* (P<0.001) and CIE b^* (P<0.001) values compared to the T0 controls, while no differences between T60 and T90 were found (P>0.05). Accordingly, chroma values of tumbled steaks were lower than the control group (P<0.001). No differences in CIE L^* (P=0.429) nor hue angle values (P=0.994) across treatments were observed. Values for CIE a^*, CIE b^*, and chroma values decreased with display duration (P<0.05), which could be expected as color deteriorates during aerobic display (Suman and Joseph, 2013). The increase in hue angle with display was just outside of statistical significance (P=0.051), and CIE L^* values did not change during display (P=0.250). As color was measured on 0d further aged samples only due to limitations in sample size, any effect further aging may have on color stability of tumbled beef cannot be fully determined. Considering the decreased CIE a^*, CIE b^*, and chroma values, it would be expected that tumbling would result in some initial oxidation or denaturation of sarcoplasmic proteins. As cooking loss of beef is generally not affected by sarcoplasmic proteins (Purslow et al., 2016), the effects of tumbling on cooking loss would likely be better explained by the disruptions to myofibrillar structures than denaturation of sarcoplasmic proteins. Regardless, additional study regarding how tumbling without brine would affect functionality and denaturation of myofibrillar and sarcoplasmic proteins would be needed. As tumbling has been shown to be exacerbate oxidative processes (Cheng and Ockerman, 2003; Bombrun et al., 2014), which may be compounded by poorer oxidative stability with extended aging (Y.H.B. Kim et al., 2018), understanding the interactive effects of fresh beef tumbling with aging duration would also be warranted.

2.4.3 Warner-Bratzler Shear Force

There was an interaction (P=0.032; Fig. 2.1) between tumbling and further aging durations for WBSF values. Tumbling resulted in an immediate decrease in WBSF values, decreasing from 34.8 N in T0 to 24.7 N and 21.6 N for T60 and T90 treatments, respectively. While further aging decreased WBSF values of steaks from the control group (P<0.05), the steaks from the T0 control group at 14d further aging maintained higher WBSF values (25.4 N) than T90 immediately after tumbling (P<0.05) and were comparable to the T60 group (P>0.05). This result clearly indicates

tumbling would have an immediate positive impact on instrumental tenderness, even without further aging. Further aging improved tenderness of the T60 steaks from 0d to 14d (P<0.05), though no decrease in WBSF with aging was found in T90 (P>0.05).

Multiple studies have found improved tenderness of tumbled meat products with an increase in tumbling duration (Dzudie and Okubanjo, 1999; Pietrasik and Shand, 2004; Moon et al., 2007), in particular with an increase in cumulative revolutions (Lin et al., 1990). However, considering the intermuscular differences regarding individual factors that influence tenderness (Rhee et al., 2004), it would be expected improvements to tenderness would be muscle-specific. Molina et al. (2005) evaluated the impacts of brine incorporation method (marinating, needle-injection, or vacuum tumbling) on palatability attributes of eight muscles from the beef chuck. It was found that tumbling could significantly reduce the amount of sensory detectable connective tissue in beef *splenius* and *serratus ventralis* muscles, though other muscles were not affected (Molina et al., 2005). Similarly, Morrow et al. (2019) found no positive effect of tumbling without brine on slice shear force values and consumer tenderness of beef *rectus abdominis* muscles. This may be attributed to the lower amount of cumulative revolutions (20 min at 10 rpm) applied by Morrow et al. (2019) relative to the present study or by the inherent differences between the flank and loin muscles. The flank muscle has larger muscle fibers compared to the loin, as well as a larger amount of connective tissue (Jeremiah et al., 2003; Couvreur et al., 2019). It could also be expected that increased cooking loss through tumbling may result in poorer cooked meat tenderness (Warner, 2017; Warner et al., 2020), though Rhee et al. (2004) reported no correlation between cooking loss and WBSF values of the beef *longissimus lumborum et thoracis.* The present study is in agreement with this finding, as no correlation between WBSF and cooking loss was observed (P=0.623; Table 4). A weak negative correlation (r=-0.34) was observed between WBSF and purge losses, which could be related to the degradation of sarcoplasmic proteins which are then more readily expelled from the myofibrillar matrix (Purslow et al., 2016).

2.4.4 Proteolysis and Muscle Ultrastructure

MFI has been identified as a strong predictor of beef loin tenderness, regardless of animal maturity and intramuscular fat (Culler et al., 1978). The present study found moderate correlations between MFI values and WBSF (r=-0.58), purge loss (r=0.58), and relative abundances of intact

(r=-0.54) and degraded (r=0.56) troponin-T (P<0.001; Table 2.3). Further, weak but significant correlations of MFI with intact (r=-0.23) and degraded (r=0.42) desmin were also observed. Both main effects of fresh beef tumbling and duration of postmortem aging influenced MFI values (P<0.001; Fig. 2.2A, B). Higher MFI values were observed in T60 (99.9) and T90 (104.0) compared to the T0 control group (93.1). This pattern was similar to that observed with aging duration, as 0d (86.3) was lower than the 7d (99.8) and 14d (110.8) groups. While both main effects of tumbling and aging were significant, the absolute differences between MFI were more pronounced between aging duration means. There was no interaction between fresh beef tumbling and aging for MFI (P=0.366).

While MFI was affected by both fresh beef tumbling and aging duration, the results of western blot analysis only partially agreed with the MFI results. This could be due to physical disruptions caused by tumbling resulting in fragmented myofibrils but not measurable proteolytic degradation. An interaction was observed in intact troponin-T (P=0.009; Fig. 2.3 & 2.4B). Relative abundance was generally unchanged across tumbling treatments at 0d and 7d, respectively. Less abundant intact troponin-T was observed in T90 at 14d, which could indicate a higher extent of proteolytic degradation through combined tumbling and aging treatment. While an interaction of tumbling and aging was observed for intact troponin-T, only further aging affected relative abundances of intact desmin (P=0.004; Table 2.4 & Fig. 2.4A), degraded desmin (P<0.001; Table 2.4 & Fig. 2.4A), and degraded troponin-T (P<0.001; Table 2.4 & Fig. 2.4B). Less intact desmin and more abundant desmin and troponin-T degradation products were observed at 7d and 14d of further aging compared to 0d, while values between 7d and 14d were comparable. As only intact troponin-T was affected by the interaction of tumbling and aging, it could be that tumbling would result in more proteolysis in the intercellular thin filament regions, rather than intermediate filaments, adjacent myofibrils, and centromeric proteins. However, as multiple myofibrillar and cytoskeletal proteins are relevant to meat tenderness (Huff Lonergan et al., 2010; Y.H.B. Kim et al., 2018; Warner et al., 2020), further research into the potential effects of tumbling on proteolysis with aging would be necessary.

Representative TEM images of beef loin muscles across tumbling and further aging treatments are presented in Fig. 2.5. As treatments were applied at 7d postmortem, a loss of certain ultrastructural features including A and I bands was apparent in all samples. There was, in general, a limited effect of further aging on the ultrastructure of T0 control samples. Samples from T60 and

T90 exhibited some lateral and longitudinal fracturing of the myofibrillar structure, although degradation of structures at the Z-line were generally similar to the respective T0 controls. Substantial degradation of the myofibrils, observed at both the Z-line and myofibril itself, was apparent in the T60 and T90 groups with 7d and 14d of further aging. This observation provides some support regarding the previously discussed measures of proteolysis. As fragmenting of the myofibrils occurred in the T60 and T90 groups at 0d of further aging, the findings of increased MFI in tumbled treatments regardless of duration of postmortem aging would also be supported.

2.5 Conclusion

This study supports that tumbling without a brine solution could be an effective strategy to improve objective tenderness of beef loin muscles. An additional 14d of further aging was required for T0 loins to reach equivalent WBSF values to the T60 group achieved immediately after tumbling with no additional aging. Primarily, the effects of tumbling would be attributable to physical disruptions caused to the muscle structure, and no decrease in WBSF values with further aging was observed in the T90 group. However, these results provide some evidence that tumbling could result in increased proteolysis with aging. While the added improvements to objective tenderness of tumbled beef loins with additional aging were negligible, these observations could be relevant to the tenderization of more inherently tough beef cuts. These findings could be utilized by the beef industry to achieve more consistent tenderness, which may eventually be applied to improve quality attributes of lower value cuts.

2.6 Acknowledgements

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2.7 Tables and Figures

Table 2.1. Effect of fresh beef tumbling and further aging on water-holding capacity attributes of beef loins (n=9).

	Fresh beef tumbling duration (min)					Further aging (days)				
	T0	T60	T90	SEM	Significance of *P*-value	0d	7d	14d	SEM	Significance of *P*-value
Purge loss (%)	1.60	1.77	1.84	0.17	0.412	0.48^{z}	2.15^{y}	2.58^{x}	0.17	<0.001
Cooking loss (%)	22.9^{b}	24.9^{a}	26.0^{a}	2.7	0.011	24.8	24.7	24.3	2.7	0.885
Display weight loss (%)	2.24	2.47	2.57	0.50	0.362	-	-	-	-	-

No two-way interactions between fresh beef tumbling and aging duration were significant ($P>0.05$).

^{a,b}Means lacking a common superscript within a row differ due to fresh beef tumbling duration ($P<0.05$).

$^{x\text{-}z}$Means lacking a common superscript within a row differ due to further aging duration ($P<0.05$).

Table 2.2. Effect of fresh beef tumbling duration and display day on instrumental color attributes of beef loins (n=9).

	CIE *L**	CIE *a**	CIE *b**	Hue angle	Chroma
T[1]					
T0	36.2	26.6[a]	20.5[a]	37.6	33.6[a]
T60	35.9	24.6[b]	19.0[b]	37.6	31.1[b]
T90	35.9	24.1[b]	18.6[b]	37.6	30.5[b]
SEM	0.7	0.5	0.4	0.3	0.7
D[2]					
1d	36.2	27.2[x]	20.8[x]	37.4	34.2[x]
4d	36.0	25.0[y]	19.2[y]	37.5	31.5[y]
7d	35.8	23.2[z]	18.1[z]	38.0	29.4[z]
SEM	0.7	0.5	0.4	0.3	0.7
Significance of *P*-value					
T	0.429	<0.001	<0.001	0.994	<0.001
D	0.250	<0.001	<0.001	0.051	<0.001
T × *D*	0.061	0.343	0.950	0.383	0.678

[1]Tumbling duration effect

[2]Display period effect

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

[x-z]Means lacking a common superscript within a column differ due to further aging duration ($P<0.05$).

Table 2.3. Pearson correlation coefficients of various traits of beef loins.

Trait[1]	COOK	PURGE	MFI	TnT1	TnT2	DES1	DES2
WBSF	0.06	-0.34**	-0.58***	0.38**	-0.39***	0.35**	-0.35**
COOK		0.00	0.15	-0.15	-0.08	0.25*	-0.22
PURGE			0.58***	-0.36***	0.20	-0.34**	0.34**
MFI				-0.54***	0.56***	-0.23*	0.42***
TnT1					-0.17	0.30**	-0.19
TnT2						-0.25*	0.19
DES1							-0.46***

[1]WBSF=Warner-Bratzler shear force; COOK=cooking loss; PURGE=purge loss; MFI = myofibril fragmentation index; TnT1 = troponin-T, intact; TnT2 = troponin-T, degraded; DES1 = desmin, intact; DES2 = desmin, degraded.

*$P<0.05$.

**$P<0.01$.

***$P<0.001$.

Table 2.4. Effect of fresh beef tumbling and further aging on quantitative abundance of desmin (intact and degraded) and troponin-T (degraded) of beef loins (n=9).

	Desmin, intact	Desmin, degraded	Troponin-T, degraded
T[1]			
T0	0.99	1.02	0.74
T60	0.95	1.06	0.77
T90	0.90	1.12	0.78
SEM	0.07	0.09	0.07
A[2]			
0d	1.06^{x}	0.90^{y}	0.66^{x}
7d	0.87^{y}	1.10^{x}	0.78^{y}
14d	0.91^{y}	1.21^{x}	0.84^{y}
SEM	0.07	0.09	0.07
Significance of *P*-value			
T	0.309	0.223	0.485
A	0.004	<0.001	<0.001
T × *A*	0.247	0.186	0.465

[1]Tumbling duration effect
[2]Aging duration effect
[x,y]Means lacking a common superscript within a column differ due to further aging duration (P<0.05).

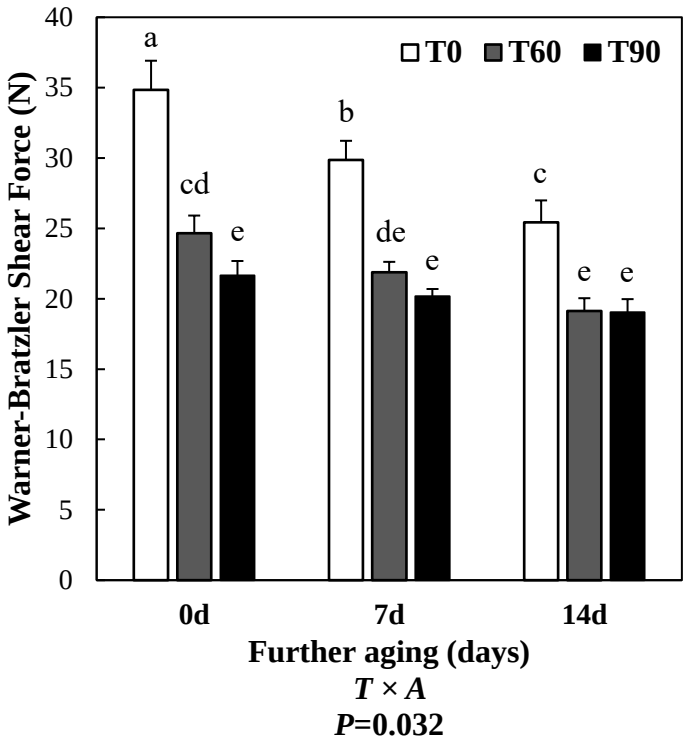
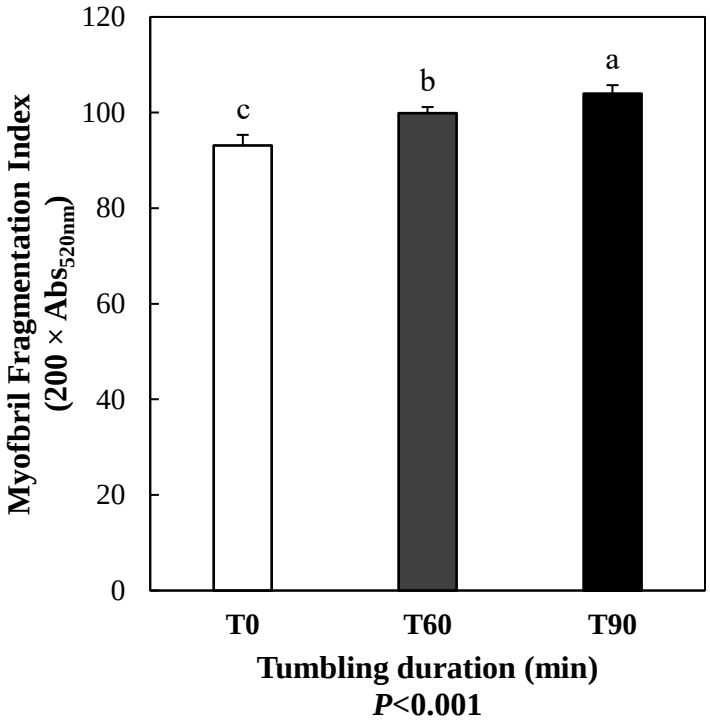
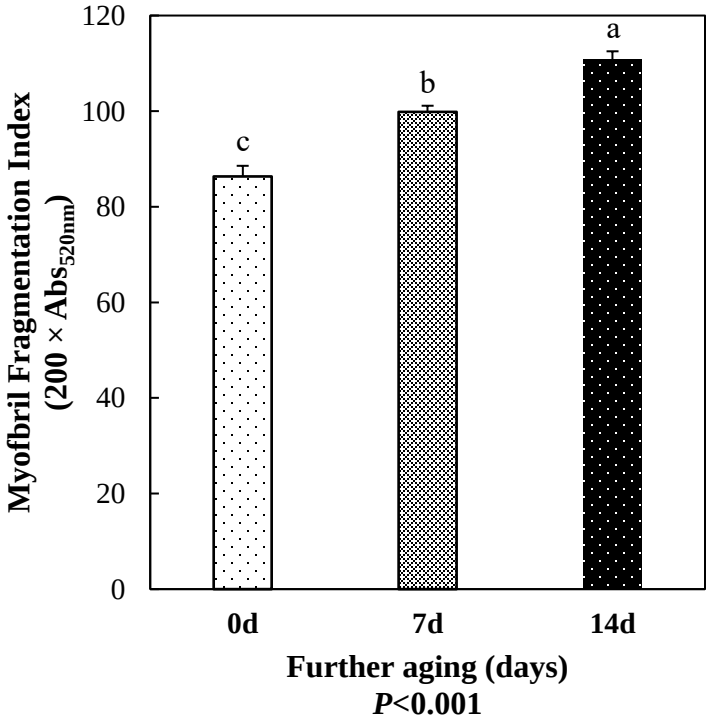


Figure 2.1. Interactive effect of fresh beef tumbling (*T*) and further aging (*A*) on Warner-Bratzler shear force (WBSF) values (N) of beef loins (n=9). Means lacking a common superscript (a-e) differ at ($P<0.05$). Error bars indicate standard error of the mean. Note: T0 indicates non-tumbled control, T60 indicates tumbled for 60 min, and T90 indicates tumbled for 90 min.

Figure 2.2. Main effects of fresh beef tumbling (A) and aging (B) on myofibril fragmentation index (MFI) values of beef loins (n=9). Means lacking a common superscript (a-c) differ at (P<0.05). Error bars indicate standard error of the mean. The two-way interaction between fresh beef tumbling and aging duration was not significant (P=0.366). Note: T0 indicates non-tumbled control, T60 indicates tumbled for 60 min, and T90 indicates tumbled for 90 min.


120
100
80
60
40
20
0
Myofbril Fragmentation Index
(200 × Abs520nm)
c
b
a
T0
T60
T90
Tumbling duration (min)
P<0.001

(A)


120
100
80
60
40
20
0
Myofbril Fragmentation Index
(200 × Abs520nm)
c
b
a
0d
7d
14d
Further aging (days)
P<0.001

(B)

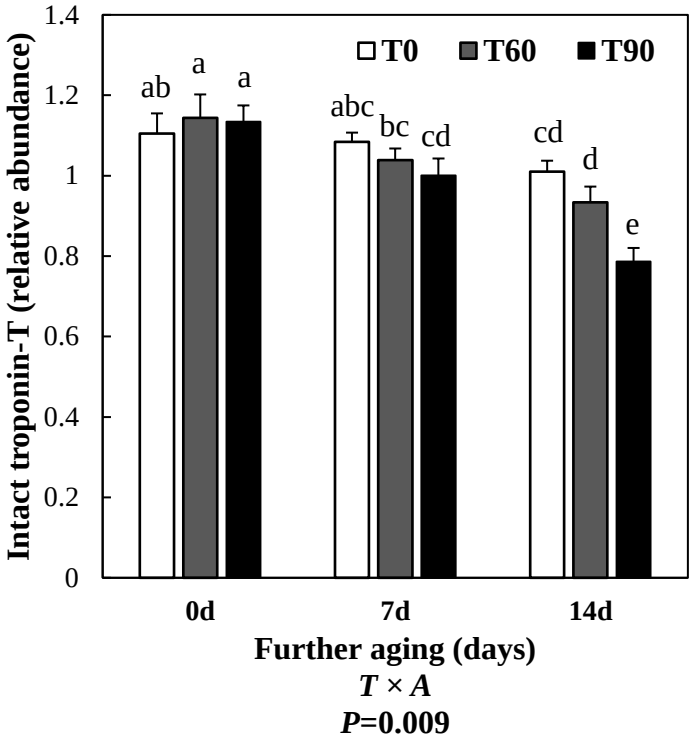


Figure 2.3. Interactive effect of fresh beef tumbling (*T*) and further aging (*A*) on relative abundance of intact troponin-T of beef loins (n=9). Means lacking a common superscript (a-e) differ at ($P<0.05$). Error bars indicate standard error of the mean. Bands were quantified by comparison of band intensity to the intact band of an internal reference sample (each same T0 control sample with 0d further aging). Note: T0 indicates non-tumbled control, T60 indicates tumbled for 60 min, and T90 indicates tumbled for 90 min.

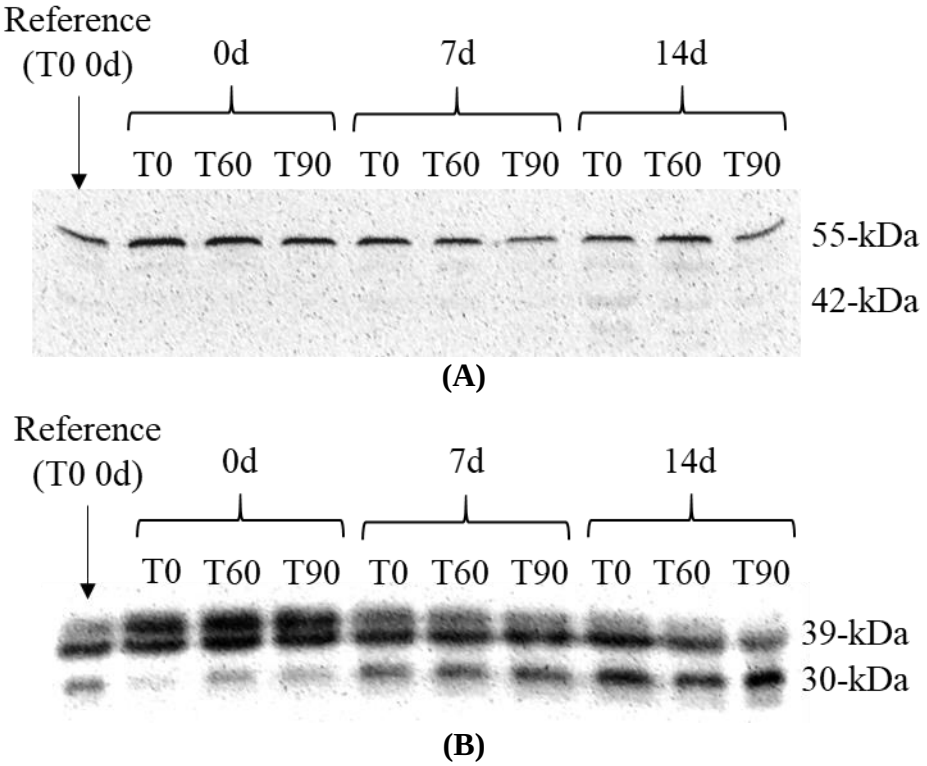
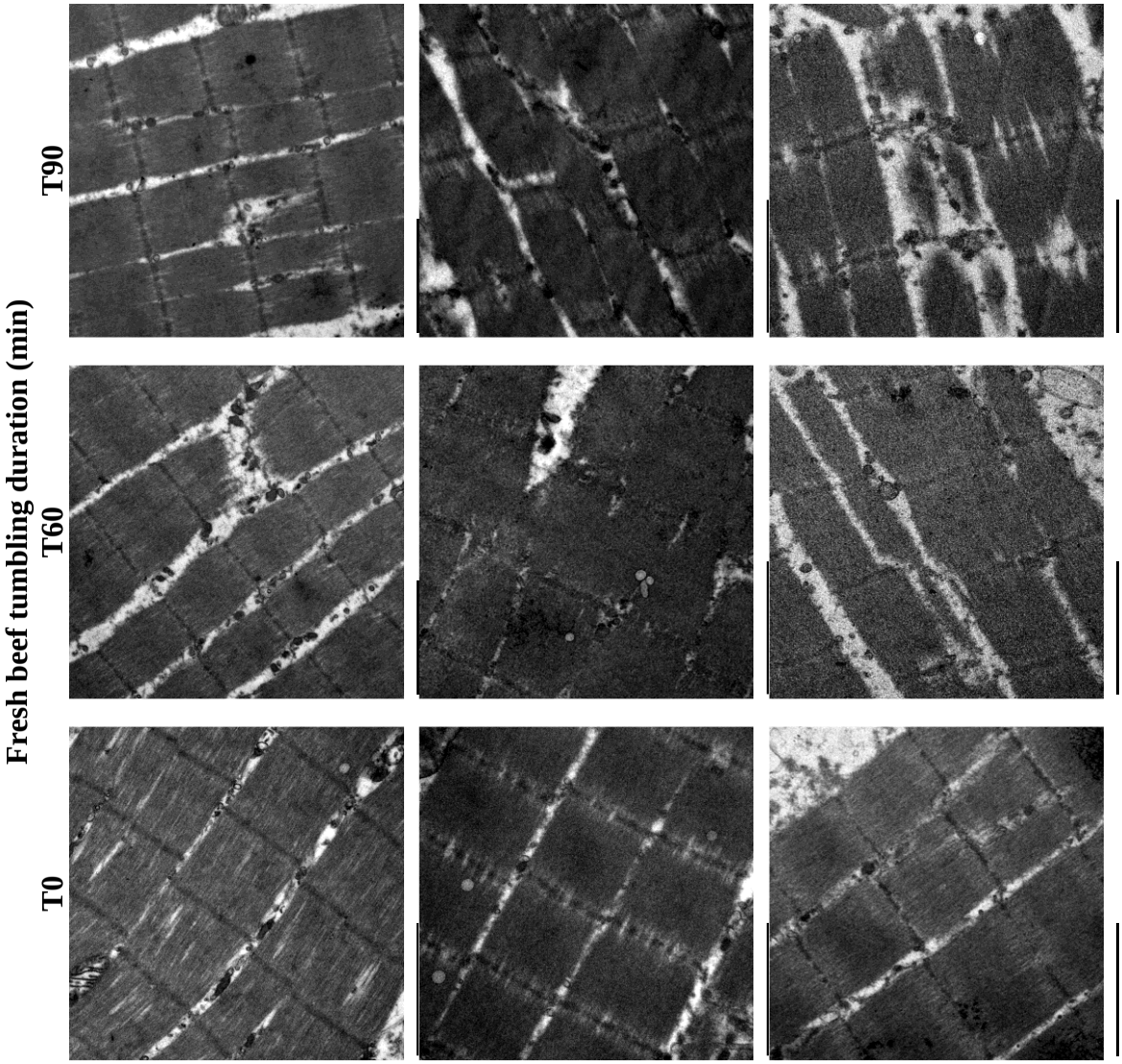


Figure 2.4. Representative western blot images of desmin (A) and troponin-T (B) from beef loins (n=9) subjected to fresh beef tumbling and aging treatment. Note: T0 indicates non-tumbled control, T60 indicates tumbled for 60 min, and T90 indicates tumbled for 90 min.

Figure 2.5. Representative transmission electron microscopy (TEM) images (11,500 × magnification) of beef loin muscle ultrastructure by fresh beef tumbling and aging duration. Note: T0 indicates non-tumbled control, T60 indicates tumbled for 60 min, and T90 indicates tumbled for 90 min.


Fresh beef tumbling duration (min)
T90
T60
T0
0d
7d
14d
Further aging (days)

2.8 References

AMSA. 2012. Meat Color Measurement Guidelines. In: American Meat Science Association, Champaign, IL, USA. p. 1–17.

AMSA. 2015. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat. In: American Meat Science Association, Champaign, IL, USA. p. 82–85.

Boleman, S. J., S. L. Boleman, R. K. Miller, J. F. Taylor, H. R. Cross, T. L. Wheeler, M. Koohmaraie, S. D. Shackelford, M. F. Miller, R. L. West, D. D. Johnson, and J. W. Savell. 1997. Consumer evaluation of beef of known categories of tenderness. J. Anim. Sci. 75:1521. doi:10.2527/1997.7561521x.

Boles, J. A., and P. J. Shand. 2002. Tumbling regime effects on the processing characteristics and tenderness of cooked roast beef. J. Muscle Foods. 13:25–35. doi:10.1111/j.1745-4573.2002.tb00318.x.

Bombrun, L., P. Gatellier, M. Carlier, and A. Kondjoyan. 2014. The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking. Meat Sci. 96:5–13. doi:10.1016/j.meatsci.2013.06.029.

Cheng, J., and H. W. Ockerman. 2003. Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. Meat Sci. 65:1353–1359. doi:10.1016/S0309-1740(03)00057-3.

Couvreur, S., G. Le Bec, D. Micol, and B. Picard. 2019. Relationships Between Cull Beef Cow Characteristics, Finishing Practices and Meat Quality Traits of Longissimus thoracis and Rectus abdominis. Foods. 8:141. doi:10.3390/foods8040141.

Culler, R. D., F. C. P. Jr, G. C. Smith, and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177–1180. doi:10.1111/j.1365-2621.1978.tb15263.x.

Dzudie, T., and A. Okubanjo. 1999. Effects of rigor state and tumbling time on quality of goat hams. J. Food. Eng. 42:103–107. doi:10.1016/S0260-8774(99)00097-7.

Glover, E. E., J. C. Forrest, H. R. Johnson, V. D. Bramblett, and M. D. Judge. 1977. Palatability and Cooking Characteristics of Mechanically Tenderized Beef. J. Food Sci. 42:871–874. doi:https://doi.org/10.1111/j.1365-2621.1977.tb12626.x.

Gonzalez, J. M., and K. J. Phelps. 2018. United States beef quality as chronicled by the National Beef Quality Audits, Beef Consumer Satisfaction Projects, and National Beef Tenderness Surveys — A review. Asian-Australas J. Anim. Sci. 31:1036–1042. doi:10.5713/ajas.18.0199.

Guelker, M. R., A. N. Haneklaus, J. C. Brooks, C. C. Carr, R. J. Delmore, D. B. Griffin, D. S. Hale, K. B. Harris, G. G. Mafi, D. D. Johnson, C. L. Lorenzen, R. J. Maddock, J. N. Martin, R. K. Miller, C. R. Raines, D. L. VanOverbeke, L. L. Vedral, B. E. Wasser, and J. W. Savell. 2013. National Beef Tenderness Survey–2010: Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. J. Anim. Sci. 91:1005–1014. doi:10.2527/jas.2012-5785.

Huff Lonergan, E., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of postmortem muscle — Lessons on mechanisms of meat tenderization. Meat Sci. 86:184–195. doi:10.1016/j.meatsci.2010.05.004.

Jeremiah, L. E., L. L. Gibson, J. L. Aalhus, and M. E. R. Dugan. 2003. Assessment of palatability attributes of the major beef muscles. Meat Sci. 65:949–958. doi:10.1016/S0309-1740(02)00307-8.

Jeremiah, L. E., L. L. Gibson, and B. Cunningham. 1999. The influence of mechanical tenderization on the palatability of certain bovine muscles. Food Res. Int. 32:585–591. doi:10.1016/S0963-9969(99)00134-9.

