THESIS

IMPACT OF EXTENDED WET-AGING ON BEEF *SEMIMEMBRANOSUS*, *GLUTEUS MEDIUS*, AND *BICEPS FEMORIS* MUSCLES

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ABSTRACT

IMPACT OF EXTENDED WET-AGING ON BEEF *SEMIMEMBRANOSUS*, *GLUTEUS MEDIUS*, AND *BICEPS FEMORIS* MUSCLES

Postmortem wet aging is the most common aging method used in the meat industry to enhance tenderness, palatability, and overall consumer acceptance of beef. Previous industry surveys have indicated that the average aging time for beef is 25.9 days, while some beef cuts can be aged for up to 102 days. During postmortem aging, several microbiological and biochemical changes occur within the muscles. Most of the previous aging studies focused on the *longissimus lumborum* muscle. It has become more common to market single muscle cuts such as the *semimembranosus* (SM), *gluteus medius* (GM), and *biceps femoris* (BF). However, few studies have been conducted on microbial and biochemical changes in these muscles during aging. Therefore, the objective of this study was to determine the impact of aging on the microbial load, microbiome (16S rRNA gene sequencing and analysis), and desmin degradation of beef SM, GM, and BF.

Beef top rounds (SM) and sirloin top butts (N = 80) were collected at a commercial beef processing facility. These subprimals were wet-aged for 14, 28, 35, 42, 49, 56, 63, or 70 days, and on each of these days, the BF and GM were separated from the top sirloin butt. Ten (n = 10) samples were analyzed from each muscle for each aging period. On each aging day, two separate 5 × 10 cm areas of the muscle surface were sampled using separate sponge samplers. Buffered peptone water was added to one set of sponge samples, followed by mechanical pummeling and analysis for total aerobic mesophilic microflora counts (AC) and lactic acid bacteria counts (LABC) using the TEMPO® system. To the second set of sponges, phosphate-buffered saline (PBS)

was added, and after mechanical pummeling, the liquid was collected and stored at -70°C for microbiome analysis. Additionally, on each aging day, the muscles were fabricated into 2.54 cm thick steaks, frozen in liquid nitrogen, powdered, and stored at -70°C for desmin degradation analysis. After extracting bacterial DNA from the PBS meat homogenate, microbiome library preparation was performed according to the Earth Microbiome Project (https://earthmicrobiome.org/), and an Illumina MiSeq was used for sequencing. Downstream analysis was performed using the Qiime2 pipeline. Desmin degradation was analyzed using a western blotting system. The protein concentration was measured using a detergent compatible protein assay and normalized with a Coomassie blue stain. The proteins were then separated using SDS-PAGE and transferred to a PVDF membrane. Desmin was visualized using primary (polyclonal rabbit anti-desmin) and secondary (goat anti-rabbit-HRP) antibodies. All data were analyzed using R, and significance was set at $\alpha = 0.05$. After downstream analysis, microbiome analysis was performed using the phyloseq, vegan, and pairwiseAdonis packages, and samples were rarefied at 4800 sequences. The relative abundance and beta diversity (Bray-Curtis and weighted UniFrac) were evaluated. The bacterial counts and desmin degradation were analyzed using the emmeans package, and the 55 kDa intact band intensity was evaluated using iBright analysis software to determine desmin degradation.

All three muscles had a low microbial load (<2 log CFU/cm^2) initially (day 14), which increased over time, as expected. In the SM, the AC did not increase ($P > 0.05$) after 28 days of aging, and LABC showed no substantial increases ($P > 0.05$) after 42 days. By day 70 of aging, the SM muscle had an AC of 5.6 log CFU/cm^2 and LABC of 6.0 log CFU/cm^2. In GM, the AC and LABC increased ($P < 0.05$) by 4.5 and 3.8 log CFU/cm^2, respectively, from day 14 to day 42 of aging. After 42 days, no significant differences ($P > 0.05$) in AC and LABC were obtained in the

GM muscle. The AC at 70 days of aging for the GM was 6.4 log CFU/cm^2, while the LABC was 6.0 log CFU/cm^2. The AC of BF did not increase ($P > 0.05$) after 42 days, whereas the LABC did not increase ($P > 0.05$) after 35 days of aging. The bacterial load of the BF muscle at 70 days of aging was 6.1 and 6.0 log CFU/cm^2 for AC and LABC, respectively. For all three muscles, the *Leuconostoc* bacterial genus had the greatest relative abundance, followed by *Carnobacterium*, when averaged over all eight aging periods. Beta diversity of all the muscles decreased ($P < 0.05$) over time, and 14 days of aging was different ($P < 0.05$) from all other aging periods. Overall, there was an initial increase ($P < 0.05$) in desmin degradation with aging for all the muscles, but as aging time increased, there was no further increase in desmin degradation. Specifically, desmin did not degrade further after 35 days for the SM and BF and 49 days for GM.

Generally, for all three muscles, the microbial load and desmin degradation increased with aging, whereas microbial diversity decreased with aging. Aside from the initial 14-day aging period, the most abundant bacterial genera were lactic acid bacteria. A higher degree of desmin degradation was observed with an increase in aging times for all the muscles, which could lead to increased tenderness. While there were some differences in microbial load, microbial diversity, and desmin degradation during early aging days, those disappeared as the aging time increased. Overall, these results suggest that a longer aging period for these muscles does not necessarily yield a higher-quality product.

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REVIEW OF LITERATURE

Beef quality, especially tenderness, is an important quality attribute for consumer eating satisfaction. The beef industry has developed several practices to improve postmortem tenderness, of which aging is the most important. Beef aging refers to holding beef at a refrigerated temperature for a certain period to improve its overall eating quality, including tenderness and flavor. In the US beef industry, there are two common aging methods: wet-aging and dry aging. Wet-aging is the process of sealing a beef primal or subprimal in a vacuum bag and storing it at a refrigerated temperature in order to improve tenderness, while during dry-aging, the meat is held without a bag (Warren and Kastner, 1992; Campbell et al., 2006; Sitz et al., 2006; Smith et al., 2008; Kim et al., 2016; Wang et al., 2025). In the US, most of the beef produced undergoes wet-aging as opposed to dry-aging.

1.1 Wet-Aging Versus Dry-Aging

While wet-aging occurs in a vacuum-sealed bag, dry-aging traditionally takes place in a controlled environment where the meat is not sealed. Dry-aged beef will develop a crust, which can have an effect on overall flavor (Warren and Kastner, 1992; Campbell et al., 2006). Warren and Kastner (1992) found that dry-aging can result in a higher intensity of nutty, brown/roasted, and beefy flavors compared to wet-aged beef. Dry-aging also causes more shrinkage than wet-aging due to surface water evaporation (Warren and Kastner, 1992; Ahnström et al., 2006; da Silva Bernardo et al., 2021; Kim et al., 2022). This is problematic as meat is sold on a weight basis, so the meat must be sold at a premium price to compensate for this loss. Regarding postmortem proteolysis, Cavender (2021) reported more desmin degradation in wet-aged strip loin steaks than in dry-aged steaks. However, Chaosap et al. (2025) found that aging method had no effect on troponin-T degradation. Multiple authors have also reported a higher amount of lipid oxidation in dry-aged beef (Kim et al., 2022; Ribeiro et al., 2024). Aerobic bacteria are also more abundant in dry-aged than wet-aged beef due to the aerophilic environment (Bischof et al., 2023; Meloni et al., 2023; de Matos et al., 2024). Additionally, Kim et al. (2022) and Meloni et al. (2023) found a greater amount of molds and yeasts in dry-aged beef. These differences between aging methods imply that wet-aged beef has the potential for a longer shelf life than dry-aged beef (Kim et al., 2022; Meloni et al., 2023).

1.2 Postmortem Aging Time

The National Beef Tenderness Survey is a national audit in the United States that benchmarks the tenderness of retail beef cuts, typically every five years. These surveys also examine postmortem aging periods and provide data on retail and foodservice aging times. The first survey was conducted in 1990, and it determined that the average postmortem aging time for retail beef in the US was 17 days (Morgan et al., 1991). The authors also reported that aging times ranged from three days to 90 days. For subprimal aging times, the average ranged from 15-20 days (Morgan et al., 1991). Chuck muscles were generally aged for the shortest amount of time, while loin cuts were aged the longest (Morgan et al., 1991).

The next tenderness survey was conducted in 1998 (Brooks et al., 2000). The average retail aging time increased from 17 days to 19 days when compared to the 1990 survey, but the aging range was more compact, with a range of two to 61 days (Morgan et al., 1991; Brooks et al., 2000). Another change from the 1990 survey was the inclusion of aging times for both retail and foodservice beef. For foodservice cuts, only loin and rib muscles were evaluated in that survey.

The average aging periods for loin cuts were 17.8 and 31.5 days for retail and foodservice beef, respectively. For the rib muscles, the average aging time for retail was 21.5 days, while foodservice beef was aged for 27 days on average (Brooks et al., 2000).

The 2006 survey also saw an increase in average retail aging time, from 19 days in the 1998 survey to 22.6 days (Brooks et al., 2000; Voges et al., 2007). The aging range was also similar to the 1990 survey, with a range of three to 83 days (Morgan et al., 1991; Voges et al., 2007). Additionally, when examining subprimals at a retail level, rib and loin muscles were generally aged longer than chuck and round muscles (Voges et al., 2007). Like the previous survey, foodservice subprimals were aged longer than their retail counterparts. For example, retail top sirloins were aged for an average of 24.4 days, while the same muscle when aged for foodservice increased to an average of 33.2 days (Voges et al., 2007).

The 2010 National Beef Tenderness Survey saw a decrease in average retail aging time from 22.6 days in 2006 to 20.5 days in 2010 (Voges et al., 2007; Guelker et al., 2013). This survey also found the largest range of aging times, spanning from one to 358 days (Guelker et al., 2013). Similar to the previous survey, rib and loin subprimals at a retail level were aged longer than the chuck and round subprimals on average (Voges et al., 2007; Guelker et al., 2013). For foodservice muscles, the average overall aging time was 28.1 days, with the rib eye, top loin, and top sirloin being aged for an average of 29.3, 29.8, and 24.7 days, respectively (Guelker et al., 2013).

The 2015 survey was the last tenderness survey that evaluated postmortem aging times, as these data were not collected in 2022 (Martinez et al., 2017; Gonzalez et al., 2024). When compared with all previous surveys, the 2015 survey had the longest average retail aging time of 25.9 days (Martinez et al., 2017). The range of aging times was also more compact than the 2010 survey, with a range of six to 102 days (Martinez et al., 2017). The average aging times for retail and foodservice loin and rib muscles also increased from the previous survey. The loin aging times were approximately 26 days for retail storage and a similar aging time of 31.1 days in foodservice. Rib aging times were approximately 28.7 and 32.2 days in retail and foodservice storage, respectively (Martinez et al., 2017).

Another tenderness survey was performed in 2022 (Gonzalez et al., 2024). However, this survey did not contain data on postmortem aging periods. The authors stated that due to the difficulty of collecting the aging data, they were unable to include this information. Additionally, no data were collected on cuts from the beef round. This was due to the desire to perform further research on improving round tenderness as opposed to comparing it to other muscles (Gonzalez et al., 2024). Tenderloins, however, were included in this survey for the first time since the 1998 National Beef Tenderness Survey in order to be used as a comparison to other muscles (Brooks et al., 2000; Gonzalez et al., 2024).

Overall, wet-aging provides a few advantages, such as a lack of water loss and extended shelf life when compared to other packaging and aging methods (Warren and Kastner, 1992; Shi et al., 2020; da Silva Bernardo et al., 2021; Kim et al., 2022). However, there is also a potential for off-flavors or off-odors to develop during extended wet-aging (Jeremiah and Gibson, 2003; Li et al., 2014; Barker et al., 2023). The changes in meat quality attributes during wet-aging will be explored in the following sections.

1.3 Warner-Bratzler Shear Force

Warner-Bratzler shear force (WBSF) refers to a method of measuring the force required to slice through a piece of meat and serves as an indicator for the level of tenderness (Bratzler, 1932). It has been well documented that beef tenderness increases during the wet-aging process, thus decreasing WBSF values (Smith et al., 1978; Smith et al., 2008; Kim et al., 2019; Shi et al., 2020;

Yu et al., 2024). Depending on the muscle, its location, and its purpose (i.e., supportive or locomotive), some muscles may need to be aged longer than others to obtain the desired improvement in WBSF (Belew et al., 2003; Gruber et al., 2006; Yar et al., 2024; Yu et al., 2024). For example, the beef *longissimus lumborum* (LL) needs to be aged for a longer period than the *psoas major* (PM), as PM is inherently more tender than LL due to the intrinsic factors found within those muscles (Yar et al., 2024; Yu et al., 2024). Belew et al. (2003) also found that when aging steaks from 40 different muscles for 14 days, steaks from the diaphragm, *M. spinalis*, and *M. infraspinatus* had the lowest shear force values, while the *M. brachialis*, *M. pectoralis profundus*, and *M. flexor digitorum superficialis* had the highest values. Bratcher et al. (2005) found that muscles from the same beef primal can also vary in shear force values during aging. When muscles in the chuck were aged for 14 days, the *rhomboideus* had significantly higher WBSF values than the other muscles examined. Molina et al. (2005) also observed differences in shear force between muscles from the same primal during wet-aging.

Quality grade can also influence shear force. Gruber et al. (2006) determined that many muscles, especially in a 14-day aging period, display lower shear force values in Choice steaks than Select steaks. Additionally, out of the 34 muscle-quality grade combinations examined (17 Choice and 17 Select), the Select grade *semimembranosus* (SM) had the highest numerical WBSF values across all four aging periods, while the Choice grade SM had statistically lower WBSF values. Derington et al. (2011) also reported that a higher quality grade resulted in a lower WBSF value as well as slice shear force value in beef strip loin steaks aged for 21 days. Conversely, Obuz et al. (2004) determined that quality grade only influenced shear force in the *longissimus lumborum* and not the *biceps femoris* or deep *pectoralis*.

1.4 Postmortem Proteolysis

One of the main objectives of wet-aging beef muscles is to improve their tenderness. During rigor mortis, the sarcomere, or the basic contractile unit of muscle, will shorten, making the meat tough. Multiple authors have reported that WBSF values will peak at approximately 40% of the sarcomere length and over time, as the sarcomere length increases, shear force values will decrease and the meat will become more tender (Marsh and Leet, 1966; Davey et al., 1967; Marsh and Carse, 1974). Proteolytic systems, such as the calpain, cathepsin, and caspase systems, can degrade proteins, potentially increasing meat tenderness (Sancho et al., 1997; Steen et al., 1997).

1.4.1 Calpains, Cathepsins, and Caspases

The calpain enzyme system, which is activated by Ca^{2+}, has been shown to be a major contributor to postmortem proteolysis and a subsequent increase in meat tenderness during aging, specifically μ-calpain (calpain 1) and m-calpain (calpain 2) (Koohmaraie et al., 1987; Geesink et al., 2006; Mohrhauser et al., 2011). Geesink et al. (2006) demonstrated the importance of the calpain system when they showed that proteins such as desmin, dystrophin, metavinculin, and troponin-T degraded significantly less in mice that had a deactivated calpain system over a three day aging period. Calpain quantity and level of activity can differ between muscles as well. For example, Ouali and Talmant (1990) determined that the concentration of calpain 2 was higher in the *masseter* than the *longissimus dorsi*. Camou et al. (2007) also observed that both calpain 1 and calpain 2 are less active in the beef *triceps brachii* than in the lumbar or thoracic *longissimus dorsi*, *semimembranosus*, and *psoas major*.

However, during wet-aging, calpain activity decreases. Huff-Lonergan et al. (1996a) showed that, when wet-aging beef *longissimus thoracis* for up to 56 days, most significant degradation of titin, nebulin, filamin, desmin, and troponin-T occurred early in wet-aging, and μ-calpain was credited with this degradation activity. Interestingly, the cessation of most major degradation activities also coincided with the final reduction of WBSF values observed in the study (Huff-Lonergan et al., 1996a). Colle and Doumit (2017) reported that calpain 1 had no detectable activity after 14 days of aging of beef *longissimus lumborum* and *semimembranosus* muscles. On the other hand, calpain 2 had a steady decrease in activity over the aging period but was still detectable at 42 days of aging for both muscles (Colle and Doumit, 2017) .

While not as significant as calpains, endogenous enzymes such as cathepsins, and caspases could also contribute to postmortem proteolysis and meat tenderness. Like calpains, they are both Ca^{2+} activated enzymes that can degrade proteins. Multiple authors have reported that cathepsins B, H, L, and D specifically play a role in postmortem proteolysis during aging (Calkins and Seideman, 1988; Wang et al., 2022). Wang et al. (2022) found that cathepsin B+L activity increased from 0 to 8 days of wet-aging in the beef *semitendinosus* muscle, unlike calpains. Likewise, Zhang et al. (2019) found that cathepsins B and D increased their activity from 0 to 168 hours of wet-aging. However, Kaur et al. (2021) found no changes in cathepsin B, H, and L activity between 4 and 14 days of wet-aging in beef briskets. Additionally, Huang et al. (2016) observed that caspases, specifically caspase-3, are functional only during early aging periods, similar to the calpain system.

1.5 Protein Degradation during Wet-Aging

Many proteins, such as titin, nebulin, and desmin, among others, are degraded during postmortem wet-aging due to the aforementioned calpain, cathepsin, and caspase systems (Koohmaraie, 1992; Huff-Lonergan et al., 1996a; Cruzen et al., 2014). The degradation of these proteins can help to improve tenderness, thus leading to a higher quality product. While many proteins have been extensively studied, this review will focus mainly on titin, nebulin, desmin, and troponin-T in wet-aged beef, as they have been identified as key contributors to improved beef tenderness.

