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Impacts of Stepwise Aging/Freezing Process and Repeated Freezing on Meat Quality, Physicochemical and Biochemical Properties, and Sensory Attributes of Beef Loins

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Abstract: The study aimed to investigate the impact of aging durations prior to and after repeated freezing/thawing cycles on the quality attributes of beef loins. At 2 d postmortem, paired beef loins from 30 carcasses were divided into aging groups (0, 2, 3, and 4 weeks at 2°C) named A0, A2, A3, and A4, respectively. Sections from each group were taken as never-frozen samples (F0T). The 4-week aged subsections were stored for an extra week as long-term aged-only controls (A5). The remaining portions were frozen for 1 to 5 weeks, creating a total aging/freezing cycle of 5 weeks. After the initial freezing periods, samples were thawed and collected as aged/frozen samples (F5T, F3T, F2T, F1T). The remaining portions were frozen again for 5 weeks and thawed as repeated frozen/thawed samples (F5T × 2, F3T × 2, F2T × 2, F1T × 2). Aging significantly reduced freeze/thaw losses, with the least losses observed in 4-week-aged, one-time frozen samples (A4F1T; $P < 0.05$). Repeatedly frozen samples exhibited lower losses compared to one-time frozen samples. Cooking loss was not different between never-frozen and aged/frozen samples ($P > 0.05$). In non-aged samples, instrumental tenderness significantly decreased with repeated freezing (A0F5T × 2), comparable to 5-week aged-only (never-frozen) samples (A5F0T; $P > 0.05$). The myofibrillar fragmentation index increased with aging coupled with freezing ($P < 0.05$). Lipid oxidation was increased in 4-week-aged, repeatedly frozen samples (A4F1T × 2, $P < 0.05$). Carbonyl content increased with aging and freezing processes ($P < 0.05$). Repeated freezing adversely affected display color stability compared to never-frozen samples. The consumer panel ($n = 90$) found no differences in eating quality attributes ($P > 0.05$), regarding liking of tenderness, flavor, juiciness, and overall liking between the aging/freezing samples. The results of the study suggest that stepwise aging/freezing can provide protective effects against moisture loss during freezing and repeatedly frozen/thawed processes, likely due to increases in muscle fragmentation. Stepwise aging/freezing can be a viable approach for the meat industry for the consistent supply of frozen beef with quality equivalent to fresh meat.

Key words: aging, freezing, repeated freezing, beef quality, sensory evaluation
Meat and Muscle Biology 8(1): 18294, 1–18 (2024) **doi:10.22175/mmb.18294**
Submitted 9 August 2024 Accepted 14 November 2024

Introduction

Freezing is one of the most widely practiced and effective methods to extend the shelf-life of meat products (Zhang et al., 2023). However, quality deterioration of frozen/thawed meat occurs due to mechanical disruption to the muscle structures, mainly associated with the formation of ice crystals during the freezing process. Several quality defects, such as an increase in purge/thaw loss, rapid discoloration (Cheng et al., 2020; Grayson et al., 2014; Kim et al., 2017), and/or a decrease in palatability attributes (Curry et al., 2023), are commonly associated with frozen/thawed meat products. Ice crystal formation during freezing causes the rupture of cell membranes, resulting in poor water-holding capacity

(WHC) upon meat thawing (Dang et al., 2021; Qian et al., 2022). Moreover, the repeated freezing and thawing experienced during transportation and/or storage at various points along the supply chain can further amplify the challenges associated with purge loss and other quality alterations (Zhang et al., 2023). Therefore, there exists a critical need for developing novel freezing practices that can ameliorate the meat quality defects associated with frozen/thawed beef.

Postmortem aging is a common industry practice to improve fresh meat quality attributes. During aging, endogenous proteolytic enzymes in meat, such as calpains and cathepsins, break down cytoskeletal myofibrillar proteins. This degradation weakens the overall structural integrity of muscle fibers and further biochemical interactions result in tenderization and enhanced flavor development (Setyabrata and Kim, 2019; Kominami et al., 2021). In addition, the potential of aging meat before freezing to alleviate the quality deterioration of frozen beef was suggested in previous studies (Kim et al., 2011, Kim et al., 2015; Li et al., 2019). During aging, proteolytic enzymes degrade muscle proteins, which can disrupt dripping channels in muscle fibers. This disruption can result in more formation of a moisture-entrapped matrix, lessening water migrations from muscle cells upon freezing and thawing (Setyabrata and Kim, 2019).