Juárez, M., I. L. Larsen, L. L. Gibson, W. M. Robertson, M. E. R. Dugan, N. Aldai, and J. L. Aalhus. 2010. Extended ageing time and temperature effects on quality of sub-primal cuts of boxed beef. Can. J. Anim. Sci. 90:361–370. doi:10.4141/CJAS09079.

Kim, H.-W., J.-H. Kim, J.-K. Seo, D. Setyabrata, and Y. H. B. Kim. 2018. Effects of aging/freezing sequence and freezing rate on meat quality and oxidative stability of pork loins. Meat Sci. 139:162–170. doi:10.1016/j.meatsci.2018.01.024.

Kim, Y. H. B., D. Ma, D. Setyabrata, M. M. Farouk, S. M. Lonergan, E. Huff-Lonergan, and M. C. Hunt. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Sci. 144:74–90. doi:10.1016/j.meatsci.2018.04.031.

Kim, Y. H. B., S. M. Lonergan, and E. Huff-Lonergan. 2010. Protein denaturing conditions in beef deep semimembranosus muscle results in limited μ-calpain activation and protein degradation. Meat Sci. 86:883–887. doi:10.1016/j.meatsci.2010.06.002.

Lin, G. C., G. S. Mittal, and S. Barbut. 1990. Effects of tumbling speed and cumulative revolutions on restructured hams' quality. J. Food Process. Preserv. 14:467–479. doi:10.1111/j.1745-4549.1990.tb00147.x.

Luchansky, J. B., R. K. Phebus, H. Thippareddi, and J. E. Call. 2008. Translocation of Surface-Inoculated Escherichia coli O157:H7 into Beef Subprimals following Blade Tenderization. J. Food Protection. 71:2190–2197. doi:10.4315/0362-028X-71.11.2190.

Martin, M. 2001. Meat Curing Technology. In: O. Young, R. Rogers, Y. Hui, and W.-K. Nip, editors. Meat Science and Applications. CRC Press. Available from: http://www.crcnetbase.com/doi/10.1201/9780203908082.ch20

Martinez, H. A., A. N. Arnold, J. C. Brooks, C. C. Carr, K. B. Gehring, D. B. Griffin, D. S. Hale, G. G. Mafi, D. D. Johnson, C. L. Lorenzen, R. J. Maddock, R. K. Miller, D. L. VanOverbeke, B. E. Wasser, and J. W. Savell. 2017. National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. Meat Muscle Biol. 1:138. doi:10.22175/mmb2017.05.0028.

McKillip, K. V., A. K. Wilfong, J. M. Gonzalez, T. A. Houser, J. A. Unruh, E. A. E. Boyle, and T. G. O'Quinn. 2017. Sensory Evaluation of Enhanced Beef Strip Loin Steaks Cooked to 3 Degrees of Doneness. Meat Muscle Biol. 1. doi:10.22175/mmb2017.06.0033.

Miller, M. F., M. A. Carr, C. B. Ramsey, K. L. Crockett, and L. C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062. doi:10.2527/2001.79123062x.

Miller, R. 2020. Drivers of Consumer Liking for Beef, Pork, and Lamb: A Review. Foods. 9. doi:10.3390/foods9040428.

Molina, M. E., D. D. Johnson, R. L. West, and B. L. Gwartney. 2005. Enhancing palatability traits in beef chuck muscles. Meat Sci. 71:52–61. doi:10.1016/j.meatsci.2005.04.004.

Moon, S. S., H. S. Yang, G. B. Park, and S. T. Joo. 2007. The Effect of Tumbling Time on the Quality and Binding Ability of Restructured Beef M. Pectoralis profundus with Alginate Binder. Asian Australas. J. Anim. Sci. 20:418–423. doi:10.5713/ajas.2007.418.

Morrow, S. J., A. J. Garmyn, N. C. Hardcastle, J. C. Brooks, and M. F. Miller. 2019. The Effects of Enhancement Strategies of Beef Flanks on Composition and Consumer Palatability Characteristics. Meat Muscle Biol. 3:457. doi:10.22175/mmb2019.07.0030.

Nair, M. N., A. C. V. C. S. Canto, G. Rentfrow, and S. P. Suman. 2019. Muscle-specific effect of aging on beef tenderness. LWT. 100:250–252. doi:10.1016/j.lwt.2018.10.038.

Ockerman, H. W., R. F. Plimpton, V. R. Cahill, and N. A. Parrett. 1978. Influence of short term tumbling, salt and phosphate on cured canned pork. J. Food Sci. 43:878–881. doi:10.1111/j.1365-2621.1978.tb02445.x.

O'Quinn, T. G., J. F. Legako, J. C. Brooks, and M. F. Miller. 2018. Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience. Transl. Anim. Sci. 2:26–36. doi:10.1093/tas/txx008.

Pearce, K. L., K. Rosenvold, H. J. Andersen, and D. L. Hopkins. 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes — A review. Meat Sci. 89:111–124. doi:10.1016/j.meatsci.2011.04.007.

Pietrasik, Z., and P. J. Shand. 2004. Effect of blade tenderization and tumbling time on the processing characteristics and tenderness of injected cooked roast beef. Meat Sci. 66:871–879. doi:10.1016/j.meatsci.2003.08.009.

Purslow, P. P., S. Oiseth, J. Hughes, and R. D. Warner. 2016. The structural basis of cooking loss in beef: Variations with temperature and ageing. Food Res. Int. 89:739–748. doi:10.1016/j.foodres.2016.09.010.

Rhee, M. S., T. L. Wheeler, S. D. Shackelford, and M. Koohmaraie. 2004. Variation in palatability and biochemical traits within and among eleven beef muscles. J. Anim. Sci. 82:534–550. doi:10.2527/2004.822534x.

Setyabrata, D., and Y. H. B. Kim. 2019. Impacts of aging/freezing sequence on microstructure, protein degradation and physico-chemical properties of beef muscles. Meat Sci. 151:64–74. doi:10.1016/j.meatsci.2019.01.007.

Setyabrata, D., J. R. Tuell, and Y. H. B. Kim. 2019. The Effect of Aging/Freezing Sequence and Freezing Rate on Quality Attributes of Beef Loins. Meat Muscle Biol. 3:488. doi:10.22175/mmb2019.08.0034.

Shackelford, S. D., T. L. Wheeler, M. K. Meade, J. O. Reagan, B. L. Byrnes, and M. Koohmaraie. 2001. Consumer impressions of Tender Select beef. J. Anim. Sci. 79:2605. doi:10.2527/2001.79102605x.

Suman, S. P., and P. Joseph. 2013. Myoglobin Chemistry and Meat Color. Annu. Rev. Food Sci. Technol. 4:79–99. doi:10.1146/annurev-food-030212-182623.

Voges, K. L., C. L. Mason, J. C. Brooks, R. J. Delmore, D. B. Griffin, D. S. Hale, W. R. Henning, D. D. Johnson, C. L. Lorenzen, R. J. Maddock, R. K. Miller, J. B. Morgan, B. E. Baird, B. L. Gwartney, and J. W. Savell. 2007. National beef tenderness survey – 2006: Assessment of Warner–Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. Meat Sci. 77:357–364. doi:10.1016/j.meatsci.2007.03.024.

Warner, R. D. 2017. Chapter 14 - The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. In: F. Toldra´, editor. Lawrie´s Meat Science (Eighth Edition). Woodhead Publishing. p. 419–459. Available from: https://www.sciencedirect.com/science/article/pii/B9780081006948000145

Warner, R., R. Miller, M. Ha, T. L. Wheeler, F. Dunshea, X. Li, R. Vaskoska, and P. Purslow. 2020. Meat Tenderness: Underlying Mechanisms, Instrumental Measurement, and Sensory Assessment. Meat Muscle Biol. 4. doi:10.22175/mmb.10489.

Yang, L., N. L. Arnold, T. Drape, R. C. Williams, T. Archibald, B. Chapman, and R. Boyer. 2021. A survey of United States consumer awareness, purchasing, and handling of mechanically tenderized beef products. Food Control. 120:107505. doi:10.1016/j.foodcont.2020.107505.

CHAPTER 3. TUMBLING AND SUBSEQUENT AGING IMPROVES TENDERNESS OF BEEF LONGISSIMUS LUMBORUM AND SEMITENDINOSUS STEAKS BY DISRUPTING MYOFIBRILLAR STRUCTURE AND ENHANCING PROTEOLYSIS

This chapter is a reprint of a previously published article with minor edits: Tuell, J. R., Nondorf, M.J., Abdelhaseib, M., Setyabrata, D., & Kim, Y. H. B. 2022. Tumbling and subsequent aging improves tenderness of beef longissimus lumborum and semitendinosus steaks by disrupting myofibrillar structure and enhancing proteolysis. Journal of Animal Science, 100(3): skac062. https://doi.org/10.1093/jas/skac062

3.1 Abstract

Tenderness is an important sensory attribute to the overall eating experience of beef. Identifying novel methods to ensure consistent tenderness, especially in inherently tough cuts, is critical for the industry. This study investigated if tumbling without brine inclusion could be an effective method to improve the quality and palatability attributes of beef longissimus lumborum (**LL**) and semitendinosus (**ST**) steaks. Furthermore, interactions with postmortem aging were evaluated to determine how tumbling might affect protein degradation and muscle ultrastructure. At 5 d postmortem, pairs of LL and ST muscles from beef carcasses (n = 16) were bisected, vacuum packaged, and tumbled for 0, 40, 80, or 120 min. Sections were divided and subsequently aged an additional 0 or 10 d at 2 °C. Tumbling for any duration improved instrumental tenderness of LL ($P < 0.001$) but not ST ($P > 0.05$) steaks, regardless of aging time. Tumbling exacerbated moisture loss in both muscles shown by greater purge and cooking losses ($P < 0.05$). Myofibrillar fragmentation was induced through tumbling in both muscles ($P < 0.001$), which was supported by transmission electron microscopy images. Tumbling for 120 min followed by 10 d of aging resulted in less abundant intact troponin-T in both LL and ST ($P < 0.05$), as well as less intact desmin in ST ($P < 0.05$); however, calpain-1 autolysis was not affected by tumbling ($P > 0.05$). No effects of tumbling, aging, nor the interaction were found for the content and solubility of collagen ($P > 0.05$). Consumer panelists (n = 120/muscle) rated LL steaks tumbled for any duration higher for tenderness and overall liking compared to control steaks ($P < 0.05$). For ST, significant interactions were found for consumer liking of tenderness and juiciness. In general, tumbling without subsequent aging resulted in poorer juiciness than non-tumbled ($P < 0.05$), while at 10 d

no differences in juiciness were found between treatments ($P > 0.05$). For ST steaks that were aged 10 d, 120 min of tumbling resulted in greater tenderness liking than non-tumbled steaks ($P < 0.05$). These results suggest that tumbling would result in myofibrillar fragmentation and may benefit the degradation of myofibrillar proteins; however, there would be negligible impacts on collagen. Accordingly, tumbling without brine inclusion alone may be sufficient to improve tenderness and overall liking of LL steaks, while combined tumbling with subsequent postmortem aging would be necessary to improve tenderness liking of ST.

Key words: beef tenderness, consumer evaluation, meat tumbling, natural processing, postmortem proteolysis, sensory panel

Abbreviations used: longissimus lumborum (**LL**); semitendinosus (**ST**); myofibril fragmentation index (**MFI**); transmission electron microscopy (**TEM**); Warner-Bratzler shear force (**WBSF**); lightness (***L****); redness (***a****); yellowness (***b****); ratio of absorbance at 630 to 580 nm (**R630/580 nm**)

3.2 Introduction

Tenderness is considered as a primary eating quality attribute influencing the overall palatability and consumer acceptance of fresh beef products (Miller, 2020). Considering the clear economic implications of beef tenderness (Miller et al., 2001; Mennecke et al., 2007), ensuring consumers are provided with consistently tender fresh beef is a high priority for the industry. Achieving this aim, however, remains a considerable challenge, as tenderness is an attribute that is well known to be highly variable between cuts. Historically, the beef longissimus lumborum (**LL**) muscle has been considered as "intermediate" in tenderness, while the semitendinosus (**ST**) muscle would be categorized as "tough" (Sullivan and Calkins, 2011). Considerable progress has been made in recent decades in improving the consistency and mean tenderness of beef LL, although muscles from the round primal continue to be rated poorly for eating quality attributes, especially tenderness (Martinez et al., 2017; Gonzalez and Phelps, 2018; Miller, 2020).

Postmortem aging is widely applied to fresh beef muscles to improve various sensory attributes, particularly for tenderness (Kim et al., 2018). However, the extent to which certain beef muscles tenderize during the aging process is inconsistent owing to factors such as lower activity of endogenous proteolytic enzymes and a large contribution of intramuscular connective tissues

(Rhee et al., 2004; Stolowski et al., 2006; Chun et al., 2020; Warner et al., 2020). In such instances where aging alone may be insufficient to achieve acceptable levels of tenderness, physical interventions such as tumbling may be employed to promote tenderization. Several studies have found tumbling with brine inclusion to improve the quality and palatability attributes of inherently tough beef muscles (Cheng and Ockerman, 2003; Molina et al., 2005; Morrow et al., 2019; Garmyn et al., 2020). At present, though, little consideration has been given to potential applications in fresh beef without brine inclusion.

Several recent studies conducted on the potential use of tumbling without brine inclusion have reported mixed results, likely due to differences in the characteristics of muscles used. For instance, Tuell et al. (2021) reported that tumbling would result in an immediate improvement in the instrumental tenderness of beef LL, which would be sustained during postmortem aging compared to non-tumbled controls. In contrast, Morrow et al. (2019) concluded that tumbling alone would be insufficient to tenderize the beef rectus abdominis muscle. While this observation may suggest tumbling without brine inclusion would be ineffective to tenderize inherently tough cuts, there is some evidence to suggest that tumbling coupled with subsequent postmortem aging could potentially promote tenderness development. Tumbling followed by aging has been shown to result in a greater extent of troponin-T degradation in beef LL (Tuell et al., 2021). Considering tumbling has been shown to result in disruptions to myofibrillar integrity and cellular membranes (Katsaras and Budras, 1993; Tyszkiewicz et al., 1997; Cheng and Ockerman, 2003), it stands to reason that the degradation of myofibrillar and stromal proteins could be promoted as has been recently shown for ultrasound damage in beef longissimus lumborum and semitendinosus muscles (Dang et al., 2022; Wang et al., 2022). However, at present, no studies have evaluated how tumbling without brine inclusion would affect proteolytic enzyme activities nor the properties of collagen, as well as their possible relevancy to tenderization. Furthermore, while an improvement in instrumental tenderness of beef LL has been shown with tumbling alone (Tuell et al., 2021), no previous studies have evaluated how the actual palatability as assessed by consumer panel would be affected in LL and ST steaks. Accordingly, this study aimed to determine how tumbling without brine inclusion would affect the quality (pH, water-holding ability, instrumental color, instrumental tenderness) and palatability (tenderness, juiciness, flavor, overall liking) attributes of beef LL and ST steaks with subsequent postmortem aging. This study also investigated the underlying mechanisms regarding how tumbling may affect tenderization during aging through

the degradation of troponin-T and desmin, calpain-1 autolysis, myofibril fragmentation index (**MFI**), collagen content and solubility, and transmission electron microscopy (**TEM**).

3.3 Materials and Methods

Institutional animal care and use committee approval was not requested for this study, as the fresh beef products were purchased from a USDA inspected commercial plant.

3.3.1 Treatment application

Paired LL and ST muscles from beef carcasses [n = 16; USDA Choice$^-$ grade (USDA, 2017)] were purchased from a commercial processor and transported at 1 d postmortem on ice to the Purdue Meat Science and Muscle Biology Laboratory in West Lafayette, IN. Muscles from eight carcasses were obtained in the first sample processing with the remaining eight at a later date. Vacuum sealed sections were stored at 2 °C until 5 d postmortem. Muscles from both sides were sectioned into two equal length sections, resulting in four sections per carcass. Each section was allocated among three tumbling treatments at 40 (**T40**), 80 (**T80**), or 120 (**T120**) min, as well as a non-tumbled (**T0**) control group, in a balanced randomized design for equal distribution of muscle positions. Each section was then divided transversely. Sub-sections were trimmed to approximately equal weights, resulting in LL sub-sections weighing 1091 ± 45 g and ST sub-sections weighing 1090 ± 44 g (reported as ± standard error of the mean). Sub-sections were randomly divided among aging durations (0 d or 10 d) and were packaged in two layers of barrier packaging (3-mil, CLARITY, Bunzl Processor Division, Riverside, MO). Weights were recorded prior to packaging. Tumbling was applied in a Lance LT-30 500 lb capacity meat tumbler (Lance Industries, Hartford, WI) set at 8.5 rpm. The diameter of the drum was 50.8 cm. Each muscle by treatment group was tumbled independently to minimize any potential impact of tumbler fill, with the fill of each repetition being 17.5 ± 0.1 kg and 17.4 ± 0.1 kg for LL and ST, respectively. Afterwards, 0 d aged sub-sections were removed from the packaging and reweighed. Sub-sections assigned to a further 10 d of aging were repackaged to ensure a vacuum seal was maintained, and aging was conducted at 2 °C. At each respective aging time, steaks (2 cm thickness) were collected for determining quality and palatability attributes. For measures of proteolysis, 8 carcasses were

selected for analysis, with 4 carcasses from each slaughter date, balanced for muscle position. Steaks were individually vacuum packaged, frozen/stored at -40 °C until analysis (up to 4 months), while samples used for biochemical analyses were frozen/stored at -80 °C. Prior to biochemical analyses, samples were trimmed of visible fat and connective tissue, frozen in liquid nitrogen, and pulverized.

3.3.2 Meat quality

pH and water-holding ability

A meat pH probe (Hanna Instrument, Inc., Warner, NH) was used to determine pH by inserting directly into the interior of the sub-sections. Before measurement, the instrument was calibrated with buffer solutions at pH 4 and 7 at 2 °C. Measurements were conducted in duplicate. Measures of water-holding ability included purge, thawing, and cooking losses in the same manner described by Tuell et al. (2021). Prior to each measurement, a paper towel was used to gently remove excess surface moisture. In brief, purge loss for 0 d aged sections was determined by the percent weight loss before and after tumbling treatments or similar duration of vacuum sealed storage for T0 controls. Purge loss of the 10 d aged group was conducted in the same manner at the completion of the aging period. At each aging duration, one steak per treatment was weighed, frozen at -40 °C, and later thawed for 24 h at 2 °C for determining thawing loss. The thawed steak was then used for cooking loss by cooking to an internal temperature of 71 °C on an open face electric griddle (GR-150, Cuisinart, Stamford, CT), flipping once at 41 °C. The griddle was set at a surface temperature of 135 °C, and internal temperature of each steak was monitored with a thermocouple (T type, Omega Engineering, Stamford, CT). Once the targeted internal temperature was reached, the steaks were held for 30 min at room temperature prior to weighing.

Instrumental tenderness

Instrumental tenderness was determined following American Meat Science Association guidelines for Warner-Bratzler shear force (**WBSF**) analysis (AMSA, 2015). Steaks cooked in the manner previously described were individually wrapped in foil and cooled for 16 h at 4 °C. A TA-

XT Plus Texture Analyzer (Stable Micro System Ltd, Godalming, Surrey, UK) was calibrated with a 5 kg load cell prior to analysis. Six cores measuring 1.27 cm in diameter were cut from each steak parallel to the direction of the myofibers. Cores were perpendicularly sheared by a V-shaped blade, and peak shear force was recorded in Newtons (N). The values of individual cores were averaged to determine the mean WBSF value for each steak.

Instrumental color

Following treatment application but prior to freezing, one steak (2 cm thickness) was cut and set aside to oxygenate for a period of 30 min at 2 °C. At three randomly determined locations per steak, CIE lightness (***L****), redness (***a****), and yellowness (***b****) values were measured using a calibrated Hunter MiniScan EZ colorimeter (HunterLab, Reston, VA). The conditions of the colorimeter were illuminant A, degree of observer of 10°, and aperture size of 25 mm diameter. Oxymyoglobin to metmyoglobin ratio was approximated by the ratio of absorbance at 630 and 580 nm wavelength (**R630/580 nm**) according to American Meat Science Association guidelines (AMSA, 2012). The hue angle [arctangent(b^*/a^*)] and chroma [$(a^{*2} + b^{*2})^{1/2}$] values were calculated in accordance with the same standards.

3.3.3 Proteolysis

Western blots

Extraction of whole muscle proteins and SDS-PAGE load checks were conducted using the protocol of Setyabrata and Kim (2019). Protein concentration of the gel sample was adjusted to a concentration of 4 µg per µL. For western blot analysis, 40 µg of protein was loaded into each lane. Western blotting of troponin-T, desmin, and calpain-1 was performed. A 5% bis-acrylamide (100:1) stacking gel was used for each protein, and the bis-acrylamide concentration of the separating gel was 15% for troponin-T, 12% for desmin, and 9% for calpain-1. Electrophoresis was conducted using a Bio-Rad PowerPac Basic system (Bio-Rad Laboratories, Hercules, CA) at 130 V for 3 h. Afterwards, proteins were transferred at 90 V for 90 min to nitrocellulose membranes (IPVH00010, Millipore, Billerica, MA). Membranes were blocked at room temperature in a solution of 5% (w/v) nonfat dry milk in PBS-tween for a duration of 1 h. Primary

antibodies and dilutions used were 1:20,000 monoclonal mouse anti-troponin-T (T6277, Sigma Aldrich, St. Louis, MO), 1:20,000 monoclonal mouse anti-desmin (D1022, Sigma Aldrich, St. Louis, MO), and 1:7,500 monoclonal mouse anti-calpain-1 (9A4H8D3, Thermo Fisher Scientific, Rockford, IL) in 3% (w/v) nonfat dry milk in PBS-tween. Membranes were incubated at 4 °C for 16 h, after which they were washed at room temperature three times in PBS-tween for 10 min each. Secondary antibody in 3% (w/v) nonfat dry milk in PBS-tween was applied at room temperature for 1 h. For each, monoclonal goat anti-mouse IgG (H+L) horseradish peroxidase conjugate (170–6516, Bio-Rad Laboratories, Hercules, CA) was used at a dilution of 1:20,000 (troponin-T), 1:15,000 (desmin), and 1:10,000 (calpain-1). Membranes were again washed three times as previously described. Pierce enhanced chemiluminescence reagents (32106, Thermo Fisher Scientific, Rockford, IL) were applied to the membranes and imaged (ChemiDoc-ItTS2, UVP, Upland, CA). The optical density of troponin-T and desmin was quantified relative to the density of the intact band of same T0 reference sample from the 0 d aged group, within each respective muscle. Subunits of calpain-1 were identified by comparison to an internal reference, and the ratios of the 80-kDa, 78-kDa, and 76-kDa bands were determined.

Myofibril fragmentation index

Determination of MFI was conducted in accordance with the method published by Culler et al. (1978) with modifications described by Tuell et al. (2021). In duplicate, 4 g of powdered muscle sample was homogenized with buffer (100 mM potassium chloride, 20 mM potassium phosphate, 1 mM egtazic acid, 1 mM magnesium chloride, and 1 mM sodium azide; pH 7.0) in a 1:10 (w/v) ratio. The samples were centrifuged for 15 min at 1,000 ×g, discarding the supernatant afterwards. The pellet was suspended in 40 mL of buffer, centrifuged for 15 min at 1,000 ×g, again discarding the supernatant. After resuspending the pellet in 10 mL of buffer, the sample was passed through a mesh strainer. The protein concentration of the strained samples was adjusted to 0.5 mg/mL by comparison to known bovine serum albumin standards. After adjustment, absorbance at 540 nm was determined (VWR UV-1600 PC, VWR International, San Francisco, CA), and MFI was calculated by multiplying absorbance by 200.

Collagen content and solubility

Content of insoluble, soluble, and total collagen, as well as solubility, was assessed in accordance to the method of Cross, Carpenter, and Smith (1973) with some modifications. In duplicate, 4 g of powdered muscle sample was stirred with 12 mL of one fourth strength Ringer's solution and incubated in a 78 °C water bath for 60 min with occasional stirring. The residual and supernatant fractions containing the insoluble and soluble portions, respectively, were separated following centrifugation for 25 min at 3,000 ×g. The pellet was transferred to a separate glass tube with two 5 mL rinses of distilled water. Each fraction hydrolyzed with a known volume of 12 N hydrochloric acid (10 mL for insoluble and 15 mL for soluble) for 16 h at 110 °C. Afterwards, samples were neutralized with 6 N sodium hydroxide (20 mL for insoluble and 25 mL for soluble) and filtered through Whatman #1 filter paper. A dilution of the filtrate was prepared by adding 200 µL of filtrate to 1.8 mL of distilled water. Afterwards, 500 µL of 7% (w/v) chloramine T hydrate in oxidant buffer [0.42 M sodium acetate trihydrate, 0.13 M trisodium citrate dihydrate, and 26.2 mM citric acid monohydrate in 38.5% (v/v) isopropanol; pH 6.0] was added to 200 µL of diluted filtrate, and the solution was allowed to react at room temperature for 20 min. The red chromophore was developed by addition of 500 µL of Ehrlich reagent, followed by incubation in a 60 °C water bath for 20 min. Samples were cooled in an ice bath for 5 min prior to plating. Absorbance at 558 nm was measured (Epoch, BioTek Instruments, Inc., Winooski, VT), and the absorbance of the reagent blank was subtracted from all sample readings. Samples were compared to known hydroxyproline standards. The determined hydroxyproline content was multiplied by a factor of 7.25 and 7.52 for the insoluble and soluble fractions, respectively, in accordance with the cited method. Values were expressed as mg per g of wet tissue. Total collagen content was determined as the sum of insoluble and soluble collagen contents. Collagen solubility was determined as soluble collagen content divided by total collagen content, expressed as on a percentage basis.

Transmission electron microscopy

Following treatment application but prior to freezing, TEM was conducted on muscles from two randomly determined carcasses. Samples from the interior of each sub-section were obtained by cutting with a razor parallel to the direction of the myofibers, approximately 2 cm from the exterior. Pre-fixation was conducted by addition of 2.5% (v/v) glutaraldehyde in 0.1 M

sodium cacodylate buffer (pH 7.4). Samples were stored at 4 °C until post-fixation. Post-fixation was conducted by addition of 1% (w/v) osmium tetroxide and 0.8% (w/v) ferricyanide. Afterwards, a series of ethanol dilutions was performed prior to infiltration and embedding with Spurr's resin. Samples were thin sectioned and mounted to copper TEM grids. Imaging was performed at 2,550× and 11,500× magnification (Tecnai T12, FEI Company, Hillsboro, OR), and images were viewed with Gatan DigitalMicrograph (3.31.2360.0, Gatan, Inc., Pleasanton, CA).

3.3.4 Consumer evaluation

The consumer evaluation was conducted in accordance to American Meat Science Association guidelines (AMSA, 2015). The protocol for the consumer panel evaluation was exempted by the Purdue Institutional Review Board (IRB exempt -2019-16). A separate panel for LL and ST muscles was conducted. For both evaluations, 120 individuals were recruited from the West Lafayette, IN area, with each panel being conducted over the course of 20 sessions with 6 unique panelists each. Prior to the session, steaks were thawed for 24 h at 2 °C. Cooking was conducted in the manner as previously described to an endpoint temperature of 71 °C. Cooked steaks were held in a foil covered metal pan for no more than 5 min in an oven set at 50 °C. Each steak was cut into cubes measuring 1.27 × 1.27 cm. Panelists in each session evaluated one randomly selected steak per treatment, thus 8 steaks were evaluated per session. Prior to evaluating these samples, a standardized warm-up sample consisting of a USDA Choice$^-$ LL or ST steak was evaluated to standardize panelists. Two cubes were presented to each panelist in a randomly predetermined order that was consistent within the session, with sample cups coded with a randomly generated 4-digit number. The order treatments were presented was randomized between panels to minimize the potential bias of serving order. Panelists evaluated the samples in red incandescent lighting to mask color differences between samples. Unsalted saltine crackers, a cup of distilled water, an expectorant cup, toothpicks, and napkins were provided. Panelists were asked to provide demographic information prior to evaluating samples, as well as questions regarding their beef consumption habits. At the completion of the session, panelists were compensated with a gift card worth $10 USD.

Attributes assessed were similar to the study published by Nyquist et al. (2018). Samples were evaluated on a continuous line scale ranging from 0 to 100. The 0-anchor represented extreme

disliking of tenderness, juiciness, flavor, or overall liking, and the 100-anchor represented extreme liking of those attributes. The 50-anchor represented the neutral midpoint of each attribute, indicated by a hatch mark. Panelists were also asked to evaluate the acceptability of each attribute (acceptable or unacceptable), as well as the perceived quality level (unsatisfactory, everyday quality, better than everyday quality, premium quality). Responses were recorded on electronic tablets (iPad, Apple Inc., Cupertino, CA) using online software (Qualtrics, Provo, UT).

3.3.5 Statistical analysis

The experimental design of this study was a balanced complete block design with each carcass serving as a block. For meat quality (pH, water-holding ability, instrumental color, and instrumental tenderness) and proteolysis (western blots, MFI, collagen content and solubility) measures, data were analyzed using the PROC GLIMMIX procedure of SAS (9.4, SAS Institute, Cary, NC). Data from LL and ST muscles were analyzed separately. The fixed effects included tumbling treatment (T0, T40, T80, and T120), aging duration (0 d and 10 d), and the interaction of tumbling × aging. Carcass was considered as a random effect. Muscles from both sides of one carcass were considered as equivalent, and sections were considered as the experimental unit. Slaughter date was not significant for any attribute ($P > 0.05$) and was omitted from the final models. For the consumer evaluation model, the fixed effects were considered the same as previously described. Random effects included panelist and session. Consumer acceptability and perceived quality data were analyzed using a binomial error distribution model created with the ILINK option of PROC GLIMMIX. Consumer demographics were summarized with the PROC FREQ procedure of SAS. Least square means were separated using an F-ratio test of each fixed effect with statistical significance designated at $P < 0.05$.

3.4 Results and Discussion

3.4.1 Meat quality

For pH, a higher value was observed for T40 LL muscles relative to other treatments ($P < 0.01$; Table 3.1); however, the magnitude of the differences between treatment means would be unlikely to have practical significance. Tumbling did not affect the pH of ST muscles ($P > 0.05$;

Table 3.2). Further aging increased the pH of ST muscles from 5.60 to 5.69 ($P < 0.001$; Table 3.2), while aging did not affect pH in LL ($P > 0.05$; Table 3.1). Regardless of treatment, the pH values of the LL and ST sections were within the normal range of 5.5 to 5.7 and were similar to several recent studies (Dang et al., 2022; Wang et al., 2022). Previous studies have reported tumbling to increase pH, although this would be primarily through the inclusion of non-meat ingredients, namely phosphates (Cheng and Ockerman, 2003; Gao et al., 2014; Garmyn et al., 2020). Cheng and Ockerman (2003) reported that while pH would increase with phosphate concentration, values between tumbled and non-tumbled beef bottom round muscles would be equivalent. This finding was corroborated by Morrow et al. (2019) who reported tumbling without a marinade would not impact pH values of beef rectus abdominis muscles. Considering tumbling treatments were applied well after the ultimate pH would be reached, it is unsurprising that negligible differences would be observed.