Titin, nebulin, desmin, and troponin-T are considered to be biochemical markers for beef tenderness during aging, and all four proteins degrade during the postmortem period (Huff-Lonergan et al., 1996a). These proteins are found in the sarcomere, the basic contractile unit of muscle. They also exhibit distinct characteristics from one another. For example, titin is the largest protein in an animal's body and it spans from the Z-line to M-line, and functions as a "molecular spring," assisting with muscle contraction and relaxation (Wang et al., 1979; Fürst et al., 1988; Tonino et al., 2017). Nebulin, another large protein, is found on the thin filament and anchors it to the Z-line, making it essential for keeping the sarcomere structurally sound and assisting in contractile regulation (Wang and Williamson, 1980; Wang and Wright, 1988). Desmin is another protein associated with the Z-line, and like nebulin, is important for the structural integrity of the sarcomere (Cooke, 1976; Lazarides and Hubbard, 1976). Troponin-T is a thin filament-associated protein, and it binds to tropomyosin (Greaser and Gergely, 1971).

Titin, nebulin, desmin, and troponin-T tend to degrade more in the early stages of aging, which is most likely due to the higher calpain activity closer to slaughter (Huff-Lonergan et al., 1995; Huff-Lonergan et al., 1996a, Wu et al., 2014; Wright et al., 2018). Huff-Lonergan et al. (1996a) also reported that, when aging both high and low shear force *longissimus thoracis* steaks for up to 56 days, intact proteins degraded faster in the low shear force samples. When comparing the low shear force samples to the high shear force samples, titin 1 (T1) was absent after 7 and 14 days, respectively. Fritz et al. (1993) also found an increase in titin degradation in *M. longissimus dorsi* steaks over 14 days. In the same study by Huff-Lonergan et al. (1996a), intact nebulin was fully degraded by 3 days postmortem. This early degradation of nebulin is also consistent with other studies on the *longissimus* muscle (Anderson and Parrish, 1989; Huff-Lonergan et al., 1995; Wu et al., 2014).

When Whipple et al. (1990) examined postmortem desmin degradation in *longissimus* muscles aged up to 14 days, they found that some desmin remained intact by the end of the aging period in cattle with *Bos indicus* genetics. Huff-Lonergan et al. (1996a) also found that a 45 kDa desmin degradation band was still visible after 14 days of aging in the high shear force *longissimus* samples, although it had disappeared in the low shear force samples by this point. Conversely, when examining beef *longissimus lumborum, semitendinosus,* and *triceps brachii* muscles wet-aged up to 14 days, Grobbel et al. (2008) found that desmin degraded in all the muscles during aging regardless of the packaging type. Moreover, the desmin degradation rate varied between the muscles. Phelps et al. (2016) also found that desmin degraded in a linear fashion in wet-aged *semimembranosus* muscles up to 70 days, indicating that desmin is still present in extended periods of wet-aging in this specific muscle. These results were similar to Hernandez et al. (2022), where desmin from beef strip loins had over 90% degradation when aged for 56 days. The results of these studies indicate that desmin degrades slower than titin and nebulin, and longer periods of aging would be necessary to achieve more desmin degradation.

Similar to desmin, Huff-Lonergan et al. (1996a) found that intact troponin-T was still present in low- and high-shear force *longissimus thoracis* samples aged 14 and 28 days, respectively. Muroya et al. (2006) also found differences in troponin-T degradation between wet-aged beef *longissimus*, *masseter*, and *diaphragm* muscles. The authors reported that more troponin-T degradation is seen in *longissimus* muscles compared to the *masseter* and *diaphragm* when aged up to 14 days. Isoforms of troponin-T were also seen throughout the aging period. Onopiuk et al. (2018) observed a steady and significant reduction in troponin-T content in beef *semitendinosus*, *longissimus thoracis*, and *psoas major* muscles over an aging period of 21 days. The *semitendinosus* also displayed the greatest amount of postmortem myofibrillar fragmentation, with most proteolysis occurring between 7 and 14 days. These authors also observed that the rate of troponin-T proteolysis differed between the muscles, and like desmin, it degrades slower than titin and nebulin.

1.6 Changes in pH During Wet Aging

pH is an intrinsic factor that can affect beef quality and decreases directly after slaughter (Spanier et al., 1997; Jeleníková et al., 2008; Hopkins et al., 2014). Generally, after this initial decline, the pH will stay relatively constant throughout the wet-aging period. For example, when wet-aging beef *semimembranosus* for up to 14 days, Spanier et al. (1997) observed a drop in pH from 6.5 (at 45 minutes postmortem) to 5.4 during the first two days of aging, and this value did not change significantly after the initial decline. Likewise, when aging *M. longissimus dorsi*, Daszkiewicz et al. (2003) found that pH only increased from 5.37 to 5.43 from days 3 to 14. Shi et al. (2020) found that pH increased in *longissimus thoracis* aged up to 14 days, but the pH only changed slightly after the initial postmortem decline. Multiple authors have also reported no significant changes in pH when wet-aging *longissimus thoracis* muscles (da Silva Bernardo et al., 2021; Sirtori et al., 2023). Similar to studies on other muscles like the *longissimus*, Main (2024) found no significant pH change in beef *semimembranosus*, *biceps femoris*, and *gluteus medius* muscles during aging for up to 70 days. This indicates that throughout different muscles, pH is generally not affected much by wet-aging after the initial postmortem decline.

1.7 Changes in Water Activity and Water Loss During Wet Aging

Water activity (a_w) refers to the ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions (FDA, 1984). The wet-aging process can impact a_w. da Silva Bernardo et al. (2021) reported that a_w increased from 0.989 to 0.995 in beef *longissimus thoracis et lumborum* from 21 to 42 days of wet-aging. However, other researchers have reported no difference in a_w when wet-aging beef for up to 60 days (Kim et al., 2022; Di Paolo et al., 2023; Meloni et al., 2023). Conversely, Santos et al. (2025) found that in dark-cutting beef from Nellore cattle, a_w decreased significantly after 28 days of wet-aging (0.9999 in non-aged samples and 0.9928 in samples wet-aged 28 days). In this case, it is possible that the characteristics of the dark-cutting beef played a role in the decrease of a_w. It is important to note that the a_w recorded in these papers was high enough to support bacterial growth. As bacteria only require a water activity of approximately 0.91 to grow, the differences found are likely not biologically significant (Allen, 2018).

The amount of water lost from the product is also impacted by wet-aging, but it results in only a slight loss of weight (Parrish Jr. et al., 1991; Warren and Kastner, 1992; Smith et al., 2008; Li et al., 2014; Shi et al., 2020). Multiple authors reported that there was no change in cooler shrink (weight reduction during storage at a refrigerated temperature) for up to 35 days of aging in steaks and subprimals (Parrish Jr. et al., 1991; Laster et al., 2008; Smith et al., 2008). Parrish Jr. et al. (1991) also found no difference in cooler shrink between different USDA quality grades. Many researchers have also measured aging loss (weight change percentage relative to initial weight; Warren and Kastner, 1992; Li et al., 2014; Shi et al., 2020). Warren and Kastner (1992) determined that when aging strip loins for 14 days, there was as little as 0.8% of total weight lost due to aging. Other authors saw slightly higher losses using *longissimus thoracis et lumborum* and *longissimus thoracis* muscles. For example, Li et al. (2014) reported a loss of 2.7% after 19 days of aging *longissimus thoracis et lumborum*, but at 8 days, they reported only a loss of 1.2%. Shi et al. (2020) also reported that the maximum aging loss at 14 days was 1.44% in beef *longissimus thoracis*.

Numerous authors have also reported purge (difference in weight before and after aging) and drip loss (a sample is weighed, suspended, patted dry, and reweighed) measurements in wet-aged beef. Greer et al. (1990) found that, in tenderloins aged for 70 days, purge loss was not significant. Kerth et al. (1995) found similar results in strip loins aged 7 and 14 days. While Smith et al. (2008) found only 1.1% purge loss after aging for up to 35 days in beef short loins, Aroeira et al. (2016) reported as much as 3.12% purge loss in *longissimus thoracis* aged 21 days. Yu et al. (2024) also found a significant increase in purge loss when aging for 3 or 28 days, and the *psoas major* had more purge loss than the *longissimus lumborum*. Yu et al. (2024) also observed that drip loss was similar in both *longissimus lumborum* and *psoas major* muscles, and the amount of drip loss also decreased over aging time.

1.8 Meat Color

Beef, when wet-aged, generally decreases in color stability over time (Madhavi and Carpenter, 1993; English et al., 2016; Nair et al., 2018). When aging *longissimus lumborum* and *psoas* major muscles, Madhavi and Carpenter (1993) found that the samples aged 4 and 7 days displayed greater color stability than samples aged 14 and 21 days. Lee et al. (2008) found similar results in the *gluteus medius*, where the steaks aged for longer periods, such as 28 or 35 days, had less color stability than those aged 7 or 14 days. When aging *longissimus lumborum* muscles for up to 62 days, English et al. (2016) also saw a decrease in *a** and chroma as the aging duration increased. Conversely, Insausti et al. (1999) found that while *L** (lightness), *a** (redness), *b** (yellowness), hue, and chroma (measured after 1 h of blooming) increased in the first 5 days of wet-aging *longissimus dorsi* muscles, these values remained relatively constant when measured on days 10 and 15. Beriain et al. (2009) found similar results in *longissimus dorsi* muscles aged up to 14 days and reported that the metmyoglobin percentage decreased as aging progressed.

Inherent differences in color stability between muscles also affect the color stability of the muscle after wet-aging. For example, Seyfert et al. (2006) found that after wet-aging for 11 days, the deep *semimembranosus* muscle had significantly higher *L** and *b** values when compared to the *psoas major*, *longissimus lumborum*, superficial *semimembranosus*, and *semitendinosus* muscles. Nair et al. (2018) also found that when aging *longissimus lumborum*, *psoas major*, and *semitendinosus* muscles for up to 21 days, the *longissimus lumborum* displayed the greatest color stability, followed by the *semitendinosus* and then the *psoas major*. Additionally, the steaks aged 7 days had the highest *a** values, with the *longissimus lumborum* and *semitendinosus* displaying a redder surface when compared to the *psoas major*. Main (2024) reported that when aging the beef *biceps femoris*, *gluteus medius*, and *semimembranosus*, there were no differences in *a**, *b**, or chroma between the *biceps femoris* samples aged 14 and 70 days, while the *gluteus medius* and *semimembranosus* had lower *a**, *b**, and chroma on day 70 than day 14. While the *biceps femoris* and *gluteus medius* saw no change in *L** values, the *semimembranosus* had lower *L** on day 70 than day 14.

1.9 Lipid Oxidation

Lipid oxidation is a reaction that causes rancidity and other off-odors and flavors such as rancid, cardboard, greasy, and dairy (Campo et al., 2006; Shahidi and Oh, 2020; Fu et al., 2022). During lipid oxidation, a compound known as malondialdehyde (MDA) is formed as the secondary oxidation product, which can be used to quantify the degree of lipid oxidation using 2-thiobarbituric acid in the thiobarbituric acid reactive substances (TBARS) assay (Tarladgis et al., 1960). Lipid oxidation can be triggered when meat is exposed to oxygen, and therefore tends to remain low in wet-aged beef as it is kept in a vacuum package (O'Grady et al., 2000; Clausen et al., 2009; Suman et al., 2010; Resconi et al., 2012; Resconi et al., 2018; Wang et al., 2023). However, lipid oxidation can still occur during aging, which can generate off-flavors, potentially leading to lower consumer flavor acceptance.

When wet-aging *longissimus lumborum* and *longissimus thoracis* muscles up to 14 days, Lee et al. (1996) found that while MDA increased over time, the average did not surpass 1 mg MDA/kg of sample. Low TBARS values are seen across muscles aside from the *longissimus* as well (Spanier et al., 1997; Colle et al., 2015; Colle et al., 2016). When aging *semimembranosus* for 14 days, Spanier et al. (1997) found no change in TBARS values over the aging period, and MDA concentration was also relatively low. In two studies by Colle et al. (2015 and 2016), when aging *longissimus lumborum, semimembranosus*, *gluteus medius*, and *biceps femoris* for up to 63 days, MDA concentration did not exceed 0.7 mg MDA/kg of meat on average for any of the muscles. Across multiple muscles and wet-aging periods, MDA is generally seen in low concentrations, implying that wet-aging greatly impedes the lipid oxidation mechanism.

1.10 Microbiological Characteristics

Meat is a highly favorable environment for microbial growth due to intrinsic factors such as pH, a_w, and nutrient availability. Due to the low levels of oxygen in vacuum packages, facultative anaerobes such as lactic acid bacteria grow well in wet-aged beef (Newton and Rigg, 1979; Newsome et al., 1984a; Lee et al., 1996; Jääskeläinen et al., 2016; Main, 2024). When wet-aging beef loins, Newsome et al. (1984b) reported that anaerobic bacterial counts increased from 2.72 log CFU/g after 1 week of aging to 6.40 log CFU/g after 5 weeks of aging. Additionally, *Lactobacillus* counts increased from 0.74 to 5.95 log CFU/g during the same time period. Lee et al. (1996) also found an increase in total plate counts, as well as counts of lactic acid bacteria, anaerobic bacteria, and psychrotrophic bacteria when aging *longissimus lumborum* and *longissimus thoracis* for 14 days; however, none of these bacterial counts exceeded 5 log CFU/cm^2.

Due to the technological capabilities of 16S rRNA gene sequencing and shotgun metagenomics, specific bacterial genera and species in wet-aged meat have been examined. Since lactic acid bacteria are the predominant bacterial group found in vacuum-sealed beef, genera such as *Lactobacillus, Lactococcus,* and *Carnobacterium* have a high relative abundance in wet-aged beef (Jääskeläinen et al., 2016; de Matos et al., 2024). When wet-aging beef for up to 26 days, Jääskeläinen et al. (2016) reported a high relative abundance of *Lactobacillus* and *Lactococcus* across the aging periods. In agreement, Setyabrata et al. (2022) found a high relative abundance of an unclassified Lactobacillales when wet-aging *longissimus lumborum* for 28 days. Additionally, a high relative abundance of a *Carnobacterium*, *Brochothrix*, and an unclassified Yersiniaceae was detected (Setyabrata et al., 2022). Similarly, de Matos et al. (2024) found an overwhelming relative abundance of *Carnobacterium* when wet-aging beef strip loins, with a relative abundance of 88.8% and 79.4% in strip loins aged 20 and 34 days, respectively.

Alpha (diversity within a sample) and beta (diversity between samples) diversity are also altered during the wet-aging process. When examining alpha diversity in strip loins aged at different temperatures and aging periods (210 days at 0°C, 120 days at 2°C, 75 days at 4°C, and 25 days at 8°C), Kaur et al. (2021) found that regardless of temperature, diversity decreased at the beginning of aging periods and plateaued as total viable microbial counts stabilized. de Matos et al. (2024) noted a similar decrease in alpha diversity as well as beta diversity in strip loins aged 20 and 34 days. When aging tenderloins from 2 to 21 days, Mansur et al. (2019) also saw a decrease in beta diversity, which suggested that across muscles, diversity decreases during wet-aging.

1.11 Sensory Characteristics

1.11.1 Flavor and Aroma

Multiple authors have reported distinct flavor or odor notes in wet-aged beef, some of which are classified as undesirable. However, these off-flavors are not always present or overwhelming in wet-aged beef. For example, when wet-aging beef strip loins and steaks for 7 days, Oreskovich et al. (1988) reported that off-flavors were generally undetectable. While off-flavors (painty, cardboard, bitter, and sour) increased and desirable flavors (cooked beef, brothy, browned/caramel, and sweet) decreased over a 14 day aging period for the *semimembranosus* muscle, Spanier et al. (1996) reported that no off-flavors exceeded 1.5 on a 15 point scale, indicating that these flavor differences were not detrimental to overall flavor. Additionally, when Muchenje et al. (2010) aged *longissimus thoracis et lumborum* for 21 days, off-flavors (animal, bloody/livery, cooked vegetable, glassy, metallic, sour, and unpleasant) were in an acceptable range, while off-odors with the same descriptors were deemed unacceptable. The authors reported that bloody/livery off-odors were the most prominent after wet aging. Dikeman et al. (2013) also reported a low off-flavor intensity in *longissimus lumborum* muscles aged for 21 days.

When aging *longissimus lumborum* samples for 46 days, O'Quinn et al. (2016) found an increase in bloody/metallic, grassy/hay-like, gamey, livery, fishy, and sour off-flavors when compared to samples aged for 14 days, but these off-flavors were all rated as less than three on a 10-point flavor desirability scale. Foraker et al. (2020) reported that off-flavors (sour, oxidized, and musty/earthy) when aging beef *longissimus* muscles up to 63 days were rated below a three on a 15-point scale for all aging periods (3, 14, 28, 35, and 49 days), indicating that off-flavors were barely present. Main (2024), when examining beef *gluteus medius*, *biceps femoris*, and *semimembranosus* muscles aged up to 70 days, found that off-flavors such as sour, musty, cardboardy, and painty, among others, were generally not noticeable in any of the muscles at any aging period.

On the other hand, Jeremiah and Gibson (2003) reported that in ribs and short loins aged either 7, 14, 21, or 28 days, 100% of the samples aged 7, 14, and 21 days and 98.9% of samples aged 28 days had an off-odor, with the majority of samples having a livery odor. Almost 100% of samples displayed a sour flavor note and most also had a bitter taste. Li et al. (2014) found similar results in *longissimus thoracis et lumborum* aged either 8 or 19 days. Animal and metallic odors, metallic flavors, and animal and metallic aftertastes were generally noticed by panelists in that study.