Additionally, while aging affects meat palatability attributes, freezing conditions can also influence meat quality. Importantly, the repeated freezing/thawing process (also known as double freezing) can occur in retail stores, food service sectors, and household settings. Meat subprimals may be frozen for storage and thawed for steak cutting or display. At the food service or household level, frozen/thawed steaks may be re-frozen after purchase, which can further degrade meat quality attributes (Curry et al., 2023). Double freezing can result in severe adverse impacts on meat quality characteristics of frozen/thawed beef due to additional cryo-damage on the extracellular and intracellular space of muscle. Considering the beneficial impact of postmortem aging in mitigating freezing-associated quality defects, it would be reasonable to hypothesize that beef muscles assigned to sufficient aging periods prior to freezing will potentially result in a lesser extent of cryo-damage in muscle fibers. This approach in turn could reduce the adverse effects of double freezing by retaining moisture within muscle cells through the degradation of myofibrillar protein networks.

While many studies have studied the effects of aging, freezing, and/or thawing on meat quality attributes, there is little to no published information regarding the impact of aging on meat quality in repeatedly frozen samples. Therefore, the objectives of this study were to determine 1) the effects of different aging durations prior to freezing (termed as "stepwise aging/freezing") on quality characteristics of beef loins and 2) the impact of initial subprimal aging prior to repeated freezing/thawing process on cryo-damage, physicochemical, and sensory quality attributes of beef loins.

Materials and Methods

Raw material and sample processing

At 2 d postmortem, paired beef loins (*M. longissimus lumborum*) from 30 beef carcasses (USDA [United States Department of Agriculture] Top Choice; total of 60 loins) were collected from a commercial abattoir. The loins were vacuum packaged and transported on ice to the Purdue Meat Science laboratory within 4 hr. Pairs of loins from every 2 carcasses were assigned to 4 aging periods (0, 2, 3, and 4 weeks) as a balanced incomplete block design (Figure 1). A posterior section of the loins subjected to aging for 4 weeks was stored for one additional week, serving as 5-week aged samples. Aging time was designated as A0, A2, A3, A4, and A5, corresponding to specific aging durations. The initial beef samples at 2 d postmortem and 5 weeks age served as non-aged control and long-term aged-only (never-frozen) control, respectively. After the completion of each assigned aging time in a 2°C cooler, three 1-inch-thick steaks were cut from each loin to serve as never-frozen samples (namely F0T). Two additional steaks (each for Warner-Bratzler shear force and sensory evaluation) were cut from 4-weeks-aged loin portion and aged for one more week serving as 5-weeks-aged sample. The remaining portions of the loins from each of the 4 aging periods were vacuum packaged, stored in cardboard boxes, and kept in a −20°C conventional blast freezer for the designated durations of 5, 3, 2, and 1 week, (F5T, F3T, F2T, and F1T, respectively; Figure 1), resulting a total of 5 weeks of aging and/or freezing periods. After each assigned freezing period, the loin samples were thawed at 2°C for 36 hr, and a total of 4 (one additional steak for sensory evaluation) 1-inch-thick steaks were collected as the first freezing/thawing samples. Remaining portions of the loins were re-packaged in vacuum bags and stored for an additional 5 weeks under the same freezing condition and then were thawed again to serve as repeatedly frozen/thawed samples, designated as F5T × 2, F3T × 2, F2T × 2, and F1T × 2.