Several measures of water-holding ability were affected by the treatments in both muscles. An interaction was observed for purge loss in LL ($P = 0.024$; Table 3.1), where less purge loss was observed in the T0 control group without additional postmortem aging compared to all other treatment combinations. The LL T0 controls further aged 10 d had comparable purge loss with tumbled samples without additional aging ($P > 0.05$), while further aging of tumbled LL sections induced more purge loss compared to T0 ($P < 0.05$). Thawing loss of LL was unaffected by tumbling and aging main effects, as well as the interaction ($P > 0.05$). Tumbling for any duration increased cooking loss of LL steaks relative to the T0 controls ($P < 0.001$; Table 3.1). The T40 treatment maintained significantly lower cooking loss (22.4%) than T80 (24.6%) and T120 (24.1%) treatments. Aging LL for 10 d lowered cooking loss (21.2%) compared to the 0 d group (24.5%) ($P < 0.001$). A significant interaction was observed for purge loss in ST (Table 3.2) No meaningful difference was observed for purge loss among tumbled ST muscles aged 0 d ($P > 0.05$). With aging, greater purge loss was induced in tumbled ST muscles compared to T0 ($P < 0.05$). Thawing loss was unaffected by the treatments ($P > 0.05$). No differences in cooking loss were found among tumbled and control ST steaks aged an additional 10 d ($P > 0.05$), although the T0 control at 0 d maintained lower cooking loss than tumbled steaks at the same postmortem time ($P < 0.05$). Previous studies have reported that tumbling would be beneficial to water-holding ability in both raw and cooked products (Cheng and Ockerman, 2003; Gao et al., 2014; Morrow et al., 2019; Garmyn et al., 2020); however, these findings would be mainly attributed to brine inclusion.

Without the addition of a brine, it has been shown that tumbling of beef rectus abdominis and LL muscles would induce greater cooking loss compared to non-tumbled controls (Morrow et al., 2019; Tuell et al., 2021), in agreement with the present study. While Tuell et al. (2021) reported that purge loss during aging would not be affected by tumbling, the present study found that tumbling would worsen purge loss in both LL and ST muscles. This finding is likely due to the migration of moisture with aging being exacerbated through tumbling expanding the extracellular spaces (Hughes et al., 2014; Sharedeh et al., 2015; N'Gatta et al., 2021). In general, lower cooking loss of aged steaks would likely be related to prior moisture loss as purge coupled with swelling of the myofibrils induced through proteolysis; however, this observation is not always consistent in the literature (Hughes et al., 2014; Warner et al., 2017).

For instrumental tenderness, tumbling LL muscles for any duration decreased WBSF values relative to the T0 control group ($P < 0.001$; Table 3.1), regardless of aging duration. There were no significant differences in WBSF among different durations of tumbling in LL. Aging for 10 d decreased WBSF values relative to 0 d ($P < 0.001$). No interaction of tumbling with aging was found for WBSF values in LL ($P > 0.05$). For WBSF values of ST steaks, no main effect of tumbling was observed, nor was there any interaction between tumbling and aging duration ($P > 0.05$; Table 3.2). Further aging decreased WBSF values of ST steaks from 41.7 N to 36.6 N ($P < 0.001$). Increased instrumental tenderness of tumbled LL steaks was in line with the study by Tuell et al. (2021). Regarding the ST, numerous studies have reported the limited aging potential of muscles from the beef round primal (Stolowski et al., 2006; Anderson et al., 2012; Nair et al., 2019; Roy et al., 2021). The study by Morrow et al. (2019) reported that 20 min of tumbling would have no impact on slice shear force values of beef rectus abdominis muscles. However, N'Gatta et al. (2021) recently reported that extended tumbling (greater than 3 h) of beef ST muscles could influence textural properties through changes to muscle micro- and ultrastructure. Accordingly, it may be that longer durations of tumbling without brine inclusion would be necessary to influence the textural properties of inherently tough cuts. Results may also be somewhat influenced by the use of packaging preventing friction between pieces, although the brief deformation phase is considered as the primary force influencing texture and would be less likely to be affected by packaging (Daudin et al., 2016). The discrepancies in the effectiveness of tumbling between the LL and ST muscles are likely attributable to intermuscular differences in the amount and properties of connective tissue (Jeremiah et al., 2003; Torrescano et al., 2003; Rhee et al., 2004; Roy et al.,

2021). Differences may also be partly due to tumbling exacerbating greater moisture losses in the ST, which would be expected to contribute to poorer tenderness (Hughes et al., 2014; Warner, 2017; Warner et al., 2020). Supporting this, Rhee et al. (2004) reported that cooking loss would be significantly correlated to instrumental tenderness of beef ST but not longissimus thoracis et lumborum muscles.

In general, tumbling did not affect instrumental color attributes of LL steaks ($P > 0.05$; Table S 3.1), nor were any significant interactions with aging observed. Main effects of aging were observed in LL for all measured color attributes ($P < 0.05$), except R630/580 nm ($P > 0.05$). The LL steaks further aged 10 d had higher CIE L^*, CIE a^*, CIE b^*, chroma, and hue angle values compared to 0 d counterparts ($P < 0.05$). Tumbling did not affect instrumental color attributes of ST steaks ($P > 0.05$; Table S 3.2), and no significant interactions between tumbling and aging were observed. Aging increased CIE L^*, CIE b^*, and hue angle values, while R630/580 nm values decreased ($P < 0.05$). Aging did not affect CIE a^* and chroma values in ST steaks ($P > 0.05$). An increase in color attributes of aged beef is consistent with previous studies (Wyrwisz et al., 2016). The findings for color attributes of tumbled muscles suggest that initial color would be mostly unaffected. Nevertheless, it would be expected that damage caused by tumbling could disrupt cellular membranes and promote the release of free radicals (Cheng and Ockerman, 2003; Dang et al., 2022). Considering the color stability of beef is well known to worsen with the duration of postmortem aging (Beriain et al., 2009; Ma et al., 2017), further study regarding how tumbling with subsequent postmortem aging would affect color stability and oxidation would be necessary.

3.4.2 Proteolysis

Several main and interactive effects were found for the degradation of troponin-T and desmin, as well as calpain-1 autolysis, with representative western blots presented in the supplementary materials (Fig. S 3.1 – S. 3.6). A tumbling by aging interaction was observed for the intact 39-kDa band of troponin-T in both LL ($P < 0.001$; Fig. 3.1) and ST ($P < 0.05$; Fig. 3.2). For the LL, less intact troponin-T was observed in the T80 and T120 groups at 10 d compared to the T0 control at the same postmortem timepoint. At 0 d, a slightly lower abundance of the intact band was observed in T0 compared to T120. For the ST, relative abundance of intact troponin-T for the T120 treatment further aged 10 d was lower than all other treatment combinations. An

increase in relative abundance of the 30-kDa degradation band was found with aging duration only in both LL ($P < 0.001$; Fig. S 3.7) and ST ($P < 0.001$; Fig. S 3.8). Similarly, desmin degradation was affected by aging only in LL ($P < 0.001$; Fig. S 3.9). While relative abundance of the 42-kDa degradation band of desmin increased with aging in ST ($P < 0.001$; Fig. S 3.10), an interaction between tumbling and aging was observed for the 55-kDa intact band ($P < 0.05$; Fig. 3.3). No differences across tumbling treatments were observed within the 0 d group, while T120 had less intact desmin at 10 d. The results were supported in part by the autolysis of calpain-1 in LL (Table 3.3) and ST (Table 3.4). For the LL, aging decreased relative abundances of the 80-kDa and 78-kDa bands, thereby increasing relative abundance of the 76-kDa band ($P < 0.001$). While aging did not affect the 80-kDa band in ST ($P > 0.05$), a similar pattern was found with aging for the 78-kDa and 76-kDa bands ($P < 0.001$). In general, there were limited effects of tumbling on calpain-1 autolysis, although tumbling appeared to decrease the relative abundance of the 78-kDa band in the ST ($P < 0.05$).

Collagen content and solubility were not affected by tumbling, aging, nor the interaction in either LL or ST ($P > 0.05$; Tables 3.5 & 3.6). It has been shown that damage caused by ultrasonication could rupture the perimysium and increase collagen solubility (Wang et al., 2022). This finding was attributed to ultrasound damage releasing cathepsin B and L from the lysosomes, allowing them to degrade collagen during postmortem aging. Considering the lack of treatment differences for collagen, the findings from this study suggest there would be limited impacts of tumbling on the degradation of collagen.

Numerous studies have observed that damage to muscle micro- and ultrastructure could enhance the activity of certain proteolytic enzymes, likely due to the release of enzymes (e.g., cathepsins from the lysosomes) or cofactors (e.g., calcium ions from the sarcoplasmic reticulum to increase calpain-1 activity) (Kim et al., 2018; Setyabrata and Kim, 2019; Tuell et al., 2021; Dang et al., 2022; Wang et al., 2022). The study by Tuell et al. (2021) reported that tumbling without brine would result in a greater extent of troponin-T but not desmin degradation during subsequent postmortem aging, largely in agreement with the present study. Although there was a significant tumbling by aging interaction observed for the disappearance of intact desmin in the ST, it is likely this was insufficient to meaningfully impact instrumental tenderness due to overriding factors. Particularly, a high amount of background toughness could inhibit tenderization of certain muscles, even if considerable proteolysis is exhibited (Rhee et al., 2004; Anderson et

al., 2012; Chun et al., 2020). While there is presently little available information in relation to the mechanism of how tumbling-induced damage could influence proteolysis, several recent studies have been conducted regarding damage induced through ultrasonication. For instance, Dang et al. (2022) reported that ultrasound damage would facilitate tenderization through increased cytosolic calcium levels enhancing the activity of calpain-1. Although it is reasonable to postulate that physical disruptions induced through tumbling would have a similar effect, it may be that the postmortem timepoint that tumbling was applied in this study was not optimal to cause measurable impacts on calpain-1 activation. However, it should be noted that while fresh beef tumbling at 5 d postmortem resulted in minimal impacts on calpain-1 autolysis, the present study still found several interactive effects of tumbling and aging on the degradation of the intact bands of troponin-T and desmin. These observations could indicate that the improved instrumental tenderness and the extent of degradation of myofibrillar proteins of fresh beef muscles upon tumbling would be likely attributed to synergistic impacts of tumbling on the disruption of the myofiber structure and ensuing easier access of proteolytic enzymes for cytoskeletal myofibrillar protein degradation during aging, rather than elevated activation of calpain-1 through more calcium ion release. Considering the findings for the MFI and TEM results collected in the present study, this postulation appears well-supported.

There were obvious tumbling effects on myofibrillar fragmentation shown by MFI values in both LL and ST muscles ($P < 0.001$; Tables 3.5 & 3.6). While MFI is typically associated with aging, myofibrillar fragmentation has also been exhibited with physical interventions that disrupt the myofibrillar structure including tumbling and mechanical tenderization (Katsaras and Budras, 1993; Tyszkiewicz et al., 1997; Tuell et al., 2021). Supporting these data, representative TEM images at 2,550× (Fig. 3.4 & 3.5 for LL and ST, respectively) and 11,500× magnification (Fig. S 3.11 & S 3.12 for LL and ST, respectively) are provided. For both muscles, ultrastructural components appear mostly intact within the T0 group with 0 d of aging. With the application of additional aging, degradation at the Z disks was clearly apparent in the LL muscle (Fig. 3.4 & S 3.11); however, no obvious differences with aging only were apparent in the ST (Fig. 3.5 & S 3.12). Regardless of aging duration and muscle, samples from the T120 treatment exhibited numerous breaks in the myofibrils at the junction of the Z disk with the I band. Further, several lateral breakages extending across multiple adjacent myofibrils were observable. Katsaras and Budras (1993) reported that tumbling would disintegrate myofibrillar structures near the external

surface of the muscle exposed to the most kinetic force, whereas portions of ultrastructural integrity could be preserved in the interior. As samples for TEM were collected at a consistent depth, it is unclear if any gradient in ultrastructural damage would be apparent. However, taken together with the MFI results, it is likely that tumbling under the conditions used in this study would result in immediate dislocation of structural integrity as well as subsequent increases in myofibrillar fragmentation throughout the entire section. As previously discussed, it is likely that fragmentation of the myofibrils through tumbling would be sufficient to cause considerable textural changes in muscles without a high contribution of background toughness (e.g., LL) but not in those with (e.g., ST).

3.4.3 Consumer evaluation

Demographic characteristics of consumer panelists are provided in Tables S3 and S4 for LL and ST, respectively. Several main effects were found for consumer sensory scores of LL steaks ($P < 0.05$; Table 3.7). The LL steaks tumbled for any duration had a higher liking of tenderness compared to T0 control steaks ($P < 0.001$), as well as greater overall liking ($P < 0.05$), regardless of aging period. No differences were found among different durations of tumbling for these attributes ($P > 0.05$). Aging of LL steaks increased tenderness ($P < 0.001$) and overall liking ($P < 0.01$), irrespective of tumbling treatment. The interaction term was not significant for any sensory score within LL, nor were tumbling and aging main effects significant for juiciness or flavor liking of LL steaks. Within the ST, tumbling did not affect flavor or overall liking ($P > 0.05$; Table 3.8). Further aging increased overall liking of the ST ($P < 0.05$). For liking of tenderness and juiciness, the interaction term was significant for the ST. Additional aging increased tenderness within the T120 treatment ($P < 0.05$), while no effect of aging was observed within other tumbling groups, as well as T0 controls ($P > 0.05$). Consequently, ST steaks from the T120 group aged 10 d had greater liking of tenderness compared to the T0 group at the same timepoint ($P < 0.05$). For juiciness, T0 steaks within the 0 d group were juicier than T40 and T120 counterparts at the same timepoint ($P < 0.05$). Juiciness increased with aging in the T40 and T120 groups ($P < 0.05$), while no differences with aging were found within the T0 and T80 groups ($P > 0.05$). Despite several differences among the fixed effects observed in consumer sensory scores, the acceptability (Tables S 3.5 & S 3.6 for LL and ST, respectively) and perceived quality (Tables

S 3.7 & S 3.8 for LL and ST, respectively) percentages were not affected in either muscle ($P > 0.05$).

The findings for consumer liking of tenderness were in general agreement with the quality and proteolysis data. Increased instrumental tenderness with tumbling was reflected by greater consumer liking of tenderness in LL steaks. To the best of our knowledge, this is the first study to evaluate consumer palatability attributes of beef LL and ST steaks tumbled without a brine. Morrow et al. (2019) found tumbling without a brine would not affect slice shear force or subjective tenderness of beef rectus abdominis muscles. Considering the significant interaction observed for subjective tenderness of the ST steaks in the present study, it may be that tumbling with subsequent postmortem aging may be able to overcome the inherent toughness of certain beef muscles as assessed by the consumer. When ST steaks were not further aged, consumer liking of tenderness was equivalent across treatments, in agreement with observations for the rectus abdominis muscle reported by Morrow et al. (2019). While WBSF was unchanged with tumbling and the tumbling by aging interaction within the ST, discrepancies between objective and subjective measures of tenderness have been reported by several previous studies (Rhee et al., 2004; Strydom et al., 2015). Strydom et al. (2015) suggested that panelists may rate steaks of equivalent WBSF values dissimilarly in subjective tenderness, attributable to differences in mouthfeel from the complex interaction of myofibrillar proteins, intramuscular connective tissues, and other factors which may not be reflected by instrumental evaluation. Although consumer liking of tenderness was influenced by the treatments, the impact on consumer acceptability and perceived quality percentages were largely unaffected. This observation would likely be due to most steaks in the present study being below the threshold to be considered acceptably tender by most consumers (Shackelford et al., 1991; Belew et al., 2003; Sullivan and Calkins, 2011). Another consideration would be the use of relatively thin steaks (2 cm thickness), which could be beneficial to the perception of tenderness when compared to steaks of a greater thickness (Miller et al., 2019).

While juiciness is generally considered as a minor component to overall beef palatability (O'Quinn et al., 2018), the ST muscle is known to rank remarkably poorly for this attribute (Jeremiah et al., 2003; Sullivan and Calkins, 2011). In fact, Jeremiah et al. (2003) reported that the ST would be rated lowest of 33 evaluated beef muscles for juiciness. Although the tumbling treatments did appear to be detrimental to water-holding, previous studies have reported inconsistencies between measures of water-holding and juiciness (Rhee et al., 2004; Hughes et al.,

2014; Warner, 2017; Morrow et al., 2019). For instance, Morrow et al. (2019) reported that juiciness of rectus abdominis steaks tumbled without a brine would be equivalent to non-tumbled controls, despite exhibiting greater cooking loss. This finding would be in good agreement with the observations for the LL where no differences in juiciness were found among treatments, despite tumbling increasing cooking loss. This observation may also be related to the relationship of subjective tenderness and juiciness. It is generally considered that the relationship between subjective tenderness and juiciness is strong (Warner, 2017), and it has also been shown that a favorable perception of one eating quality attribute (e.g., tenderness) may result in others being more positively perceived as well (Roeber et al., 2000). For the ST steaks, it may be that consumers would perceive steaks within the 0 d aged group as drier due to poorer water-holding with no benefit to tenderness. Conversely, within the 10 d group, equivalent juiciness between tumbled and control ST steaks could be related to an improvement in the perception of tenderness among the tumbled treatments.

Taken together, the findings of this study support that tumbling without brine inclusion would result in considerable structural dislocation and fragmentation of the myofibrils, which may in turn facilitate more degradation of myofibrillar proteins over the course of postmortem aging. However, tumbling would not influence the instrumental tenderness of the ST, despite obvious myofibrillar fragmentation. This observation would likely be attributable to tumbling having negligible impacts on the properties of collagen. Accordingly, for beef muscles where tenderness is not primarily driven by a high contribution of intramuscular connective tissues (e.g., LL), tumbling without brine inclusion alone may be sufficient to improve tenderness and overall liking. For muscles with considerable background toughness (e.g., ST), tumbling followed by subsequent postmortem aging may be an effective strategy to improve consumer liking of tenderness; however, further study would be necessary to optimize the process. These findings demonstrate the feasibility of tumbling without brine inclusion as a potential method to ensure consumers are provided fresh beef steaks with consistently high quality and palatability attributes.

3.5 Acknowledgements

Appreciation is extended to Dr. Chris Gilpin and Robert Seiler of the Whistler Life Science Microscopy Facility for assistance in transmission electron microscopy.

3.6 Tables and Figures

Table 3.1. Effect of tumbling and aging durations on meat quality attributes of longissimus lumborum muscles (n = 16)

Treatment		pH	Purge loss, %	Thawing loss, %	Cooking loss, %	WBSF, N
T[1]						
T0		5.58[b]	0.9	3.1	20.3[c]	29.6[a]
T40		5.60[a]	2.2	2.7	22.4[b]	25.0[b]
T80		5.58[b]	2.0	2.9	24.6[a]	25.7[b]
T120		5.56[b]	2.1	2.8	24.1[a]	24.1[b]
SEM		0.01	0.2	0.2	0.7	1.3
A[2]						
0 d		5.58	1.0	2.9	24.5[x]	28.0[x]
10 d		5.58	2.6	2.9	21.2[y]	24.2[y]
SEM		0.01	0.1	0.1	0.6	1.2
$T \times A$						
T0	0 d	5.57	0.4[C]	2.9	21.0	31.6
	10 d	5.58	1.4[B]	3.3	19.6	27.5
T40	0 d	5.60	1.0[B]	2.7	24.0	26.7
	10 d	5.59	3.3[A]	2.7	20.9	23.4
T80	0 d	5.59	1.2[B]	3.1	26.9	27.5
	10 d	5.57	2.8[A]	2.8	22.4	24.0
T120	0 d	5.56	1.3[B]	2.8	26.1	26.1
	10 d	5.57	2.9[A]	2.8	22.1	22.0
SEM		0.01	0.2	0.2	0.9	1.5
Significance of P-value						
T		0.005	<0.001	0.081	<0.001	<0.001
A		0.760	<0.001	0.717	<0.001	<0.001
$T \times A$		0.239	0.024	0.202	0.201	0.981

[1]Tumbling duration (min)

[2]Aging duration (days)

[a-c]Means lacking a common superscript within a column differ at ($P < 0.05$) due to tumbling main effect.

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

[A-C]Means lacking a common superscript within a column differ at ($P < 0.05$) due to the interaction of tumbling and aging.

Table 3.2. Effect of tumbling and aging durations on meat quality attributes of semitendinosus muscles (n = 16)

Treatment		pH	Purge loss, %	Thawing loss, %	Cooking loss, %	WBSF, N
T[1]						
T0		5.65	1.4	3.8	25.9	40.4
T40		5.66	3.0	4.0	29.1	39.2
T80		5.65	2.6	3.8	28.2	38.6
T120		5.63	3.0	3.7	29.1	38.4
SEM		0.01	0.2	0.3	0.8	1.4
A[2]						
0 d		5.60^{y}	0.8	4.0	30.2	41.7^{x}
10 d		5.69^{x}	4.2	3.7	26.0	36.6^{y}
SEM		0.01	0.2	0.2	0.7	1.1
$T \times A$						
T0	0 d	5.62	0.5^{D}	3.8	26.5^{B}	42.2
	10 d	5.67	2.3^{C}	3.8	25.3^{B}	38.6
T40	0 d	5.61	0.8^{D}	4.4	31.7^{A}	43.1
	10 d	5.70	5.2^{A}	3.7	26.5^{B}	35.2
T80	0 d	5.61	0.9^{D}	4.2	30.7^{A}	40.3
	10 d	5.69	4.3^{B}	3.5	25.8^{B}	36.9
T120	0 d	5.58	1.1^{D}	3.6	31.7^{A}	41.1
	10 d	5.68	5.0^{AB}	3.8	26.5^{B}	35.6
SEM		0.02	0.3	0.4	0.9	1.7
Significance of P-value						
T		0.373	<0.001	0.799	<0.001	0.545
A		<0.001	<0.001	0.251	<0.001	<0.001
$T \times A$		0.401	<0.001	0.457	0.013	0.421

[1]Tumbling duration (min)

[2]Aging duration (days)

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

[A-D]Means lacking a common superscript within a column differ at ($P < 0.05$) due to the interaction of tumbling and aging.

Table 3.3. Effect of tumbling and aging durations on relative abundance of calpain-1 of longissimus lumborum muscles (n = 8)

Treatment	80-kDa	78-kDa	76-kDa
T[1]			
T0	0.124	0.208	0.668
T40	0.122	0.200	0.678
T80	0.112	0.209	0.679
T120	0.113	0.198	0.690
SEM	0.027	0.020	0.044
A[2]			
0 d	0.128[x]	0.253[x]	0.619[y]
10 d	0.108[y]	0.155[y]	0.738[x]
SEM	0.026	0.018	0.043
Significance of P-value			
T	0.169	0.835	0.745
A	<0.001	<0.001	<0.001
$T \times A$	0.465	0.972	0.967

[1]Tumbling duration (min)

[2]Aging duration (days)

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Table 3.4. Effect of tumbling and aging durations on relative abundance of calpain-1 of semitendinosus muscles (n = 8)

Treatment	80-kDa	78-kDa	76-kDa
T[1]			
T0	0.234	0.295[a]	0.471
T40	0.238	0.275[b]	0.487
T80	0.232	0.273[b]	0.495
T120	0.229	0.269[b]	0.502
SEM	0.007	0.009	0.014
A[2]			
0 d	0.237	0.309[x]	0.454[y]
10 d	0.229	0.247[y]	0.524[x]
SEM	0.006	0.008	0.012
Significance of P-value			
T	0.662	0.033	0.073
A	0.119	<0.001	<0.001
$T \times A$	0.610	0.932	0.869

[1]Tumbling duration (min)

[2]Aging duration (days)

[a,b]Means lacking a common superscript within a column differ at ($P < 0.05$) due to tumbling main effect.

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Table 3.5. Effect of tumbling and aging durations on collagen content and solubility and myofibril fragmentation index values of longissimus lumborum muscles (n = 8)

Treatment	Insoluble collagen, mg/g tissue	Soluble collagen, mg/g tissue	Total collagen, mg/g tissue	Collagen solubility, %	Myofibril fragmentation index
T[1]					
T0	4.23	0.58	4.81	11.57	50.3[d]
T40	4.47	0.78	5.25	13.27	64.5[c]
T80	3.96	0.49	4.44	10.49	82.8[b]
T120	3.95	0.46	4.41	9.68	103.5[a]
SEM	0.20	0.11	0.28	1.64	3.9
A[2]					
0 d	4.28	0.66	4.94	11.98	62.8[y]
10 d	4.02	0.50	4.52	10.52	87.8[x]
SEM	0.16	0.08	0.20	1.25	3.5
Significance of *P*-value					
T	0.125	0.192	0.112	0.370	<0.001
A	0.142	0.173	0.128	0.334	<0.001
T × *A*	0.894	0.736	0.820	0.883	0.122

[1]Tumbling duration (min)

[2]Aging duration (days)

[a-d]Means lacking a common superscript within a column differ at ($P < 0.05$) due to tumbling main effect.

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Table 3.6. Effect of tumbling and aging durations on collagen content and solubility and myofibril fragmentation index values of semitendinosus muscles (n = 8)

Treatment	Insoluble collagen, mg/g tissue	Soluble collagen, mg/g tissue	Total collagen, mg/g tissue	Collagen solubility, %	Myofibril fragmentation index
T[1]					
T0	7.20	0.57	7.78	7.28	43.9[d]
T40	6.73	0.48	7.22	6.61	57.1[c]
T80	6.42	0.48	6.90	6.84	71.6[b]
T120	6.21	0.49	6.70	7.13	86.9[a]
SEM	0.31	0.09	0.36	1.04	1.7
A[2]					
0 d	6.52	0.52	7.04	7.16	55.1[y]
10 d	6.76	0.50	7.26	6.77	74.5[x]
SEM	0.23	0.08	0.26	1.02	1.2
Significance of *P*-value					
T	0.134	0.234	0.137	0.402	<0.001
A	0.448	0.632	0.525	0.193	<0.001
T × *A*	0.631	0.896	0.680	0.861	0.067

[1]Tumbling duration (min)

[2]Aging duration (days)

[a-d]Means lacking a common superscript within a column differ at ($P < 0.05$) due to tumbling main effect.

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Table 3.7. Effect of tumbling and aging durations on consumer (n = 120) sensory scores for palatability traits of longissimus lumborum muscles (n = 16)

Treatment	Tenderness liking	Juiciness liking	Flavor liking	Overall liking
T[1]				
T0	65.4[b]	67.7	65.9	65.2[b]
T40	71.9[a]	70.6	67.4	69.4[a]
T80	74.4[a]	71.7	68.9	70.5[a]
T120	73.5[a]	68.1	68.4	69.4[a]
SEM	1.7	1.7	1.7	1.6
A[2]				
0 d	68.1[y]	68.4	66.5	66.6[y]
10 d	74.5[x]	70.7	68.8	70.5[x]
SEM	1.4	1.4	1.5	1.4
Significance of P-value				
T	<0.001	0.102	0.276	0.019
A	<0.001	0.081	0.052	0.001
$T \times A$	0.412	0.202	0.897	0.658

[1]Tumbling duration (min)

[2]Aging duration (days)

[a,b]Means lacking a common superscript within a column differ at ($P < 0.05$) due to tumbling main effect.

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Note: The 0-anchor represented extreme disliking of tenderness, juiciness, flavor, or overall liking. The 100-anchor represented extreme liking of tenderness, juiciness, flavor, or overall liking.

Table 3.8. Effect of tumbling and aging durations on consumer (n = 120) sensory scores for palatability traits of semitendinosus muscles (n = 16)

Treatment		Tenderness liking	Juiciness liking	Flavor liking	Overall liking
T[1]					
T0		58.6	61.4	59.1	59.7
T40		60.4	56.8	59.7	59.4
T80		59.7	61.2	60.5	61.2
T120		60.5	57.2	58.7	58.9
SEM		2.2	2.0	1.9	1.9
A[2]					
0 d		58.4	57.7	58.6	58.2^{y}
10 d		61.2	60.6	60.3	61.4^{x}
SEM		1.8	1.7	1.7	1.7
$T \times A$					
T0	0 d	61.2^{AB}	64.4^{A}	59.5	61.3
	10 d	56.0^{B}	58.3^{AB}	58.6	58.0
T40	0 d	58.5^{AB}	53.4^{B}	58.1	55.8
	10 d	62.2^{AB}	60.2^{A}	61.3	63.1
T80	0 d	57.7^{B}	59.7^{A}	60.0	59.4
	10 d	61.7^{AB}	62.7^{A}	61.0	62.9
T120	0 d	56.1^{B}	53.3^{B}	57.0	56.3
	10 d	65.0^{A}	61.1^{A}	60.4	61.5
SEM		2.7	2.6	2.2	2.4
Significance of P-value					
T		0.847	0.067	0.757	0.691
A		0.113	0.087	0.203	0.038
$T \times A$		0.037	0.011	0.565	0.055

[1]Tumbling duration (min)

[2]Aging duration (days)

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

[A,B]Means lacking a common superscript within a column differ at ($P < 0.05$) due to the interaction of tumbling and aging.

Note: The 0-anchor represented extreme disliking of tenderness, juiciness, flavor, or overall liking. The 100-anchor represented extreme liking of tenderness, juiciness, flavor, or overall liking.

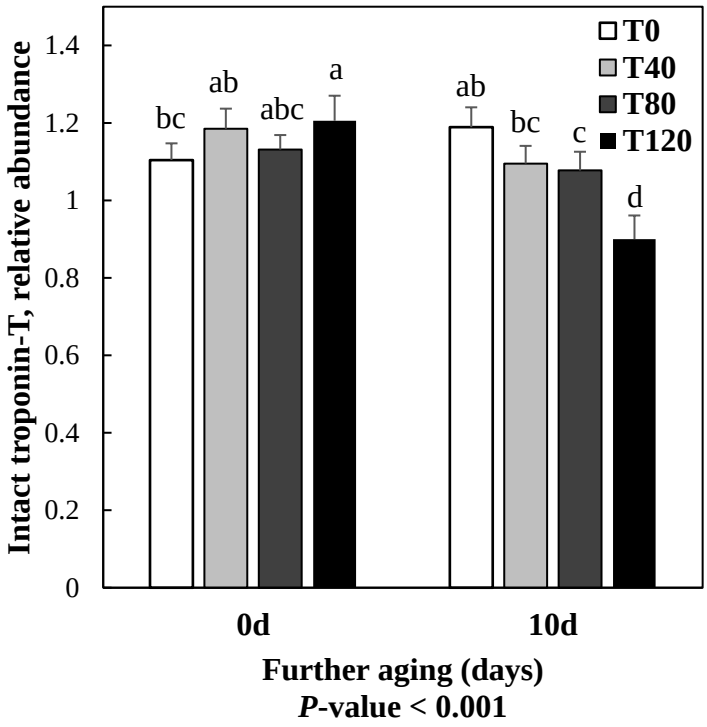


Figure 3.1. Interactive effect of tumbling and aging durations on relative abundance of intact troponin-T of longissimus lumborum muscles (n = 8). Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Means lacking a common superscript differ due to the interaction of tumbling and aging. Error bars indicate standard error of the mean.