1.11.2 Tenderness

Tenderness is another aspect of palatability affected by wet-aging. Like other factors affected by wet-aging, there are also differences between muscles. For example, Carmack et al. (1995) determined that *psoas major*, *infraspinatus*, and *longissimus lumborum* aged 7 days were more tender than *biceps femoris*, *semimembranosus*, and *pectoralis profundus* aged for the same period when evaluated by a panel. Brewer and Novakafski (2008) found that in loins aged 0, 7, and 14 days, tenderness likability increased from 0 to 7 days, but no differences were seen between 7 and 14 days. Dikeman et al. (2013) found that on an 8-point scale, tenderness was rated as 5.98 in *longissimus lumborum* muscles aged for 21 days, indicating high likeability. Li et al. (2014) also found that tenderness increased from 8 to 19 days of aging in *longissimus thoracis et lumborum* muscles. Similarly, Foraker et al. (2020) reported that in *longissimus* muscles, tenderness increased initially but plateaued with longer aging periods, and results from Lepper-Billie et al. (2016) were in agreement with this finding. However, when aging beef *gluteus medius* muscles, Main (2024) reported that no significant differences in tenderness were seen between samples aged for 14, 28, 35, 42, 49, and 56 days. Similarly, in the *biceps femoris* and *semimembranous* muscles sampled, no increase in tenderness was seen during aging from 14 to 70 days.

1.11.3 Juiciness

Dikeman et al. (2013) reported that *longissimus lumborum* wet-aged 21 days had a high score for juiciness acceptability compared to dry-aged samples. Differences in juiciness may also be seen between muscles. For instance, the *serratus ventralis*, *infraspinatus*, and *psoas major* have been determined to be juicier than the *biceps femoris*, *semitendinosus*, and *semimembranosus* when evaluated by consumers at 7 days of aging (Carmack et al., 1995). Conversely, Barker et al. (2023) found no differences in juiciness between *psoas major*, *longissimus lumborum*, and *gluteus medius* muscles aged up to 56 days. These authors also reported that juiciness was not affected by aging time. Other authors have reported similar results across different muscles and aging periods (Jeremiah and Gibson, 2003; Brewer and Novakofski, 2008; Foraker et al., 2020; Main, 2024).

1.12 Volatile Compounds

Volatile compounds, which affect odor, are also produced during the wet-aging process. As the concentration of some specific volatile compounds increase, off-odor can also increase. Gorraiz et al. (2002) reported that in *longissimus dorsi* aged from 2 to 7 days, off-odor compounds such as 2,3,4-trimethyl-pentane, 2,3,3-trimethyl-pentane, 3-methyl-2-heptene, 2,5-dimethyl-heptane, and 2,2,4,6,6-penta-methylheptane increased with aging time. Mansur et al. (2019) also found that an increase of certain acids (acetic and butanoic acid), alcohols (2,3-butanediol), ketones (2-butanone and 3,3-dimethylbutan-2-one), and sulfur compounds (methyl thioacetate) also increases off-odors in tenderloins aged up to 21 days. Setyabrata et al. (2022) performed volatile compound analysis on beef loins wet-aged for 28 days and reported that hexanal (n-aldehyde), ethanol (alcohol), and 2-propanone (ketone) had the highest concentrations of any compounds with 119.12, 59.84, and

42.12 ng/g of sample, respectively. The authors suggested that these compounds could potentially be responsible for the unique odor profile of wet-aged beef.

1.13 Metabolites

Metabolites produced in the meat can be influenced by postmortem wet-aging. Lactate and creatine were identified as having high signal intensity in the NMR spectrum of beef *longissimus dorsi* muscles aged for 3, 7, 14, and 21 days, with the highest intensity on day 21 (Graham et al., 2010). These authors also saw an increase in adenine/hypoxanthine, adenosine/inosine, and xanthine in 21-day samples compared to 3-day-aged samples. Additionally, in samples aged 14 and 21 days, more amino acids, sugars, and nucleosides, with the exception of ATP and ADP, were seen when compared to samples aged 3 and 7 days. Ercolini et al. (2011) found similar results for lactate and creatine in beef chops (boneless tender beef) aged from 0 to 45 days, with a noticeable increase in creatine over the aging period. Over this time, glycogen, ADP, IMP, and methionine also decreased.

When aging *semimembranosus* and *longissimus dorsi* muscles in Hanwoo and Chikso cattle, Lee et al. (2023) also observed a higher abundance of lactate and creatine, as well as carnosine in 28-day-aged samples compared to non-aged samples. In the Chikso *semimembranosus*, an increase in both lactate and carnosine was observed over the aging period (up to 28 days), while the only change seen in the *longissimus dorsi* was a decrease in creatine. In the Hanwoo *semimembranosus* and *longissimus dorsi*, no change was seen in the three metabolites when comparing the day 0 samples to the samples from day 28, indicating that there may be a muscular as well as breed influence on metabolites. Ueda et al. (2024) also found muscular differences in metabolites from *longissimus thoracis* and *adductor* aged 40 days. Glutaric acid, 2,3-bisphosphate-glyceric acid, citrulline, maleic acid, and ethylmalonic acid were only found in *longissimus thoracis*, and trehalose, mannose, xylitol, and nicotinic acid were only detected in the *adductor*. For both muscles, glutamic acid, tryptophan, phenylalanine, acetyl-lysine, xylulose, citric acid, and hypoxanthine increased from 0 to 40 days of aging, while creatinine decreased.

In summary, the wet-aging of beef affects a multitude of factors such as postmortem proteolysis, flavor, color, microbial characteristics, and tenderness, among others. The length of aging is also a great contributor to beef quality during wet-aging. Tenderness, for example, generally increases with aging, although most change may happen at the beginning of aging. While a great number of studies have focused on wet-aging, many of these examined *longissimus* muscles at a shorter aging period, usually 21 days or fewer. In comparison, the available literature on extended wet-aging of at least 35 days on other muscles is sparse. Therefore, this research was performed during extended aging periods of up to 70 days on microbial enumerations, microbial community and diversity, and desmin degradation of *biceps femoris*, *gluteus medius*, and *semimembranosus* muscles in an effort to bridge this research gap.

CHAPTER 2

IMPACT OF EXTENDED WET-AGING ON BEEF *BICEPS FEMORIS*, *SEMIMEMBRANOSUS*, AND *GLUTEUS MEDIUS* MUSCLES

2.1 Introduction

Wet-aging is the most common method of aging beef to improve tenderness, palatability, and overall consumer acceptance. A recent national beef tenderness survey reported that the average aging time for beef retail muscles was 25.9 days, with some muscles getting aged up to 102 days (Martinez et al., 2017). Previous research has shown that bacterial growth occurs on the meat surface during aging. For example, Bischof et al. (2023) showed that aerobic and lactic acid bacteria counts of the beef *M. longissimus thoracis et lumborum* muscle increased over a 28-day aging period. Flavor and consumer acceptance of beef can also be influenced by the presence or growth of bacteria. For example, some *Lactobacillus* spp. can cause ‘cheesy’ or ‘buttery’ odors (Dainty and Mackey, 1992; Whitfield, 1998), whereas *Pseudomonas fragi*, another common organism found in beef, can also produce compounds responsible for off-flavors and off-odors (Dainty et al., 1985). In vacuum-packaged beef, some of the dominant genera during aging are *Lactococcus* and *Lactobacillus*, with species including *Lactococcus piscium* and *Lactobacillus sakei* (Jääskeläinen et al., 2016), with the latter capable of producing compounds responsible for ‘sulfide’ off-odors (Egan et al., 1989).

Recently, O’Quinn et al. (2018) reported that tenderness accounted for 43.4% of overall palatability, whereas flavor accounted for 49.4% of overall palatability. Additionally, multiple authors have reported an increase in tenderness with increased aging times (Gruber et al., 2006; Li et al., 2014; Yar et al., 2024). One contributing factor to improved tenderness is postmortem proteolysis, which degrades proteins. One such protein is desmin, a structural protein that, when degraded, assists in improving tenderness (Hwan and Bandman, 1989; King et al., 2009; Lindahl et al., 2010; Phelps et al., 2016; Hernandez et al., 2022).

While the impact of extended wet-aging on beef *longissimus lumborum* (LL) has been examined extensively (Warren and Kastner, 1992; DeGeer et al., 2009; Dikeman et al., 2013), other muscles have not been studied in depth. It has become more common to market single muscle cuts such as the *semimembranosus* (SM), *gluteus medius* (GM), and *biceps femoris* (BF). However, few studies have been conducted on microbial and biochemical changes in these muscles during aging. Therefore, the objective of this study was to determine the impact of aging on the microbial load, microbiome (16S rRNA gene sequencing and analysis), and desmin degradation of beef SM, GM, and BF.

2.2 Materials and Methods

2.2.1 Sample Collection

Beef top sirloin butts (IMPS 184) and top rounds (IMPS 168; SM) were collected (N = 80) from A maturity beef carcasses with marbling scores between Modest 00 and Modest 100 from a commercial beef processing facility. The products were collected over two trips with N = 40 per collection. The subprimals were vacuum packaged and randomly assigned an aging treatment (14, 28, 35, 42, 49, 56, 63, or 70 days) and were stored at 2.2°C ± 0.2°C for each aging duration. Prior to sampling on each aging day, the fabrication table surfaces and packaging surfaces were sprayed with 70% ethanol. On each sampling day, the top sirloin butts were separated into BF and GM and fabricated into 2.54 cm thick steaks, whereas SM was fabricated from the top round. Ten (n =10)

BF, GM, and SM were evaluated for microbial population levels and 16S rRNA gene sequences on each aging day (i.e., five replicates per muscle in each of the two trials of the study). Five samples were evaluated for desmin degradation (n = 5). Steaks designated for desmin degradation analysis were frozen in liquid nitrogen and homogenized using a 600W Nutribullet blender (Nutribullet, Los Angeles, CA). Sample powders were stored at -80°C at Texas Tech University (TTU) and were then shipped to Colorado State University (CSU), where they were stored at -70°C until analysis.

2.2.2 Culture-based Enumeration of Microbial Populations

At each of the eight aging periods, the muscle surface was sampled before the muscles were fabricated into steaks. These samples were analyzed for total aerobic mesophilic microflora counts (designated as aerobic counts) and lactic acid bacteria counts using the TEMPO® system (bioMerieux, Marcy I'Etolie, France). For sample collection, the packaging was opened aseptically using a sterile scalpel. A sterile USDA poultry template (5 × 10 cm; Neogen, Lansing, MI) was then placed on the surface, and the 50 cm^2 area was swabbed using an EZ Reach sponge prehydrated with 10 mL buffered peptone water (BPW; World Bioproducts, Woodinville, WA). To each sponge sample, 15 mL of additional BPW was added. The samples were then mechanically pummeled for 30 seconds at 230 rpm (Seward Stomacher 400C, Bohemia, NY), and the resulting liquid was serially diluted as necessary using BPW. Glass vials containing dehydrated culture media for the TEMPO® AC (aerobic count) and TEMPO® LAB (lactic acid bacteria count) tests were reconstituted with 3 mL of autoclave-sterilized water. Then, either 1 mL or 0.1 mL from the sample dilution tube was pipetted into the vial containing the reconstituted culture medium. The sample-medium solution was then transferred, using the TEMPO® Filler (bioMerieux), into TEMPO® AC and TEMPO® LAB cards. The TEMPO® LAB cards were incubated for 40 to 48 hours at 30°C in an anaerobic environment, while TEMPO® AC cards were incubated for 22 to 28 hours at 35°C. After incubation, the cards were read with the TEMPO® Reader (bioMerieux). Before statistical analysis, the bacterial count results were converted to $\log_{10}$ CFU/cm^2

2.2.3 16S Sample Collection

A separate sterile USDA poultry template (5 × 10 cm) was placed on the surface of the muscle to be sampled at the same time as microbial count samples were collected, and the area was swabbed in the same manner as the microbial count samples. The sponges were placed in sterile bags, and samples were kept cold before further processing. After sample collection, 30 mL of cold, phosphate-buffered saline (PBS) was added to each bag, and the contents were mechanically pummeled for 2 minutes at 200 rpm. The resulting liquid was transferred to a 50 mL conical tube and stored at -80°C until further processing. Prior to DNA extractions, samples were centrifuged (Sorvall Legend X1R, Thermo Fisher Scientific, Karlsruhe, Germany) at 4280 × *g* for 20 minutes at 4°C.

2.2.4 DNA Extractions, Library Preparation, and DNA Sequencing

DNA extractions were performed using a DNeasy PowerFood Microbial Kit (Qiagen, Germantown, MD) following the manufacturer's instructions with minor modifications. Modifications included resuspending the sample pellet in 1 mL of PBS, vortexing, and centrifuging at 13,000 × *g* for 1 minute. This process was repeated a second time before extraction began. Samples were also heated in a water bath at 65°C for 10 minutes, as well as eluted in 40 µL of elution buffer. Negative and positive controls were also utilized during extractions. Library preparation was performed according to the Earth Microbiome Project (https://earthmicrobiome.org/) 16S Illumina amplification protocol. Samples were sent to the University of Colorado Boulder and sequenced using the Illumina MiSeq sequencer (Illumina, San

Diego, CA). Sequences were demultiplexed using the Qiime2 pipeline (version 2023.5; Bolyen et al., 2019) and denoised using the DADA2 pipeline. Reads were truncated at 230 base pairs. The feature table, taxonomy classification, and phylogenetic tree were generated using the SILVA 128 taxonomic database. Samples were rarefied at 4800 sequences, and mitochondria and chloroplasts were filtered out. Excluding controls, sequence counts ranged from 2,148 to 113,100 with an average of 23,456 sequences.

2.2.5 Protein Extractions

From the 10 samples analyzed per muscle at each aging period, five samples (n = 5) were selected for desmin degradation analysis based on median Warner-Bratzler shear force values reported by Main (2024). To extract proteins, 10 mL of whole muscle buffer [5 mM NaH_2PO_4, 5 mM Na_2HPO_4, 69 mM sodium dodecyl sulfate (SDS)] was added into a glass tissue grinder containing 0.5 g of the powdered sample and homogenized until fully mixed. Samples were centrifuged (Sorvall Legend X1R) at 1,500 × *g* for 30 minutes at 22°C. Protein concentration was determined using a detergent-compatible (DC) protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin (BSA) standards according to the manufacturer's instructions. Samples were adjusted to 6.4 mg/mL using whole muscle buffer and then further diluted to 4 mg/mL according to Johnson et al. (2023a). After dilution, samples were vortexed, heated for 15 minutes at 55°C, and stored at -70°C until further analysis.

Normalization was performed according to Johnson et al. (2023a) with minor modifications. Briefly, the modifications included loading 5 µg of each sample into each lane of the gel, and 15% polyacrylamide resolving gels as well as 5% polyacrylamide stacking gels were made using 37.5:1 (wt/wt) acrylamide: *N*, *N'*- bis – methylene acrylamide. The first lane of each gel was also loaded with Precision Plus® Protein Standards (Bio-Rad Laboratories). A mini-

PROTEAN® Tetra system (Bio-Rad Laboratories) with a gel size of 8.3 × 7.3 cm was also used for protein fractionation. Fractionation was performed using a constant voltage of 130 V for 260 V/h. Gels were imaged using an iBright 750 imager (Thermo Fisher Scientific) and analyzed using the proprietary software (iBright Analysis Software; Thermo Fisher Scientific).

2.2.6 Western Blotting

Western blotting was performed according to Johnson et al. (2023b) with some modifications. Briefly, the reference sample was prepared by combining 15 µL of each sample. Protein fractionation was performed under the same conditions used for normalization. Proteins were then transferred to an Immun-Blot PVDF membrane (Bio-Rad Laboratories) using a mini Trans-Blot® cell (Bio-Rad Laboratories) with transfer buffer made of 25 mM Tris, 192 mM glycine, and 15% (v/v) methanol and run at a constant 90 V for 90 minutes. The membranes were blocked for 1 hour in a blocking buffer [PBS-Tween: 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, and 0.1% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20); and 5% (wt/vol) nonfat dry milk). After blocking, membranes were incubated overnight at 4°C with a polyclonal rabbit anti-desmin primary antibody from Iowa State University (Huff-Lonergan et al., 1996a; Huff-Lonergan et al., 1996b). The antibody was diluted in PBS-Tween at a ratio of 1:40,000. After overnight incubation, the membranes were washed three times for 15 minutes each in PBS-Tween. A goat anti-rabbit-HRP secondary antibody (Thermo Fisher Scientific), diluted in PBS-Tween at a ratio of 1:20,000, was used, and the membranes were incubated with the antibody for 1 hour at room temperature. A chemiluminescent detection kit (ECL Prime western blotting detection kit; GE Healthcare, Piscataway, NJ) was used for imaging. Imaging was performed identically as it was for normalization. The intensity of the 55 kDa intact desmin band was analyzed against the reference sample band.

2.2.7 Data Analysis

Data were analyzed using R (version 4.3.1). Relative abundance for the 16S rRNA gene sequencing results was determined using the phyloseq package (version 1.46.0, McMurdie and Holmes, 2013). The phyloseq package was also utilized for determining beta diversity. Diversity (Bray-Curtis and weighted UniFrac) was analyzed using the ADONIS function from the vegan package (version 2.6.6.1, Oksanen et al., 2024), and pairwise comparisons were made using the pairwiseAdonis package (version 0.4.1). The Benjamini-Hochberg p-value adjustment was also used. Microbial count and desmin degradation data were analyzed using the emmeans (version 1.8.9) package using R (version 4.3.1), and days of aging and muscle were used as fixed effects, while carcass was used as a random variable. Microbial count data were blocked by trial, and desmin degradation data had an additional random effect of gel. Significance was set at $\alpha = 0.05$ for all analyses.