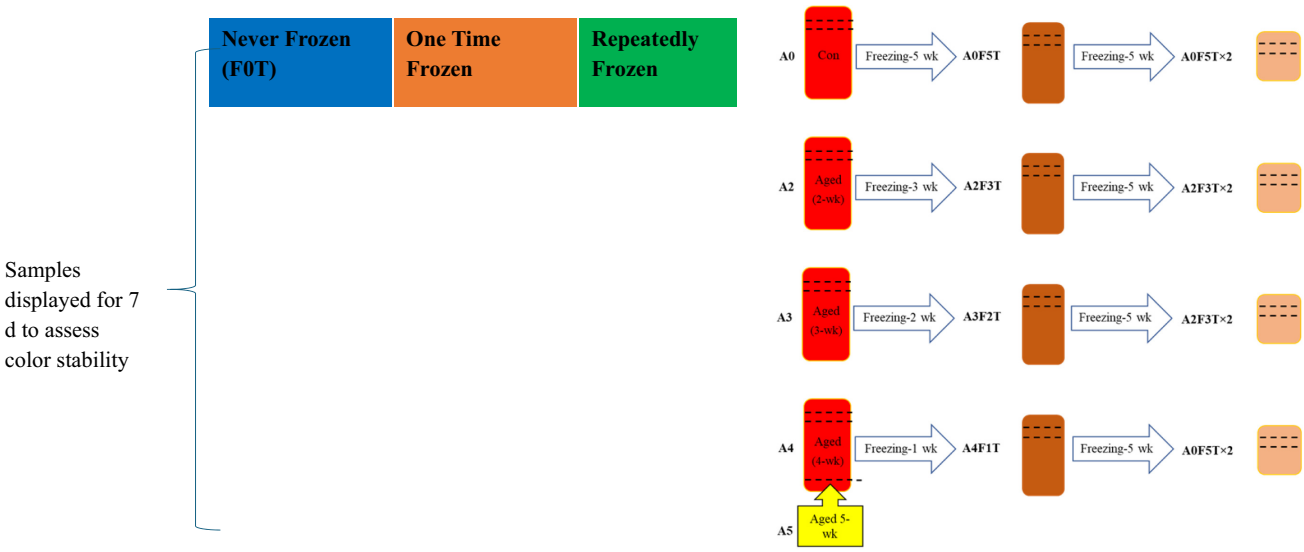

Figure 1. Schematic diagram illustrates the experimental design involving 13 aging/freezing combinations applied to beef strip loins. The combinations encompass various aging periods, freezing durations, and repeated freeze/thaw cycles. Loins highlighted in red represent those subjected to aging without freezing, while the remaining portions undergo distinct freezing/thawing processes. Dark brown indicates a single freeze-thaw cycle, and pale orange denotes repeated cycles. Each aging/freezing combination is systematically labeled based on aging duration, freezing time, and the repetition of freeze/thaw cycles. A0–A5 specifies the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

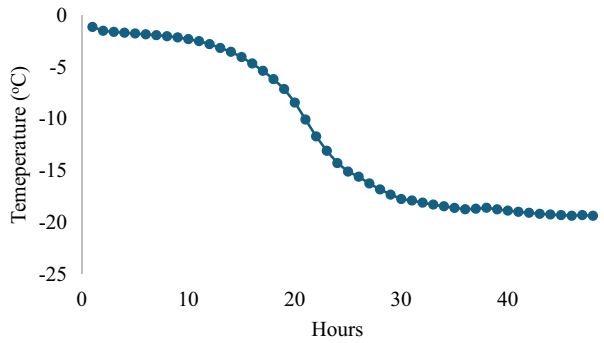

Figure 2. Temperature decline rate of loin muscles during blast freezing (−20°C).

Three 1-inch-thick steaks from sections were collected. The temperature changes during freezing and thawing were monitored using extra loins with T-type thermocouples (Omega Engineering, Stamford, CT, USA), which were plugged into an OctTemp 2000 data logger (Madge Tech, Inc., Warner, NH, USA). In brief, the loin samples took about 30 h to reach their core temperatures of −18°C (Figure 2), after which the temperature remained stable at −19.60 ± 0.49°C during frozen storage periods.

Moisture loss

Moisture losses during multiple post-handling processes including aging, freeze/thawing, displaying, and cooking, were measured as aging loss, freeze/thaw loss, display loss, and cooking loss to determine the extent of WHC. The percentage of aging loss for never-frozen loins was measured by recording the initial and final weights after the aging period, with purge removed and the surface blotted dry. The percentage freeze/thaw loss was calculated by measuring the weight of the loin before and after each freezing time. Similarly, for display loss of frozen/thawed samples, steaks cut for the retail display were weighed before being placed in a Styrofoam tray with a soaking pad and overwrap-polyvinylchloride (PVC) film packaged. After 1 week of retail display, each steak was weighed again to calculate the percentage display weight loss. In order to evaluate cooking loss (%), another steak (the same used for shear force analysis, described in a later section) samples were cooked on a clamshell griddle (Griddler GR-150, Cuisinart, Glendale, AZ, USA) at 180°C until the center temperature reached 71°C. Afterwards, they were cooled to 2°C, and to determine the cooking loss, the initial and final weights of samples were recorded. A T-type thermocouple and a data logger (OctTemp 2000, Madge Tech, Inc., Warner, NH, USA) were used to track the internal temperature.

Color measurements

Samples were packaged in oxygen-permeable PVC film and displayed under fluorescent light (1800 lx) for one week. Color stability of steaks

samples was assessed using a Hunter MiniScan EZ colorimeter (Hunter, Reston, VA, USA) equipped with a 25-mm (diameter) aperture. The colorimeter was calibrated using black and white calibration tiles, and the illuminant A with 10° standard observer was used. Commission Internationale de l'Éclairage (International Commission on Illumination) (CIE) L^*, a^*, and b^* values were recorded from 3 random locations on the surface of the beef muscles each day of the week. Collected CIE L^*, a^*, and b^* values were used to calculate chroma $[(a^{*2} + b^{*2})^{1/2}]$ and hue angle $[(b^*/a^*)\tan^{-1}]$ values (King et al., 2023).