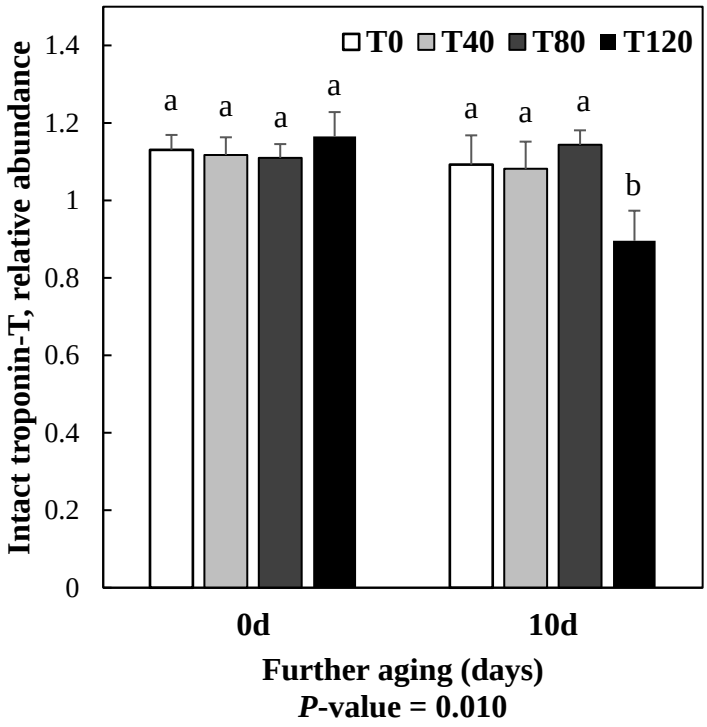


Figure 3.2. Interactive effect of tumbling and aging durations on relative abundance of intact troponin-T of semitendinosus muscles (n = 8). Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Means lacking a common superscript differ due to the interaction of tumbling and aging. Error bars indicate standard error of the mean.

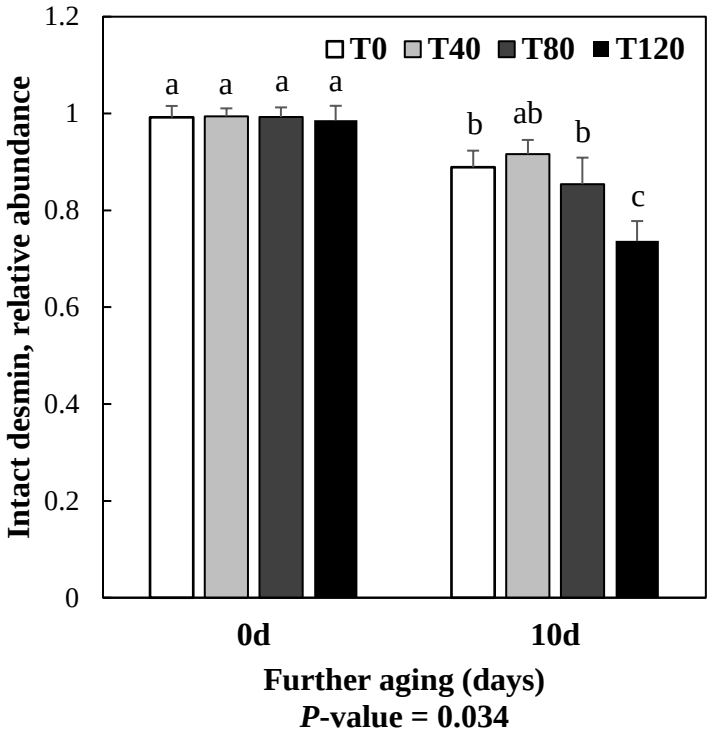


Figure 3.3. Interactive effect of tumbling and aging durations on relative abundance of intact desmin of semitendinosus muscles (n = 8). Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Means lacking a common superscript differ due to the interaction of tumbling and aging. Error bars indicate standard error of the mean.

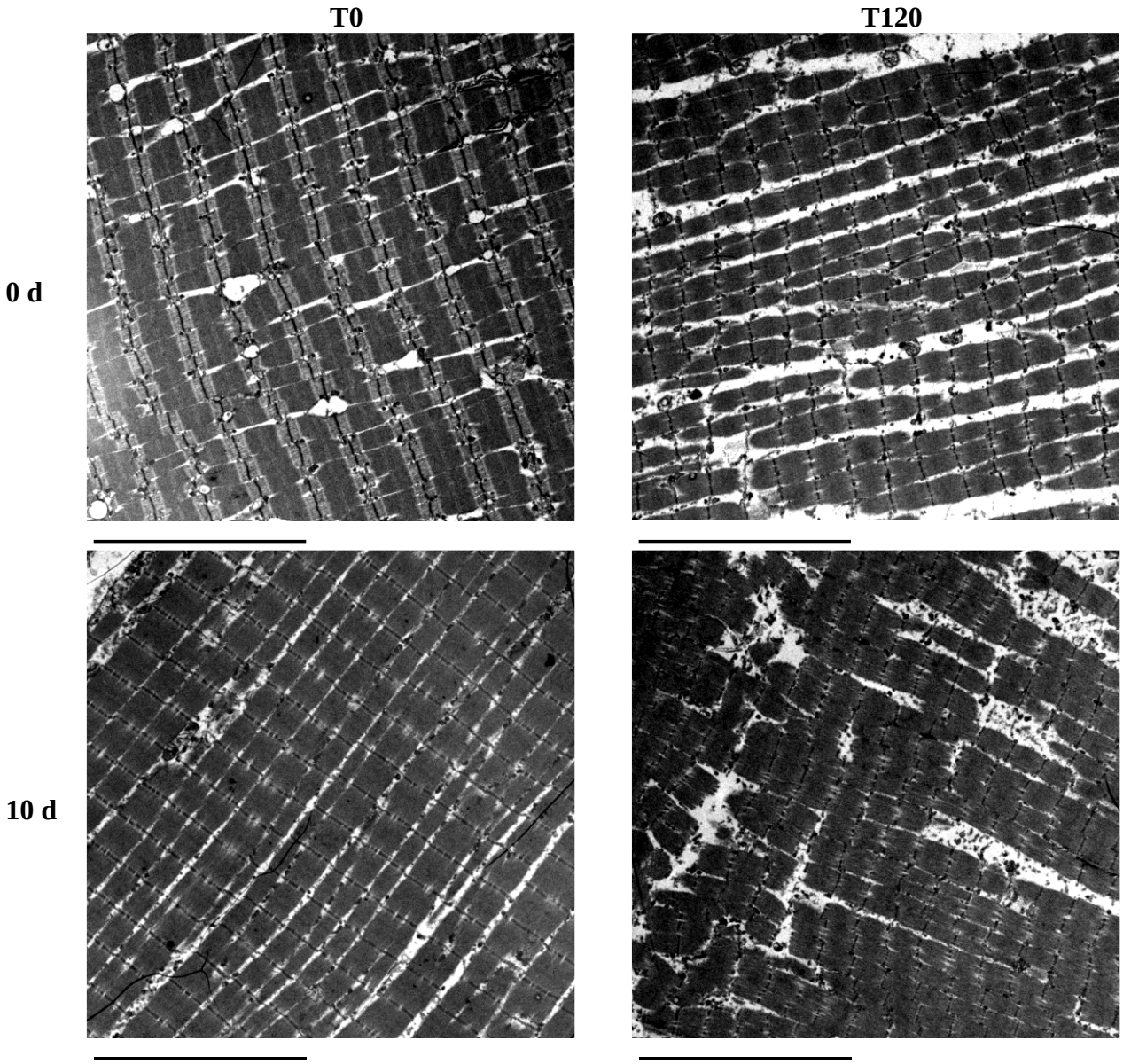


Figure 3.4. Representative transmission electron microscopy images at 2,550× magnification of longissimus lumborum muscles from different tumbling and aging treatments.

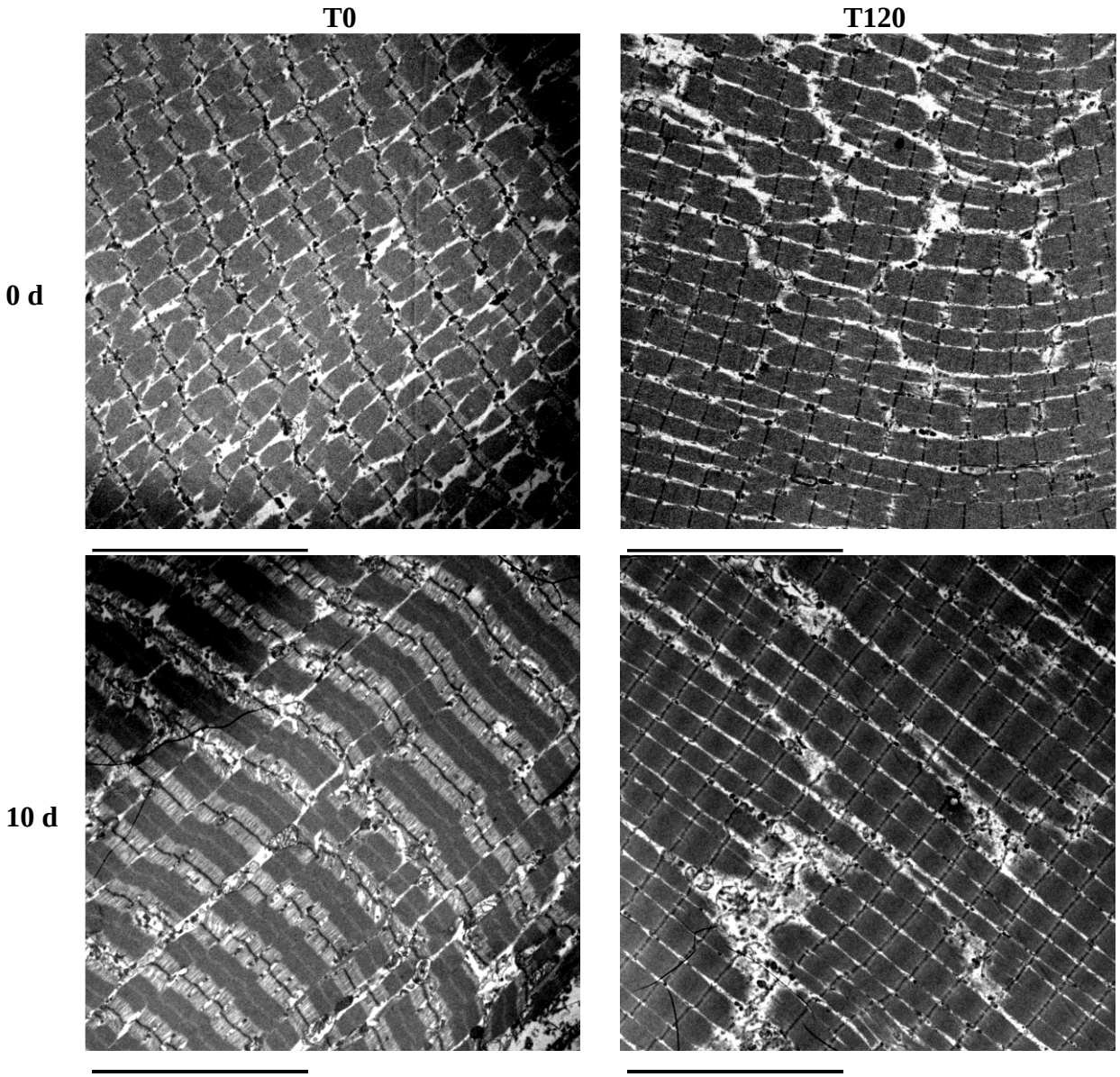


Figure 3.5. Representative transmission electron microscopy images at 2,550× magnification of semitendinosus muscles from different tumbling and aging treatments.

Table S 3.1. Effect of tumbling and aging durations on instrumental color attributes of longissimus lumborum muscles (n = 16)

Treatment	CIE L^*	CIE a^*	CIE b^*	Chroma	Hue angle	R630/580 nm
T[1]						
T0	42.7	26.6	20.0	33.3	36.9	5.3
T40	43.5	26.3	19.9	33.0	37.1	5.1
T80	43.1	26.0	19.4	32.4	36.8	5.1
T120	43.9	26.7	20.2	33.5	37.1	5.2
SEM	0.7	0.3	0.2	0.3	0.2	0.1
A[2]						
0 d	42.6^{y}	26.1^{y}	19.5^{y}	32.6^{y}	36.7^{y}	5.1
10 d	43.9^{x}	26.6^{x}	20.2^{x}	33.4^{x}	37.2^{x}	5.2
SEM	0.6	0.2	0.2	0.3	0.2	0.1
Significance of P-value						
T	0.086	0.097	0.057	0.075	0.190	0.198
A	<0.001	0.038	<0.001	0.006	<0.001	0.234
$T \times A$	0.770	0.313	0.474	0.358	0.979	0.419

[1]Tumbling duration (min)

[2]Aging duration (days)

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Table S 3.2. Effect of tumbling and aging durations on instrumental color attributes of semitendinosus muscles (n = 16)

Treatment	CIE L^*	CIE a^*	CIE b^*	Chroma	Hue angle	R630/580 nm
T[1]						
T0	45.0	27.5	21.5	34.9	38.1	5.2
T40	44.8	27.1	21.0	34.3	37.8	5.2
T80	45.3	27.1	21.1	34.4	37.8	5.1
T120	44.8	27.1	20.9	34.2	37.7	5.2
SEM	0.6	0.3	0.3	0.5	0.2	0.1
A[2]						
0 d	43.9^{y}	27.1	20.9^{y}	34.2	37.5^{y}	5.2^{x}
10 d	46.0^{x}	27.3	21.4^{x}	34.7	38.1^{x}	5.1^{y}
SEM	0.5	0.3	0.3	0.4	0.2	0.1
$T \times A$						
Significance of P-value						
T	0.795	0.702	0.415	0.550	0.492	0.746
A	<0.001	0.614	0.046	0.224	<0.001	0.022
$T \times A$	0.445	0.808	0.962	0.887	0.891	0.104

[1]Tumbling duration (min)

[2]Aging duration (days)

[x,y]Means lacking a common superscript within a column differ at ($P < 0.05$) due to aging main effect.

Table S 3.3. Demographic characteristics and beef consumption habits of consumers (n = 120) who evaluated longissimus lumborum steaks

Characteristic	Response	% of consumers
Sex	Male	43.33
	Female	56.67
	Other	0.00
	Prefer not to disclose	0.00
Household size	1 person	37.50
	2 people	25.83
	3 people	12.50
	4 people	15.00
	5 people	6.67
	>5 people	1.67
	Prefer not to disclose	0.83
Marital status	Single	59.17
	Married	39.17
	Prefer not to disclose	1.67
Age	<20 years old	2.50
	20 – 29 years old	50.00
	30 – 39 years old	29.17
	40 – 49 years old	8.33
	50 – 59 years old	5.83
	≥60 years old	3.33
	Prefer not to disclose	0.83
Ethnic origin	African-American	5.00
	Asian	17.50
	Caucasian/White	65.00
	Hispanic	5.83
	Native American	0.00
	Mixed Race	0.00
	Other	5.00
	Prefer not to disclose	1.67
Annual household income (USD)	<$25,000	37.50
	$25,000 - $34,999	9.17
	$35,000 - $49,999	10.00
	$50,000 - $74,999	6.67
	$75,000 - $99,999	3.33
	$100,000 - $149,999	15.00
	$150,000 - $199,999	5.00
	>$199,999	1.67
	Prefer not to disclose	11.67
When eating beef, which palatability trait is the most important to you?	Flavor	56.67
	Juiciness	9.17
	Tenderness	34.17

Table S 3.3, continued

When eating beef, what degree of doneness do you prefer?	Rare	1.67
	Medium-Rare	25.00
	Medium	24.17
	Medium-Well	26.67
	Well done	22.50
	Not sure	0.00
Beef consumption, meals per week[1]	-	3.83 ± 0.24

[1]Values are expressed as meals per week, not on a percentage basis. Participants were asked how many times per week they consumed beef using a line scale ranging from 0 to 20.

Table S 3.4. Demographic characteristics and beef consumption habits of consumers (n = 120) who evaluated semitendinosus steaks

Characteristic	Response	% of consumers
Sex	Male	50.00
	Female	49.17
	Other	0.83
	Prefer not to disclose	0.00
Household size	1 person	32.50
	2 people	26.67
	3 people	18.33
	4 people	10.00
	5 people	10.00
	>5 people	1.67
	Prefer not to disclose	0.83
Marital status	Single	53.33
	Married	45.00
	Prefer not to disclose	1.67
Age	<20 years old	0.83
	20 – 29 years old	47.50
	30 – 39 years old	32.50
	40 – 49 years old	9.17
	50 – 59 years old	7.50
	≥60 years old	2.50
	Prefer not to disclose	0.00
Ethnic origin	African-American	6.67
	Asian	17.50
	Caucasian/White	59.17
	Hispanic	10.00
	Native American	0.83
	Mixed Race	1.67
	Other	3.33
	Prefer not to disclose	0.83
Annual household income (USD)	<$25,000	30.00
	$25,000 - $34,999	16.67
	$35,000 - $49,999	6.67
	$50,000 - $74,999	9.17
	$75,000 - $99,999	8.33
	$100,000 - $149,999	20.00
	$150,000 - $199,999	4.17
	>$199,999	1.67
	Prefer not to disclose	3.33
When eating beef, which palatability trait is the most important to you?	Flavor	51.67
	Juiciness	14.17
	Tenderness	34.17

Table S 3.4, continued

When eating beef, what degree of doneness do you prefer?	Rare	4.17
	Medium-Rare	30.00
	Medium	25.83
	Medium-Well	21.67
	Well-done	18.33
	Not sure	0.00
Beef consumption, meals per week[1]	-	3.55 ± 0.23

[1]Values are expressed as meals per week, not on a percentage basis. Participants were asked how many times per week they consumed beef using a line scale ranging from 0 to 20.

Table S 3.5. Effect of tumbling and aging durations on consumer (n = 120) sensory acceptability percentages of longissimus lumborum muscles (n = 16)

Treatment	Tenderness acceptability, %	Juiciness acceptability, %	Flavor acceptability, %	Overall liking acceptability, %
T[1]				
T0	82.0	89.5	84.6	80.9
T40	91.0	89.4	85.2	87.5
T80	92.6	92.0	87.7	87.2
T120	90.5	86.8	85.7	85.3
SEM	7.0	6.0	6.4	7.0
A[2]				
0 d	84.2	89.0	84.6	82.9
10 d	93.3	90.1	87.0	87.6
SEM	4.7	4.0	4.5	4.8
Significance of P-value				
T	0.577	0.932	0.987	0.875
A	0.126	0.831	0.705	0.455
$T \times A$	0.952	0.979	1	0.973

[1]Tumbling duration (min)

[2]Aging duration (days)

Note: Values reflect the percentage of consumers rating the particular attribute as “acceptable”

Table S 3.6. Effect of tumbling and aging durations on consumer (n = 120) sensory acceptability percentages of semitendinosus muscles (n = 16)

Treatment	Tenderness acceptability, %	Juiciness acceptability, %	Flavor acceptability, %	Overall liking acceptability, %
T[1]				
T0	76.1	82.7	80.3	78.0
T40	83.4	74.9	80.5	77.7
T80	79.9	82.3	85.5	82.7
T120	81.3	73.7	83.1	79.6
SEM	7.6	7.9	7.0	7.5
A[2]				
0 d	74.8	76.1	80.9	76.1
10 d	84.9	81.1	83.9	82.7
SEM	5.4	5.6	4.9	5.4
Significance of P-value				
T	0.911	0.750	0.941	0.959
A	0.175	0.503	0.666	0.368
$T \times A$	0.673	0.670	0.998	0.760

[1]Tumbling duration (min)

[2]Aging duration (days)

Note: Values reflect the percentage of consumers rating the particular attribute as “acceptable”

Table S 3.7. Effect of tumbling and aging durations on consumer (n = 120) perceived quality percentages of longissimus lumborum muscles (n = 16)

Treatment	Unacceptable quality, %	Everyday quality, %	Better than everyday quality, %	Premium quality, %
T[1]				
T0	17.7	40.9	30.7	10.5
T40	13.4	44.7	27.2	14.8
T80	9.9	41.0	36.2	12.2
T120	14.6	38.0	32.0	12.9
SEM	6.8	8.8	8.5	6.5
A[2]				
0 d	15.7	42.3	31.3	9.6
10 d	11.9	40.0	31.5	16.1
SEM	4.6	6.2	5.9	4.7
Significance of P-value				
T	0.847	0.961	0.895	0.963
A	0.541	0.788	0.984	0.296
$T \times A$	0.988	0.995	0.896	0.738

[1]Tumbling duration (min)
[2]Aging duration (days)

Table S 3.8. Effect of tumbling and aging durations on consumer (n = 120) perceived quality percentages of semitendinosus muscles (n = 16)

Treatment	Unacceptable quality, %	Everyday quality, %	Better than everyday quality, %	Premium quality, %
T[1]				
T0	21.0	44.4	25.1	8.9
T40	19.9	49.5	25.2	4.2
T80	16.5	48.7	26.8	6.6
T120	21.3	49.2	22.1	7.4
SEM	7.3	4.0	7.9	5.0
A[2]				
0 d	22.8	47.0	23.6	5.9
10 d	16.7	49.0	26.0	7.3
SEM	5.3	3.1	5.5	3.3
Significance of P-value				
T	0.962	0.737	0.978	0.913
A	0.398	0.584	0.753	0.775
$T \times A$	0.828	0.989	0.849	0.869

[1]Tumbling duration (min)
[2]Aging duration (days)

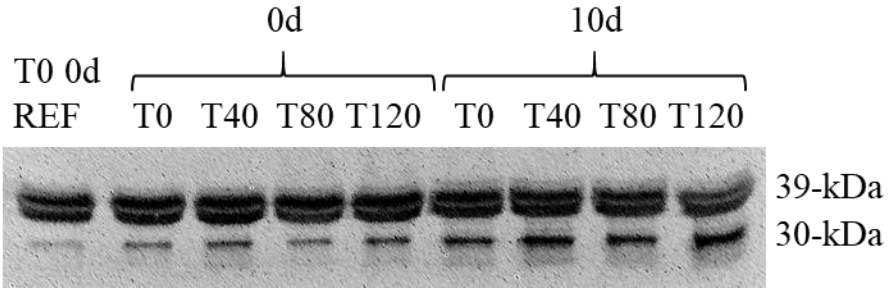


Figure S 3.1. A representative blot of troponin-T from beef longissimus lumborum muscles subjected to combined tumbling and aging treatments. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference (REF) sample.

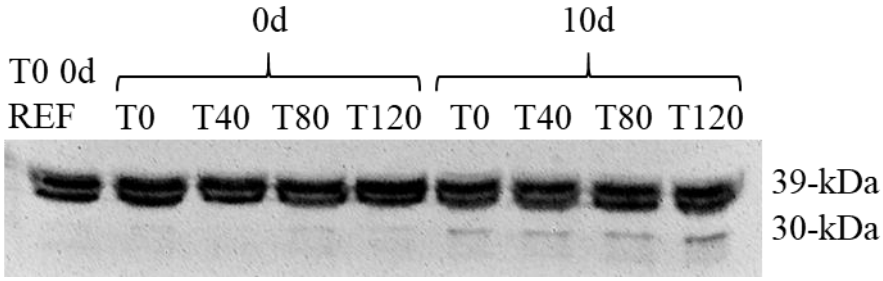


Figure S 3.2. A representative blot of troponin-T from beef semitendinosus muscles subjected to combined tumbling and aging treatments. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference (REF) sample.

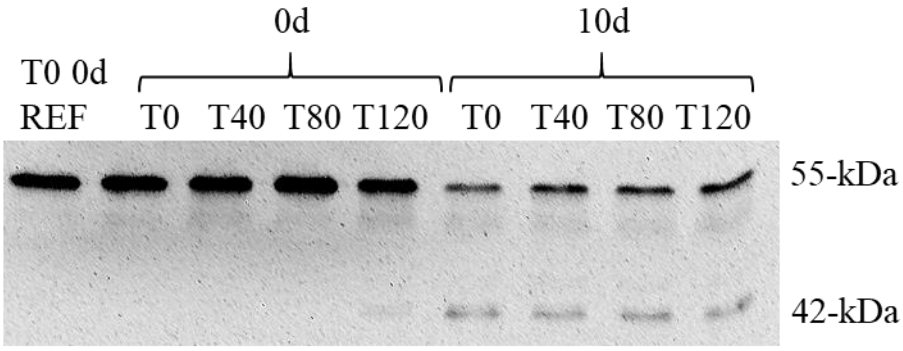


Figure S 3.3. A representative blot of desmin from beef longissimus lumborum muscles subjected to combined tumbling and aging treatments. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference (REF) sample.

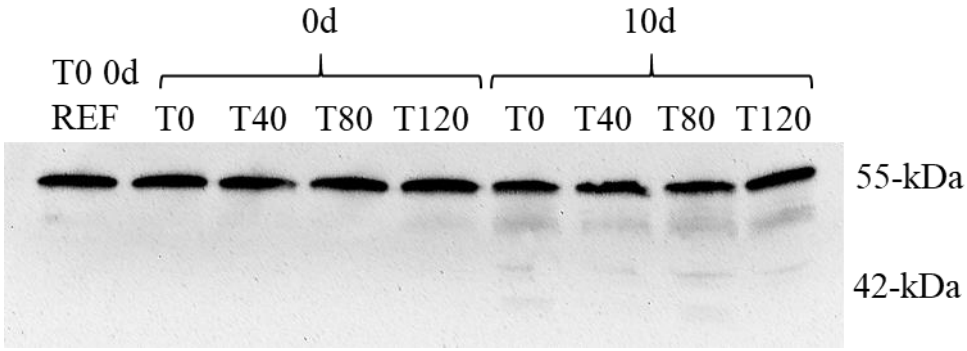


Figure S 3.4. A representative blot of desmin from beef semitendinosus muscles subjected to combined tumbling and aging treatments. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference (REF) sample.

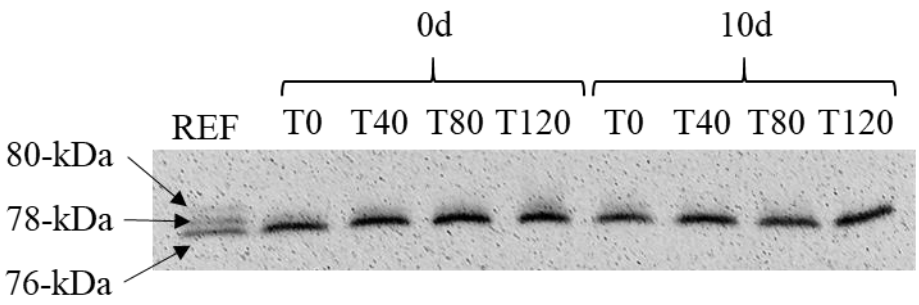


Figure S 3.5. A representative blot of calpain-1 from beef longissimus lumborum muscles subjected to combined tumbling and aging treatments. Reference (REF) sample was beef longissimus lumborum muscle obtained at 1 d postmortem.

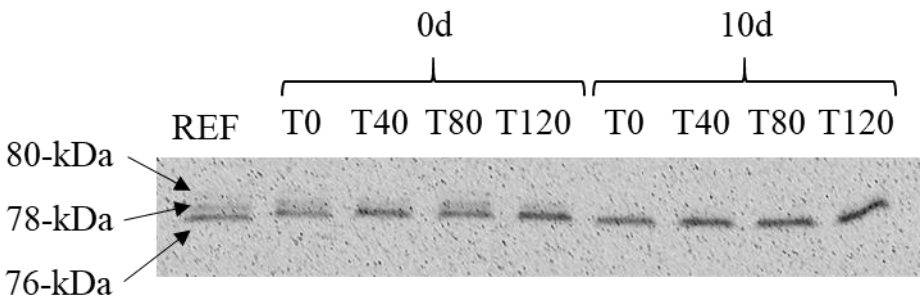


Figure S 3.6. A representative blot of calpain-1 from beef semitendinosus muscles subjected to combined tumbling and aging treatments. Reference (REF) sample was beef longissimus lumborum muscle obtained at 1 d postmortem.

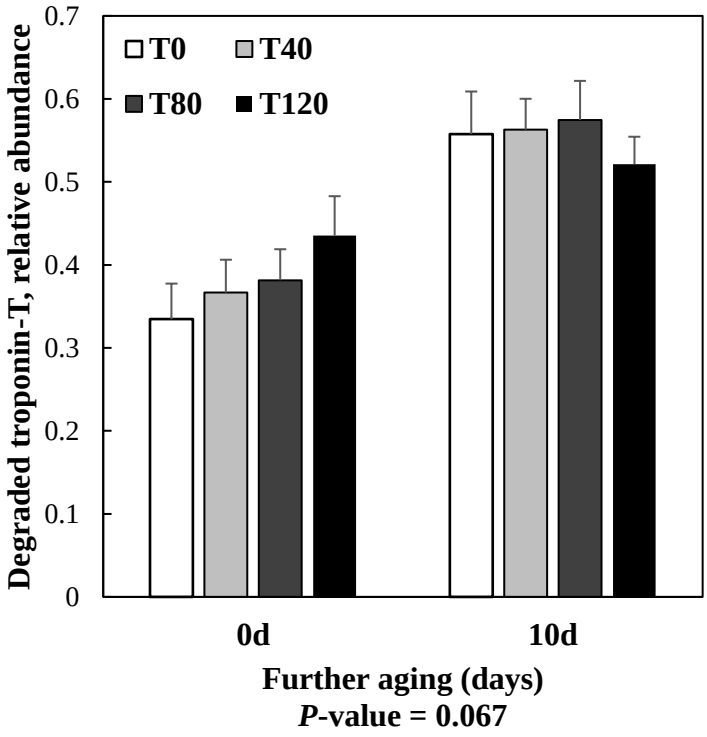


Figure S 3.7. Interactive effect of tumbling and aging durations on relative abundance of degraded troponin-T of longissimus lumborum muscles (n = 8). Only the main effect of aging was significant ($P < 0.001$) in degraded troponin-T with relative abundance of 0.380 and 0.554 for 0 d and 10 d, respectively. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Means lacking a common superscript differ due to the interaction of tumbling and aging. Error bars indicate standard error of the mean.