2.3 Results

2.3.1 Culture-based Enumeration of Microbial Populations

The results of the culture-based enumeration of aerobic (AC) and lactic acid bacteria (LABC) populations for SM, GM, and BF are presented in Figures 2.1, 2.2, and 2.3, respectively. For the SM muscle (Figure 2.1), the AC of the 14-day aging period was lower ($P < 0.05$) than the AC of all the other aging periods, whose microbial counts were similar to each other ($P > 0.05$), whereas LABCs at 14 and 28 days of aging were similar ($P > 0.05$). The LABC for the 35-day aging period was also similar ($P > 0.05$) to 42, 49, 56, and 63 days of aging. However, there was no change ($P > 0.05$) in LABC from 42 days of aging.

Similar to SM, the AC of the GM muscle (Figure 2.2) aged for 14 days was lower ($P < 0.05$) than the AC of all the other aging periods. While the AC slightly increased ($P > 0.05$) with aging, there were no changes ($P < 0.05$) after 42 days of aging. The LABC was similar ($P > 0.05$) between samples aged 14 and 28 days. However, similar to SM, there was no change ($P > 0.05$) in LABC after 42 days.

Like the SM and GM muscles, BF aged for 14 days had the lowest ($P < 0.05$) AC (Figure 2.3). The 28 and 35 days of aging samples had similar ($P > 0.05$) aerobic microbial loads and no significant increase ($P > 0.05$) in AC after 42 days. There were no changes ($P > 0.05$) in LABCs during the initial (14, 28, and 35) aging days. Moreover, there was no change ($P > 0.05$) in LABC from day 35 until day 63 of aging.

Overall, from day 14 to 70, the AC increased from 1.1 to 5.6 log CFU/cm^2 for the SM muscle, from 1.5 to 6.5 log CFU/cm^2 for the GM muscle, and from 2.0 to 6.1 log CFU/cm^2 for the BF muscle. The LABC for the SM muscle increased from 0.7 to 6.0 log CFU/cm^2 during aging (14 to 70 days). Similarly, the LABC for the GM muscle increased from 1.2 to 6.0 log CFU/cm^2, while in the BF muscle it increased from 1.5 to 6.0 log CFU/cm^2.

2.3.2 Relative Abundance and Beta Diversity

Relative abundance and taxonomy were assessed at the genus level within each muscle. For the SM (Figure 2.4), the most abundant genera across all aging periods on average were *Leuconostoc* (61.90%), *Carnobacterium* (7.58%), and *Lactobacillus* (9.90%). Results for the GM muscle were similar (Figure 2.5), with the most abundant genera across all aging periods being *Leuconostoc* (39.08%), *Carnobacterium* (26.71%), and *Lactobacillus* (10.35%). Similar to the other two muscles, the two most abundant genera across all aging periods in the BF muscle (Figure 2.6) were *Leuconostoc* (39.85%) and *Carnobacterium* (16.66%). However, *Serratia* also had a high relative abundance (13.76%) in the BF. For all three muscles, the samples aged for 14 days had a relative abundance of more than 10% for *Ralstonia*, *Sediminibacterium*, and *Sphingomonas*.

However, by day 28 of aging, all three of these genera had a relative abundance of less than 1%, indicating that genera such as *Leuconostoc* outcompeted these bacteria.

Beta diversity results for all three muscles are presented in Figures 2.7-2.9. Within the SM (Figure 2.7) and BF (Figure 2.9), the beta diversity at 14-days of aging was different ($P < 0.05$) to the other aging periods using a weighted unique fraction metric (UniFrac) and Bray-Curtis dissimilarity. The weighted UniFrac for the GM (Figure 2.8) showed differences ($P < 0.05$) between 14 days and all other aging periods. However, 28 days of aging were also different ($P < 0.05$) from 63 and 70 days, samples aged 35 days were different ($P < 0.05$) from 56 days, and 42- and 56-day samples were different ($P < 0.05$) from 70 days.

2.3.3 Desmin Degradation

The results of desmin degradation are presented in Figure 2.10. Desmin degradation in the SM muscle (Figure 2.10) aged for 14 days was similar ($P < 0.05$) to that obtained for the 28-day aging period. After 35 days of aging, there were no differences ($P > 0.05$) between band intensities with further aging. For the BF muscle (Figure 2.10), there were no differences ($P > 0.05$) in the band intensities of muscles aged 14, 28, 35, and 42 days. Similarly to the SM muscle, there was no increase ($P > 0.05$) in desmin degradation after 35 days of aging. The GM muscle (Figure 2.10) showed similar ($P < 0.05$) band intensities from 14 to 42 days of aging. Unlike the other two muscles, band intensities increased ($P < 0.05$) until 49 days of aging, where they were similar ($P < 0.05$) until 70 days of aging.

2.4 Discussion

2.4.1 Culture-based Enumeration of Microbial Populations

It has been reported that off-odors develop in vacuum-packaged beef when the microbial load reaches 7 to 8 log CFU/cm^2, while slime may form at 8 log CFU/cm^2 (Sutherland et al., 1975).

In this study, microbial counts were, on average, below this level. There was a steady increase in AC and LABC during aging. Similar to our results, Cholle et al. (2015 and 2016) also observed an increase in aerobic plate counts for SM, GM, and BF muscles when aged from 2 to 63 days. Our results are also in agreement with Hernandez et al. (2022) and Ribeiro et al. (2024), where microbial loads of both AC and LABC were well below the spoilage threshold in strip loins wet-aged for up to 56 and 42 days, respectively. Conversely, some authors have reported microbial loads above the spoilage threshold in beef sirloin and strip loins aged for 42 days (da Silva Bernardo et al., 2021) and 60 days (Kim et al., 2022). The differences between our study and these studies could be due to the variations in experimental conditions such as initial bacterial load, chilling, and packaging conditions.

2.4.2 Relative Abundance and Beta Diversity

In the current study, across all muscles, *Leuconostoc* was the most abundant genus. On the other hand, de Matos et al. (2024) reported that *Carnobacterium* was the most abundant genus in strip loins wet-aged for 20 and 34 days, with a relative abundance of 88.8% and 79.4%, respectively, while *Leuconostoc* only had an abundance of 2.1% for 20 days and 5% for 34 days. Moreover, Jääskeläinen et al. (2016) found that *Lactobacillus* was the dominant genus in vacuum-packaged beef after 26 days, while *Leuconostoc* had a low abundance. However, *Leuconostoc* was abundant in the high-oxygen packaging (Jääskeläinen et al., 2016). The differences in the results observed in the current study could be due to muscle differences, processing environment, or sampling methods.

The results of this study showed that beta diversity, or the diversity between samples, decreased as aging time increased. Specifically, muscles aged for 14 days were different ($P < 0.05$) from all other aging periods, indicating that the diversity decreases rapidly from 14 to 28 days of wet-aging. Multiple authors have investigated the differences in microbial diversity during wet aging of beef (Kaur et al., 2021; Yang et al., 2021; Zhang et al., 2022; de Matos et al., 2024). When analyzing both wet- and dry-aged beef strip loins, de Matos et al. (2024) reported a significant decrease in beta diversity when aging for up to 34 days. Likewise, Yang et al. (2021) found a decrease in beta diversity in beef ribeye that was wet-aged for up to 180 days. The most likely explanation for the decrease in diversity is resource competition between bacteria. Lactic acid bacteria, the dominant bacterial group within our wet-aged muscles, are able to thrive in a wet-aging environment, allowing them to become more abundant than other genera (Egan et al., 1989; Jääskeläinen et al., 2016; Esteves et al., 2021; de Matos et al., 2024).

2.4.3 Desmin Degradation

Desmin is a structural protein located at the Z-line of the sarcomere that degrades during postmortem proteolysis (Lazarides and Hubbard, 1976; Hwan and Bandman, 1989; de Oliveira et al., 2019). Desmin degradation has also been shown to have a positive impact on beef tenderness (Whipple et al., 1990; Taylor et al., 1995; Hou et al., 2020). During beef aging, the amount of intact desmin has been shown to decrease over time in multiple muscles (Hwan and Bandman, 1989; King et al., 2009; Bhat et al., 2018; de Oliveira et al., 2019). In our current study, there was no further desmin degradation in the SM and BF after 35 days of aging, and after 42 days of aging in the GM. Only a few studies have conducted desmin degradation analysis in extended aging periods such as 56 or 70 days (Phelps et al., 2016; Hernandez et al., 2022). Using wet-aged beef strip loins, Hernandez et al. (2022) determined that desmin does not degrade after 28 days of aging at 4°C, and 42 days at 0°C. While strip loins were not examined in the current study, the results are consistent with these findings. Phelps et al. (2016) also reported that desmin degraded linearly in the SM up to 70 days of aging, whereas in the current study, desmin degradation remained stagnant after 35 days of aging. When examining GM steaks wet-aged for 12, 26, and 40 days, King et al. (2009) observed differences in the percentage of desmin degraded between all three aging periods. However, in the current study, no differences were seen between the 14-, 28-, 35-, and 42-day aging periods for GM. It is possible that this discrepancy could be due to the differences in analysis (band intensity vs percent degradation) as well as aging conditions.

2.5 Conclusions

In general, for all three muscles examined in the current study, the microbial load and desmin degradation increased with aging, whereas microbial diversity decreased. Aside from the initial 14-day aging period, the most abundant bacterial genera were lactic acid bacteria, which could potentially contribute to any souring or off-flavors as beef ages. A higher degree of desmin degradation was observed with increasing aging times initially for all muscles, which could lead to increased tenderness during the initial aging days. While there were some differences in microbial load, microbial diversity, and desmin degradation during early aging days, those disappeared as the aging time increased. Overall, these results suggest that microbial and biochemical changes occur in a muscle-specific manner during extended aging.

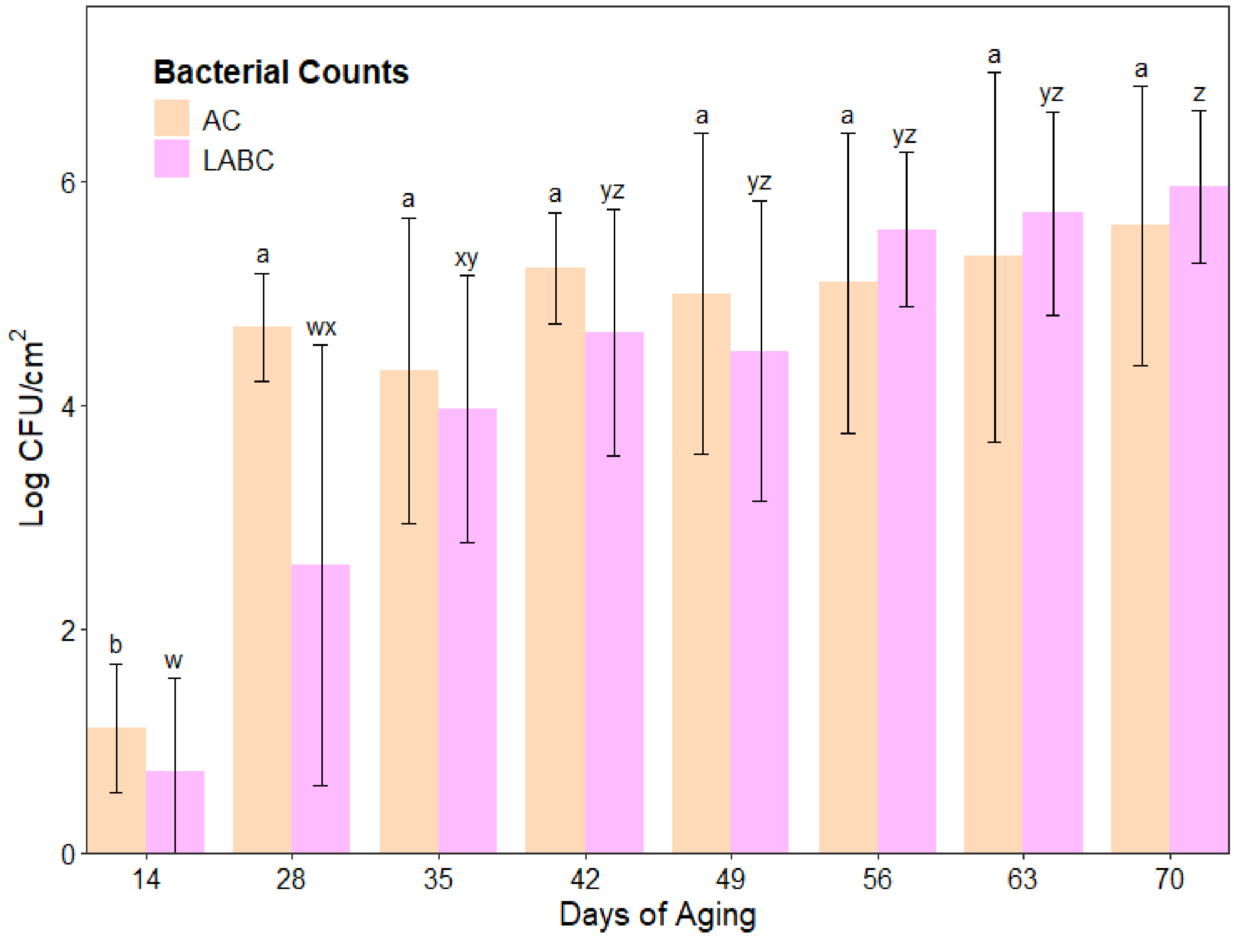

Figure 2.1. Marginal means ± standard deviation (log CFU/cm^2) of total aerobic mesophilic microflora counts (AC) and lactic acid bacteria counts (LABC) for beef *semimembranosus* (n = 10) over eight aging periods. Marginal means within bacterial count type with different letters (a, b, w-z) are different ($P < 0.05$).

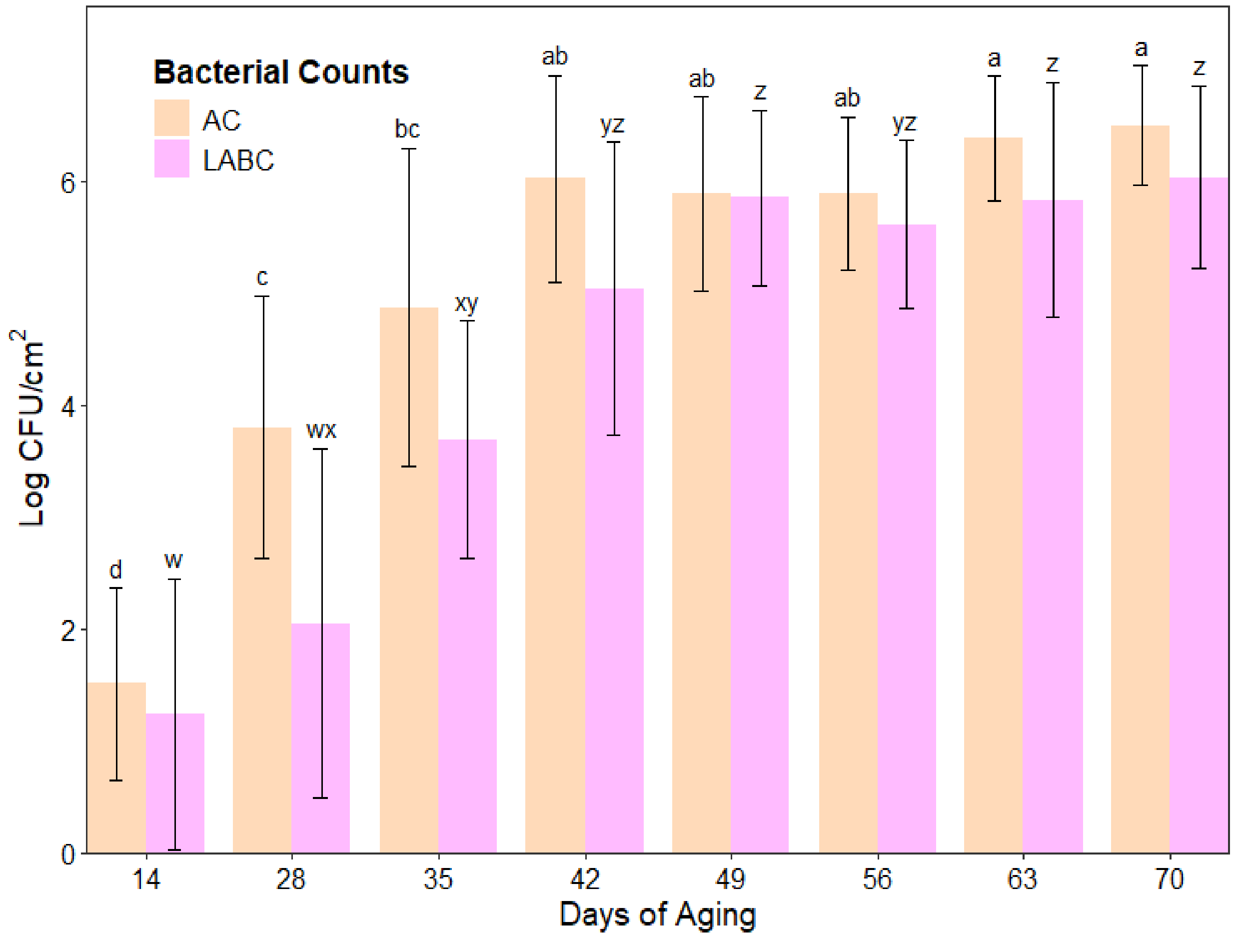

Figure 2.2. Marginal means ± standard deviation (log CFU/cm^2) of total aerobic mesophilic microflora counts (AC) and lactic acid bacteria counts (LABC) for beef *gluteus medius* (n = 10) over eight aging periods. Marginal means within bacterial count type with different letters (a-d, w-z) are different ($P < 0.05$).