Thiobarbituric acid reactive substances

Lipid oxidation of beef samples was determined by measuring thiobarbituric acid reactive substances (TBARS) following the published methods (Buege and Aust, 1978; H. Kim et al., 2018). Briefly, 5 g of powdered beef samples was homogenized with 15 mL of distilled water and 50 µL of 10% butylated hydroxyanisole. Then, 1 mL homogenate was vortexed with 2 mL of 20 mM 2-thiobarbituric acid in 15% trichloroacetic acid solution. After thoroughly mixing, samples were heated in a water bath set at 80°C for 15 min. Following cooling in ice water for 10 min, the samples were vortexed once more and then centrifuged at 2,000 *g* for 10 min. The supernatant was filtered through a Whatman Filter paper # 4 (Cytiva, Marlborough, MA, USA). The Epoch™ Microplate Spectrophotometer (BioTek Instrument Inc., Winooski, VT, USA) was used to read the absorbance of filtered samples at 531 nm. The TBARS value was calculated by multiplying the absorbance value with factor 5.54 and reported as mg malondialdehyde/kg meat.

Carbonyl content

Carbonyl content of samples was measured by using the methods described by Levine et al. (1994) and Vossen and Smet (2015) with modifications. Briefly, the powdered beef sample (2 g) was homogenized with 20 mL of the 20 mM phosphate buffer (pH 6.5) containing 0.6 M NaCl and 1 mM EDTA. To precipitate protein, 4 aliquots from each sample were taken and treated with ice-cold 750 µL 10% TCA for 15 min. Subsequently, the homogenates were centrifuged at 10,000 *g* for 15 min and the supernatant was removed. Two aliquots were treated with 500 µL 10 mM DNPH, and the other 2 were used as blank with the addition of 500 µL 3.0 M HCl. After thoroughly vortexing, samples were kept in a shaker for 1 h. Afterward, 250 µL of 40% TCA was vortexed thoroughly, kept in an ice bath for 15 min, and centrifuged at 10,000 *g* for 10 min. Pellets were washed twice with ethanol/ethyl acetate solution (1:1, v/v) and centrifuged at 10,000 *g* for 8 min. After washing, samples were left for 15 min under a hood for evaporation. Then, pellets were vortexed after adding 500 µL of 6 M guanidine-HCl in 20 mM phosphate buffer (pH 6.5) and were centrifuged at 10,000 *g* for 8 min. Absorbance was measured at 280 and 370 nm. Carbonyl content was then calculated by using the following equation:

$$C_{Hydrazone}/C_{Protein} = [A_{370}/(22000 M^{-1}cm^{-1} \times (A_{280} - A_{370} \times 0.43)] \times 10^6$$

Warner-Bratzler shear force

After the cooking loss determination, the cooked samples were used for the Warner-Bratzler shear force (WBSF) measurement. For this analysis, the method reported by Corrette et al. (2024) was used. Briefly, the samples were stored overnight in a 2°C cold room prior to the measurement. Parallel to the muscle fiber direction, a minimum of 6 cores (1.27 cm) were taken from each steak, avoiding any visible fat or connective tissue. A TA-XT Plus Texture Analyzer (Stable Micro System Ltd., Godalming, UK) adjusted for the WBSF measurement was used to shear collected cores perpendicularly at the speed of 3 mm/sec and the average peak shear force (*N*) was calculated.

Myofibrillar fragmentation index

To measure the myofibrillar fragmentation index (MFI), a modified version of the procedure by Culler et al. (1978) was used. Two grams of powdered beef samples was homogenized for 45 s in 20 mL MFI buffer (100 mM KCl, 20 mM potassium phosphate, 1 mM EGTA, 1 mM $MgCl_2$, and 1 mM NaN_3). The homogenized solution was centrifuged twice at 4°C at 1000 *g* for 15 min. The supernatant was removed, and the pellet was resuspended in 5 mL MFI buffer for a second centrifugation. Then, 5 mL MFI buffer was added again and vortexed to dissolve the pellet. Connective tissues in the solution were removed through a no. 18 polyethylene strainer. Protein quantification of beef samples was, further prepared in duplicate (0.25 mL beef MFI solution, 0.75 mL of MFI buffer, and 4 mL of biuret solution [1.5 g copper (II) sulphate pentahydrate, 6 g sodium potassium tartrate, 500 mL water, 300 mL 10% (w/v) sodium hydroxide, and 1 g potassium iodide]). The absorbances were read at 540 nm using a microplate spectrophotometer (Epoch, BioTek Instruments, Inc., Winooski, VT, USA) and compared

to a known concentration of bovine serum albumin (Fisher, USA) standard. Protein concentration was adjusted to 0.5 mg/mL by adding MFI buffer and absorbance was measured at 540 nm. Absorbance readings were multiplied by 200 to calculate the MFI value.