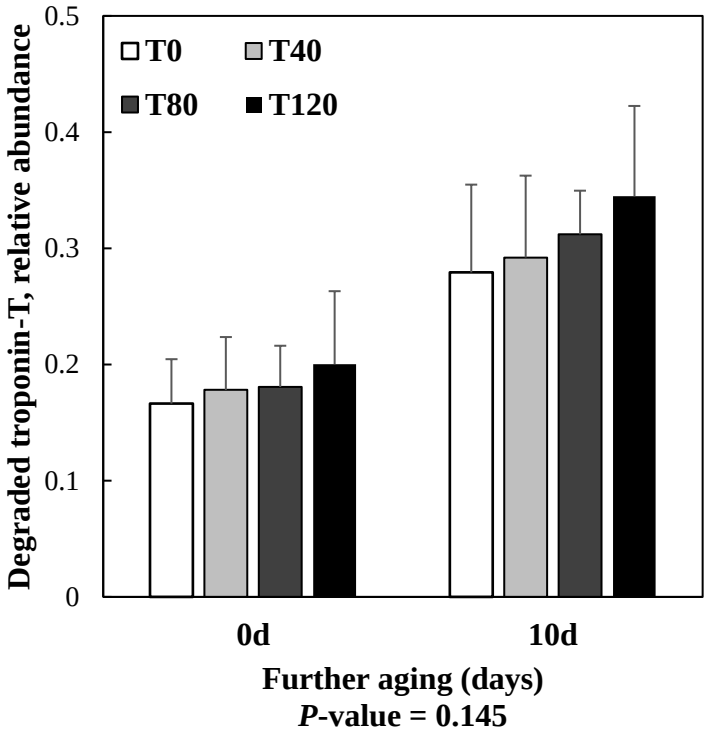
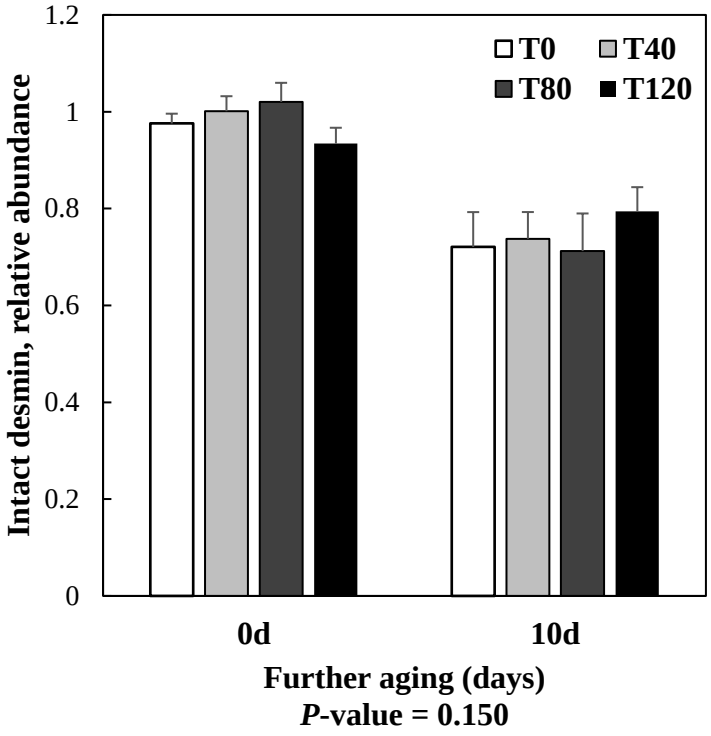
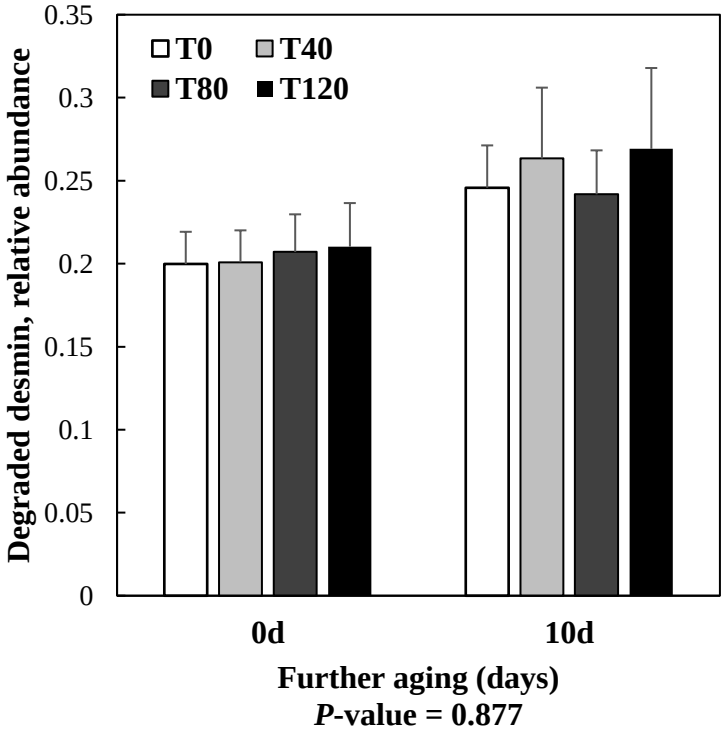


Figure S 3.8. Interactive effect of tumbling and aging durations on relative abundance of degraded troponin-T of semitendinosus muscles (n = 8). Only the main effect of aging was significant ($P < 0.001$) in degraded troponin-T with relative abundance of 0.181 and 0.307 for 0 d and 10 d, respectively. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Means lacking a common superscript differ due to the interaction of tumbling and aging. Error bars indicate standard error of the mean.

Figure S 3.9. Interactive effect of tumbling and aging durations on relative abundance of intact (A) and degraded (B) desmin of longissimus lumborum muscles (n = 8). Only the main effect of aging was significant in intact ($P < 0.001$) and degraded ($P < 0.001$) desmin. Relative abundance of intact desmin was 0.983 and 0.741 for 0 d and 10 d, respectively. Relative abundance of degraded desmin was 0.205 and 0.255 for 0 d and 10 d, respectively. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Error bars indicate standard error of the mean.


1.2
1
0.8
0.6
0.4
0.2
0
Intact desmin, relative abundance
T0
T40
T80
T120
0d
10d
Further aging (days)
P-value = 0.150

(A)


0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Degraded desmin, relative abundance
T0
T40
T80
T120
0d
10d
Further aging (days)
P-value = 0.877

(B)

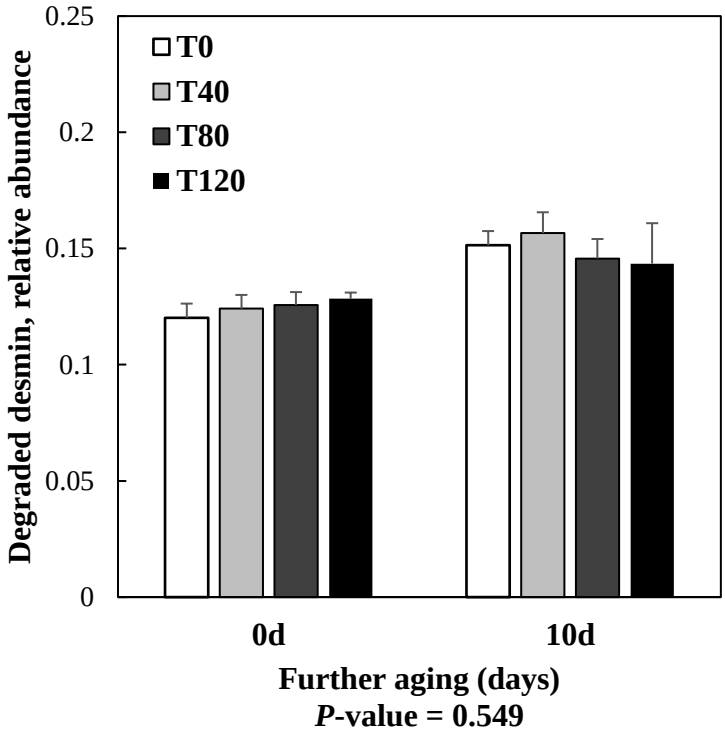


Figure S 3.10. Interactive effect of tumbling and aging durations on relative abundance degraded desmin of semitendinosus muscles (n = 8). Only the main effect of aging was significant (P < 0.001) in degraded desmin with relative abundance of 0.125 and 0.149 for 0 d and 10 d, respectively. Relative abundance was quantified by comparison to the intact band of the same T0 0 d reference sample. Means lacking a common superscript differ due to the interaction of tumbling and aging. Error bars indicate standard error of the mean.

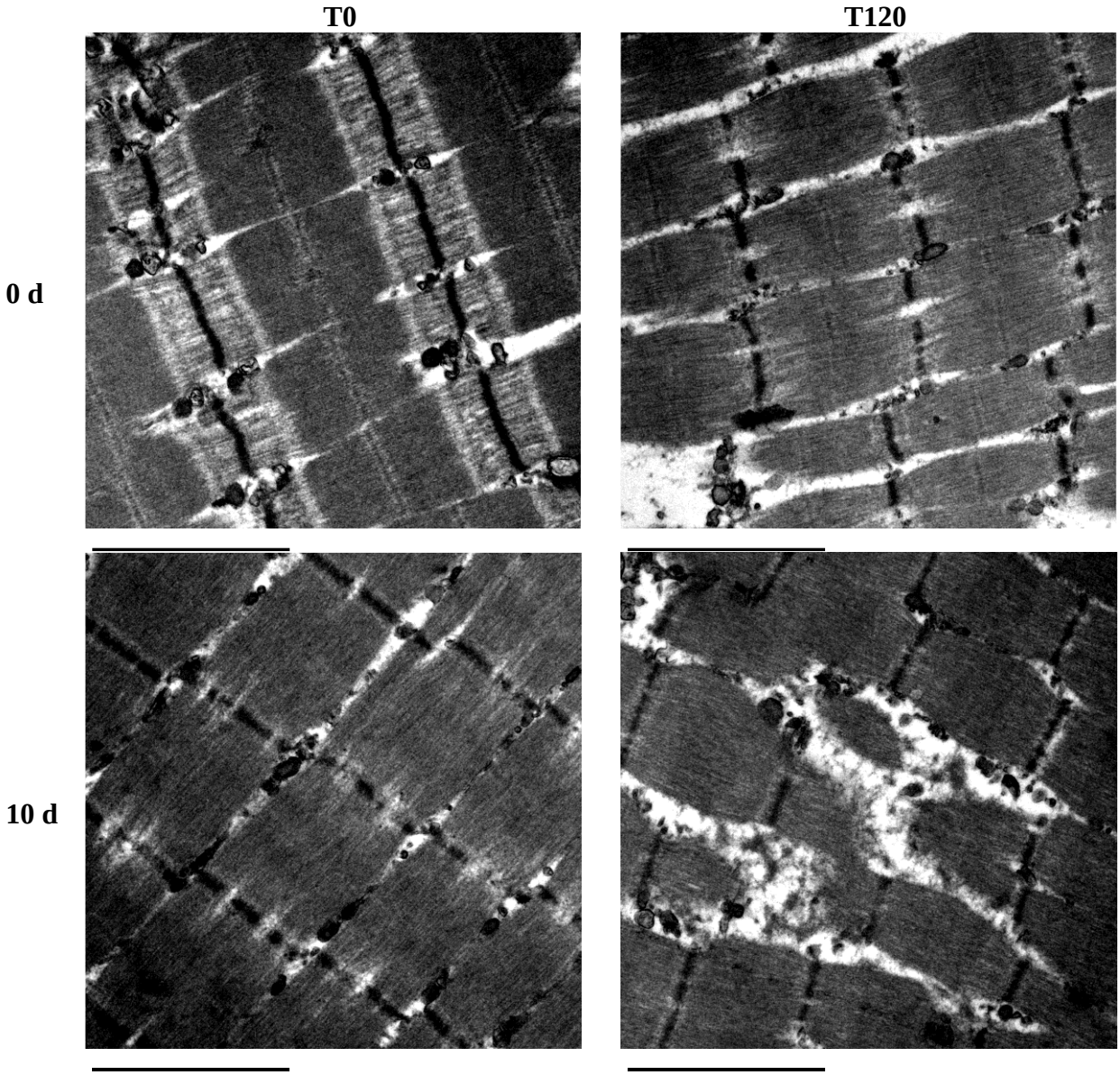


Figure S 3.11. Representative transmission electron microscopy images at 11,500× magnification of longissimus lumborum muscles from different tumbling and aging treatments.

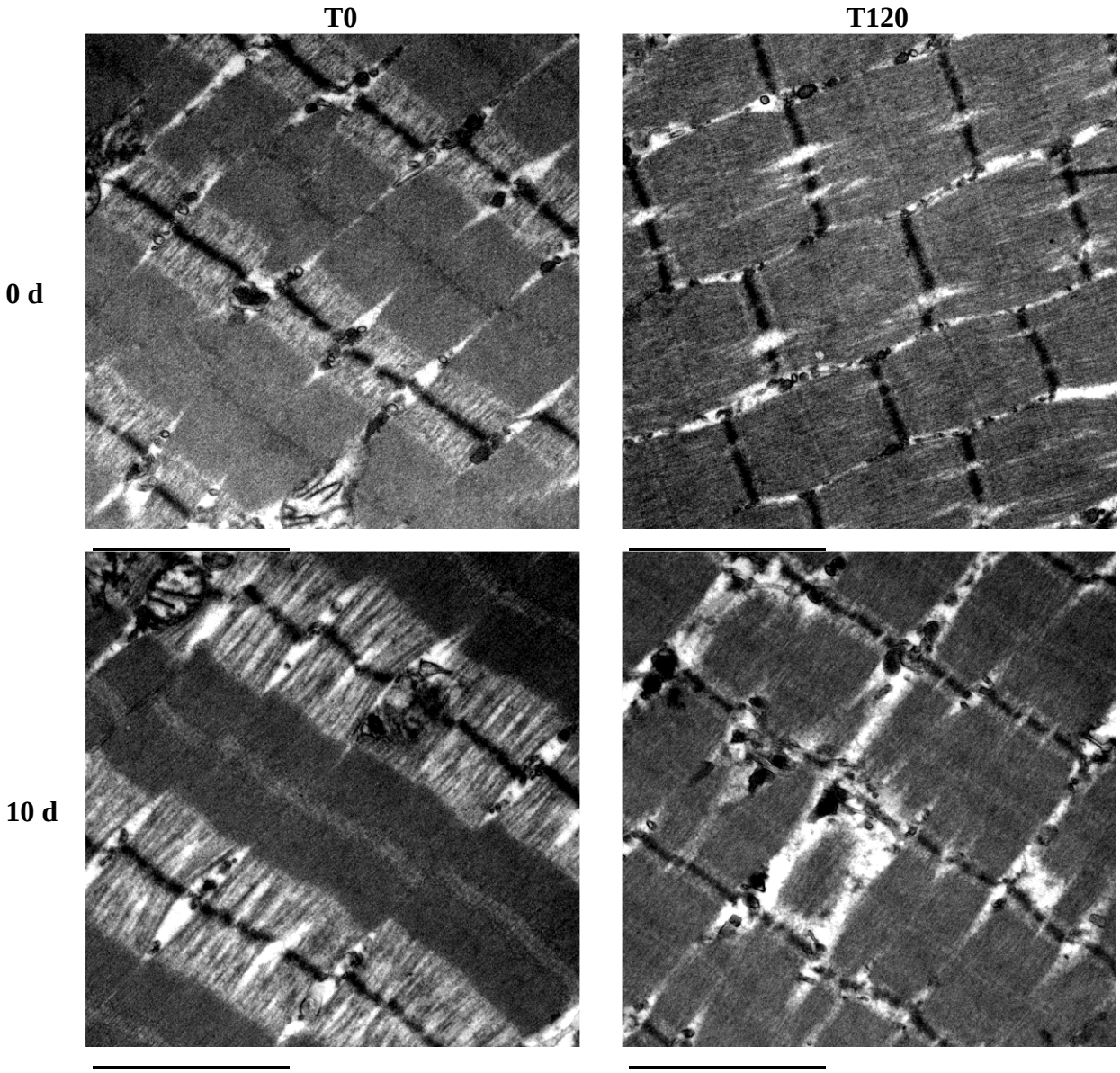


Figure S 3.12. Representative transmission electron microscopy images at 11,500× magnification of semitendinosus muscles from different tumbling and aging treatments.

3.7 References

AMSA. 2012. Meat Color Measurement Guidelines. In: American Meat Science Association, Champaign, IL, USA.

AMSA. 2015. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat. In: American Meat Science Association, Champaign, IL, USA.

Anderson, M. J., S. M. Lonergan, C. A. Fedler, K. J. Prusa, J. M. Binning, and E. Huff-Lonergan. 2012. Profile of biochemical traits influencing tenderness of muscles from the beef round. Meat Sci. 91:247–254. doi:10.1016/j.meatsci.2012.01.022.

Belew, J. B., J. C. Brooks, D. R. McKenna, and J. W. Savell. 2003. Warner–Bratzler shear evaluations of 40 bovine muscles. Meat Sci. 64:507–512. doi:10.1016/S0309-1740(02)00242-5.

Beriain, M. J., M. V. Goñi, G. Indurain, M. V. Sarriés, and K. Insausti. 2009. Predicting Longissimus dorsi myoglobin oxidation in aged beef based on early post-mortem colour measurements on the carcass as a colour stability index. Meat Sci. 81:439–445. doi:10.1016/j.meatsci.2008.09.009.

Cheng, J., and H. W. Ockerman. 2003. Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. Meat Sci. 65:1353–1359. doi:10.1016/S0309-1740(03)00057-3.

Chun, C. K. Y., W. Wu, A. A. Welter, T. G. O'Quinn, G. Magnin-Bissel, D. L. Boyle, and M. D. Chao. 2020. A preliminary investigation of the contribution of different tenderness factors to beef loin, tri-tip and heel tenderness. Meat Sci. 170:108247. doi:10.1016/j.meatsci.2020.108247.

Cross, H. R., Z. L. Carpenter, and G. C. Smith. 1973. Effects of Intramuscular Collagen and Elastin on Bovine Muscle Tenderness. J. Food Sci. 38:998–1003. doi:10.1111/j.1365-2621.1973.tb02133.x.

Culler, R. D., F. C. P. Jr, G. C. Smith, and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177–1180. doi:10.1111/j.1365-2621.1978.tb15263.x.

Dang, D. S., C. D. Stafford, M. J. Taylor, J. F. Buhler, K. J. Thornton, and S. K. Matarneh. 2022. Ultrasonication of beef improves calpain-1 autolysis and caspase-3 activity by elevating cytosolic calcium and inducing mitochondrial dysfunction. Meat Sci. 183:108646. doi:10.1016/j.meatsci.2021.108646.

Daudin, J.-D., Sharedeh, D., Favier, R., Portanguen, S., Auberger, J.-M., & Kondjoyan, A. (2016). Design of a new laboratory tumbling simulator for chunked meat: Analysis, reproduction and measurement of mechanical treatment. J. Food Eng. 170, 83–91. https://doi.org/10.1016/j.jfoodeng.2015.09.020

Gao, T., J. Li, L. Zhang, Y. Jiang, R. Ma, L. Song, F. Gao, and G. Zhou. 2014. Effect of Different Tumbling Marination Treatments on the Quality Characteristics of Prepared Pork Chops. Asian Australas. J. Anim. Sci. 28:260–267. doi:10.5713/ajas.14.0511.

Garmyn, A., N. Hardcastle, C. Bendele, R. Polkinghorne, and M. Miller. 2020. Exploring Consumer Palatability of Australian Beef Fajita Meat Enhanced with Phosphate or Sodium Bicarbonate. Foods. 9:177. doi:10.3390/foods9020177.

Gonzalez, J. M., and K. J. Phelps. 2018. United States beef quality as chronicled by the National Beef Quality Audits, Beef Consumer Satisfaction Projects, and National Beef Tenderness Surveys — A review. Asian-Australas. J. Anim. Sci. 31:1036–1042. doi:10.5713/ajas.18.0199.

Hughes, J. M., S. K. Oiseth, P. P. Purslow, and R. D. Warner. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Sci. 98:520–532. doi:10.1016/j.meatsci.2014.05.022.

Jeremiah, L. E., L. L. Gibson, J. L. Aalhus, and M. E. R. Dugan. 2003. Assessment of palatability attributes of the major beef muscles. Meat Sci. 65:949–958. doi:10.1016/S0309-1740(02)00307-8.

Katsaras, K., and K.-D. Budras. 1993. The Relationship of the Microstructure of Cooked Ham to its Properties and Quality. LWT. 26:229–234. doi:10.1006/fstl.1993.1050.

Kim, Y. H. B., D. Ma, D. Setyabrata, M. M. Farouk, S. M. Lonergan, E. Huff-Lonergan, and M. C. Hunt. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Sci. 144:74–90. doi:10.1016/j.meatsci.2018.04.031.

Ma, D., Y. H. B. Kim, B. Cooper, J.-H. Oh, H. Chun, J.-H. Choe, J. P. Schoonmaker, K. Ajuwon, and B. Min. 2017. Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. J. Agric. Food Chem. 65:6708–6716. doi:10.1021/acs.jafc.7b02175.

Martinez, H. A., A. N. Arnold, J. C. Brooks, C. C. Carr, K. B. Gehring, D. B. Griffin, D. S. Hale, G. G. Mafi, D. D. Johnson, C. L. Lorenzen, R. J. Maddock, R. K. Miller, D. L. VanOverbeke, B. E. Wasser, and J. W. Savell. 2017. National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. Meat Muscle Biol. 1:138. doi:10.22175/mmb2017.05.0028.

Mennecke, B. E., A. M. Townsend, D. J. Hayes, and S. M. Lonergan. 2007. A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. J. Anim. Sci. 85:2639–2659. doi:10.2527/jas.2006-495.

Miller, M. F., M. A. Carr, C. B. Ramsey, K. L. Crockett, and L. C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062. doi:10.2527/2001.79123062x.

Miller, R. 2020. Drivers of Consumer Liking for Beef, Pork, and Lamb: A Review. Foods. 9. doi:10.3390/foods9040428.

Miller, R. K., Kerth, C. R., Berto, M. C., Laird, H. L., & Savell, J. W. (2019). Steak Thickness, Cook Surface Temperature and Quality Grade Affected Top Loin Steak Consumer and Descriptive Sensory Attributes. Meat Muscl. Biol, 3(1), Article 1. https://doi.org/10.22175/mmb2018.07.0020

Molina, M. E., D. D. Johnson, R. L. West, and B. L. Gwartney. 2005. Enhancing palatability traits in beef chuck muscles. Meat Sci. 71:52–61. doi:10.1016/j.meatsci.2005.04.004.

Morrow, S. J., A. J. Garmyn, N. C. Hardcastle, J. C. Brooks, and M. F. Miller. 2019. The Effects of Enhancement Strategies of Beef Flanks on Composition and Consumer Palatability Characteristics. Meat Muscle Biol. 3:457. doi:10.22175/mmb2019.07.0030.

Nair, M. N., A. C. V. C. S. Canto, G. Rentfrow, and S. P. Suman. 2019. Muscle-specific effect of aging on beef tenderness. LWT. 100:250–252. doi:10.1016/j.lwt.2018.10.038.

N'Gatta, K. C. A., A. Kondjoyan, R. Favier, J. Rouel, A. Vénien, T. Astruc, D. Gruffat, and P.-S. Mirade. 2021. Impact of Tumbling Process on the Toughness and Structure of Raw Beef Meat Pieces. Foods. 10:2802. doi:10.3390/foods10112802.

Nyquist, K. M., T. G. O'Quinn, L. N. Drey, L. W. Lucherk, J. C. Brooks, M. F. Miller, and J. F. Legako. 2018. Palatability of beef chuck, loin, and round muscles from three USDA quality grades1. J. Anim. Sci. 96:4276–4292. doi:10.1093/jas/sky305.

O'Quinn, T. G., J. F. Legako, J. C. Brooks, and M. F. Miller. 2018. Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience1. Transl. Anim. Sci. 2:26–36. doi:10.1093/tas/txx008.

Rhee, M. S., T. L. Wheeler, S. D. Shackelford, and M. Koohmaraie. 2004. Variation in palatability and biochemical traits within and among eleven beef muscles. J. Anim. Sci. 82:534–550. doi:10.2527/2004.822534x.

Roeber, D. L., R. C. Cannell, K. E. Belk, R. K. Miller, J. D. Tatum, and G. C. Smith. 2000. Implant strategies during feeding: impact on carcass grades and consumer acceptability. J. Anim. Sci. 78:1867. doi:10.2527/2000.7871867x.

Roy, B. C., C. Das, J. L. Aalhus, and H. L. Bruce. 2021. Relationship between meat quality and intramuscular collagen characteristics of muscles from calf-fed, yearling-fed and mature crossbred beef cattle. Meat Sci. 173:108375. doi:10.1016/j.meatsci.2020.108375.

Setyabrata, D., and Y. H. B. Kim. 2019. Impacts of aging/freezing sequence on microstructure, protein degradation and physico-chemical properties of beef muscles. Meat Sci. 151:64–74. doi:10.1016/j.meatsci.2019.01.007.

Shackelford, S. D., J. B. Morgan, H. R. Cross, and J. W. Savell. 1991. Identification of Threshold Levels for Warner-Bratzler Shear Force in Beef Top Loin Steaks. J. Muscle Foods. 2:289–296. doi:10.1111/j.1745-4573.1991.tb00461.x.

Sharedeh, D., P.-S. Mirade, A. Venien, and J.-D. Daudin. 2015. Analysis of salt penetration enhancement in meat tissue by mechanical treatment using a tumbling simulator. J. Food Eng. 166:377–383. doi:10.1016/j.jfoodeng.2015.06.023.

Stolowski, G. D., B. E. Baird, R. K. Miller, J. W. Savell, A. R. Sams, J. F. Taylor, J. O. Sanders, and S. B. Smith. 2006. Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. Meat Sci. 73:475–483. doi:10.1016/j.meatsci.2006.01.006.

Strydom, P. E., N. Strydom, L. Frylinck, A. Hugo, M. Hope-Jones, E. Moholisa, and I. van Heerden. 2015. The interrelationship between sensory tenderness and Warner Bratzler shear force of three muscles as influenced by various production and post-harvest conditions. In: Proc. 61st Int. Congr. Meat Sci. Technol., Clermont-Ferrand, France. p. 5.

Sullivan, G. A., and C. R. Calkins. 2011. Ranking Beef Muscles for Warner–Bratzler Shear Force and Trained Sensory Panel Ratings from Published Literature. J. Food Qual. 34:195–203. doi:https://doi.org/10.1111/j.1745-4557.2011.00386.x.

Torrescano, G., A. Sánchez-Escalante, B. Giménez, P. Roncalés, and J. A. Beltrán. 2003. Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics. Meat Sci. 64:85–91. doi:10.1016/S0309-1740(02)00165-1.

Tuell, J. R., Q. Yu, and B. Kim. 2021. Can Tumbling without Brine Improve Tenderness and Proteolysis of Beef Loin Muscles? Meat Muscle Biol. 5(1):45:1–11. doi:10.22175/mmb.13044.

Tyszkiewicz, I., B. M. Kłossowska, U. Wieczorek, and A. Jakubiec-Puka. 1997. Mechanical Tenderisation of Pork Meat: Protein and Water Release due to Tissue Damage. J. Sci. Food Agric. 73:179–185. doi:10.1002/(SICI)1097-0010(199702)73:2<179::AID-JSFA699>3.0.CO;2-#.

USDA. 2017. United States Standards for Grades of Carcass Beef. https://www.ams.usda.gov/sites/default/files/media/CarcassBeefStandard.pdf

Wang, L., J. Li, S. Teng, W. Zhang, P. P. Purslow, and R. Zhang. 2022. Changes in collagen properties and cathepsin activity of beef M. semitendinosus by the application of ultrasound during post-mortem aging. Meat Sci. 185:108718. doi:10.1016/j.meatsci.2021.108718.

Warner, R. 2017. Chapter 14 - The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. In: F. Toldra´, editor. Lawrie´s Meat Science (Eighth Edition). Woodhead Publishing. p. 419–459.

Warner, R., R. Miller, M. Ha, T. L. Wheeler, F. Dunshea, X. Li, R. Vaskoska, and P. Purslow. 2020. Meat Tenderness: Underlying Mechanisms, Instrumental Measurement, and Sensory Assessment. Meat Muscle Biol. 4. doi:10.22175/mmb.10489.

Wyrwisz, J., M. Moczkowska, M. Kurek, A. Stelmasiak, A. Półtorak, and A. Wierzbicka. 2016. Influence of 21days of vacuum-aging on color, bloom development, and WBSF of beef semimembranosus. Meat Sci. 122:48–54. doi:10.1016/j.meatsci.2016.07.018.

CHAPTER 4. BEEF QUALITY, BIOCHEMICAL ATTRIBUTES, AND DESCRIPTIVE SENSORY SCORES OF *GLUTEUS MEDIUS, BICEPS FEMORIS,* AND *TENSOR FASCIAE LATAE* MUSCLES SUBJECTED TO COMBINED TUMBLING AND POSTMORTEM AGING

This chapter is a reprint a manuscript currently under review for consideration for publication: Tuell, J. R., Nondorf, M. J., Abdelhaseib, M., Setyabrata, D., Legako, J., & Kim, Y. H. B. Beef Quality, Biochemical Attributes, and Descriptive Sensory Scores of Gluteus Medius, Biceps Femoris, and Tensor Fasciae Latae Muscles Subjected to Combined Tumbling and Postmortem Aging.

4.1 Abstract

This study assessed how fresh beef tumbling without brine inclusion combined with aging would affect quality, biochemical attributes, and descriptive sensory scores of sirloin muscles. *Gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 16) at 5 days postmortem were assigned to 0 or 120 min of tumbling, after which sections were aged 0 or 10 days. Tumbled GM ($p < 0.001$) and TFL ($p < 0.01$) muscles had increased objective tenderness compared to respective controls. Greater cook and initial purge losses were induced in all muscles with tumbling ($p < 0.05$), while thawing loss and purge loss with aging were similar ($p > 0.05$). Fragmentation of myofibrils was increased with tumbling and aging main effects ($p < 0.001$), although degradation of troponin T and desmin were primarily affected by aging only. In general, neither tumbling nor aging affected properties of collagen. Trained panelists assessed muscles aged 10 days for descriptive sensory scores including tenderness (myofibrillar, connective tissue, overall), flavor (beef flavor identity, bloody/serumy, fat-like, liver-like, oxidized, umami, metallic, sour), and juiciness (overall). Tumbled GM had greater myofibrillar tenderness than the control group ($p < 0.05$). Most sensory scores were unaffected by tumbling; however, tumbling increased oxidized and liver-like flavors of GM and TFL, respectively, as well as decreased overall juiciness of BF ($p < 0.05$). These findings indicate tumbling combined with postmortem aging can improve tenderness of certain sirloin muscles like GM, although some impairments to flavor and juiciness could also occur.