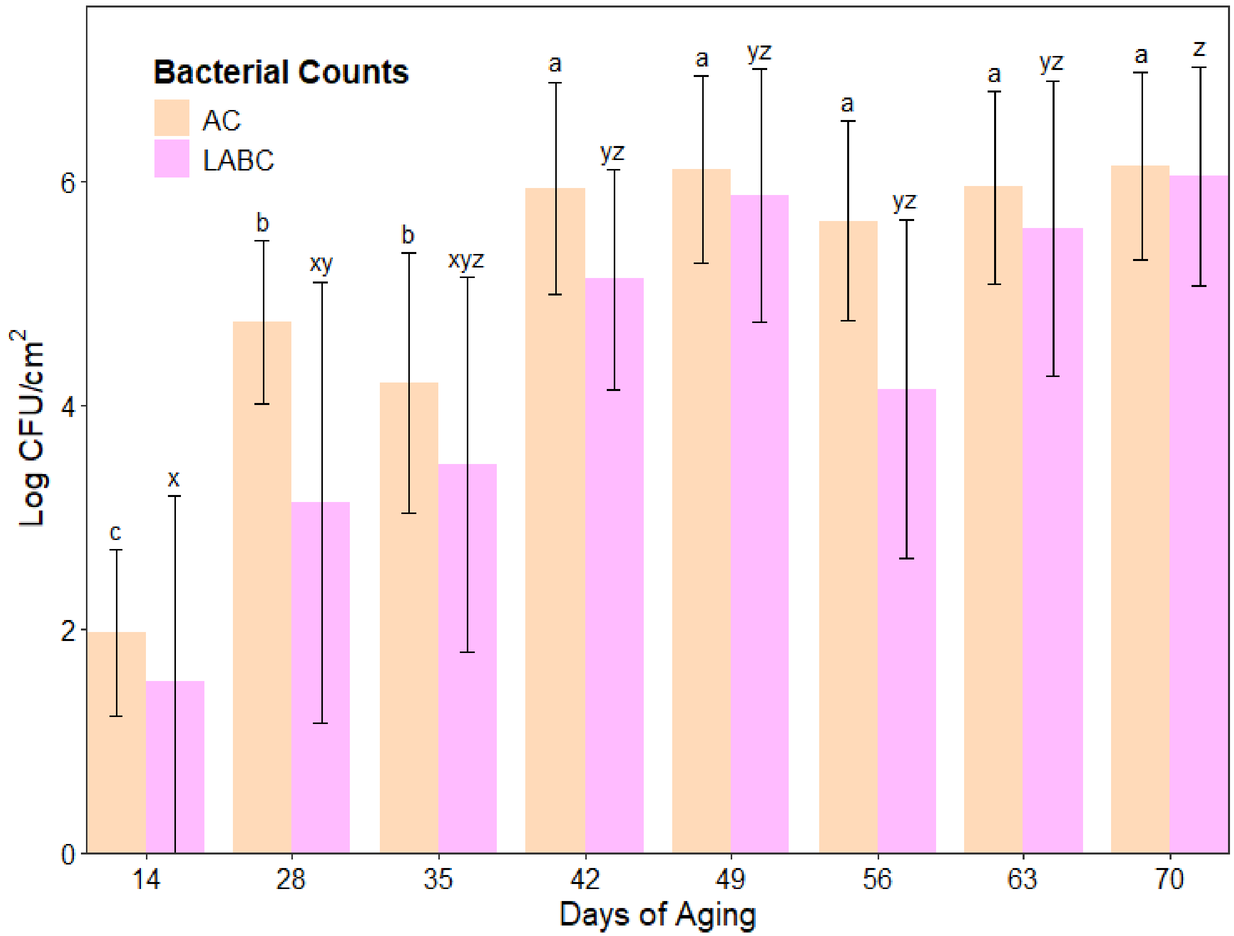

Figure 2.3. Marginal means ± standard deviation (log CFU/cm^2) of total aerobic mesophilic microflora counts (AC) and lactic acid bacteria counts (LABC) for beef *biceps femoris* (n = 10) over eight aging periods. Marginal means within bacterial count type with different letters (a-c, x-z) are different ($P < 0.05$).

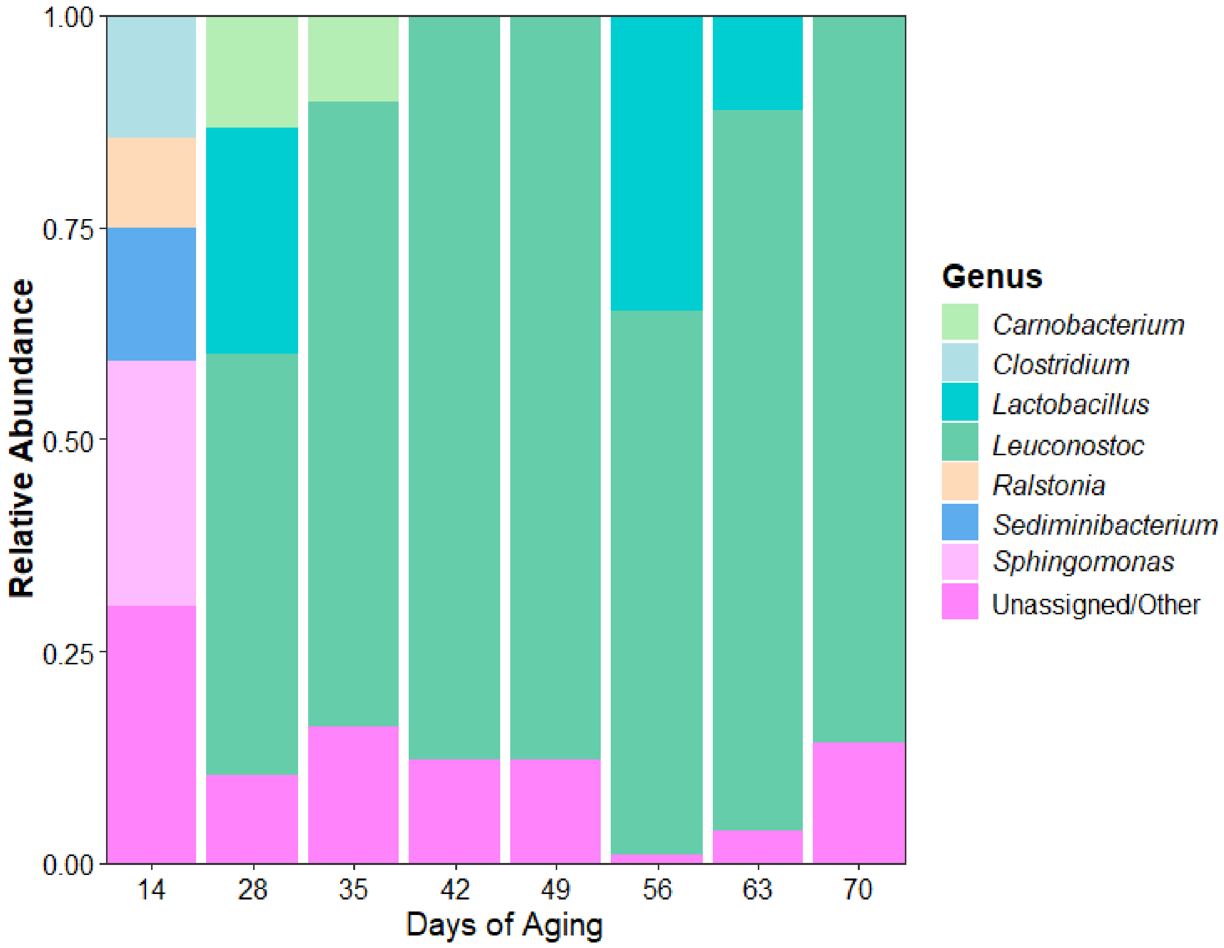

Figure 2.4. Relative abundance at the genus level of beef *semimembranosus* (n = 10) over eight aging periods.

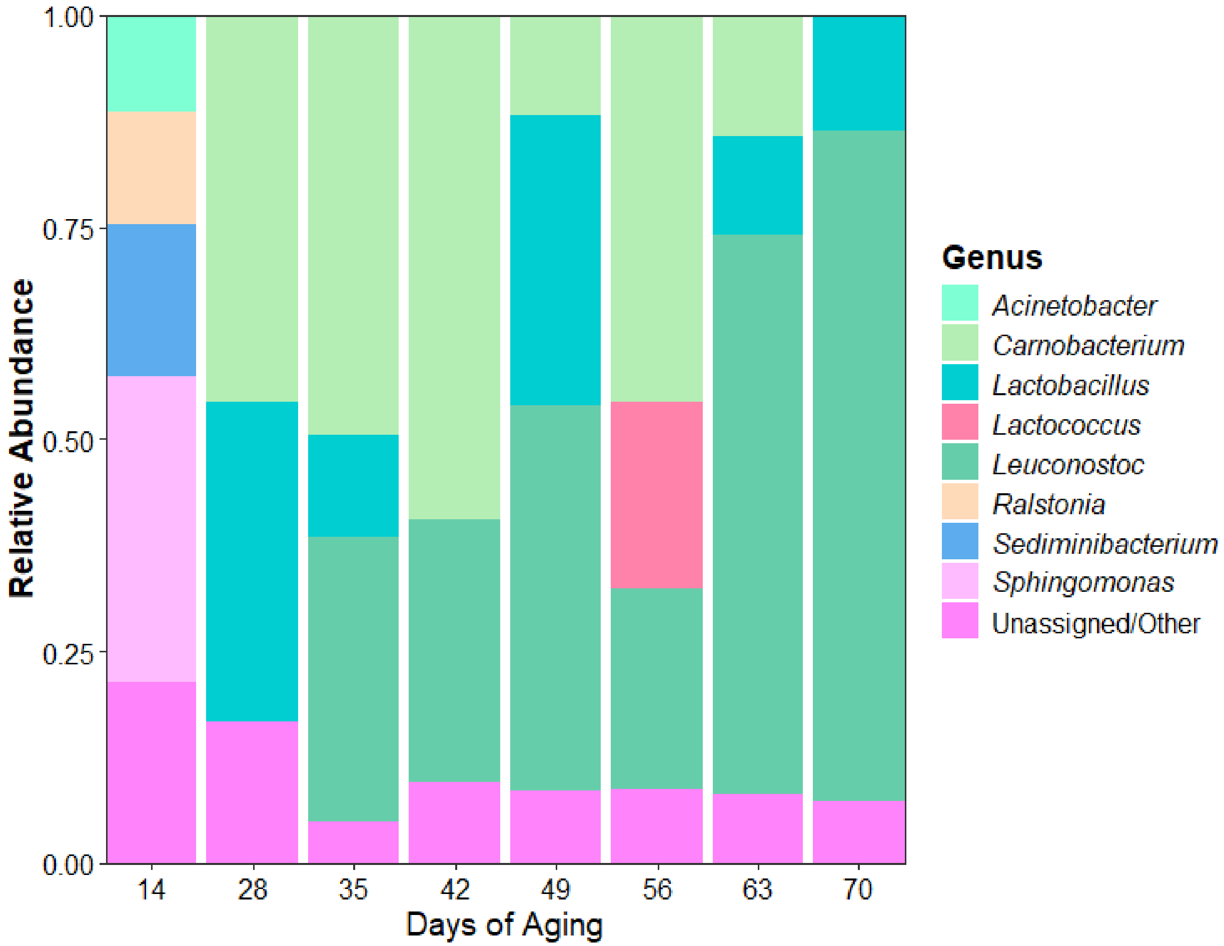

Figure 2.5. Relative abundance at the genus level of beef *gluteus medius* (n = 10) over eight aging periods.

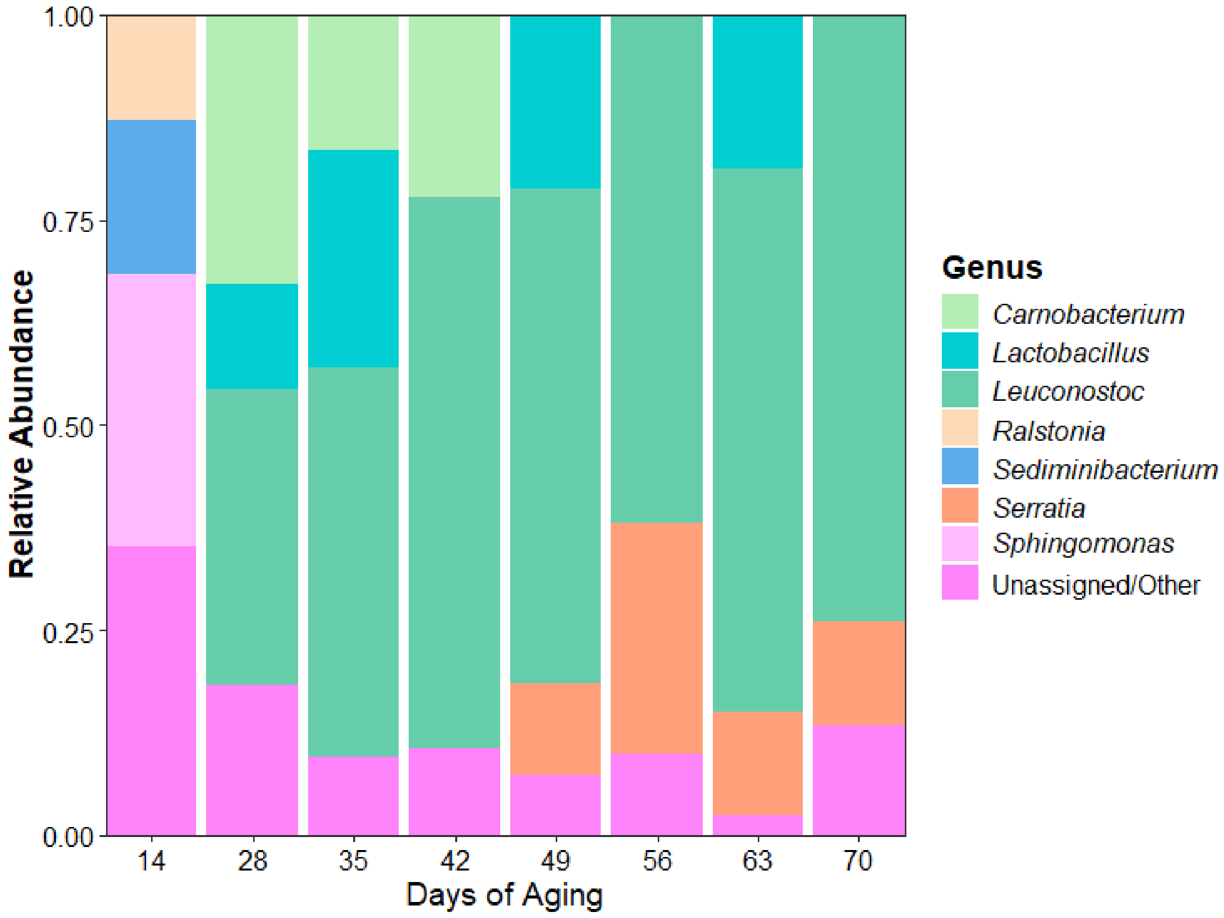

Figure 2.6. Relative abundance at the genus level of beef *biceps femoris* (n = 10) over eight aging periods.

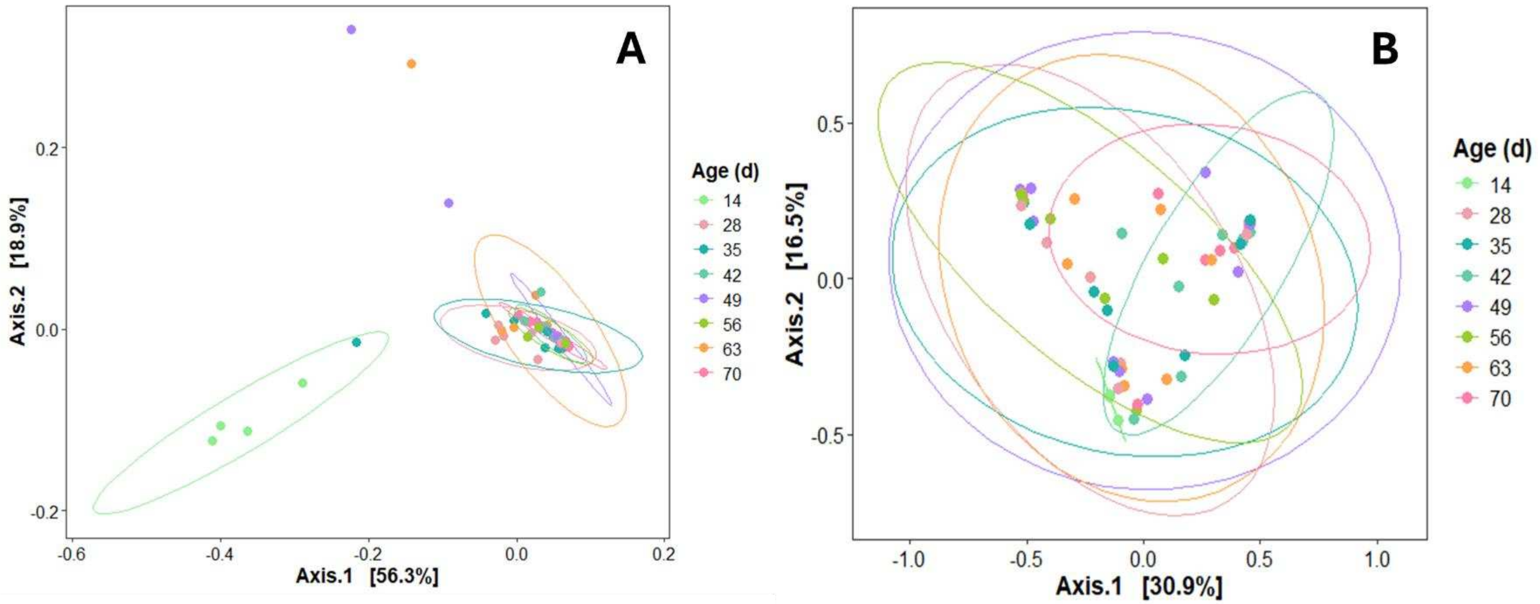

Figure 2.7. Beta diversity as a weighted UniFrac (**A**) and Bray-Curtis Dissimilarity (**B**) principal coordinate analysis (PCoA) for beef *semimembranosus* (n = 10) over eight aging periods.