Western blotting

The extent of intact and degradation bands of desmin and troponin-T was measured using western blot. Instead of conducting western blotting for all 13 aging/freezing combinations, we focused on specific aging/freezing combinations of interest: including frozen/thawed with no aging (A0F0T, A0F5T, A0F5T × 2) and 4-weeks-aged samples (A4F0T, A4F1T, A4F1T × 2). SDS-PAGE and western blot were performed as described by Setyabrata and Kim (2019) with minor modifications. For SDS-PAGE, the polyacrylamide stacking gels (5%) were made using 100:1 acrylamide:bisacrylamide, 4 mL 1 M Tris-HCl buffer (pH 6.8), 120 µL ammonium persulfate (10% wt/vol) and 20 µL tetra-methyl-ethylene-diamine (TEMED). Polyacrylamide separating gels (15%) were made using 100:1 acrylamide:bisacrylamide, 10.5 mL 2 M Tris-HCl buffer (pH 8.8), 300 µL ammonium persulfate (10% wt/vol) and 30 µL TEMED. Gels were loaded with samples having 40 µg of protein per lane, along with an internal reference consisting of 4-week-aged beef samples. Electrophoresis was run at 130 V for 3 h (Hoefer Inc., Richmond, CA, USA), followed by protein transfer to polyvinylidene fluoride membranes (Millipore Corporation, Billerica, MA, USA) at 90 V for 1.5 h.

Membrane blockage was done by 5% nonfat dry milk powder dissolved in Phosphate-Buffered Saline with Tween (PBS-Tween) solution for 1 h (14 h for troponin-T at 4°C) prior to incubation with primary antibodies. Further, primary antibodies were diluted in PBS-Tween solution at 1:10,000 for monoclonal mice anti-desmin and anti-troponin-T (Sigma Aldrich, St. Louis, MO, USA) antibodies. The membranes were allowed to incubate for 16 h and 1 h for desmin and troponin-T, respectively at 4°C in the primary antibody. The membranes were rinsed 3 times for 10 min with PBS-Tween solution. These membranes were incubated with a secondary antibody (goat anti-mouse IgG [H + L] horseradish peroxidase conjugate; Bio-Rad, Hercules, CA, USA) in 3 PBS-Tween solutions (1:10,000) for 1 h. The membranes were then rinsed again 3 times in 10-min intervals. Pierce ECL western blotting reagents (ThermoFisher Scientific, Waltham, MA, USA) were used to recreate the protein bands. The bands were visualized and quantified using ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA). The ratio of band intensities of samples to those of the internal reference was calculated to determine the extent of protein abundance.

Consumer panel sensory evaluation

Consumer panel sensory evaluation ($n = 90$) was performed to evaluate the impact of stepwise aging/freezing on consumers' liking and acceptability Institutional Review Board (IRB exempt – 2023-16). Consumers were recruited from Purdue University's faculty, staff, and students. Five specific aging/freezing combinations, including A0F5T, A2F3T, A3F2T, A4F1T, and A5F0T (never-frozen control), were selected for the sensory evaluation. The steaks were cooked following the same procedure as cooking loss described above. After cooking, cubes (1 cm^3) were cut from each steak. The steak cubes (a minimum of 2 from each aging/freezing sample) were placed in 60 mL serving cups labeled with a 4-digit code pre-assigned to a given group and served in random order. In addition to the steak sample, each panelist was given water and unsalted crackers to cleanse the palate between samples. The sensory evaluation consisted of 15 sessions (6 panelists/session) and was performed under a red light. Panelists were asked to evaluate the samples for flavor, tenderness, juiciness, and overall liking (AMSA, 2015). Panelists used a linear hedonic test procedure (0 to 100 points) for likeness, with 0 as dislike extremely, 50 as neither like nor dislike, and 100 as like extremely. Acceptability of flavor, tenderness, juiciness, and overall was recorded as "yes" or "no," and the results were reported as the acceptability percentage, calculated as the number of "yes" responses out of the total number of panelists in each session. These percentages were then averaged across all sessions to determine the acceptability percentage of each attribute. Panelists were asked to categorize meat quality as "unsatisfactory," "everyday quality," "better than everyday quality," or "premium quality," with corresponding percent frequencies reported. The panelists were also asked to provide demographic information and beef consumption habits. Survey responses were recorded on electronic tablets (iPad, Apple Inc., Cupertino, CA, USA) via an online generated survey software (Qualtrics, Provo, UT, USA).