4.2 Introduction

Modern consumers are increasingly interested in the production methods of their food (Asioli et al., 2017). For meat products, consumers may discriminate against products with non-meat ingredients perceived to be unhealthy or unnatural. The study by Ardeshiri et al. (2019) reported that US consumers would prefer and be willing to pay a premium for beef products labeled as natural. Although numerous strategies have been developed to improve beef quality post-harvest such as mechanical tenderization, injection enhancement, inclusion of exogenous enzymes, among others (Bhat et al., 2018), many rely upon the use of non-meat ingredients or have negative connotations to consumers. For instance, while mechanical tenderization may improve the tenderness of certain beef cuts (Jeremiah et al., 1999), some consumers may perceive the process adversely and avoid purchasing such products (Yang et al., 2021). Accordingly, the development of natural, post-harvest processes to consistently improve beef eating quality is of great interest to the industry.

Postmortem aging is a natural method used to improve fresh beef quality, particularly tenderness (Kim et al., 2018). During aging, considerable improvements in meat tenderness can be made through myofibrillar protein degradation via naturally occurring endogenous enzymes (Kim et al., 2018). Postmortem aging, however, is a time-consuming process. Consequently, beef steaks may remain undesirably tough and have an inferior aging response due to high amounts of intramuscular connective tissues (Chun et al., 2020; Rhee et al., 2004; Stolowski et al., 2006). This is regularly apparent in muscles from the beef sirloin such as the *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL), which are ranked by consumers and trained panelists as less tender and palatable than steaks from the loin and rib primals (Martinez et al., 2017; Sullivan & Calkins, 2011). Hence, sirloin cuts are generally less economically valuable than those with superior tenderness and overall palatability (USDA, 2021).

Tumbling with brine inclusion has been shown to be effective in improving palatability of various beef cuts (Garmyn et al., 2020; Molina et al., 2005; Morrow et al., 2019). As previously discussed, however, modern consumers tend to prefer natural beef with no added non-meat ingredients (Ardeshiri et al., 2019; Asioli et al., 2017). The recent study by Garmyn et al. (2020) examined the use of "clean label" brines containing only sodium chloride and sodium bicarbonate. It was found that beef muscles tumbled in the "clean label" brine would have similar or superior

eating quality to those tumbled in a brine containing phosphate (Garmyn et al., 2020). Several recent studies have explored tumbling in the absence of a brine solution entirely (Morrow et al., 2019; N'Gatta et al., 2021; Nondorf & Kim, 2022; Tuell et al., 2021; Tuell & Kim, 2021). Notably, tumbling without brine inclusion could contribute to considerable improvements in beef tenderization via physical disruptions to the muscle tissue coupled with greater degradation of myofibrillar proteins with subsequent aging (Nondorf & Kim, 2022; Tuell et al., 2021; Tuell & Kim, 2021). However, it is also known that the effectiveness would differ between cuts, being most effective in the *longissimus lumborum* (Nondorf & Kim, 2022; Tuell et al., 2021; Tuell & Kim, 2021) and less effective in more inherently tough cuts like the *rectus abdominis* and *semitendinosus* (Morrow et al., 2019; N'Gatta et al., 2021; Tuell & Kim, 2021). While it is reasonable to postulate that fresh beef tumbling could accelerate the tenderization of cuts from the beef sirloin, it is also known that individual sirloin muscles vary considerably regarding tenderness development (Chun et al., 2020; Rhee et al., 2004; Stolowski et al., 2006; Sullivan & Calkins, 2011). This study aimed to determine how tumbling coupled with additional postmortem aging would influence the beef quality, biochemical attributes, and descriptive sensory scores of GM, BF, and TFL muscles.

4.3 Materials and Methods

4.3.1 Sample procurement and processing

Muscles utilized in this study included the GM [top sirloin butt, center-cut; Institutional Meat Purchase Specifications (IMPS) 184D], BF (top sirloin cap; IMPS 184D), and TFL (bottom sirloin butt, tri-tip; IMPS 185D) (USDA, 2014). Over the course of three harvest dates, muscles (n = 16) were collected from both sides of Choice$^-$ carcasses (USDA, 2017) at 5 days postmortem. Visible external fat was trimmed from each muscle. Muscles from either side of an individual carcass were considered equivalent for assignment to tumbling treatment groups. These groups consisted of either 120 min of tumbling (T120) at 8.5 rpm in a meat tumbler (LT-30 500 lb; Lance Industries, Hartford, WI, USA) or a non-tumbled (T0) control. Prior to tumbling, each muscle was transversely sectioned, and sections were allocated in a balanced manner among postmortem aging treatments (an additional 0 or 10 days) within a tumbling time. The weights of each section after

trimming and sectioning were 1643 ± 34 g for GM, 897 ± 35 g for BF, and 938 ± 31 g for TFL. Each section was anaerobically packaged in a double layer of 3 mil packaging (CLARITY, Bunzl Processing Division, Riverside, MO, USA). After the tumbling treatment was complete, sections were removed from the packaging. Sections to be aged an additional 10 days at 2 °C were repackaged to maintain a vacuum seal. At each aging time, steaks (2 cm thickness) were collected by cutting perpendicular to the direction of the myofibers. Collected steaks were frozen and subsequently stored at -40 °C. Biochemical measures were conducted on sections from 8 randomly determined carcasses. These samples for the biochemical analyses were submerged in liquid nitrogen until frozen, pulverized into a fine powder, and stored at -80 °C.

4.3.2 Meat quality

Water-holding ability

Prior to all measurements of water-holding ability, the surface of each sample was gently blotted to remove moisture on the meat surface. Measures of water-holding ability were similar to the study published by Yu et al. (2021). Briefly, purge loss at 0 days was considered as the weight loss prior to and after tumbling or similar duration of vacuum storage for T120 and T0, respectively. Purge loss at 10 days was calculated by the weight loss between the weight after tumbling treatment application and the weight after 10 days of aging. At each aging time point, one steak was weighed and frozen at -40 °C. The weight loss of this steak after a 24 h period of thawing at 2 °C was considered as thawing loss. This thawed steak was then cooked to an endpoint temperature of 71 °C using a nonstick griddle (GR-150, Cuisinart, Stamford, CT, USA) at 135 °C, flipping once at 41 °C. Temperature of the geometrical center was checked throughout with a thermocouple (Omega Engineering, Stamford, CT, USA). At the endpoint temperature, steaks were removed from the griddle and cooled at room temperature for a period of 30 min prior to weighing. Afterwards, cook loss was calculated as the weight loss between raw and cooked steaks.

Objective tenderness

Steaks cooked in the manner previously described were held overnight in a 4 °C cooler, individually wrapped in aluminum foil. Warner-Bratzler shear force (WBSF) measurement was

used to determine objective tenderness (AMSA, 2015). A V-shaped WBSF blade attachment was used to measure peak force necessary to shear each core using a texture analyzer (TA-XT Plus Texture Analyser, Stable Micro Systems Ltd., Godalming, Surrey, UK). For each steak, six cores were collected by cutting parallel to the direction of the myofibers. Peak forces of individual cores were averaged to determine the mean WBSF (N) per steak.

Objective color

Color attributes were determined after treatment application, prior to freezing. Each steak was allowed a 30 min period for oxygenation at 2 °C. A colorimeter (4500L, HunterLab MiniScan EZ, Reston, VA, USA) with 25 mm viewed area was used to determine the A_{10} color attributes for Commission Internationale de l´Eclairage (CIE) *L**, CIE *a**, and CIE *b**. The absorbance at 630 nm and 580 nm was recorded to approximate the ratio of oxymyoglobin to metmyoglobin (R630/580 nm) (AMSA, 2012). Chroma and hue angle values were determined (AMSA, 2012). Each steak was measured in triplicate at three randomly determined locations.

4.3.3 Biochemical analyses

Whole muscle protein extraction

Extraction of whole muscle proteins was conducted in the manner described by Setyabrata et al. (2019). Protein concentration of extracts was determined using a spectrometer (Epoch, BioTek Instruments, Inc., Winooski, VT, USA) to determine absorbance at 280 nm. Protein extracts were then diluted to an adjusted concentration of 6.4 mg/mL. Gel samples were made in accordance with the cited protocol. After heating, samples were stored at -80 °C. Prior to analysis, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) load checks were performed to ensure consistency across samples. The SDS-PAGE was conducted by loading 10% bis-acrylamide (100:1) separating gels with 5% bis-acrylamide (100:1) stacking gel with 40 µg of protein. Electrophoresis was performed using a Bio-Rad PowerPac Basic (Hoefer Inc., Richmond, CA, USA) at 130 V for 3 h. After separation, gels were stained once with 0.1% (w/v) R-250 Coomassie blue stain and de-stained twice to allow for visual comparison of band densities.

Immunoblotting

Immunoblotting of troponin T and desmin was performed in accordance to the cited protocol (Setyabrata et al., 2019). The following modifications were made. For troponin T and desmin, the concentration of the bis-acrylamide (100:1) separating gels were 15% and 12%, respectively. For each, 40 µg of protein was inserted into lanes, and electrophoresis was conducted as previously described. On each gel, an internal reference within each muscle from the T0 treatment at 0 days of further aging was included for comparison. After electrophoresis, proteins were transferred to nitrocellulose membranes (IPVH00010, Millipore Corporation, Billerica, MA, USA) at 90 V for 1.5 h. After transfer, blocking in 5% (w/v) nonfat dry milk in phosphate buffered saline-tween (PBST) for a period of 1 h at room temperature was performed. Afterwards, incubation with primary antibodies was conducted. The primary antibody solutions consisted of a 1:20,000 dilution of monoclonal mouse anti-troponin T (T6277, Sigma Aldrich, St. Louis, MO, USA) or monoclonal mouse anti-desmin (D1022, Sigma Aldrich, St. Louis, MO, USA) in 3% (w/v) nonfat dry milk in PBST. The incubation was conducted for 16 h at 4 °C. Afterwards, PBST was applied three times each for 10 min prior to incubation with the secondary antibody. For the secondary, in 3% (w/v) nonfat dry milk in PBST, a dilution at 1:20,000 (troponin T) or 1:15,000 (desmin) of monoclonal goat anti-mouse IgG (H+L) horseradish peroxidase conjugate (170-6516, Bio-Rad Laboratories, Hercules, CA, USA) was prepared. The incubation with the secondary antibody was conducted at room temperature for a duration of 1 h, after which three washes in PBST were performed as previously described. Imaging was performed using the cited protocol (Setyabrata et al., 2019), and bands were quantified by comparison to intact band of the same reference sample within each muscle.

Myofibril fragmentation index

Myofibril fragmentation index (MFI) values were measured using the method of Culler et al. (1978) with some adjustments as explained by Tuell et al. (2021). Measurement of MFI was performed in duplicate on samples that had previously been powdered. Values for MFI were determined by multiplying absorbance at 540 nm (VWR UV-1600 PC, VWR International, Radnor, PA, USA) by 200.

Collagen content and solubility

Collagen content and solubility were determined using the protocol by Cross et al. (1973) with modifications. Measures were conducted in duplicate. Briefly, 4 g of powdered muscle was mixed with 12 mL of ¼ strength Ringer's solution at 78 °C. The incubation occurred over the course of 1 h with occasional stirring. Subsequently, centrifugation at 3,000 ×g for 25 min was performed to separate the insoluble (pelleted) and soluble (supernatant) fractions. The pelleted fraction was transferred to a clean tube using a known quantity of distilled water. Then, 10 mL (insoluble) or 15 mL (soluble) of 12 N HCl was added. Hydrolysis occurred over the course of 16 h at 110 °C, after which fractions were neutralized with 20 mL (insoluble) and 25 mL (soluble) of 6 N NaOH. Filtration was performed using Whatman #1 filter paper. Sample filtrates were diluted by combining 200 µL of filtrate to 1.8 mL of distilled water. To the diluted filtrate, 500 µL of 7% (w/v) chloramine T hydrate was added and incubated at room temperature for 20 min. After, an addition of 500 µL of 4-dimethylamino-benzaldehyde reagent was performed, after which they were incubated in a 60 °C water bath for 20 min. Prior to plating, samples were cooled for 5 min in an ice bath. Using a spectrometer (Epoch), absorbance at 580 nm was measured. Known hydroxyproline standards were used to determine concentration of the sample. Collagen content was calculated with a hydroxyproline factor of 7.25 (insoluble) and 7.52 (soluble), and values were expressed as mg collagen per g of tissue. Total collagen content was calculated as the sum of insoluble and soluble collagen, and solubility was determined by dividing soluble by total collagen contents.

4.3.4 Trained descriptive sensory scores

A trained panel consisting of 8 panelists was conducted to evaluate descriptive sensory scores of steaks from the 10 day further aging group. Each panelist had been previously trained using guidelines for trained panel evaluation (AMSA, 2015). The training consisted of approximately 28 h of objectively evaluating the intensity of multiple descriptive sensory scores, similar to Adhikari et al. (2011). Attributes for tenderness included myofibrillar, connective tissue, and overall tenderness. Attributes for flavor consisted of beef flavor identity, bloody/serumy, fat-like, liver-like, oxidized, umami, metallic, and sour. Juiciness was determined by overall juiciness. Each attribute was evaluated on an unstructured line scale ranging from 0 (extreme toughness,

non-existence of a flavor, or extreme dryness) to 100 (extreme tenderness, extreme intensity of a flavor, or extreme juiciness). Samples were randomly served over the course of 12 sessions with no more than 8 samples evaluated per session.

Prior to serving, steaks were cooked in a similar manner as previously described. Endpoint temperature of each steak was measured (Thermapen, Classic Super-Fast, Thermoworks, American Works, UT, USA). After cooking, steaks were held in a holding pan (214UPCH400, Webstraurant Store, Litiz, PA, USA) at 50 - 55 °C for a period lasting no longer than 5 min prior to serving. Each steak was cut into uniform cubes (1.27 × 1.27 cm). Two cubes were provided to each panelist in a plastic sample cup. Panelists were provided with toothpicks, napkins, expectorant cup, unsalted saltines, distilled water, and diluted apple juice and between samples were instructed to cleanse their palate. Samples were evaluated in red incandescent lighting to mask any variations in product color. Responses were collected using Qualtrics software (Qualtrics, Provo, UT, USA) on digital tablets (iPad, Apple Inc., Cupertino, CA, USA).

4.3.5 Statistical analysis

Data were analyzed with SAS software (9.4, SAS Institute Inc., Cary, NC, USA). For meat quality and biochemical measures, the PROC GLIMMIX procedure was utilized. The experimental design was considered as a balanced complete block. A model to analyze the fixed effects of tumbling (T0 or T120), further aging (0 or 10 days), and the interaction term was created. Individual carcasses were included as a random effect. Data from individual muscles were analyzed independently. For the trained descriptive sensory scores, the PROC GLIMMIX procedure (SAS) was used. These data were analyzed in a split-plot design with the whole plot as individual carcass and the sub-plot as tumbling treatment. Endpoint temperature of individual steaks was considered as a covariate. Degrees of freedom for the denominator were determined with the Kenward-Rodgers adjustment. Least square means were separated using an F test with significance defined as $p < 0.05$. Pearson correlation coefficients were calculated with the PROC CORR procedure (SAS). As descriptive sensory scores were conducted at only one aging time, only data from the 10 day further aging group were included.

4.4 Results and Discussion

4.4.1 Meat quality

Several main effects of tumbling treatments and aging were observed for measures of water-holding ability across muscles ($p < 0.05$; Table 4.1); however, the interaction terms were non-significant. For all muscles, the application of the T120 treatment induced higher purge loss immediately after tumbling compared to the T0 group subjected to a similar duration of vacuum packaging ($p < 0.001$). However, the magnitude of these differences between tumbling groups ranged from only 0.19% in the TFL to 0.28% in the BF. When sections were further aged an additional 10 days, purge losses were comparable within each muscle ($p > 0.05$). Greater cook loss was observed in the T120 groups for each muscle compared to respective T0 controls ($p < 0.05$). Further aging increased cook loss of the GM and TFL muscles ($p < 0.05$), while cook loss of the BF was similar across aging times ($p > 0.05$). Thawing loss was lower in the muscles aged an additional 10 days ($p < 0.05$), likely owing to prior purge loss during the aging process. Tumbling treatments did not affect thawing loss in any muscle ($p > 0.05$).

Tumbling without brine inducing greater purge loss with additional aging in *longissimus lumborum* and *semitendinosus* muscles has been previously reported (Tuell & Kim, 2021). The findings of this study suggest that while tumbling may induce some purge loss immediately after tumbling, additional purge loss with aging may not be exacerbated in sirloin muscles. Colle et al. (2015) reported a similar effect of aging on cook loss of beef GM muscles, where additional aging would result in increased cook loss. Despite cooking to a similar degree of doneness, the study by Hunt et al. (2014) reported lower cook loss of beef GM muscles than the present study. This may owe to steaks in this study being previously frozen and thawed, which would be expected to negatively impact water-holding (Setyabrata et al., 2019). As such, it cannot be fully determined how tumbling without brine and additional aging might affect water-holding attributes of never frozen beef sirloin muscles. However, increased cook loss of GM, TFL, and BF muscles tumbled without a brine was in line with multiple previous studies (Morrow et al., 2019; Nondorf & Kim, 2022; Tuell et al., 2021; Tuell & Kim, 2021). While it is generally considered that cook loss has a close relationship with sensory juiciness (Warner, 2017), it has also been reported that greater cook loss induced through tumbling may not cause inferior subjective juiciness scores (Morrow et al.,

2019; Tuell & Kim, 2021). The implications of the findings for water-holding on sensory traits will be discussed in greater detail with those data.

The T120 treatment increased instrumental tenderness shown by decreased WBSF values in the GM and TFL muscles ($p < 0.01$; Table 4.1), while WBSF was similar across tumbling treatments in the BF ($p > 0.05$). This decrease was equivalent to 14.1% and 10.9% lower WBSF of T120 GM and TFL muscles, respectively, relative to the respective T0 controls. Aging had no effect on WBSF values in any muscle ($p > 0.05$), nor was the interaction term significant. In general, the WBSF values of the GM were similar to values previously reported (Colle et al., 2015; Guelker et al., 2013; Martinez et al., 2017). Instrumental tenderness of the BF was considerably greater in this study compared to Martinez et al. (2017), which can likely be attributed to the dorsal portion of the BF that comprises the sirloin cap being substantially more tender than rest of the muscle (Beyer et al., 2021; Hosch et al., 2013; King et al., 2021). However, it has also been noted that the sirloin cap would be highly variable in the amount of collagen relative to other beef muscles (Jeremiah et al., 2003a). Previous studies have reported that tumbling without brine inclusion could increase objective tenderness of the *longissimus lumborum* but not the *semitendinosus* and *rectus abdominis* (Morrow et al., 2019; Tuell et al., 2021; Tuell & Kim, 2021). The present study is in partial agreement, where tumbling may decrease WBSF values of GM and TFL but not BF. N'Gatta et al. (2021) recently reported that extended tumbling (greater than 3 h) could influence textural properties of the *semitendinosus*, which may suggest a longer duration of tumbling may be necessary to tenderize some inherently tough muscles.

Several instrumental color attributes were affected by tumbling and aging main effects in each muscle ($p < 0.05$; Table 4.2). Color attributes of the GM were comparable between the T0 and T120 groups ($p > 0.05$). However, additional aging increased CIE L^*, hue angle, and R630/580 nm ($p < 0.01$), regardless of tumbling treatment. For the BF, the T120 treatment increased CIE a^*, CIE b^*, chroma, and hue angle ($p < 0.05$) compared to T0, while CIE L^* and R630/580 nm values were similar ($p > 0.05$). In general, aging had a similar effect as tumbling on color of the BF, where muscles aged an additional 10 days had greater CIE L^*, CIE a^*, CIE b^*, chroma, and hue angle values ($p < 0.01$), while R630/580 nm was not affected ($p > 0.05$). Aging did not affect color attributes of the TFL ($p > 0.05$). T120 TFL steaks had greater CIE a^*, CIE b^*, and chroma ($p < 0.05$) values compared to controls, while other color attributes were comparable ($p > 0.05$). The effects of tumbling without a brine on color attributes of beef have been inconsistent (Tuell et al.,

2021; Tuell & Kim, 2021). The present study found tumbling to not affect color attributes of the GM, similar to the findings for the *longissimus lumborum* and *semitendinosus* reported by Tuell and Kim (2021). The findings for the TFL and BF muscles are in disagreement with Tuell et al. (2021) who reported tumbling would decrease CIE *a**, CIE *b**, and chroma values of *longissimus lumborum* steaks. These differences could owe to tumbling affecting the muscle structure differently between muscles, which would be expected to influence color (Hughes et al., 2014). Greater color attributes of aged beef steaks have been previously reported, attributable to more rapid oxygenation (Wyrwisz et al., 2016).

4.4.2 Biochemical analyses

In general, most measures of proteolysis were affected by additional aging, rather than tumbling or the interaction of tumbling and aging. For the GM, additional aging increased the relative abundances of the troponin T and desmin degradation products ($p < 0.05$; Table 4.3, Figures 4.1 & 4.2). While the intact band of desmin decreased with aging in the GM ($p < 0.05$), the intact band of troponin T was outside of statistical significance. Similarly, additional aging decreased the relative abundance of intact desmin within BF ($p < 0.05$); however, significant interactions were observed for intact and degraded troponin T. Least intact troponin T was observed in the T120 tumbling group aged an additional 10 days compared to other treatment groups. However, the T0 treatment at 10 days of aging had the greatest degraded troponin T, while T120 at both 0 and 10 days was intermediate. Significant main effects of aging for intact and degraded troponin T and desmin were observed within TFL, with no tumbling or interaction effects found ($p > 0.05$). Rhee et al. (2004) previously found a high extent of desmin degradation in the BF muscle over the course of 14 days of aging relative to the GM. However, desmin degradation was not correlated with WBSF values of the BF, while a significant correlation was reported within the GM (Rhee et al., 2004). As previously noted, this could be affected by the dorsal portion of the BF exhibiting different properties than the muscle as a whole (Beyer et al., 2021; Hosch et al., 2013; King et al., 2021). However, discrepancies between protein degradation and tenderization in muscles from the beef round have been reported (Anderson et al., 2012). Similarly, Chun et al. (2020) reported that although the TFL would exhibit a high extent of troponin T degradation from 5 to 21 days of aging, the muscle would remain tougher than the *longissimus lumborum*. This

finding can likely be attributable to greater collagen content and crosslinking in the TFL (Chun et al., 2020). It has been previously reported that tumbling could facilitate postmortem proteolysis during aging to an extent in beef *longissimus lumborum* muscles (Tuell et al., 2021). This study is in partial agreement, as a significant tumbling by aging interaction was observed for troponin T within the BF muscle only.

No significance was found for tumbling, aging, nor the interaction term for collagen content and solubility of the GM and TFL ($p > 0.05$; Table 4.4). Additional aging was shown to increase the amount of soluble collagen, thereby increasing percent soluble collagen of the BF ($p < 0.05$), although this was not affected by tumbling treatment. It has been previously reported that the amount of connective tissue would be highly correlated with instrumental tenderness of beef GM and BF muscles; however, the relationship with collagen specifically would be weaker in general (Rhee et al., 2004). Jeremiah et al. (2003b) stated that beef tenderness would have a stronger relationship with insoluble collagen content, rather than total or soluble collagen. This may help to explain in part the lack of differences between aging times for instrumental tenderness of the BF, despite variations in collagen solubility. It has also been suggested that aging would decrease the amount of deoxypyridinoline collagen crosslinks in the *longissimus lumborum* but not the TFL, resulting in the latter muscle maintaining greater toughness (Chun et al., 2020). In general, the findings of this study support that tumbling, regardless of aging, would have a negligible effect on the amount and properties of collagen.

While most other measures of proteolysis were affected by aging only, MFI values were affected by the main effects of both tumbling and aging in all muscles ($p < 0.001$; Table 4.4). Specifically, significantly greater MFI values were observed in T120 treatments relative to T0 controls, as well as in the muscles aged an additional 10 days compared to those that were not further aged. This finding is in good agreement with previous literature regarding tumbling without a brine (Tuell et al., 2021). In particular, Tuell et al. (2021) reported that tumbling would increase MFI in *longissimus lumborum* muscles, supported by transmission electron microscopy images exhibiting considerable disruption to myofibrillar structure. However, as previously discussed, the extent to which fragmentation of the myofibrils may influence tenderness could differ between muscles (Chun et al., 2020; Rhee et al., 2004; Warner et al., 2020). Considering the discrepancy between WBSF and MFI values in the BF, this may indicate that tenderness of the BF would be less attributable to myofibrillar fragmentation than other factors such as background toughness.

4.4.3 Trained descriptive sensory scores

For trained panel descriptive sensory scores, it was found that T120 GM muscles would have greater myofibrillar tenderness than the respective T0 controls ($p < 0.05$; Table 4.5). However, connective tissue and overall tenderness scores would be similar within the GM ($p > 0.05$). No measures of tenderness were affected by the tumbling treatments for BF and TFL muscles ($p > 0.05$). In general, most attributes evaluated were unaffected by the treatments. However, it was observed that T120 GM and TFL muscles would have greater oxidized and liver-like flavors, respectively, compared to respective controls ($p < 0.05$). Overall juiciness of the GM and TFL was similar between treatments ($p > 0.05$), although decreased juiciness of the T120 BF muscles was found ($p < 0.05$). As panelists assessed the muscles from the group aged an additional 10 days only, it may be that differences in tenderness would be negligible by this time point. Likewise, the increases in oxidized and liver-like flavors could be related to poorer oxidative stability of aged beef (Ma et al., 2017), which could be worsened with prior tumbling. Previously, Cheng and Ockerman (2003) suggested that tumbling without the use of phosphates would exacerbate oxidation through the release of free radicals and disruptions to cellular membranes. As such, it is reasonable to expect that this process would be compounded by postmortem aging, contributing to off-flavor development. Despite this, Tuell and Kim (2021) reported that consumer liking of flavor would be unaffected by tumbling and the interaction of tumbling by aging for beef *longissimus lumborum* and *semitendinosus* muscles. Differences in this study could owe to intermuscular differences in flavor and oxidative stability, as well as trained panelists having a greater ability to identify specific off-flavors. Similarly, although several main effects of tumbling were found for measures of water-holding, only overall juiciness of the BF was decreased with tumbling. This could be partly attributed to intermuscular differences in the relationship between water-holding and sensory juiciness, where the BF has been reported to have a stronger correlation between cook loss and juiciness than the GM (Rhee et al., 2004).

To quantify the relationship between various meat quality and descriptive sensory scores, Pearson correlation coefficients were determined (Table 4.6). When pooling all muscles, WBSF was not significantly correlated with any other trait ($p > 0.05$), aside from a weak positive correlation with cook loss ($p < 0.01$). Rhee et al. (2004) found that there would be a strong negative correlation with WBSF and tenderness evaluated by trained panelists when all eleven beef muscles

evaluated were pooled. However, it was also demonstrated that the strength of this relationship would noticeably differ when considering muscles individually (Rhee et al., 2004). In this study, within the GM muscle, WBSF had a moderate negative correlation with both myofibrillar and overall tenderness scores ($p < 0.01$). However, for the BF and TFL, WBSF was not related to any tenderness score. Within all muscles, significant moderate to high positive correlations were observed between individual tenderness scores. Overall juiciness was not correlated to any water-holding ability attribute within the GM muscles ($p > 0.05$), which may help explain why overall juiciness was unaffected by tumbling despite greater cook and initial purge losses. Purge loss with additional aging and thaw loss were negatively correlated within the BF and TFL muscles ($p < 0.05$), likely owing to greater purge losses with aging leaving less moisture available to leave the myofibrillar matrix as purge upon thawing. Considering the observed positive relationship between overall juiciness with myofibrillar and overall tenderness scores of the BF and TFL ($p < 0.01$), minimizing possible negative impacts of tumbling on juiciness would be critical to improving overall eating quality. Furthermore, ensuring tumbling does not exacerbate oxidative processes resulting in off-flavor development would also be important. Highlighting this, O'Quinn et al. (2018) reported flavor, rather than tenderness, would be more salient to the overall eating experience of beef steaks. It would also be important to note that panelists evaluated samples that had previously been frozen, which would be expected to worsen oxidative stability compared to fresh, never frozen steaks. Accordingly, further study on the effects of combined tumbling and aging on oxidative stability and the generation of off-flavors in never frozen beef steaks would be warranted.

4.5 Conclusion

The findings of this study suggest that tumbling without brine inclusion could improve the quality and palatability of beef sirloin steaks; however, there would be considerable intermuscular differences. For example, tumbling increased instrumental tenderness of the GM muscle, thereby increasing myofibrillar tenderness scores. However, although the T120 treatment decreased WBSF values of the TFL, no measures of sensory tenderness were affected. Within the BF, neither instrumental nor trained panel descriptive tenderness scores were affected by the treatments. Improvements in objective tenderness would be primarily attributed to the disruptions caused to

the muscle structure, resulting in considerable fragmentation of the myofibrils. However, myofibrillar fragmentation may not necessarily result in improved tenderness, which may owe to intermuscular differences in the amount and properties of intramuscular connective tissues. The results of this study also suggest that tumbling without brine inclusion could result in the generation of some off-flavors with the application of additional postmortem aging. Therefore, this study supports that tumbling may be effective to improve the tenderness of certain beef sirloin muscles like the GM, although ensuring potential detriments to flavor and juiciness do not outweigh gains in tenderness would be important.

4.6 Acknowledgements

Authors thank Samantha Barker of the Texas Tech University Department of Animal and Food Sciences for assistance in coordinating and conducting the trained panel evaluation.

4.7 Tables and Figures

Table 4.1. Main and interaction effects of tumbling and aging treatments on water-holding capacity attributes (purge losses, thawing loss, cook loss) and Warner-Bratzler shear force (WBSF) values in *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 16).