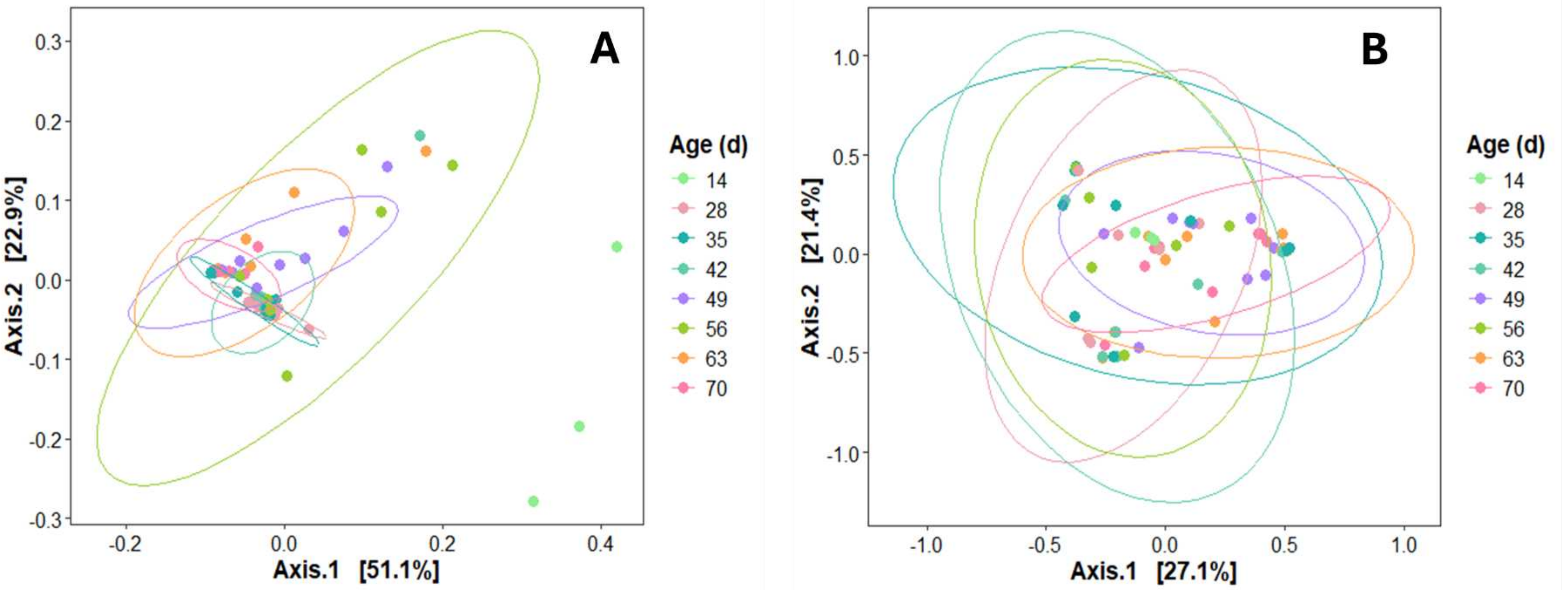

Figure 2.8. Beta diversity as a weighted UniFrac (**A**) and Bray-Curtis Dissimilarity (**B**) principal coordinate analysis (PCoA) for beef *gluteus medius* (n = 10) over eight aging periods.

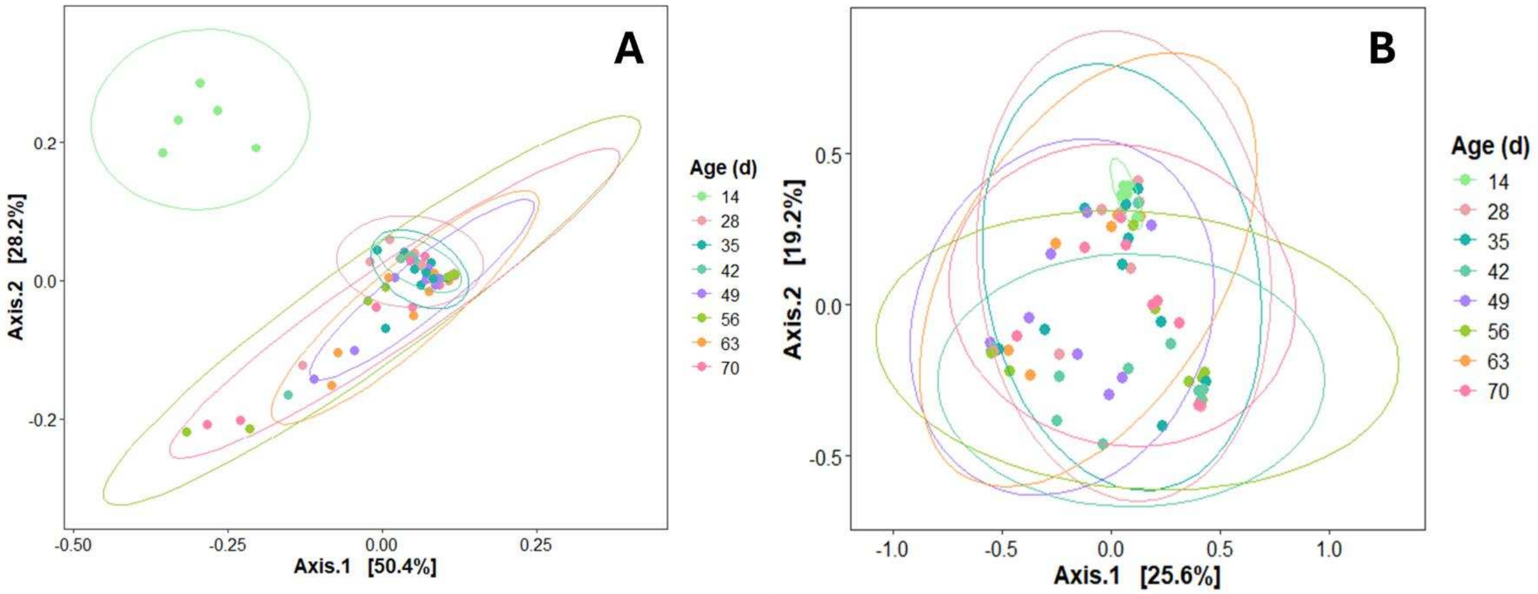

Figure 2.9. Beta diversity as a weighted UniFrac (**A**) and Bray-Curtis Dissimilarity (**B**) principal coordinate analysis (PCoA) for beef *biceps femoris* (n = 10) over eight aging periods.

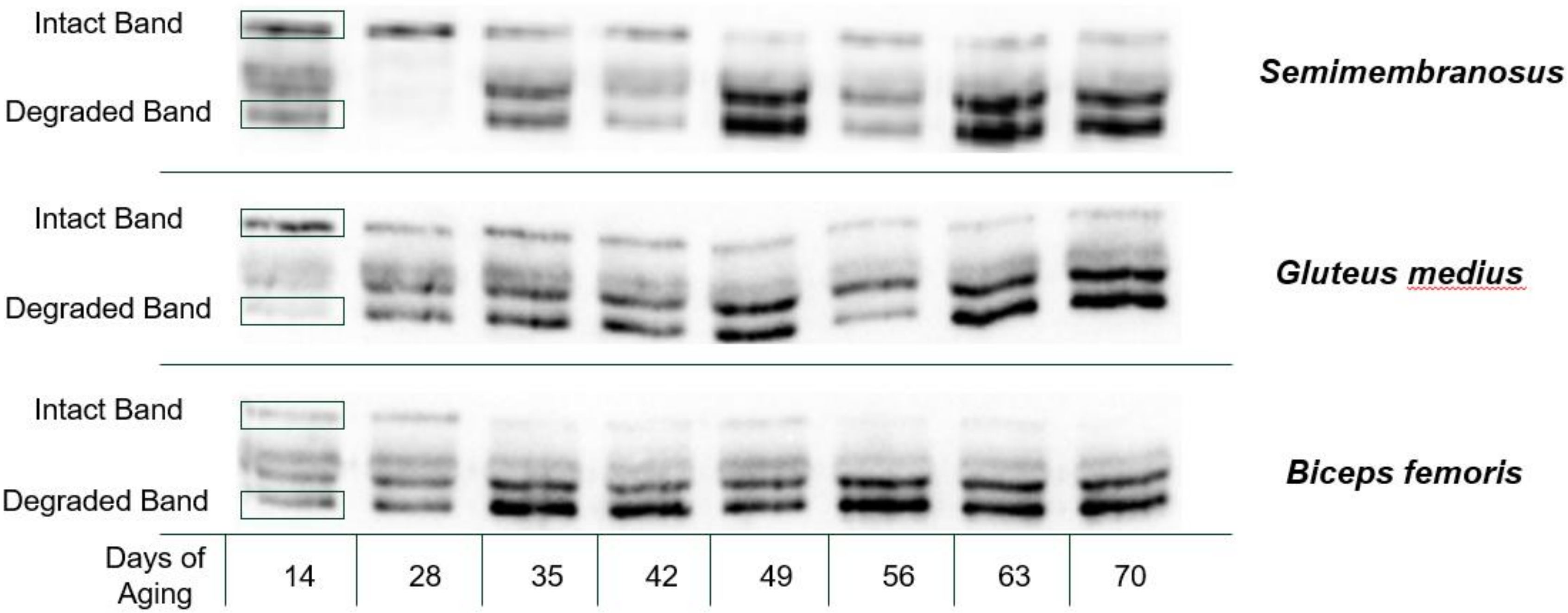

Figure 2.10. Representative image of intact (55 kDa) and degraded (37 kDa) desmin of beef *semimembranosus, gluteus medius,* and *biceps femoris* over eight aging periods.

REFERENCES

Ahnström, M. L., M. Seyfert, M. C. Hunt, and D. E. Johnson. 2006. Dry aging of beef in a bag highly permeable to water vapour. *Meat Sci.* 73:674–679. doi:10.1016/j.meatsci.2006.03.006.

Allen, L. V. Jr. 2018. Quality Control: Water Activity Considerations for Beyond-use Dates. *Int. J. Pharm. Compd.* 22:288–293.

Anderson, T. J., and F. C. Parrish Jr. 1989. Postmortem Degradation of Titin and Nebulin of Beef Steaks Varying in Tenderness. *J. Food Sci.* 54:748–749. doi:10.1111/j.1365-2621.1989.tb04695.x.

Aroeira, C. N., R. A. Torres Filho, P. R. Fontes, L. A. M. Gomide, A. L. S. Ramos, M. M. Ladeira, and E. M. Ramos. 2016. Freezing, thawing and aging effects on beef tenderness from *Bos indicus* and *Bos taurus* cattle. *Meat Sci.* 116:118–125. doi:10.1016/j.meatsci.2016.02.006.

Barker, S. N., J. C. Brooks, J. T. Bachler, D. R. Woerner, and J. F. Legako. 2023. Flavor Development of Individually Vacuum-Packaged Beef Steaks During Extended Wet Aging. *Meat Muscle Biol.* 7. doi:10.22175/mmb.16192.

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.

Bhat, Z. F., J. D. Morton, S. L. Mason, and A. E. A. Bekhit. 2018. Calpain activity, myofibrillar protein profile, and physicochemical properties of beef *Semimembranosus* and *Biceps femoris* from culled dairy cows during aging. *J. Food Process. Pres.* 42:e13835. doi:10.1111/jfpp.13835.

Bischof, G., F. Witte, T. Sieksmeyer, E. Januschweski, N. Terjung, C. Hertel, V. Heinz, A. Juadjur, and M. Gibis. 2023. Metabolic and microbial analyses of the surface and inner part of wet-aged and dry-aged beef. *J. Food Sci.* 88:4375–4387. doi:10.1111/1750-3841.16761.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C.

Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol*. 37:852–857. doi:10.1038/s41587-019-0209-9.

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

Bratzler, L. J. 1932. Measuring the tenderness of meat by means of a mechanical shear. M. S. Thesis, Kansas State Univ, Manhattan, KS. (https://krex.k-state.edu/bitstream/handle/2097/18440/LD2668T41932B71.pdf)

Brewer, S., and J. Novakofski. 2008. Consumer Sensory Evaluations of Aging Effects on Beef Quality. *J. Food Sci*. 73:S78–S82. doi:10.1111/j.1750-3841.2007.00575.x.

Brooks, J. C., J. B. Belew, D. B. Griffin, B. L. Gwartney, D. S. Hale, W. R. Henning, D. D. Johnson, J. B. Morgan, F. C. Parrish Jr., J. O. Reagan, and J. W. Savell. 2000. National Beef Tenderness Survey–1998. *J. Anim. Sci*. 78:1852–1860. doi:10.2527/2000.7871852x.

Calkins, C. R., and S. C. Seideman. 1988. Relationships among Calcium-Dependent Protease, Cathepsins B and H, Meat Tenderness and the Response of Muscle to Aging. *J. Anim. Sci*. 66:1186. doi:10.2527/jas1988.6651186x.

Camou, J. P., J. A. Marchello, V. F. Thompson, S. W. Mares, and D. E. Goll. 2007. Effect of postmortem storage on activity of μ- and m-calpain in five bovine muscles. *J. Anim. Sci*. 85:2670–2681. doi:10.2527/jas.2007-0164.

Campbell, R. E., M. C. Hunt, P. Levis, and E. Chambers IV. 2006. Dry-Aging Effects on Palatability of Beef Longissimus Muscle. *J. Food Sci*. 66:196–199. doi:10.1111/j.1365-2621.2001.tb11315.x.

Campo, M. M., G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson. 2006. Flavour perception of oxidation in beef. *Meat Sci*. 72:303–311. doi:10.1016/j.meatsci.2005.07.015.

Carmack, C. F., C. L. Kastner, M. E. Dikeman, J. R. Schwenke, and C. M. García Zepeda. 1995. Sensory evaluation of beef-flavor-intensity, tenderness, and juiciness among major muscles. *Meat Sci*. 39:143–147. doi:10.1016/0309-1740(95)80016-6.

Cavender, A. M. 2021. Dry aging: understanding the implications of aging on different USDA quality grade beef short loins. M.S. thesis. Univ. of Nevada, Reno, NV, USA. (https://www.proquest.com/docview/2563495441?pq-origsite=gscholar&fromopenview=true&sourcetype=Dissertations%20&%20Theses).

Chaosap, C., S. Chokcharoen, A. Nilwatthana, K. D. Adeyemi, and R. Limsupavanich. 2025. Effects of aging method on meat quality, troponin T degradation, collagen solubility, fatty acids, ribonucleotides and free amino acids in Wagyu Sahiwal crossbred beef loin. *J. Food Sci. Technol*. doi:10.1007/s13197-025-06282-3.

Clausen, I., M. Jakobsen, P. Ertbjerg, and N. T. Madsen. 2009. Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef. *Packag. Technol. Sci*. 22:85–96. doi:10.1002/pts.828.

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

Colle, M. J., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the *biceps femoris* and *semimembranosus*. *Meat Sci*. 119:110–117. doi:10.1016/j.meatsci.2016.04.028.

Colle, M. J., and M. E. Doumit. 2017. Effect of extended aging on calpain-1 and -2 activity in beef *longissimus lumborum* and *semimembranosus* muscles. *Meat Sci*. 131:142–145. doi:10.1016/j.meatsci.2017.05.014.

Cooke, P. 1976. A filamentous cytoskeleton in vertebrate smooth muscle fibers. *J. Cell Biol.* 68:539–556. doi:10.1083/jcb.68.3.539.

Cruzen, S. M., P. V. R. Paulino, S. M. Lonergan, and E. Huff-Lonergan. 2014. Postmortem proteolysis in three muscles from growing and mature beef cattle. *Meat Sci*. 96:854–861. doi:10.1016/j.meatsci.2013.09.021.

Dainty, R. H., R. A. Edwards, and C. M. Hibbard. 1985. Time course of volatile compound formation during refrigerated storage of naturally contaminated beef in air. *J. Appl. Bacteriol*. 59:303–309. doi:10.1111/j.1365-2672.1985.tb03324.x.