Statistical analysis

The experimental design was a split-plot with a balanced incomplete block design for the aging time and freeze-thawing frequency allocation. Each carcass

served as a block, and the aging times were randomly assigned to 4 loins from each of the 2 carcasses ($n = 15$) in which the whole loin underwent aging representing the main plot and the remaining portion of loins underwent freezing-thawing representing the sub-plots. The data analysis was conducted by using the PROC GLIMMIX procedure by SAS 9.4 (SAS Institute, Inc.; Cary, NC, USA). The interaction of aging time and freezing frequency was analyzed. Sensory data were analyzed using one-way analysis of variation. Additionally, animal (session for sensory) was included as a random effect. Significance (F-test, $P < 0.05$) was determined, and least-squares means were distinguished using least significant differences by Tukey's adjustment with the lines function.

Results and Discussion

Moisture loss

Across aging/freezing combinations, the freeze/thaw losses generally decreased with extended aging (Figure 3). The greatest freeze/thaw losses occurred in samples that were frozen and thawed without aging (A0F5T) and in those that were repeatedly frozen and thawed (A0F5T × 2; $P < 0.01$). In contrast, samples that were aged first and then frozen/thawed consistently showed reduced freeze/thaw losses with increased aging time ($P < 0.05$). Repeated freezing exhibited lower freeze/thaw losses until 2 weeks of aging times, but no difference was found between freezing frequency afterward ($P > 0.05$).

Display loss of frozen/thawed beef samples showed a similar pattern to the freeze/thaw loss results (Figure 4). The greatest display loss was observed in the frozen/thawed samples with no aging (A0F5T) among given aging/freezing combinations ($P < 0.05$). With an increase in aging times, however, the display loss of the frozen/thawed beef loins was decreased, regardless of the freeze/thaw frequency ($P < 0.05$). This observation reflects that during retail display, there would be a likelihood of greater release of exudate from steaks that were cut from frozen/thawed loins; however, steaks from aged-first then frozen/thawed loins had a considerably lower display loss compared to never-aged counterparts, suggesting the improvement of WHC of aged/frozen/thawed loins.

The cooking loss among groups is illustrated in Figure 5. No significant differences were found between never-frozen samples (A0F0T, A2F0T, A3F0T, A4F0T, and A5F0T), regardless of the aging periods ($P > 0.05$), indicating no impact of aging on cook loss. The same trend was observed in repeatedly frozen/thawed samples. The cooking loss in repeatedly frozen samples did not exhibit differences across age groups ($P > 0.05$).

Water-holding capacity is an important meat quality attribute, which is closely related to physicochemical attributes of meat, such as myofilament structure and net charge within muscle cells (Decker et al., 2024; Sales et al., 2020; Zhang et al., 2022). The freezing process is known to exacerbate water retention challenges (Lawrence et al., 2023) due to the formation of ice crystals, which rupture muscle cell membranes, compromising WHC (Setyabrata and Kim, 2019). In contrast, aging has a transformative influence on

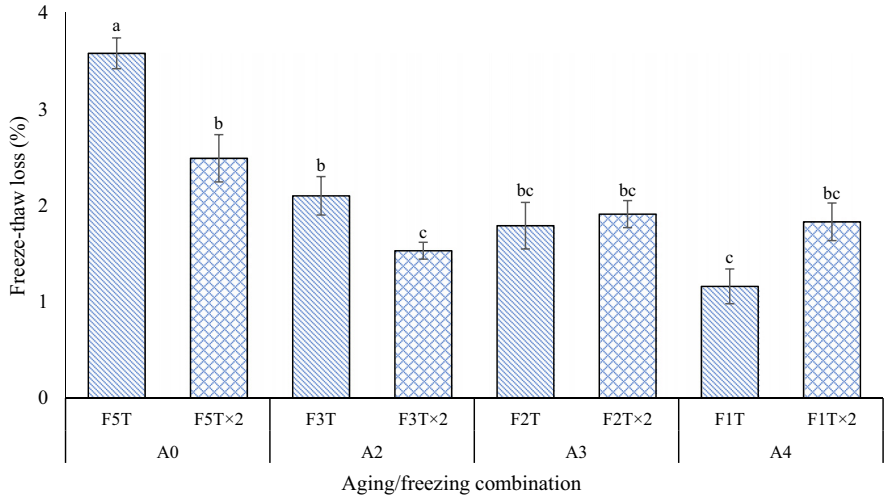

Figure 3. Impact of aging and freezing combinations on freeze/thaw loss percentage in beef strip loins. [a–c]Different letters show significant differences ($P < 0.05$) among aging/freezing combinations and error bars indicate standard error. A0–A4 specifies the number of weeks the samples underwent aging. F1–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