	Treatment	Purge loss, 0d (%)	Purge loss, 10d (%)	Thawing loss (%)	Cook loss (%)	WBSF (N)
GM	*T*[1]					
	T0	0.46[b]	5.24	4.81	29.1[b]	28.1[a]
	T120	0.70[a]	4.60	4.62	31.3[a]	24.4[b]
	SEM	0.04	0.35	0.27	0.7	0.9
	A[2]					
	0d	-	-	6.24[x]	29.6[y]	27.1
	10d	-	-	3.19[y]	30.8[x]	25.4
	SEM	-	-	0.27	0.7	0.9
	p value					
	T	<0.001	0.152	0.386	<0.001	<0.001
	A	-	-	<0.001	0.033	0.058
	T × *A*	-	-	0.107	0.368	0.404
BF	*T*					
	T0	0.38[b]	4.33	5.28	28.5[b]	26.9
	T120	0.66[a]	5.27	4.78	30.2[a]	25.5
	SEM	0.04	0.47	0.37	0.6	1.1
	A					
	0d	-	-	6.22[x]	28.7	26.6
	10d	-	-	3.84[y]	30.0	25.8
	SEM	-	-	0.37	0.6	1.1
	p value					
	T	<0.001	0.175	0.075	0.048	0.260
	A	-	-	<0.001	0.103	0.477
	T × *A*	-	-	0.111	0.085	0.334
TFL	*T*					
	T0	0.28[b]	2.24	2.53	24.6[b]	26.2[a]
	T120	0.47[a]	2.68	2.60	27.1[a]	23.5[b]
	SEM	0.03	0.23	0.17	0.8	0.8
	A					
	0d	-	-	2.88[x]	25.2[y]	25.6
	10d	-	-	2.25[y]	26.6[x]	24.2
	SEM	-	-	0.17	0.8	0.8
	p value					
	T	<0.001	0.208	0.737	<0.001	0.002
	A	-	-	0.004	0.026	0.073
	T × *A*	-	-	0.284	0.346	0.707

[1]Tumbling duration (min)
[2]Aging duration (days)

Table 4.1, continued

[a,b]Means lacking a common superscript within a column differ at ($p < 0.05$) due to tumbling main effect within same muscle.

[x,y]Means lacking a common superscript within a column differ at ($p < 0.05$) due to aging main effect within same muscle.

Table 4.2. Main and interaction effects of tumbling and aging treatments on A_{10} instrumental color attributes of *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 16).

	Treatment	CIE *L**	CIE *a**	CIE *b**	Chroma	Hue angle	R630/580 nm[1]
GM	*T*[2]						
	T0	46.2	27.7	21.3	34.9	37.6	5.17
	T120	45.9	27.6	21.2	34.9	37.5	5.21
	SEM	0.5	0.3	0.3	0.4	0.2	0.07
	A[3]						
	0d	45.3[y]	27.7	21.0	34.8	37.2[y]	5.32[x]
	10d	46.8[x]	27.6	21.5	35.0	37.9[x]	5.07[y]
	SEM	0.5	0.3	0.3	0.4	0.2	0.07
	p value						
	T	0.537	0.963	0.695	0.852	0.271	0.611
	A	0.002	0.789	0.061	0.545	<0.001	0.003
	T × *A*	0.472	0.592	0.767	0.654	0.570	0.658
BF	*T*						
	T0	42.4	26.1[b]	19.2[b]	32.4[b]	36.4[b]	5.07
	T120	42.7	26.6[a]	19.8[a]	33.2[a]	36.7[a]	5.18
	SEM	0.4	0.2	0.1	0.2	0.1	0.07
	A						
	0d	41.9[y]	26.1[y]	19.1[y]	32.3[y]	36.2[y]	5.15
	10d	43.2[x]	26.6[x]	19.9[x]	33.2[x]	36.8[x]	5.11
	SEM	0.4	0.2	0.1	0.2	0.1	0.07
	p value						
	T	0.404	0.004	0.001	0.002	0.041	0.118
	A	0.001	0.005	<0.001	<0.001	<0.001	0.604
	T × *A*	0.246	0.404	0.079	0.209	0.073	0.293
TFL	*T*						
	T0	45.5	22.3[b]	17.4[b]	28.3[b]	38.0	3.90
	T120	46.7	23.2[a]	18.1[a]	29.4[a]	38.0	4.02
	SEM	0.6	0.3	0.2	0.4	0.2	0.09
	A						
	0d	46.2	22.9	17.9	29.1	38.0	3.98
	10d	46.0	22.5	17.6	28.6	38.0	3.94
	SEM	0.6	0.3	0.2	0.4	0.2	0.09
	p value						
	T	0.114	0.018	0.024	0.017	0.966	0.226
	A	0.790	0.252	0.259	0.242	0.942	0.691
	T × *A*	0.465	0.987	0.818	0.921	0.715	0.788

[1]Ratio of absorbance at 630 to 580 nm
[2]Tumbling duration (min)
[3]Aging duration (days)

Table 4.2, continued

[a,b]Means lacking a common superscript within a column differ at ($p < 0.05$) due to tumbling main effect within same muscle.

[x,y]Means lacking a common superscript within a column differ at ($p < 0.05$) due to aging main effect within same muscle.

Table 4.3. Main and interaction effects of tumbling and aging treatments on relative abundance of troponin T and desmin of *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 8).

	Treatment		Intact troponin T	Degraded troponin T	Intact desmin	Degraded desmin
GM	*T*[1]					
	T0		1.07	0.13	1.19	0.66
	T120		1.04	0.13	1.07	0.66
	SEM		0.03	0.01	0.07	0.07
	A[2]					
	0d		1.09	0.12^{y}	1.25^{x}	0.64^{y}
	10d		1.03	0.14^{x}	1.01^{y}	0.68^{x}
	SEM		0.04	0.01	0.07	0.07
	p value					
	T		0.284	0.981	0.148	0.977
	A		0.081	0.043	0.007	0.045
	T × *A*		0.641	0.067	0.920	0.549
BF	*T*					
	T0		1.03	0.13	0.97	0.43
	T120		0.98	0.13	0.91	0.43
	SEM		0.03	0.01	0.10	0.06
	A					
	0d		1.04	0.12	1.11^{x}	0.41
	10d		0.96	0.14	0.76^{y}	0.45
	SEM		0.03	0.01	0.10	0.06
	T × *A*					
	T0	0d	1.03^{A}	0.10^{C}	1.15	0.40
		10d	1.02^{A}	0.15^{A}	0.78	0.46
	T120	0d	1.06^{A}	0.13^{B}	1.07	0.42
		10d	0.90^{B}	0.13^{B}	0.74	0.44
	SEM		0.04	0.01	0.11	0.06
	p value					
	T		0.089	0.957	0.408	0.956
	A		0.010	0.001	<0.001	0.240
	T × *A*		0.020	0.006	0.782	0.515
TFL	*T*					
	T0		1.11	0.10	1.01	0.36
	T120		1.14	0.12	0.94	0.37
	SEM		0.02	0.01	0.03	0.05
	A					
	0d		1.17^{x}	0.08^{y}	1.08^{x}	0.32^{y}
	10d		1.08^{y}	0.13^{x}	0.86^{y}	0.42^{x}
	SEM		0.02	0.01	0.03	0.05
	p value					
	T		0.359	0.130	0.053	0.470
	A		0.002	<0.001	<0.001	<0.001

Table 4.3, continued

$T \times A$	0.896	0.761	0.471	0.713

[1]Tumbling duration (min)

[2]Aging duration (days)

[x,y]Means lacking a common superscript within a column differ at ($p < 0.05$) due to aging main effect within same muscle.

[A-C]Means lacking a common superscript within a column differ at ($p < 0.05$) due to interaction of tumbling and aging effects within same muscle.

Table 4.4. Main and interaction effects of tumbling and aging treatments on collagen content and solubility and myofibril fragmentation index values of *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 8).

	Treatment	Insoluble collagen (mg/g wet tissue)	Soluble collagen (mg/g wet tissue)	Total collagen (mg/g wet tissue)	Collagen solubility (%)	Myofibril fragmentation index (200 * Abs_{520nm})
GM	*T*[1]					
	T0	4.03	0.53	4.56	11.25	62.2[b]
	T120	3.78	0.47	4.24	10.36	101.5[a]
	SEM	0.17	0.09	0.24	1.42	5.5
	A[2]					
	0d	3.97	0.53	4.49	11.15	69.4[y]
	10d	3.84	0.47	4.31	10.46	94.3[x]
	SEM	0.17	0.09	0.24	1.42	5.5
	p value					
	T	0.162	0.488	0.188	0.533	<0.001
	A	0.492	0.529	0.456	0.625	<0.001
	T × *A*	0.883	0.813	0.992	0.851	0.394
BF	*T*					
	T0	4.58	0.52	5.10	10.36	65.7[b]
	T120	5.01	0.65	5.65	11.38	100.5[a]
	SEM	0.31	0.05	0.34	0.64	5.4
	A					
	0d	4.68	0.51[y]	5.19	9.77[y]	72.8[y]
	10d	4.90	0.67[x]	5.57	11.96[x]	93.5[x]
	SEM	0.31	0.05	0.34	0.64	5.4
	p value					
	T	0.330	0.080	0.258	0.269	<0.001
	A	0.619	0.026	0.425	0.022	<0.001
	T × *A*	0.323	0.542	0.327	0.893	0.650
TFL	*T*					
	T0	5.72	0.84	6.56	12.22	58.2[b]
	T120	5.64	0.88	6.52	12.95	94.5[a]
	SEM	0.26	0.11	0.35	0.81	3.2
	A					
	0d	5.76	0.96	6.72	13.55	64.3[y]
	10d	5.61	0.75	6.36	11.62	88.4[x]
	SEM	0.26	0.11	0.35	0.81	3.2
	p value					
	T	0.829	0.789	0.939	0.526	<0.001
	A	0.678	0.182	0.476	0.102	<0.001
	T × *A*	0.779	0.985	0.838	0.842	0.268

[1]Tumbling duration (min)
[2]Aging duration (days)

Table 4.4, continued

[a,b]Means lacking a common superscript within a column differ at ($p < 0.05$) due to tumbling main effect within same muscle.

[x,y]Means lacking a common superscript within a column differ at ($p < 0.05$) due to aging main effect within same muscle.

Table 4.5. Main effects of tumbling treatment on descriptive sensory scores of *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 16).

Trait	GM		SEM	p value
	T0[1]	T120		
Beef flavor identity	49.7	50.0	1.3	0.922
Bloody/serumy	7.8	7.6	0.8	0.894
Fat-like	5.5	5.6	0.6	0.891
Liver-like	3.3	2.4	0.8	0.539
Oxidized	0.4[b]	1.3[a]	0.2	0.047
Umami	10.0	10.4	0.5	0.650
Metallic	10.7	10.7	0.8	0.928
Sour	7.0	8.0	0.9	0.506
Myofibrillar tenderness	50.3[b]	57.1[a]	1.6	0.023
Connective tissue tenderness	51.2	55.1	1.4	0.128
Overall tenderness	50.2	55.5	1.7	0.117
Overall juiciness	48.7	47.1	1.3	0.486
	BF		SEM	p value
	T0	T120		
Beef flavor identity	49.3	50.8	1.8	0.611
Bloody/serumy	8.2	6.9	0.6	0.205
Fat-like	6.7	8.1	0.7	0.291
Liver-like	3.6	2.5	1.0	0.501
Oxidized	1.6	1.3	0.4	0.589
Umami	10.9	10.9	0.3	0.978
Metallic	9.5	9.3	0.6	0.841
Sour	4.3	5.4	0.7	0.511
Myofibrillar tenderness	61.3	64.1	1.8	0.377
Connective tissue tenderness	57.3	64.4	2.3	0.091
Overall tenderness	59.2	64.2	2.0	0.159
Overall juiciness	53.4[a]	51.4[b]	0.6	0.046
	TFL		SEM	p value
	T0	T120		
Beef flavor identity	50.3	50.6	0.9	0.842
Bloody/serumy	7.2	7.3	0.7	0.936
Fat-like	12.7	9.9	1.1	0.203
Liver-like	0.9[b]	2.1[a]	0.3	0.030
Oxidized	1.2	1.7	0.6	0.624
Umami	11.6	12.1	0.5	0.455
Metallic	7.7	7.5	0.7	0.843
Sour	2.7	3.3	0.5	0.366
Myofibrillar tenderness	55.5	56.8	2.5	0.759

Table 4.5, continued

Connective tissue tenderness	49.9	51.8	3.8	0.773
Overall tenderness	53.7	54.3	3.0	0.905
Overall juiciness	55.3	52.1	0.8	0.051

[1]T0 and T120 refer to non-tumbled and 120 min of tumbling, respectively.
[a,b]Means lacking a common superscript within a row differ at ($p < 0.05$) due to tumbling main effect within same muscle.

Table 4.6. Pearson correlation coefficients of various meat quality and descriptive sensory scores of *gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) muscles (n = 16) from the 10 day further aging group.

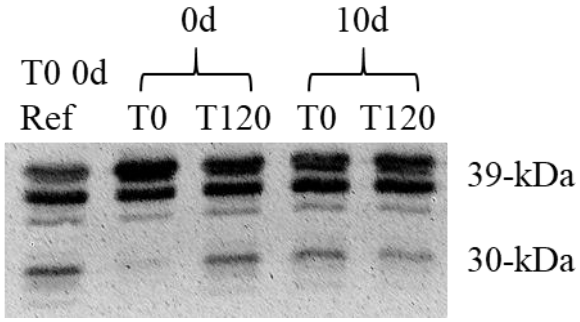
	Trait[1]	MYO	CONN	TEND	COOK	THAW	PURGE	JUI
All muscles	WBSF	-0.07	-0.03	-0.06	0.31**	-0.05	0.05	0.02
	MYO		0.76***	0.93***	-0.09	0.04	0.00	0.42***
	CONN			0.89***	0.08	0.06	0.23*	0.16
	TEND				-0.05	0.03	0.05	0.38***
	COOK					0.20*	0.23*	-0.26**
	THAW						0.09	-0.03
	PURGE							-0.33**
GM		MYO	CONN	TEND	COOK	THAW	PURGE	JUI
	WBSF	-0.53**	-0.33	-0.48**	0.38*	-0.01	0.00	-0.23
	MYO		0.61***	0.91***	-0.28	-0.39*	-0.17	0.00
	CONN			0.82***	-0.26	-0.60***	0.01	0.01
	TEND				-0.32	-0.49**	-0.16	0.09
	COOK					-0.09	-0.22	-0.05
	THAW						0.10	0.04
	PURGE							0.07
BF		MYO	CONN	TEND	COOK	THAW	PURGE	JUI
	WBSF	0.12	-0.10	-0.02	0.50**	-0.22	-0.10	0.33
	MYO		0.69***	0.85***	0.14	0.01	0.16	0.47**
	CONN			0.84***	0.09	-0.09	0.27	0.10
	TEND				0.06	-0.05	0.14	0.36*
	COOK					0.02	-0.14	0.13
	THAW						-0.37*	0.21
	PURGE							-0.23
TFL		MYO	CONN	TEND	COOK	THAW	PURGE	JUI
	WBSF	0.10	0.06	0.11	-0.03	-0.13	0.05	0.12
	MYO		0.81***	0.92***	-0.10	-0.09	-0.07	0.60***
	CONN			0.94***	0.02	-0.13	-0.09	0.39*
	TEND				-0.05	-0.13	-0.10	0.54**
	COOK					0.09	0.14	-0.24
	THAW						-0.42*	0.00
	PURGE							-0.03

[1]WBSF=Warner-Bratzler shear force; MYO=myofibrillar tenderness; CONN=connective tissue tenderness; TEND=overall tenderness; COOK=cooking loss; THAW=thawing loss; PURGE=purge loss; JUI=overall juiciness.

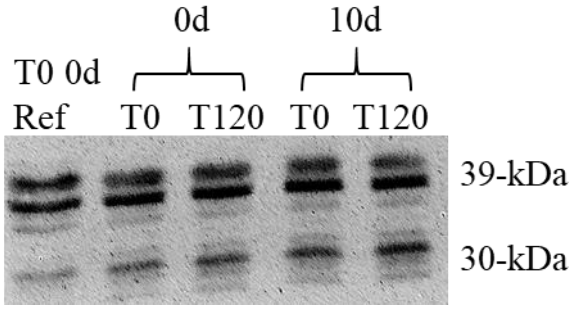
*$p < 0.05$.

**$p < 0.01$.

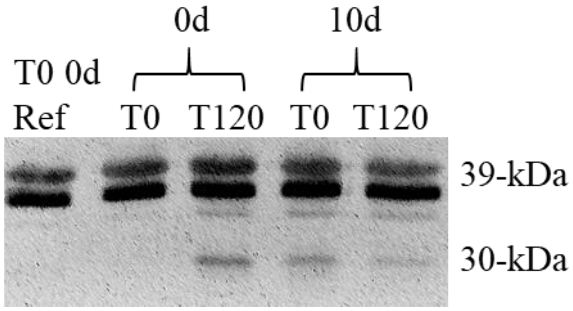
***$p < 0.001$.



(A)

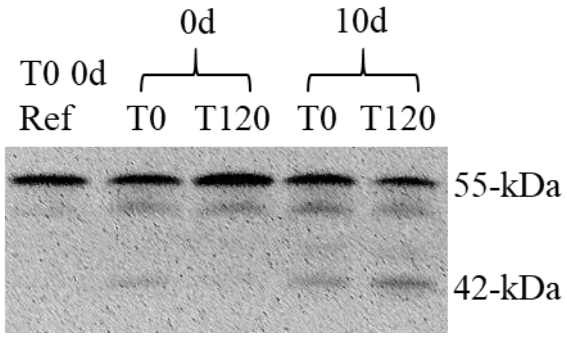


(B)

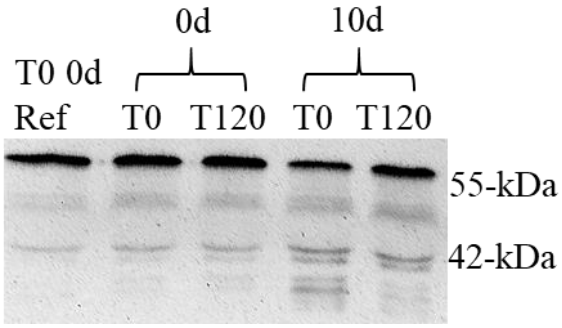


(C)

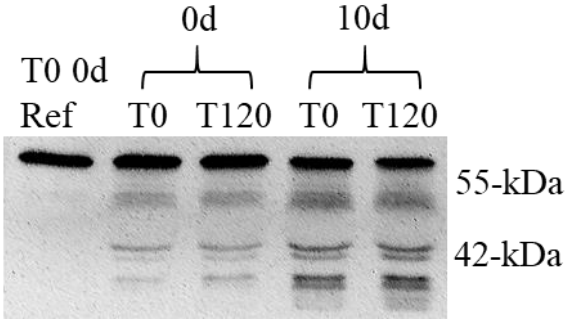
Figure 4.1. A representative blot of troponin T from beef *gluteus medius* (A), *biceps femoris* (B), and *tensor fasciae latae* (C) muscles from combined tumbling and aging treatments. Relative abundance was quantified by comparison to the intact band of the same T0 reference (Ref) sample within the 0 day further aged group. *Note*: T0 and T120 refer to non-tumbled and 120 min of tumbling, respectively. 0d and 10d refer to 0 and 10 days of additional postmortem aging, respectively.



(A)



(B)



(C)

Figure 4.2. A representative blot of desmin from beef *gluteus medius* (A), *biceps femoris* (B), and *tensor fasciae latae* (C) muscles from combined tumbling and aging treatments. Relative abundance was quantified by comparison to the intact band of the same T0 reference (Ref) sample within the 0 day further aged group. *Note*: T0 and T120 refer to non-tumbled and 120 min of tumbling, respectively. 0d and 10d refer to 0 and 10 days of additional postmortem aging, respectively.

4.8 References

Adhikari, K., Chambers Iv, E., Miller, R., Vázquez-Araújo, L., Bhumiratana, N., & Philip, C. (2011). Development of a Lexicon for Beef Flavor in Intact Muscle. *Journal of Sensory Studies*, *26*(6), 413–420. https://doi.org/10.1111/j.1745-459X.2011.00356.x

AMSA. (2012). *Meat Color Measurement Guidelines*. American Meat Science Association. Champaign, IL, USA.

AMSA. (2015). *Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat*. American Meat Science Association. Champaign, IL, USA.

Anderson, M. J., Lonergan, S. M., Fedler, C. A., Prusa, K. J., Binning, J. M., & Huff-Lonergan, E. (2012). Profile of biochemical traits influencing tenderness of muscles from the beef round. *Meat Science*, *91*(3), 247–254. https://doi.org/10.1016/j.meatsci.2012.01.022

Ardeshiri, A., Sampson, S., & Swait, J. (2019). Seasonality effects on consumers' preferences over quality attributes of different beef products. *Meat Science*, *157*, 107868. https://doi.org/10.1016/j.meatsci.2019.06.004

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., & Varela, P. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International*, *99*, 58–71. https://doi.org/10.1016/j.foodres.2017.07.022

Beyer, E. S., Harr, K. M., Olson, B. A., Rice, E. A., Jones, C. K., Chao, M. D., Vipham, J. L., Zumbaugh, M. D., & O'Quinn, T. G. (2021). Palatability Traits of Sirloin Cap Steaks From Four USDA Quality Grades. *Meat and Muscle Biology*, *5*(1), Article 1. https://doi.org/10.22175/mmb.12424

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E.-D. A. (2018). Applied and Emerging Methods for Meat Tenderization: A Comparative Perspective: Advanced methods for meat tenderization…. *Comprehensive Reviews in Food Science and Food Safety*, *17*(4), 841–859. https://doi.org/10.1111/1541-4337.12356

Cheng, J., & Ockerman, H. W. (2003). Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. *Meat Science*, *65*(4), 1353–1359. https://doi.org/10.1016/S0309-1740(03)00057-3

Chun, C. K. Y., Wu, W., Welter, A. A., O'Quinn, T. G., Magnin-Bissel, G., Boyle, D. L., & Chao, M. D. (2020). A preliminary investigation of the contribution of different tenderness factors to beef loin, tri-tip and heel tenderness. *Meat Science*, *170*, 108247. https://doi.org/10.1016/j.meatsci.2020.108247

Colle, M. J., Richard, R. P., Killinger, K. M., Bohlscheid, J. C., Gray, A. R., Loucks, W. I., Day, R. N., Cochran, A. S., Nasados, J. A., & Doumit, M. E. (2015). Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. *Meat Science*, *110*, 32–39. https://doi.org/10.1016/j.meatsci.2015.06.013

Cross, H. R., Carpenter, Z. L., & Smith, G. C. (1973). Effects of Intramuscular Collagen and Elastin on Bovine Muscle Tenderness. *Journal of Food Science*, *38*(6), 998–1003. https://doi.org/10.1111/j.1365-2621.1973.tb02133.x

Culler, R. D., Jr, F. C. P., Smith, G. C., & Cross, H. R. (1978). Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. *Journal of Food Science*, *43*(4), 1177–1180. https://doi.org/10.1111/j.1365-2621.1978.tb15263.x

Garmyn, A., Hardcastle, N., Bendele, C., Polkinghorne, R., & Miller, M. (2020). Exploring Consumer Palatability of Australian Beef Fajita Meat Enhanced with Phosphate or Sodium Bicarbonate. *Foods*, *9*(2), 177. https://doi.org/10.3390/foods9020177

Guelker, M. R., Haneklaus, A. N., Brooks, J. C., Carr, C. C., Delmore, R. J., Griffin, D. B., Hale, D. S., Harris, K. B., Mafi, G. G., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Martin, J. N., Miller, R. K., Raines, C. R., VanOverbeke, D. L., Vedral, L. L., Wasser, B. E., & Savell, J. W. (2013). National Beef Tenderness Survey–2010: Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. *Journal of Animal Science*, *91*(2), 1005–1014. https://doi.org/10.2527/jas.2012-5785

Hosch, J. J., Varnold, K., Senaratne, L. S. S., Semler, M. E., Chao, M. D., & Calkins, C. R. (2013). An Evaluation of the Extended Sirloin Cap Coulotte. *Nebraska Beef Cattle Reports*, *748*, 4.

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, *98*(3), 520–532. https://doi.org/10.1016/j.meatsci.2014.05.022

Hunt, M. R., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., Legako, J. F., Rathmann, R. J., Brooks, J. C., & Miller, M. F. (2014). Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. *Meat Science*, *98*(1), 1–8. https://doi.org/10.1016/j.meatsci.2014.04.004

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L., & Gibson, L. L. (2003a). Assessment of the chemical and cooking properties of the major beef muscles and muscle groups. *Meat Science*, *65*(3), 985–992. https://doi.org/10.1016/S0309-1740(02)00308-X

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L., & Gibson, L. L. (2003b). Assessment of the relationship between chemical components and palatability of major beef muscles and muscle groups. *Meat Science*, *65*(3), 1013–1019. https://doi.org/10.1016/S0309-1740(02)00309-1

Jeremiah, L. E., Gibson, L. L., & Cunningham, B. (1999). The influence of mechanical tenderization on the palatability of certain bovine muscles. *Food Research International*, *32*(8), 585–591. https://doi.org/10.1016/S0963-9969(99)00134-9

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. *Meat Science*, *144*, 74–90. https://doi.org/10.1016/j.meatsci.2018.04.031

King, D. A., Shackelford, S. D., & Wheeler, T. L. (2021). Postmortem Aging Time and Marbling Class Effects on Flavor of Three Muscles From Beef Top Loin and Top Sirloin Subprimals. *Meat and Muscle Biology*, *5*(1), Article 1. https://doi.org/10.22175/mmb.10939

Ma, D., Kim, Y. H. B., Cooper, B., Oh, J.-H., Chun, H., Choe, J.-H., Schoonmaker, J. P., Ajuwon, K., & Min, B. (2017). Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. *Journal of Agricultural and Food Chemistry*, *65*(31), 6708–6716. https://doi.org/10.1021/acs.jafc.7b02175

Martinez, H. A., Arnold, A. N., Brooks, J. C., Carr, C. C., Gehring, K. B., Griffin, D. B., Hale, D. S., Mafi, G. G., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Miller, R. K., VanOverbeke, D. L., Wasser, B. E., & Savell, J. W. (2017). National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. *Meat and Muscle Biology*, *1*(1), 138. https://doi.org/10.22175/mmb2017.05.0028

Molina, M. E., Johnson, D. D., West, R. L., & Gwartney, B. L. (2005). Enhancing palatability traits in beef chuck muscles. *Meat Science*, *71*(1), 52–61. https://doi.org/10.1016/j.meatsci.2005.04.004

Morrow, S. J., Garmyn, A. J., Hardcastle, N. C., Brooks, J. C., & Miller, M. F. (2019). The Effects of Enhancement Strategies of Beef Flanks on Composition and Consumer Palatability Characteristics. *Meat and Muscle Biology*, *3*(1), 457. https://doi.org/10.22175/mmb2019.07.0030

N'Gatta, K. C. A., Kondjoyan, A., Favier, R., Rouel, J., Vénien, A., Astruc, T., Gruffat, D., & Mirade, P.-S. (2021). Impact of Tumbling Process on the Toughness and Structure of Raw Beef Meat Pieces. *Foods*, *10*(11), 2802. https://doi.org/10.3390/foods10112802

Nondorf, M. J., & Kim, Y. H. B. (2022). Fresh beef tumbling at different post-mortem times to improve tenderness and proteolytic features of M. longissimus lumborum. *International Journal of Food Science & Technology*. https://doi.org/10.1111/ijfs.15627

O'Quinn, T. G., Legako, J. F., Brooks, J. C., & Miller, M. F. (2018). Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience1. *Translational Animal Science*, *2*(1), 26–36. https://doi.org/10.1093/tas/txx008

Rhee, M. S., Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2004). Variation in palatability and biochemical traits within and among eleven beef muscles. *Journal of Animal Science*, *82*(2), 534–550. https://doi.org/10.2527/2004.822534x

Setyabrata, D., Tuell, J. R., & Kim, Y. H. B. (2019). The Effect of Aging/Freezing Sequence and Freezing Rate on Quality Attributes of Beef Loins. *Meat and Muscle Biology*, *3*(1), 488. https://doi.org/10.22175/mmb2019.08.0034

Stolowski, G. D., Baird, B. E., Miller, R. K., Savell, J. W., Sams, A. R., Taylor, J. F., Sanders, J. O., & Smith, S. B. (2006). Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. *Meat Science*, *73*(3), 475–483. https://doi.org/10.1016/j.meatsci.2006.01.006

Sullivan, G. A., & Calkins, C. R. (2011). Ranking Beef Muscles for Warner–Bratzler Shear Force and Trained Sensory Panel Ratings from Published Literature. *Journal of Food Quality*, *34*(3), 195–203. https://doi.org/10.1111/j.1745-4557.2011.00386.x

Tuell, J. R., & Kim, Y. H. (2021). Smart Tumbling Improved Quality and Palatability Attributes of Fresh Beef M. Longissimus Lumborum and M. Semitendinosus. *Meat and Muscle Biology*, *5*(2), 70. https://doi.org/doi:10.22175/mmb.11683

Tuell, J. R., Yu, Q., & Kim, B. (2021). Can Tumbling without Brine Improve Tenderness and Proteolysis of Beef Loin Muscles? *Meat and Muscle Biology*. https://doi.org/10.22175/mmb.13044

USDA. (2014). *Institutional Meat Purchase Specifications: Fresh Beef Series 100*. 71.

USDA. (2017). *United States standards for Grades of Carcass Beef*. Agricultural Marketing Service, Washington, DC.

USDA. (2021). *National Retail Report Beef. Advertised Prices for Beef at Major Retail Supermarket Outlets ending during the period of 10/29 thru 11/04.* https://www.ams.usda.gov/mnreports/lswbfrtl.pdf

Warner, R. (2017). Chapter 14—The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. In F. Toldra´ (Ed.), *Lawrie´s Meat Science (Eighth Edition)* (pp. 419–459). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100694-8.00014-5

Warner, R., Miller, R., Ha, M., Wheeler, T. L., Dunshea, F., Li, X., Vaskoska, R., & Purslow, P. (2020). Meat Tenderness: Underlying Mechanisms, Instrumental Measurement, and Sensory Assessment. *Meat and Muscle Biology*, *4*(2). https://doi.org/10.22175/mmb.10489

Wyrwisz, J., Moczkowska, M., Kurek, M., Stelmasiak, A., Półtorak, A., & Wierzbicka, A. (2016). Influence of 21days of vacuum-aging on color, bloom development, and WBSF of beef semimembranosus. *Meat Science*, *122*, 48–54. https://doi.org/10.1016/j.meatsci.2016.07.018

Yang, L., Arnold, N. L., Drape, T., Williams, R. C., Archibald, T., Chapman, B., & Boyer, R. (2021). A survey of United States consumer awareness, purchasing, and handling of mechanically tenderized beef products. *Food Control*, *120*, 107505. https://doi.org/10.1016/j.foodcont.2020.107505

Yu, Q., Cooper, B., Sobreira, T., & Kim, Y. (2021). Utilizing Pork Exudate Metabolomics to Reveal the Impact of Aging on Meat Quality. *Foods*, *10*(3), 668. https://doi.org/10.3390/foods10030668

CHAPTER 5. CONCLUSION AND FUTURE DIRECTIONS

5.1 Conclusions

The research presented in this dissertation demonstrates the feasibility of using tumbling in the absence of a brine solution, combined with additional postmortem aging, as a strategy to improve palatability attributes of fresh beef steaks. In Chapter 2, the process was applied to the beef LL muscle, a cut with a high aging potential and relatively low amount of intramuscular connective tissues (Chun et al., 2020; Nair et al., 2019; Rhee et al., 2004). The results of this study indicated that tumbling for 60 or 90 minutes would result in an immediate improvement in objective tenderness shown by a substantial decrease in WBSF values, which was maintained throughout the aging period. While the immediate improvement in tenderness would suggest tenderization primarily occurred via myofibrillar fragmentation (which was supported by increased MFI and obvious fragmentation shown by TEM), there was also some evidence to suggest proteolysis shown by troponin-T degradation would be enhanced over the course of postmortem aging.