Dainty, R. H., and B. M. Mackey. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J. Appl. Bacteriol*. 73:103s–114s. doi:10.1111/j.1365-2672.1992.tb03630.x.

da Silva Bernardo, A. P., F. M. S. Ferreira, A. C. M. da Silva, F. S. Prestes, V. C. Francisco, R. T. Nassu, M. da Silva do Nascimento, and S. B. Pflanzer. 2021. Dry-aged and wet-aged beef: effects of aging time and temperature on microbiological profile, physicochemical characteristics, volatile compound profile and weight loss of meat from Nellore cattle (*Bos indicus*). *Anim. Prod. Sci*. 61:1497–1509. doi:10.1071/AN20120.

Daszkiewicz, T., S. Wajda, and P. Matusevičius. 2003. Changing of beef quality in the process of storage. *Vet. Zootec*. 21(43):62–65.

Davey, C. L., H. Kuttel, and K. V. Gilbert. 1967. Shortening as a factor in meat ageing. *Int. J. Food Sci. Tech*. 2:53–56. doi:10.1111/j.1365-2621.1967.tb01325.x.

DeGeer, S. L., M. C. Hunt, C. L. Bratcher, B. A. Crozier-Dodson, D. E. Johnson, and J. F. Stika. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. *Meat Sci*. 83:768–774. doi:10.1016/j.meatsci.2009.08.017.

de Matos, L. G., A. C. da Silva Abreu, V. P. P. Alonso, J. L. Gonçalves, M. da Silva do Nascimento, S. B. Pflanzer Jr, J. H. Rezende-de-Souza, C. Gini, N. F. Murad, M. M. Brandão, and N. C. C. Silva. 2024. Comparison of bacterial diversity in wet- and dry-aged beef using traditional microbiology and next generation sequencing. *Microbe*. 2:100035. doi:10.1016/j.microb.2024.100035.

de Oliveira, L. G., E. F. Delgado, E. M. Steadham, E. Huff-Lonergan, and S. M. Lonergan. 2019. Association of calpain and calpastatin activity to *postmortem* myofibrillar protein degradation and sarcoplasmic proteome changes in bovine *Longissiumus lumborum* and *Triceps brachii*. *Meat Sci*. 155:50–60. doi:10.1016/j.meatsci.2019.04.015.

Derington, A. J., J. C. Brooks, A. J. Garmyn, L. D. Thompson, D. B. Wester, and M. F. Miller. 2011. Relationships of slice shear force and Warner-Bratzler shear force of beef strip loin steaks as related to the tenderness gradient of the strip loin. *Meat Sci*. 88:203–208. doi:10.1016/j.meatsci.2010.12.030.

Dikeman, M. E., E. Obuz, V. Gök, L. Akkaya, and S. Stroda. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef *Longissimus lumborum* steaks. *Meat Sci*. 94:228–233. doi:10.1016/j.meatsci.2013.02.002.

Di Paolo, M., R. L. Ambrosio, C. Lambiase, V. Vuoso, A. Salzano, G. Bifulco, C. M. A. Barone, and R. Marrone. 2023. Effects of the Aging Period and Method on the Physicochemical, Microbiological and Rheological Characteristics of Two Cuts of Charolais Beef. *Foods*. 12:531. doi:10.3390/foods12030531.

Egan, A. F. 1983. Lactic acid bacteria of meat and meat products. *Antonie van Leeuwenhoek.* 49:327–336. doi:10.1007/BF00399507.

Egan, A. F., B. J. Shay, and P. J. Rogers. 1989. Factors affecting the production of hydrogen sulphide by Lactobacillus sake L13 growing on vacuum-packaged beef. *J. Appl. Bacteriol*. 67:255–262. doi:10.1111/j.1365-2672.1989.tb02493.x.

English, A. R., G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016. Effects of extended aging and modified atmospheric packaging on beef top loin steak color. *J. Anim. Sci*. 94:1727–1737. doi:10.2527/jas.2015-0149.

Ercolini, D., I. Ferrocino, A. Nasi, M. Ndagijimana, P. Vernocchi, A. La Storia, L. Laghi, G. Mauriello, M. E. Guerzoni, and F. Villani. 2011. Monitoring of Microbial Metabolites and Bacterial Diversity in Beef Stored under Different Packaging Conditions. *Appl. Environ. Microb*. 77:7372–7381. doi:10.1128/AEM.05521-11.

Esteves, E., P. Whyte, J. Mills, G. Brightwell, T. B. Gupta, and D. Bolton. 2021. An investigation into the anaerobic spoilage microbiota of beef carcass and rump steak cuts using high-throughput sequencing. *FEMS Microbiol. Lett*. 368:fnab109. doi:10.1093/femsle/fnab109.

FDA. 1984. Water activity (a_w) in foods. United States Food and Drug Administration. Silver Spring, MD.

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

Fritz, J. D., M. C. Mitchell, B. B. Marsh, and M. L. Greaser. 1993. Titin content of beef in relation to tenderness. *Meat Sci*. 33:41–50. doi:10.1016/0309-1740(93)90092-V.

Fu, Y., S. Cao, L. Yang, and Z. Li. 2022. Flavor formation based on lipid in meat and meat products: A review. *J. Food Biochem.* 46:e14439. doi:10.1111/jfbc.14439.

Fürst, D. O., M. Osborn, R. Nave, and K. Weber. 1988. The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: a map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. *J. Cell Biol*. 106:1563–1572. doi:10.1083/jcb.106.5.1563.

Geesink, G. H., S. Kuchay, A. H. Chishti, and M. Koohmaraie. 2006. μ-Calpain is essential for postmortem proteolysis of muscle proteins. *J. Anim. Sci*. 84:2834–2840. doi:10.2527/jas.2006-122.

Gonzalez, A. A., E. P. Williams, T. E. Schwartz, A. N. Arnold, D. B. Griffin, R. K. Miller, K. B. Gehring, J. C. Brooks, J. F. Legako, C. C. Carr, G. G. Mafi, C. L. Lorenzen, R. J. Maddock, and J. W. Savell. 2024. National Beef Tenderness Survey—2022: Consumer Sensory Panel Evaluations and Warner-Bratzler Shear Force of Beef Steaks From Retail and Foodservice. *Meat Muscle Biol*. 8. doi:10.22175/mmb.16997.

Gorraiz, C., M. J. Beriain, J. Chasco, and K. Insausti. 2002. Effect of Aging Time on Volatile Compounds, Odor, and Flavor of Cooked Beef from Pirenaica and Friesian Bulls and Heifers. *J. Food Sci*. 67:916–922. doi:10.1111/j.1365-2621.2002.tb09428.x.

Graham, S. F., T. Kennedy, O. Chevallier, A. Gordon, L. Farmer, C. Elliott, and B. Moss. 2010. The application of NMR to study changes in polar metabolite concentrations in beef longissimus dorsi stored for different periods post mortem. *Metabolomics*. 6:395–404. doi:10.1007/s11306-010-0206-y.

Greaser, M. L., and J. Gergely. 1971. Reconstitution of Troponin Activity from Three Protein Components. *J. Biol. Chem*. 246:4226–4233. doi:10.1016/S0021-9258(18)62075-7.

Greer, G. G., S. D. M. Jones, B. D. Dilts, and W. M. Robertson. 1990. Effect of Spray-Chilling on the Quality, Bacteriology and Case Life of Aged Carcasses and Vacuum Packaged Beef. *Can. Inst. F. Sci. Tech. J.* 23:82–86. doi:10.1016/S0315-5463(90)70206-4.

Grobbel, J. P., M. E. Dikeman, M. C. Hunt, and G. A. Milliken. 2008. Effects of different packaging atmospheres and injection-enhancement on beef tenderness, sensory attributes, desmin degradation, and display color. *J. Anim. Sci*. 86:2697–2710. doi:10.2527/jas.2007-0824.

Gruber, S. L., J. D. Tatum, J. A. Scanga, P. L. Chapman, G. C. Smith, and K. E. Belk. 2006. Effects of postmortem aging and USDA quality grade on Warner-Bratzler shear force values of seventeen individual beef muscles. *J. Anim. Sci*. 84:3387–3396. doi:10.2527/jas.2006-194.

Guelker, M. R., A. N. Haneklaus, J. C. Brooks, C. C. Carr, R. J. Delmore Jr., 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 establishments1. *J. Anim. Sci*. 91:1005–1014. doi:10.2527/jas.2012-5785.

Hernandez, M. S., D. R. Woerner, J. C. Brooks, T. L. Wheeler, and J. F. Legako. 2022. Influence of Aging Temperature and Duration on Spoilage Organism Growth, Proteolytic Activity, and Related Chemical Changes in Vacuum-Packaged Beef Longissimus. *Meat Muscle Biol*. 6. doi:10.22175/mmb.13724.

Hopkins, D. L., E. N. Ponnampalam, R. J. van de Ven, and R. D. Warner. 2014. The effect of pH decline rate on the meat and eating quality of beef carcasses. *Anim. Prod. Sci*. 54:407–413. doi:10.1071/AN12314.

Hou, Q., C. Zhang, W. Zhang, R. Liu, H. Tang, and G. Zhou. 2020. Role of protein S-nitrosylation in regulating beef tenderness. *Food Chem*. 306:125616. doi:10.1016/j.foodchem.2019.125616.

Huang, F., M. Huang, H. Zhang, C. Zhang, D. Zhang, and G. Zhou. 2016. Changes in apoptotic factors and caspase activation pathways during the postmortem aging of beef muscle. *Food Chem*. 190:110–114. doi:10.1016/j.foodchem.2015.05.056.

Huff-Lonergan, E., F. C. Parrish Jr., and R. M. Robson. 1995. Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *J. Anim. Sci*. 73:1064–1073. doi:10.2527/1995.7341064x.

Huff-Lonergan, E., T. Mitsuhashi, D. D. Beekman, F. C. Parrish Jr., D. G. Olson, and R. M. Robson. 1996a. Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *J. Anim. Sci.* 74:993–1008. doi:10.2527/1996.745993x.

Huff-Lonergan, E., T. Mitsuhashi, F. C. Parrish Jr., and R. M. Robson. 1996b. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blotting comparisons of purified myofibrils and whole muscle preparations for evaluating titin and nebulin in postmortem bovine muscle. *J. Anim. Sci.* 74:779–785. doi:10.2527/1996.744779x.

Hwan, S. F., and E. Bandman. 1989. Studies of Desmin and α-Actinin Degradation in Bovine Semitendinosus Muscle. *J. Food Sci.* 54:1426–1430. doi:10.1111/j.1365-2621.1989.tb05126.x.

Insausti, K., M. J. Beriain, A. Purroy, P. Alberti, L. Lizaso, and B. Hernandez. 1999. Colour stability of beef from different Spanish native cattle breeds stored under vacuum and modified atmosphere. *Meat Sci*. 53:241–249. doi:10.1016/S0309-1740(99)00063-7.

Jääskeläinen, E., J. Hultman, J. Parshintsev, M.-L. Riekkola, and J. Björkroth. 2016. Development of spoilage bacterial community and volatile compounds in chilled beef under vacuum or high oxygen atmospheres. *Int. J. Food Microbiol.* 223:25–32. doi:10.1016/j.ijfoodmicro.2016.01.022.

Jeleníková, J., P. Pipek, and L. Staruch. 2008. The influence of ante-mortem treatment on relationship between pH and tenderness of beef. *Meat Sci*. 80:870–874. doi:10.1016/j.meatsci.2008.04.004.

Jeremiah, L. E., and L. L. Gibson. 2003. The effects of postmortem product handling and aging time on beef palatability. *Food Res. Int.* 36:929–941. doi:10.1016/S0963-9969(03)00102-9.

Johnson, L. G., C. Zhai, L. M. Reever, K. J. Prusa, M. N. Nair, E. Huff-Lonergan, and S. M. Lonergan. 2023a. Characterizing the sarcoplasmic proteome of aged pork chops classified by purge loss. *J. Anim. Sci.* 101:skad046. doi:10.1093/jas/skad046.

Johnson, L. G., C. Zhai, E. M. Steadham, L. M. Reever, K. J. Prusa, M. N. Nair, E. Huff-Lonergan, and S. M. Lonergan. 2023b. Distinct myofibrillar sub-proteomic profiles are associated with the instrumental texture of aged pork loin. *J. Anim. Sci.* 101:skad327. doi:10.1093/jas/skad327.

Kaur, M., M. Williams, A. Bissett, T. Ross, and J. P. Bowman. 2021. Effect of abattoir, livestock species and storage temperature on bacterial community dynamics and sensory properties of vacuum packaged red meat. *Food Microbiol.* 94:103648. doi:10.1016/j.fm.2020.103648.

Kerth, C. R., M. F. Miller, and C. B. Ramsey. 1995. Improvement of beef tenderness and quality traits with calcium chloride injection in beef loins 48 hours postmortem. *J. Anim. Sci.* 73:750–756. doi:10.2527/1995.733750x.

Kim, Y. H. B., R. Kemp, and L. M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. *Meat Sci.* 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Kim, M., J. Choe, H. J. Lee, Y. Yoon, S. Yoon, and C. Jo. 2019. Effects of Aging and Aging Method on Physicochemical and Sensory Traits of Different Beef Cuts. *Food Sci. Anim. Resour.* 39:54–64. doi:10.5851/kosfa.2019.e3.

Kim, S., G. Kim, C. Moon, K. Ko, Y. Choi, J. Choe, and Y. Ryu. 2022. Effects of Aging Methods and Periods on Quality Characteristics of Beef. *Food Sci. Anim. Resour.* 42:953–967. doi:10.5851/kosfa.2022.e63.

King, D. A., T. L. Wheeler, S. D. Shackelford, K. D. Pfeiffer, R. Nickelson, and M. Koohmaraie. 2009. Effect of blade tenderization, aging time, and aging temperature on tenderness of beef longissimus lumborum and gluteus medius. *J. Anim. Sci.* 87:2952–2960. doi:10.2527/jas.2009-1803.

Koohmaraie, M., S. C. Seidemann, J. E. Schollmeyer, T. R. Dutson, and J. D. Crouse. 1987. Effect of post-mortem storage on Ca^{++}-dependent proteases, their inhibitor and myofibril fragmentation. *Meat Sci.* 19:187–196. doi:10.1016/0309-1740(87)90056-8.

Koohmaraie, M. 1992. The role of Ca^{2+}-dependent proteases (calpains) in *post mortem* proteolysis and meat tenderness. *Biochimie.* 74:239–245. doi:10.1016/0300-9084(92)90122-U.

Laster, M. A., R. D. Smith, K. L. Nicholson, J. D. W. Nicholson, R. K. Miller, D. B. Griffin, K. B. Harris, and J. W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer sensory attribute evaluations of steaks from ribeyes, strip loins, and top sirloins from two quality grade groups. *Meat Sci.* 80:795–804. doi:10.1016/j.meatsci.2008.03.024.

Lazarides, E., and B. D. Hubbard. 1976. Immunological characterization of the subunit of the 100 Å filaments from muscle cells. *P. Natl. Acad. Sci. USA*. 73:4344–4348. doi:10.1073/pnas.73.12.4344.

Lee, M., J. Sebranek, and F. C. Parrish Jr. 1996. Accelerated Postmortem Aging of Beef Utilizing Electronbeam Irradiation and Modified Atmosphere Packaging. *J. Food Sci*. 61:133–136. doi:10.1111/j.1365-2621.1996.tb14742.x.

Lee, M. S., J. K. Apple, J. W. S. Yancey, J. T. Sawyer, and Z. B. Johnson. 2008. Influence of vacuum-aging period on bloom development of the beef *gluteus medius* from top sirloin butts. *Meat Sci*. 80:592–598. doi:10.1016/j.meatsci.2008.02.006.

Lee, D., H. J. Kim, A. Ismail, S. S. Kim, D. G. Yim, and C. Jo. 2023. Evaluation of the physicochemical, metabolomic, and sensory characteristics of Chikso and Hanwoo beef during wet aging. *Anim. Biosci*. 36:1101–1119. doi:10.5713/ab.23.0001.

Lepper-Blilie, A. N., E. P. Berg, D. S. Buchanan, and P. T. Berg. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. *Meat Sci*. 112:63–68. doi:10.1016/j.meatsci.2015.10.017.

Li, X., J. Babol, W. L. P. Bredie, B. Nielsen, J. Tománková, and K. Lundström. 2014. A comparative study of beef quality after ageing *longissimus* muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. *Meat Sci*. 97:433–442. doi:10.1016/j.meatsci.2014.03.014.

Lindahl, G., Å. Lagerstedt, P. Ertbjerg, S. Sampels, and K. Lundström. 2010. Ageing of large cuts of beef loin in vacuum or high oxygen modified atmosphere – Effect on shear force, calpain activity, desmin degradation and protein oxidation. *Meat Sci*. 85:160–166. doi:10.1016/j.meatsci.2009.12.020.

Madhavi, D. L., and C. E. Carpenter. 1993. Aging and Processing Affect Color, Metmyoglobin Reductase and Oxygen Consumption of Beef Muscles. *J. Food Sci*. 58:939–942. doi:10.1111/j.1365-2621.1993.tb06083.x.

Main, A. J. 2024. Impact of extended aging on 3 beef muscles and 9-10-11 rib dissections to predict red meat yield. M.S. Thesis, Texas Tech Univ, Lubbock, TX. (https://ttu-ir.tdl.org/items/e3f27d28-a892-465a-bdea-0d1040a62f1d)

Mansur, A. R., E. J. Song, Y. S. Cho, Y. D. Nam, Y. S. Choi, D. O. Kim, D. H. Seo, and T. G. Nam. 2019. Comparative evaluation of spoilage-related bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging. *Food Microbiol*. 77:166–172. doi:10.1016/j.fm.2018.09.006.