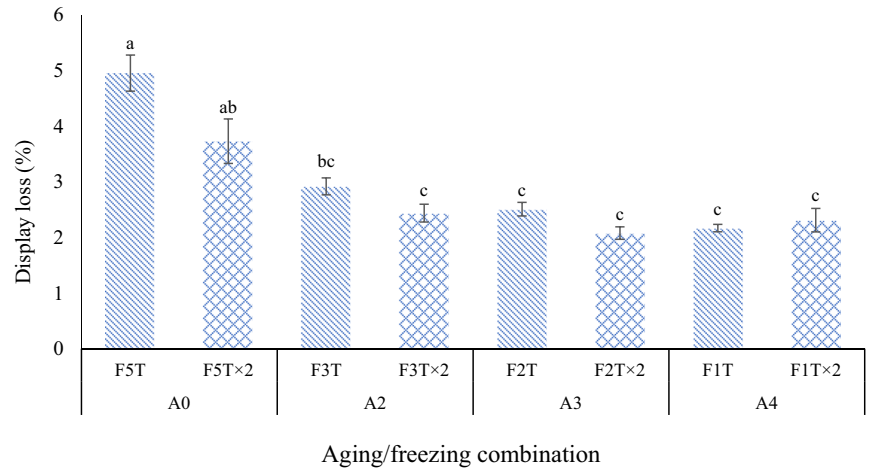

Figure 4. Impact of aging and freezing combinations on display loss percentage in beef strip loins. [a–c]Different letters show significant differences ($P < 0.05$) among aging/freezing combinations and error bars indicate standard error. A0-A4 specifies the number of weeks the samples underwent aging. F1–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

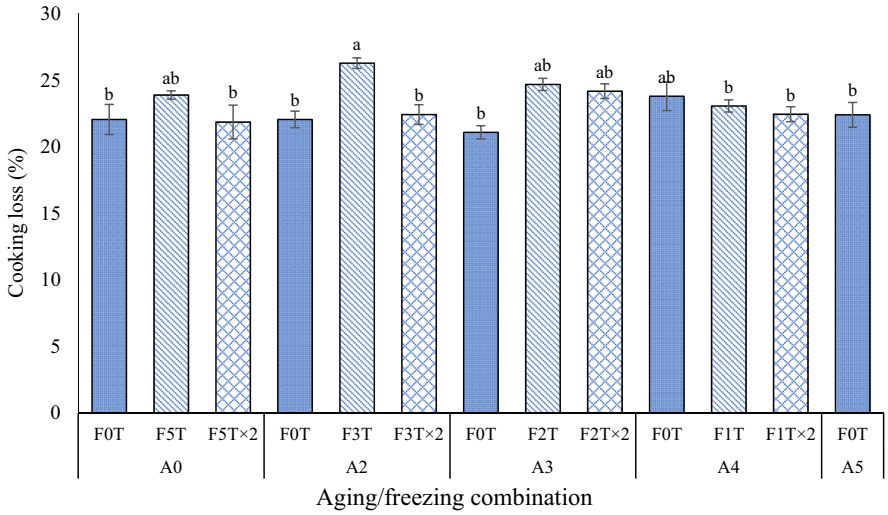

Figure 5. Impact of aging and freezing combinations on cooking loss percentage in beef strip loins. Distinct letters (a and b) indicate significant differences ($P < 0.05$) among the aging/freezing combinations and error bars indicate standard error. A0–A5 specifies the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

muscle fibers, creating a more moisture-entrapped matrix, which in turn improves WHC (Aroeira et al., 2016; Kim et al., 2020; Kominami et al., 2021). Results of our study also suggested that samples that were aged prior to freezing had a lower freeze/thaw and display loss. The proteolytic activity of endogenous enzymes during aging contributes to what is often termed a "spongy effect" in which these enzymes break drip channels responsible for water migration for drip loss, thereby promoting enhanced water retention (Farouk et al., 2012). Additionally, the microstructural modifications induced by aging, including increased spacing between muscle fibers, can impede the movement of water molecules, curtailing their ability to escape during freezing and thawing (Aroeira et al., 2016). The improvement of WHC of beef samples from the aged/frozen samples in the current study (shown by freeze/thaw loss and display loss results) would be likely attributed to the fragmentation of structural myofibrillar proteins with aging (Huff-Lonergan and Lonergan, 2005). After approximately 2 weeks of aging, the protein structures in the meat may reach a stabilized state. The modifications that occur during aging (e.g., protein degradation and subsequent changes in the muscle fiber matrix) may optimize the meat's ability to retain moisture. This stabilization

might explain why the freeze/thaw loss remains consistently low, regardless of further aging beyond 2 weeks.