While the LL would be a muscle that generally achieves acceptable tenderness prior to consumption (Martinez et al., 2017), the greatest need for physical interventions to enhance tenderization would be in cuts categorized as intermediate or tough. Further, considering Chapter 2 only assessed objective meat quality measures, elucidating what the process may do to impact eating quality attributes was warranted. In Chapter 3, tumbling with subsequent aging was applied to the beef LL and ST muscles. Validating the findings of Chapter 2, it was found that tumbling, regardless of the duration of postmortem aging, would result in considerable fragmentation of the myofibrils, therefore improving instrumental tenderness of the LL. However, it was also found that tumbling would not impact WBSF values of the ST, despite myofibrillar fragmentation also occurring. This observation would likely be attributed to tumbling having minimal impacts on intramuscular connective tissues like collagen, thus cuts with high background toughness would show limited tenderization. Despite this, there was some evidence to suggest that tumbling combined with subsequent postmortem aging could influence consumer perceptions of tenderness. This would likely owe to differences in mouthfeel reflected by subjective evaluation but not instrumental measures (Strydom et al., 2015). Consumer panelists found tumbling to improve

tenderness and overall liking of LL steaks with no detriments to juiciness and flavor. Accordingly, the process may be advantageous over other methods that are either directly (e.g., through physical cutting of myofibers) or indirectly (e.g., through necessitating cooking to a higher degree of doneness) detrimental to these eating quality attributes (Jeremiah et al., 1999; McKillip et al., 2017; Yang et al., 2021).

In Chapter 4, the process was applied to three muscles from the beef sirloin: the GM, BF, and TFL. Generally, these cuts would be less tender than the LL but more tender than the ST (Chun et al., 2020; Rhee et al., 2004; Sullivan & Calkins, 2011; Voges et al., 2007). In agreement with the previous studies, tumbling resulted in myofibrillar fragmentation but had negligible effects on collagen. Hence, objective tenderness was improved in the GM and TFL but not in the BF, a muscle known to be highly variable in the amount of intramuscular connective tissues (Jeremiah et al., 2003). Descriptive sensory scores were in general agreement with these findings, where greater myofibrillar tenderness in tumbled GM steaks was found. However, no measures of tenderness were found to be impacted in the TFL, which could be related to the tenderness of this muscle also being primarily determined by collagen content and cross-linking (Chun et al., 2020). As trained panelists evaluated muscles that had been both tumbled and aged, some off-flavors such as oxidized and liver-like were increased. This observation would be in general agreement with previous observations regarding how physical processes would disrupt cellular membranes and release free radicals (Cheng & Ockerman, 2003; Dang et al., 2022; Peña-Gonzalez et al., 2019).

5.2 Future Directions

The current beef industry generally supplies consumers with fresh beef steaks that are considered acceptably tender, especially for cuts from the loin and rib primals (Martinez et al., 2017). However, there are still multiple situations in which the method described in this dissertation could be applied to improve consumer satisfaction and add value. For instance, while the most recent NBTS indicated that virtually all beef loin steaks would be categorized as very tender or tender, considerable lengths of postmortem aging are used to achieve this (approximately 25.9 and 31.5 days for retail and foodservice steaks, respectively) (Martinez et al., 2017). As such, it is reasonable to postulate that a post-harvest strategy that could drastically shorten the necessary duration of aging to achieve acceptable tenderness would be desirable to the industry. Further,

Martinez et al. (2017) reported that mechanical tenderization and enhancement could have been applied to steaks. Considering some consumers view such processes as “unnatural” (Yang et al., 2021) combined with a preference for beef without added non-meat ingredients (Ardeshiri et al., 2019), it may be that tumbling and subsequent aging could be a viable alternative for industry application.

In addition to shortening the required duration of postmortem aging, it is also reasonable that tumbling without brine inclusion could divert some cuts from mature carcasses from being sold as ground product to being merchandized intact at a higher price. For example, the study by Alvarenga et al. (2021) recently demonstrated that 72.1% of LL muscles from culled Angus cows would be categorized as tender by 28 days of aging. It is reasonable to speculate considering the findings presented in this dissertation of tumbling improving the effectiveness of postmortem aging via myofibrillar fragmentation and enhanced proteolysis that an even greater percentage would reach acceptable levels of tenderness. Further, considering the primary reason for poorer tenderization of beef from *Bos indicus* relative to *Bos taurus* cattle would be a low extent of proteolysis rather than high extent of background toughness (Rodrigues et al., 2017; Whipple et al., 1990), it is reasonable to expect that tumbling alone would be an effective method of tenderization. While there was evidence to suggest tumbling would enhance proteolysis during the aging process, the primary mechanism through which tenderization is achieved would be direct fragmentation of the myofibrillar structure.

With that considered, there were clear intermuscular differences in the effectiveness of the tumbling and aging process on objective and subjective measures of beef quality. This finding was most likely due to the tumbling and aging conditions employed in these studies being insufficient to affect the connective tissues. Hence, the process was most effective in cuts with a relatively low amount of collagen (e.g., LL and GM) compared to those with a greater amount (e.g., ST, BF, TFL) (Chun et al., 2020; Rhee et al., 2004). However, it has also been recently demonstrated that physical damage induced through ultrasound could result in considerable disruption to the perimysium and an increase in collagen solubility, therefore increasing tenderness of the ST (Wang et al., 2022). While the studies in this dissertation provided no evidence that tumbling would favor the degeneration of intramuscular connective tissues, it may be that more rigorous tumbling or a longer duration of postmortem aging would be necessary to do so. Supporting this, N’Gatta et al. (2021) found that extended tumbling durations (greater than 3 hours) would influence the

toughness of the connective tissues of beef ST muscles. For muscles with a higher contribution of intramuscular connective tissues, future studies should consider extending the duration of tumbling or employing more aggressive tumbling strategies (e.g., intermittent tumbling, increased speed, increasing drop distance, etc.). While Chapter 1 examined how these strategies would influence quality attributes in processed meats, there is presently little information regarding how these factors would influence quality in fresh beef, as well as how they may interact with postmortem aging.

Likely the greatest unknowns that must be addressed prior to industry application would be the potential effects the method may have on microbiological properties and oxidative stability. Much of the discussion regarding the advantages of tumbling over mechanical tenderization has been under the assumption that the former would be non-invasive, hence cooking to a higher degree of doneness would not be necessary. However, considering tumbling would facilitate the migration of moisture into the product interior in processed meat applications (Sharedeh et al., 2015), it is reasonable to expect that even in the absence of a brine solution the product would no longer be considered truly intact. At present, tumbled meat products are not designated as being mechanically tenderized and are therefore not subject to the associated labeling requirements (FSIS, 2015). This does not mean that tumbling would not present similar risks of translocating pathogens from the product surface to the interior, rather that there is presently insufficient information regarding this phenomenon (FSIS, 2015). Additionally, the data presented in Chapter 4 suggested that tumbling with subsequent postmortem aging could result in the generation of off-flavors. This is unsurprising considering previous literature regarding how physical disruptions could result in the generation of free radicals (Cheng & Ockerman, 2003; Dang et al., 2022; Peña-Gonzalez et al., 2019). However, considering recent literature suggests flavor has surpassed tenderness as the primary attribute determining consumer acceptance of beef (O'Quinn et al., 2018), it is critical that potential off-flavor generation be minimized. As shown in Chapter 3, consumer panelists rated flavor liking as similar in beef LL and ST muscles that had been both tumbled and aged, suggesting any potential detriments would be slight. However, if more rigorous tumbling regimes were employed or the duration of postmortem aging were extended, as would likely be necessary to tenderize more inherently tough cuts, it is reasonable to expect that oxidation would be exacerbated. Furthermore, while the previous chapters found little evidence that initial bloom color would be affected by tumbling, color stability was not assessed. As fresh beef in the

US is generally merchandized in aerobic packaging, thus is highly susceptible to discoloration (Suman et al., 2014), minimizing discoloration at the retail and consumer levels would be critical. While it is well-established that improved tenderness would have substantial economic value (Ward et al., 2008), it is also known that beef discoloration results in considerable economic losses for the industry (Smith et al., 2000). Future studies should determine the oxidative and color stability of beef under different tumbling and aging regimes, specifically examining intermuscular differences.

Taken together, the studies presented in this dissertation suggest that tumbling without brine inclusion followed by postmortem aging would be an effective strategy to improve objective and subjective measures of beef quality under some circumstances. The advantages of the method would include improving the objective and subjective tenderness of cuts without a high amount of background toughness such as the LL and GM with minimal negative impacts on other eating quality attributes. However, the process may be limited in its effectiveness of tenderizing cuts categorized as tough. The potential drawbacks of the process would be poorer water-holding attributes (though this may not necessarily contribute to poorer juiciness), as well as the potential for the introduction of biological hazards combined with the exacerbation of oxidative processes. However, these would warrant further study to allow processors to better understand the pros and cons of utilizing this strategy. While tumbling without brine inclusion was never compared to tumbling with, these studies add to the existing literature by demonstrating that the physical disruptions alone without myofibrillar swelling through brine inclusion can improve tenderness. Further, when combined with subsequent postmortem aging, the degradation of myofibrillar proteins be enhanced with great implications for postmortem tenderization.

5.3 References

Alvarenga, T. I. R. C., Palendeng, M., Thennadil, S., McGilchrist, P., Cafe, L. M., Almeida, A. K., & Hopkins, D. L. (2021). Is meat from cull cows tougher? *Meat Science*, *177*, 108498. https://doi.org/10.1016/j.meatsci.2021.108498

Ardeshiri, A., Sampson, S., & Swait, J. (2019). Seasonality effects on consumers' preferences over quality attributes of different beef products. *Meat Science*, *157*, 107868. https://doi.org/10.1016/j.meatsci.2019.06.004

Cheng, J., & Ockerman, H. W. (2003). Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. *Meat Science*, *65*(4), 1353–1359. https://doi.org/10.1016/S0309-1740(03)00057-3

Chun, C. K. Y., Wu, W., Welter, A. A., O'Quinn, T. G., Magnin-Bissel, G., Boyle, D. L., & Chao, M. D. (2020). A preliminary investigation of the contribution of different tenderness factors to beef loin, tri-tip and heel tenderness. *Meat Science*, *170*, 108247. https://doi.org/10.1016/j.meatsci.2020.108247

Dang, D. S., Stafford, C. D., Taylor, M. J., Buhler, J. F., Thornton, K. J., & Matarneh, S. K. (2022). Ultrasonication of beef improves calpain-1 autolysis and caspase-3 activity by elevating cytosolic calcium and inducing mitochondrial dysfunction. *Meat Science*, *183*, 108646. https://doi.org/10.1016/j.meatsci.2021.108646

FSIS. (2015, May 18). *Descriptive Designation for Needle- or Blade-Tenderized (Mechanically Tenderized) Beef Products*. Federal Register. https://www.federalregister.gov/documents/2015/05/18/2015-11916/descriptive-designation-for-needle--or-blade-tenderized-mechanically-tenderized-beef-products

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L., & Gibson, L. L. (2003). Assessment of the chemical and cooking properties of the major beef muscles and muscle groups. *Meat Science*, *65*(3), 985–992. https://doi.org/10.1016/S0309-1740(02)00308-X

Jeremiah, L. E., Gibson, L. L., & Cunningham, B. (1999). The influence of mechanical tenderization on the palatability of certain bovine muscles. *Food Research International*, *32*(8), 585–591. https://doi.org/10.1016/S0963-9969(99)00134-9

Martinez, H. A., Arnold, A. N., Brooks, J. C., Carr, C. C., Gehring, K. B., Griffin, D. B., Hale, D. S., Mafi, G. G., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Miller, R. K., VanOverbeke, D. L., Wasser, B. E., & Savell, J. W. (2017). National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. *Meat and Muscle Biology*, *1*(1), 138. https://doi.org/10.22175/mmb2017.05.0028

McKillip, K. V., Wilfong, A. K., Gonzalez, J. M., Houser, T. A., Unruh, J. A., Boyle, E. A. E., & O'Quinn, T. G. (2017). Sensory Evaluation of Enhanced Beef Strip Loin Steaks Cooked to 3 Degrees of Doneness. *Meat and Muscle Biology*, *1*(1). https://doi.org/10.22175/mmb2017.06.0033

Nair, M. N., Canto, A. C. V. C. S., Rentfrow, G., & Suman, S. P. (2019). Muscle-specific effect of aging on beef tenderness. *LWT*, *100*, 250–252. https://doi.org/10.1016/j.lwt.2018.10.038

N'Gatta, K. C. A., Kondjoyan, A., Favier, R., Rouel, J., Vénien, A., Astruc, T., Gruffat, D., & Mirade, P.-S. (2021). Impact of Tumbling Process on the Toughness and Structure of Raw Beef Meat Pieces. *Foods*, *10*(11), 2802. https://doi.org/10.3390/foods10112802

O'Quinn, T. G., Legako, J. F., Brooks, J. C., & Miller, M. F. (2018). Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience1. *Translational Animal Science*, *2*(1), 26–36. https://doi.org/10.1093/tas/txx008

Peña-Gonzalez, E., Alarcon-Rojo, A. D., Garcia-Galicia, I., Carrillo-Lopez, L., & Huerta-Jimenez, M. (2019). Ultrasound as a potential process to tenderize beef: Sensory and technological parameters. *Ultrasonics Sonochemistry*, *53*, 134–141. https://doi.org/10.1016/j.ultsonch.2018.12.045

Rhee, M. S., Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2004). Variation in palatability and biochemical traits within and among eleven beef muscles. *Journal of Animal Science*, *82*(2), 534–550. https://doi.org/10.2527/2004.822534x

Rodrigues, R. T. de S., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros, E., Busato, K. C., Gomes, R. A., Ladeira, M. M., & Martins, T. da S. (2017). Differences in Beef Quality between Angus (Bos taurus taurus) and Nellore (Bos taurus indicus) Cattle through a Proteomic and Phosphoproteomic Approach. *PLOS ONE*, *12*(1), e0170294. https://doi.org/10.1371/journal.pone.0170294

Sharedeh, D., Mirade, P.-S., Venien, A., & Daudin, J.-D. (2015). Analysis of salt penetration enhancement in meat tissue by mechanical treatment using a tumbling simulator. *Journal of Food Engineering*, *166*, 377–383. https://doi.org/10.1016/j.jfoodeng.2015.06.023

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D., & Williams, S. N. (2000). Economic implications of improved color stability in beef. In *Antioxidants in muscle foods: Nutritional strategies to improve quality* (pp. 397–426). John Wiley and Sons.

Strydom, P. E., Strydom, N., Frylinck, L., Hugo, A., Hope-Jones, M., Moholisa, E., & van Heerden, I. (2015). The interrelationship between sensory tenderness and Warner Bratzler shear force of three muscles as influenced by various production and post-harvest conditions. *Proceedings of the 61st International Congress of Meat Science and Technology, 23-28th August, 2015, Clermont-Ferrand, France*, 5.

Sullivan, G. A., & Calkins, C. R. (2011). Ranking Beef Muscles for Warner–Bratzler Shear Force and Trained Sensory Panel Ratings from Published Literature. *Journal of Food Quality*, *34*(3), 195–203. https://doi.org/10.1111/j.1745-4557.2011.00386.x

Suman, S. P., Hunt, M. C., Nair, M. N., & Rentfrow, G. (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science*, *98*(3), 490–504. https://doi.org/10.1016/j.meatsci.2014.06.032

Voges, K. L., Mason, C. L., Brooks, J. C., Delmore, R. J., Griffin, D. B., Hale, D. S., Henning, W. R., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Miller, R. K., Morgan, J. B., Baird, B. E., Gwartney, B. L., & Savell, J. W. (2007). National beef tenderness survey – 2006: Assessment of Warner–Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. *Meat Science*, *77*(3), 357–364. https://doi.org/10.1016/j.meatsci.2007.03.024

Wang, L., Li, J., Teng, S., Zhang, W., Purslow, P. P., & Zhang, R. (2022). Changes in collagen properties and cathepsin activity of beef M. semitendinosus by the application of ultrasound during post-mortem aging. *Meat Science*, *185*, 108718. https://doi.org/10.1016/j.meatsci.2021.108718

Ward, C. E., Lusk, J. L., & Dutton, J. M. (2008). Implicit Value of Retail Beef Product Attributes. *Journal of Agricultural and Resource Economics*, *33*(3), 364–381.

Whipple, G., Koohmaraie, M., Dikeman, M. E., Crouse, J. D., Hunt, M. C., & Klemm, R. D. (1990). Evaluation of attributes that affect longissimus muscle tenderness in bos taurus and bos indicus cattle2. *Journal of Animal Science*, *68*(9), 2716–2728. https://doi.org/10.2527/1990.6892716x

Yang, L., Arnold, N. L., Drape, T., Williams, R. C., Archibald, T., Chapman, B., & Boyer, R. (2021). A survey of United States consumer awareness, purchasing, and handling of mechanically tenderized beef products. *Food Control*, *120*, 107505. https://doi.org/10.1016/j.foodcont.2020.107505

APPENDIX A. CURRICULUM VITAE

Jacob R. Tuell

Education

Purdue University, *West Lafayette, Indiana* Expected Summer 2022
Master of Public Health
GPA **4.00**/4.00

Purdue University, *West Lafayette, Indiana* Expected Spring 2022
Ph.D. in Meat Science and Muscle Biology
GPA **4.00**/4.00

Purdue University, *West Lafayette, Indiana* May 2018
Bachelor of Science in Animal Sciences, Animal Products; minor in Wildlife Sciences
GPA **4.00**/4.00 – Honors Recognition, Highest Distinction

Relevant Experience

Teaching Assistant – ANSC 35100 (Meat Science) [Spring 2021]

- Assisted in instruction, guest lecturing on the role of meat in the diet
- Worked closely with students to research current topics in the field of meat science including alternative proteins and health impacts
- Created exams and assignments through Brightspace platform

Teaching Assistant – ANSC 35101 (Meat Science Lab) [Spring 2018]

- Assisted in laboratory instruction and enforced laboratory rules to ensure students are following good manufacturing practices
- Communicated effectively with students both inside and outside of class and encouraged students to actively participate and ask questions
- Graded all assignments and compiled data on a grade spreadsheet

Graduate Mentor – ANSC 49100 (Special Projects) [Fall 2018 – Spring 2019]

- Acted as a graduate mentor for two undergraduate students working in the Meat Science and Muscle Biology laboratory
- Provided guidance in their writings and presentations, in which the one student received first place in the Purdue Undergraduate Research Symposium for the College of Agriculture and second place in the American Meat Science Association Undergraduate Research Competition

Research Assistant – Meat Science and Muscle Biology [Spring 2017 - Present]

- Assessed meat quality through instrumental tenderness, color evaluation, consumer panel, water-holding ability, and other assays
- Trained in specialized assays such as transmission electron microscopy, fatty acid profiling via gas chromatography, secondary lipid oxidation, and western blotting
- Published 7 peer-reviewed publications and 5 abstracts as a first author in journals such as *Meat and Muscle Biology, Meat Science,* and *Food Chemistry*
- Presented findings at national and international conferences including the American Meat Science Association Reciprocal Meat Conference and International Congress of Meat Science and Technology

Leadership Activities:

University and Department Organizations:

[Spring 2020-Spring 2021] Animal Sciences Representative to the Purdue College of Agriculture Graduate Student Advisory Committee
[Fall 2019-Fall 2021] Purdue Department of Animal Sciences Graduate Student Association – Student Representative
[Spring 2019-Spring 2020] Purdue Meat Science Academic Quiz Bowl - Coach
[Spring 2018] Purdue Meat Science Academic Quiz Bowl - Team Captain
[Spring 2017-Summer 2018] Purdue Meat Science Academic Quiz Bowl
[Fall 2016-Present] Purdue Meat Science Club - Member

Professional Associations:

[Spring 2022-Present] American Meat Science Association Intercollegiate BBQ Task Force
[Fall 2021-Present] American Meat Science Association Youth Programs Committee
[Spring 2021-Present] Institute of Food Technologists – Muscle Foods Division Senior Student Representative
[Fall 2020-Present] Indiana Rural Health Association
[Spring 2020-Spring 2021] Institute of Food Technologists – Muscle Foods Division Junior Student Representative
[Spring 2017-Present] American Meat Science Association

Certifications:

[Fall 2021] American Meat Science Association Food Safety and Science Certification
[Fall 2019] Responsible Conduct of Research Training – Faculty, Postdoctoral, and Graduate Students – CITI Program (Credential ID 32888493)
[Fall 2019] Social Behavioral Research Investigators and Key Personnel – CITI Program (Credential ID 32888492)

Honors:

[2021, Summer] Reciprocal Meat Conference PhD Research Competition – 1st Place
[2021, Summer] AMSA Virtual Processed Meats Judging – 3rd Place Graduate
[2021, Spring] Purdue Department of Animal Sciences Book Harmon Leadership Scholarship
[2021, Spring] Purdue Department of Animal Sciences LOUJA Graduate Research Competition – Winner
[2021, Spring] Purdue Graduate School InnovatED Magazine – Featured Publication
[2020, Fall] Purdue Graduate School 3MT Competition – Finalist
[2020, Summer] International Congress on Meat Science and Technology/Reciprocal Meat Conference Chairmen's Selected Abstract
[2020, Summer] International Congress on Meat Science and Technology/Reciprocal Meat Conference PhD Research Competition – 2nd Place in Meat Processing and Technology Division
[2020, Spring] Purdue Department of Animal Sciences Featherston Early Graduate Career Award
[2020, Spring] Purdue Center for Animal Welfare Science – Profiles in Animal Welfare Science
[2019, Spring] Purdue Department of Animal Sciences LOUJA Graduate Research Competition – Winner
[2019, Spring] Institute of Food Technologists – Muscle Foods Division – Student Spotlight
[2018, Summer] Reciprocal Meat Conference Undergraduate Research Competition – 1st Place
[2018, Summer] AMSA Processed Meats Judging – 1st Place Undergraduate
[2018, Summer] American Meat Science Association (AMSA) Intercollegiate Quiz Bowl – 3rd Place
[2018, Spring] Purdue Honors College Graduate – with Highest Distinction
[2018, Spring] Purdue College of Agriculture Dean's Scholars Program Graduate
[2018, Spring] Frederick N. Andrews Fellowship – Purdue University Fellowship
[2018, Spring] Outstanding Undergraduate (Senior) Award – Purdue Department of Animal Sciences
[2018, Spring] C. Boyd Ramsey RMC Scholar Award – American Meat Science Association
[2018, Spring] Office of Undergraduate Research Scholarship
[2018, Spring] Undergraduate Research and Creative Endeavors Scholarship – Purdue Honors College
[2017, Fall] Al Piccetti Scholarship – North American Meat Institute
[2017, Summer] American Meat Science Association (AMSA) Intercollegiate Quiz Bowl – 5th Place
[2017, Summer] AMSA Iron Chef Product Development – 1st Place
[2017, Summer] AMSA Processed Meats Judging – 2nd Place Undergraduate Novice
[2017, Summer] Top 10 Outstanding Research Poster – Summer Undergraduate Research Fellowship
[2015, Summer] Purdue Big Move Short Term Scholarship

[8 semesters] Purdue Dean's List
[2014, Fall] Purdue Access and Success Incentive Grant
[2014, Fall] J. Bonner Wampler Alumni Scholarship
[2014, Fall] William E. Morris Scholarship
[2014, Fall] Kelly and Margaret O'Neall Scholarship
[2014, Fall] Purdue Trustees Scholar
[2014, Spring] Purdue Veterinary Scholar
[2014, Spring] Lilly Endowment Community Scholar

Publications

Accepted Publications: (8 total; 7 as first author)

Tuell, J.R., Nondorf, M.J., Abdelhaseib, M., Setyabrata, D., Kim, Y.H.B. Tumbling and subsequent aging improves tenderness of beef longissimus lumborum and semitendinosus steaks by disrupting myofibrillar structure and enhancing proteolysis. *Journal of Animal Science, accepted manuscript*.

Tuell, J.R., Yu, Q., Kim, Y.H.B. (2021). Can Tumbling without Brine Improve Tenderness and Proteolysis of Beef Loin Muscles? *Meat and Muscle Biology*. doi: 10.22175/mmb.13044

Tuell, J.R., Nondorf, M., Maskal, J., Johnson, J., Kim, Y.H.B. (2021). Carcass and Meat Quality Traits of Market Weight Gilts Exposed to Gestational Heat Stress. *Animals,* 11(3), 717. doi: https://doi.org/10.3390/ani11030717

Tuell, J.R., Kim, H.-W., Zhang, J., Guedes, J., Seo, J.-K., Schoonmaker, J.P., Kim, Y.H.B. (2021). Arginine supplementation may improve color and redox stability of beef loins through delayed onset of mitochondrial-mediated apoptotic processes. *Food Chemistry*, 343(1), 128552. doi: https://doi.org/10.1016/j.foodchem.2020.128552

Tuell, J.R., Seo, J.-K., Kim, Y.H.B. (2020). Combined Impacts of Initial Freezing Rate of Pork Ham Muscles (*M. biceps femoris* and *M. semitendinosus*) and Subsequent Freezing on Quality Characteristics of Pork Patties. *Meat Science*, 170:108248. doi: https://doi.org/10.1016/j.meatsci.2020.108248

Tuell, J.R., Park, J.-Y., Wang, W., Cooper, B., Sobreira, T., Cheng, H.-W., Kim, Y.H.B. (2020). Effects of Photoperiod Regime on Meat Quality, Oxidative Stability, and Metabolites of Postmortem Broiler Fillet (*M. Pectoralis major*) Muscles. *Foods*, 9:215. doi: https://doi.org/10.3390/foods9020215

Tuell, J.R., Park, J.-Y., Wang, W., Cheng, H.-W., Kim, Y.H.B. Functional/Physicochemical Properties and Oxidative Stability of Ground Meat from Broilers Reared under Different Photoperiods. *Poultry Science*, 99(7):3761-3768. doi: https://doi.org/10.1016/j.psj.2020.04.021

Setyabrata, D., **Tuell, J.R**., Kim, Y.H.B. (2019). The Effect of Aging/Freezing Sequence and Freezing Rate on Quality Attributes of Beef Loins (*M. longissimus lumborum*). *Meat and Muscle Biology*, 3(1):1-12. doi: https://doi.org/10.22175/mmb.11234

Manuscripts in Preparation: (2 total; 1 as first author)

Tuell, J.R. Nondorf, M.J., Abdelhaseib, M., Setyabrata, D., Legako, J., Kim, Y.H.B. Beef quality, biochemical attributes, and descriptive sensory scores of *gluteus medius, biceps femoris,* and *tensor fasciae latae* muscles subjected to combined tumbling and postmortem aging
Xue, S., Park, J.Y., **Tuell, J.R.,** Johnson, J.S., Dinh, T., Kim, Y.H.B. In utero heat-stress has minimal impacts on processed pork products: A comparative study.

Published Abstracts/Proceeding Papers: (8 total; 5 as first author)
Tuell, J.R. Nondorf, M.J., Abdelhaseib, M., Setyabrata, D., Legako, J., Kim, Y.H.B. (2021). Muscle-specific Impacts of Fresh Beef Tumbling on Meat Quality, Palatability, and Proteolytic Attributes of Sirloin Muscles. *Meat and Muscle Biology*, 35.
Tuell, J.R. and Kim, Y.H.B. (2020). Smart Tumbling Improved Quality and Palatability of Fresh Beef *M. Longissimus lumborum* and *M. Semitendinosus*. *International Congress of Meat Science and Technology/Reciprocal Meat Conference*, 70. Chairmen's Selected Abstract.
Tuell, J.R., Yu, Q., and Kim, Y.H.B. (2020). Impacts of Smart Tumbling on Muscle Ultrastructure, Proteolysis and Quality Attributes of Fresh Beef Loins (*M. Longissimus lumborum*). *International Congress of Meat Science and Technology/Reciprocal Meat Conference*, 114.
Nondorf, M.J., **Tuell, J.R.,** Maskal, J., Johnson, J.S., and Kim, Y.H.B. (2020). Impacts of In Utero Heat Stress on Carcass and Meat Quality Traits of Pork Loins. *International Congress of Meat Science and Technology/Reciprocal Meat Conference*, 95.
Tuell, J.R., Ma, D., Kim, Y.H.B. (2019). Metabolomics Approach to Improve Meat Quality and Value. *Meat and Muscle Biology*.
Park, J.Y., **Tuell, J.R.,** Wang, W., Cheng, H.W., Kim, Y.H.B. 2019. Functional/Physicochemical Properties and Oxidative Stability of Ground Meat from Broilers Exposed to Different Photoperiods. *Meat and Muscle Biology*, 3(2):103-103. doi:10.2134/mmb2019.0103
Will, E., **Tuell, J.R.,** Park, J.Y., Wang, W., Cheng, H.W., Kim, Y.H.B. (2019). Effect of Different Photoperiods on Quality Attributes and Oxidative Stability of Breast Meat (*M. Pectoralis Major*) from Broilers. *Meat and Muscle Biology*, 3(2):181-181. doi:10.2134/mmb2019.0181
Tuell, J., Kim, H.W., Guedes, J., Seo, J.K., Schoonmaker, J., Kim, Y.H.B. (2018). Supplemental Effects of Ruminal Bypass Arginine and Lysine for Improving Meat Quality and Oxidative Stability of Aged Beef Loins. *Meat and Muscle Biology*, 2(2). doi: https://doi.org/10.221751/rmc2018.160

Other Publications/Patents: (4 popular press as first author; 1 provisional patent)
Tuell, J.R. Yu, Q., and Kim, Y.H.B. Tumbling without Brine Inclusion: A New Strategy for Tenderizing Beef? To be published in the *National Provisioner.*
Tuell, J.R. TLC for Tough Steaks. (2021). Published in *InnovatED* – Purdue's premier graduate research magazine.

Tuell, J.R. and Kim, Y.H.B. AMSA Exclusive: Developing smart tumbling – a simple and readily applicable method of fresh beef tenderization (2021). Published in *MeatingPlace*.
Tuell, J.R., Ma, D., and Kim, Y.H.B. (2019). AMSA Exclusive: Metabolomics approach to improve meat quality and value. Published in *MeatingPlace*.
Tuell, J., Kim, H.W., Guedes, J., Seo, J.K., Schoonmaker, J., Kim, Y.H.B. (2018). Ruminal bypass arginine and beef product discoloration. Published in the *National Provisioner.*
Kim, Y.H.B., **Tuell, J.R.,** and Yu, Q. A Simple and Novel Strategy to Improve Beef Quality Attributes in a Consistent and Natural Manner. Provisional Patent. PRF Reference No: 2019-KIM-68560.