Marsh, B. B., and N. G. Leet. 1966. Studies in Meat Tenderness. III. The Effects of Cold Shortening on Tenderness. *J. Food Sci*. 31:450–459. doi:10.1111/j.1365-2621.1966.tb00520.x.

Marsh, B. B., and W. A. Carse. 1974. Meat tenderness and the sliding-filament hypothesis. *Int. J. Food Sci. Tech*. 9:129–139. doi:10.1111/j.1365-2621.1974.tb01756.x.

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. doi:10.22175/mmb2017.05.0028.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS One*. 8:e61217. doi:10.1371/journal.pone.0061217.

Meloni, M. P., F. Piras, G. Siddi, R. Sanna, R. Lai, F. Simbula, D. Cabras, M. Maurichi, G. Asara, E. P. L. De Santis, and C. Scarano. 2023. Preliminary data on the microbial profile of dry and wet aged bovine meat obtained from different breeds in Sardinia. *Ital. J. Food Saf*. 12:11060. doi:10.4081/ijfs.2023.11060.

Mohrhauser, D. A., K. R. Underwood, and A. D. Weaver. 2011. In vitro degradation of bovine myofibrils is caused by μ-calpain, not caspase-31. *J. Anim. Sci*. 89:798–808. doi:10.2527/jas.2010-3149.

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.

Morgan, J. B., J. W. Savell, D. S. Hale, R. K. Miller, D. B. Griffin, H. R. Cross, and S. D. Shackelford. 1991. National beef tenderness survey. *J. Anim. Sci.* 69:3274–3283. doi:10.2527/1991.6983274x.

Muchenje, V., M. Chimonyo, K. Dzama, P. E. Strydom, T. Ndlovu, and J. G. Raats. 2010. Relationship Between Off-Flavor Descriptors and Flavor Scores in Beef from Cattle Raised on Natural Pasture. *J. Muscle Foods*. 21:424–432. doi:10.1111/j.1745-4573.2009.00192.x.

Muroya, S., I. Nakajima, M. Oe, and K. Chikuni. 2006. Difference in postmortem degradation pattern among troponin T isoforms expressed in bovine longissimus, diaphragm, and masseter muscles. *Meat Sci*. 72:245–251. doi:10.1016/j.meatsci.2005.07.008.

Nair, M. N., S. Li, C. M. Beach, G. Rentfrow, and S. P. Suman. 2018. Changes in the Sarcoplasmic Proteome of Beef Muscles with Differential Color Stability during Postmortem Aging. *Meat Muscle Biol*. 2. doi:10.22175/mmb2017.07.0037.

bNewsome, R. L., B. E. Langlois, W. G. Moody, N. Gay, and J. D. Fox. 1984a. Effect of Time and Method of Aging on the Composition of the Microflora of Beef Loins and Corresponding Steaks. *J. Food Protect*. 47:114–119. doi:10.4315/0362-028X-47.2.114.

Newsome, R. L., B. E. Langlois, W. G. Moody, N. Gay, and J. D. Fox. 1984b. Effect of Time and Method of Aging on the Microbiological Quality of Beef Loins and Corresponding Steaks. *J. Food Protect*. 47:122–125. doi:10.4315/0362-028X-47.2.122.

Newton, K. G., and W. J. Rigg. 1979. The Effect of Film Permeability on the Storage Life and Microbiology of Vacuum-packed meat. *J. Appl. Bacteriol.* 47:433–441. doi:10.1111/j.1365-2672.1979.tb01204.x.

Obuz, E., M. E. Dikeman, J. P. Grobbel, J. W. Stephens, and T. M. Loughin. 2004. Beef longissimus lumborum, biceps femoris, and deep pectoralis Warner–Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. *Meat Sci*. 68:243–248. doi:10.1016/j.meatsci.2004.03.003.

O'Grady, M. N., F. J. Monahan, R. M. Burke, and P. Allen. 2000. The effect of oxygen level and exogenous α-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. *Meat Sci*. 55:39–45. doi:10.1016/S0309-1740(99)00123-0.

Oksanen, J., G. L. Simpson, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, P. Solymos, M. H. H. Stevens, E. Szoecs, H. Wagner, M. Barbour, M. Bedward, B. Bolker, D. Borcard, G. Carvalho, M. Chirico, M. D. Caceres, S. Durand, H. B. A. Evangelista, R. FitzJohn, M. Friendly, B. Furneaux, G. Hannigan, M. O. Hill, L. Lahti, D. McGlinn, M.-H. Ouellette, E. R. Cunha, T. Smith, A. Stier, C. J. F. T. Braak, and J. Weedon. 2024. vegan: Community Ecology Package. Available from: https://cran.r-project.org/web/packages/vegan/index.html

Onopiuk, A., A. Półtorak, and A. Wierzbicka. 2018. The impact of muscle and aging time on meat tenderness in the carcasses of Limousin × Holstein-Friesian crossbred bulls. *J. Food Process. Pres*. 42:e13619. doi:10.1111/jfpp.13619.

O'Quinn, T. G., D. R. Woerner, T. E. Engle, P. L. Chapman, J. F. Legako, J. C. Brooks, K. E. Belk, and J. D. Tatum. 2016. Identifying consumer preferences for specific beef flavor characteristics in relation to cattle production and postmortem processing parameters. *Meat Sci*. 112:90–102. doi:10.1016/j.meatsci.2015.11.001.

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.

Oreskovich, D. C., F. K. McKeith, T. R. Carr, J. Novakofski, and P. J. Bechtel. 1988. Effects of Different Aging Procedures on the Palatability of Beef. *J. Food Qual*. 11:151–158. doi:10.1111/j.1745-4557.1988.tb00875.x.

Ouali, A., and A. Talmant. 1990. Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. *Meat Sci*. 28:331–348. doi:10.1016/0309-1740(90)90047-A.

Parrish Jr., F. C., J. A. Boles, R. E. Rust, and D. G. Olson. 1991. Dry and Wet Aging Effects on Palatability Attributes of Beef Loin and Rib Steaks from Three Quality Grades. *J. Food Sci*. 56:601–603. doi:10.1111/j.1365-2621.1991.tb05338.x.

Phelps, K. J., J. S. Drouillard, M. B. Silva, L. D. F. Miranda, S. M. Ebarb, C. L. Van Bibber-Krueger, T. G. O'Quinn, and J. M. Gonzalez. 2016. Effect of extended postmortem aging and steak location on myofibrillar protein degradation and Warner-Bratzler shear force of beef M. semitendinosus steaks. *J. Anim. Sci*. 94:412–423. doi:10.2527/jas.2015-9862.

Resconi, V. C., A. Escudero, J. A. Beltrán, J. L. Olleta, C. Sañudo, and M. del Mar Campo. 2012. Color, Lipid Oxidation, Sensory Quality, and Aroma Compounds of Beef Steaks Displayed under Different Levels of Oxygen in a Modified Atmosphere Package. *J. Food Sci*. 77:S10–S18. doi:10.1111/j.1750-3841.2011.02506.x.

Resconi, V. C., M. Bueno, A. Escudero, D. Magalhaes, V. Ferreira, and M. M. Campo. 2018. Ageing and retail display time in raw beef odour according to the degree of lipid oxidation. *Food Chem.* 242:288–300. doi:10.1016/j.foodchem.2017.09.036.

Ribeiro, F. A., S. K. Lau, R. A. Furbeck, N. J. Herrera, M. L. Henriott, N. A. Bland, S. C. Fernando, J. Subbiah, S. B. Pflanzer, T. T. Dinh, R. K. Miller, G. A. Sullivan, and C. R. Calkins. 2024. Effects of relative humidity on dry-aged beef quality. *Meat Sci.* 213:109498. doi:10.1016/j.meatsci.2024.109498.

Sancho, R., I. Jaime, J. A. Beltran, and P. Roncales. 1997. Degradation of Actin by Cathepsins in Beef Fibers Stored at 20C. *J. Muscle Foods*. 8:137–146. doi:10.1111/j.1745-4573.1997.tb00623.x.

Santos, M. F. C., A. K. Silva, J. H. Rezende-de-Souza, T. Monaretto, C. S. M. Rezende, M. S. M. Karwowski, R. E. F. Macedo, C. S. Prado, S. B. Pflanzer, L. M. Lião, and C. F. Tormena. 2025. Assessment of the effects of dry and wet aging on dark-cutting Nellore beef meat by TD-NMR and 1H NMR approaches. *Food Res. Int*. 205:115920. doi:10.1016/j.foodres.2025.115920.

Setyabrata, D., K. Vierck, T. R. Sheets, J. F. Legako, B. R. Cooper, T. A. Johnson, and Y. H. B. Kim. 2022. Characterizing the Flavor Precursors and Liberation Mechanisms of Various Dry-Aging Methods in Cull Beef Loins Using Metabolomics and Microbiome Approaches. *Metabolites*. 12:472. doi:10.3390/metabo12060472.

Seyfert, M., R. A. Mancini, M. C. Hunt, J. Tang, C. Faustman, and M. Garcia. 2006. Color Stability, Reducing Activity, and Cytochrome c Oxidase Activity of Five Bovine Muscles. *J. Agric. Food Chem*. 54:8919–8925. doi:10.1021/jf061657s.

Shahidi, F., and W. Y. Oh. 2020. Lipid-derived flavor and off-flavor of traditional and functional foods: an overview. *J. Food Bioact*. 10. doi:10.31665/JFB.2020.10224.

Shi, Y., W. Zhang, and G. Zhou. 2020. Effects of Different Moisture-Permeable Packaging on the Quality of Aging Beef Compared with Wet Aging and Dry Aging. *Foods*. 9:649. doi:10.3390/foods9050649.

Sirtori, F., S. Parrini, M. C. Fabbri, C. Aquilani, A. Dal Prà, A. Crovetti, G. Brajon, and R. Bozzi. 2023. Influence of Wet Ageing on Beef Quality Traits. *Animals*. 13:58. doi:10.3390/ani13010058.

Sitz, B. M., C. R. Calkins, D. M. Feuz, W. J. Umberger, and K. M. Eskridge. 2006. Consumer sensory acceptance and value of wet-aged and dry-aged beef steaks. *J. Anim. Sci*. 84:1221–1226. doi:10.2527/2006.8451221x.

Smith, G. C., G. R. Culp, and Z. L. Carpenter. 1978. Postmortem Aging of Beef Carcasses. *J. Food Sci*. 43:823–826. doi:10.1111/j.1365-2621.1978.tb02430.x.

Smith, R. D., K. L. Nicholson, J. D. W. Nicholson, K. B. Harris, R. K. Miller, D. B. Griffin, and J. W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. *Meat Sci*. 79:631–639. doi:10.1016/j.meatsci.2007.10.028.

Spanier, A. M., M. Flores, K. W. McMillin, and T. D. Bidner. 1997. The effect of post-mortem aging on meat flavor quality in Brangus beef. Correlation of treatments, sensory, instrumental and chemical descriptors. *Food Chem*. 59:531–538. doi:10.1016/S0308-8146(97)00003-4.

Steen, D., E. Claeys, L. Uytterhaegen, S. De Smet, and D. Demeyer. 1997. Early post-mortem conditions and the calpain/calpastatin system in relation to tenderness of double-muscled beef. *Meat Sci*. 45:307–319. doi:10.1016/S0309-1740(96)00124-6.

Suman, S. P., R. A. Mancini, P. Joseph, R. Ramanathan, M. K. R. Konda, G. Dady, and S. Yin. 2010. Packaging-specific influence of chitosan on color stability and lipid oxidation in refrigerated ground beef. *Meat Sci*. 86:994–998. doi:10.1016/j.meatsci.2010.08.006.

Sutherland, J. P., J. T. Patterson, and J. G. Murray. 1975. Changes in the Microbiology of Vacuum-packaged Beef. *J. Appl. Bacteriol*. 39:227–237. doi:10.1111/j.1365-2672.1975.tb00568.x.

Tarladgis, B. G., B. M. Watts, M. T. Younathan, and L. Dugan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc*. 37:44–48. doi:10.1007/BF02630824.

Taylor, R. G., G. H. Geesink, V. F. Thompson, M. Koohmaraie, and D. E. Goll. 1995. Is Z-disk degradation responsible for postmortem tenderization? *J. Anim. Sci.* 73:1351–1367. doi:10.2527/1995.7351351x.

Tonino, P., B. Kiss, J. Strom, M. Methawasin, J. E. Smith, J. Kolb, S. Labeit, and H. Granzier. 2017. The giant protein titin regulates the length of the striated muscle thick filament. *Nat. Commun*. 8:1041. doi:10.1038/s41467-017-01144-9.

Ueda, S., Y. Yoshida, B. Kebede, C. Kitamura, R. Sasaki, M. Shinohara, I. Fukuda, and Y. Shirai. 2024. New Implications of Metabolites and Free Fatty Acids in Quality Control of Crossbred Wagyu Beef during Wet Aging Cold Storage. *Metabolites*. 14:95. doi:10.3390/metabo14020095.

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.

Wang, D., H. Xiao, X. Lyu, H. Chen, and F. Wei. 2023. Lipid oxidation in food science and nutritional health: A comprehensive review. *Oil Crop Sci*. 8:35–44. doi:10.1016/j.ocsci.2023.02.002.

Wang, K., J. McClure, and A. Tu. 1979. Titin: major myofibrillar components of striated muscle. *P. Natl. Acad. Sci. USA*. 76:3698–3702. doi:10.1073/pnas.76.8.3698.

Wang, K., and C. L. Williamson. 1980. Identification of an N_2 line protein of striated muscle. *P. Natl. Acad. Sci. USA*. 77:3254–3258. doi:10.1073/pnas.77.6.3254.

Wang, K., and J. Wright. 1988. Architecture of the sarcomere matrix of skeletal muscle: immunoelectron microscopic evidence that suggests a set of parallel inextensible nebulin filaments anchored at the Z line. *J. Cell Biol*. 107:2199–2212. doi:10.1083/jcb.107.6.2199.

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.

Wang, S., C. Dahlgren, D. Devost-Burnett, C. Lemley, K. Virellia To, X. Zhang, A. Patrick, M. W. Schilling, and T. Dinh. 2025. Effects of wet aging on water-soluble flavor compounds and descriptive sensory attributes for USDA select beef strip steaks. *Meat Sci*. 225:109821. doi:10.1016/j.meatsci.2025.109821.

Warren, K. E., and C. L. Kastner. 1992. A Comparison of Dry-Aged and Vacuum-Aged Beef Strip Loins. *J. Muscle Foods*. 3:151–157. doi:10.1111/j.1745-4573.1992.tb00471.x.

Whipple, G., M. Koohmaraie, M. E. Dikeman, J. D. Crouse, M. C. Hunt, and R. D. Klemm. 1990. Evaluation of attributes that affect longissimus muscle tenderness in bos taurus and bos indicus cattle. *J. Anim. Sci*. 68:2716–2728. doi:10.2527/1990.6892716x.

Whitfield, F. B. 1998. Microbiology of food taints. *Int. J. Food Sci. Tech.* 33:31–51. doi:10.1046/j.1365-2621.1998.00156.x.

Wright, S. A., P. Ramos, D. D. Johnson, J. M. Scheffler, M. A. Elzo, R. G. Mateescu, A. L. Bass, C. C. Carr, and T. L. Scheffler. 2018. Brahman genetics influence muscle fiber properties, protein degradation, and tenderness in an Angus-Brahman multibreed herd. *Meat Sci*. 135:84–93. doi:10.1016/j.meatsci.2017.09.006.

Wu, G., M. M. Farouk, S. Clerens, and K. Rosenvold. 2014. Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. *Meat Sci*. 98:637–645. doi:10.1016/j.meatsci.2014.06.010.

Yang, X., H. Wang, S. Hrycauk, and M. D. Klassen. 2021. Effects of Peroxyacetic Acid Spray and Storage Temperature on the Microbiota and Sensory Properties of Vacuum-Packed Subprimal Cuts of Meat. *Appl. Environ. Microb*. 87:e03143-20. doi:10.1128/AEM.03143-20.

Yar, M. K., M. H. Jaspal, S. Ali, I. H. Badar, M. Ijaz, and J. Hussain. 2024. Muscle-Specific Effects of Genotype, Animal Age, and Wet Aging Duration on Beef Color, Tenderness, and Sensory Characteristics. *Animals*. 14:3593. doi:10.3390/ani14243593.

Yu, Q., X. Gu, Q. Liu, R. Wen, and C. Sun. 2024. Effect of wet-aging on meat quality and exudate metabolome changes in different beef muscles. *Food Res. Int*. 184:114260. doi:10.1016/j.foodres.2024.114260.

Zhang, J., M. Li, Q. Yu, L. Han, and Z. Ma. 2019. Effects of Lysosomal–Mitochondrial Apoptotic Pathway on Tenderness in Post-Mortem Bovine Longissimus Muscle. *J. Agric. Food Chem*. 67:4578–4587. doi:10.1021/acs.jafc.9b00894.

Zhang, P., E. Ruan, D. B. Holman, and X. Yang. 2022. Effects of a *Carnobacterium maltaromaticum* strain at natural contamination levels on the microbiota of vacuum-packaged beef steaks during chilled storage. *LWT*. 168:113944. doi:10.1016/j.lwt.2022.113944.

Zhang, R., M. J. Y. Yoo, A. B. Ross, and M. M. Farouk. 2022. Mechanisms and strategies to tailor dry-aged meat flavour. *Trends Food Sci. Tech*. 119:400–411. doi:10.1016/j.tifs.2021.12.023