Color attributes

In general, decreases in CIE L^*, a^*, b^*, and chroma values, accompanied by an increase in the hue angle of all samples, were found as the display time progressed (Table S1). Frozen/thawed samples exhibited reduced color stability compared to unfrozen samples, with hue angle values ($P < 0.05$), increasing significantly over time, particularly in repeatedly frozen samples.

The color of beef is closely linked to the myoglobin conversion process, which transforms it into deoxymyoglobin (DMb; purplish-red), oxymyoglobin (OMb; cherry-red), and metmyoglobin (MMb; brown) states (Aroeira et al., 2017). In vacuum-packaged meat, DMb predominates due to limited oxygen availability; however, during the blooming process, oxygen binds to myoglobin, resulting in the prevalence of OMb, which appears a cherry-red color that signifies freshness and color intensity (Cheng et al., 2020; Sales et al., 2020). As myoglobin oxidizes, OMb transitions to MMb, and the rate of this conversion influences color stability (Jeong et al., 2011).

In the context of frozen meat samples, a single freezing cycle during the initial days of display significantly increases a^* values, particularly in the A4F1T samples compared to A4F0T and A4F1T × 2 ($P <$ 0.05). This increase in redness is likely due to freezing-induced structural alterations, resulting in enhanced blooming (Kim et al., 2024); however, as display time progressed, redness diminished consistently across all samples. This effect was more pronounced in 2- and 3-weeks-aged frozen samples (A2F3T, A2F3T × 2, A3F2T, and A3F2T × 2) where a^* values decreased significantly more than non-frozen (A2F0T, and A3F0T) samples across 7 d ($P < 0.05$). Prolonged aging could lead to increased levels of MMb due to extended exposure to oxygen and a decreased rate of reduction conversion from MMb back to OMb. Freezing and/or thawing can degrade heme-containing proteins and disrupt enzymatic activities like metmyoglobin-reducing enzymes, impeding the reduction of MMb and contributing to discoloration (Jeong et al., 2011).

Thiobarbituric acid reactive substances

The lipid oxidation measured by TBARS values significantly increased with the increase in freeze/thawing cycles regardless of the aging time (Figure 6). Although freezing is a common method to preserve food in general, freezing-induced cryodamage initiates a cascade reaction on lipid oxidation. It may include the formation of ice crystals, rupture of muscle cells, exposure of the cell matrix, and the release of oxidants. These combined factors contribute to the acceleration of lipid oxidation of frozen beef (Y. Kim et al., 2018).

Also, within never-frozen samples, with an increase in aging time, TBARS values increased and significant differences were found between A0F0T,

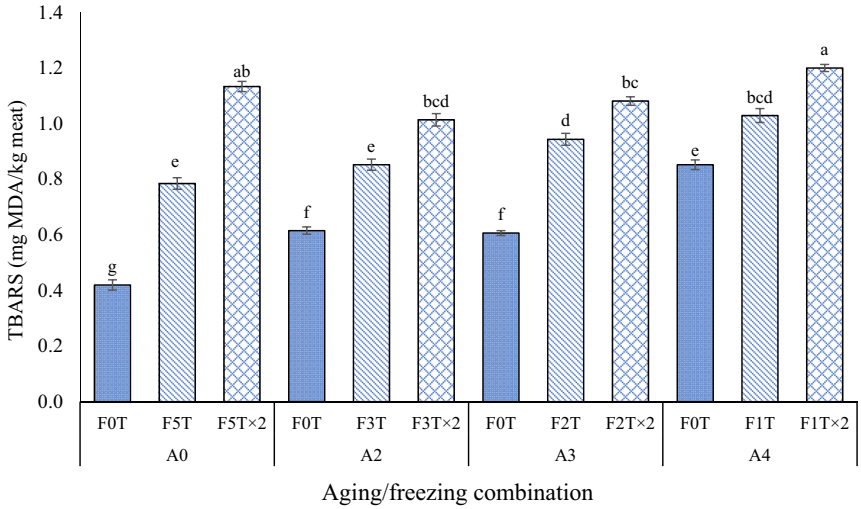

Figure 6. Effect of aging and freezing combinations on thiobarbituric acid reactive substances (TBARS) in beef strip loins. Distinct letters (a–g) indicate significant differences ($P < 0.05$) among the aging/freezing combinations and error bars indicate standard error. A0–A4 specifies the number of weeks the samples underwent aging. F0–F5 Specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

A2F0T/A3F0T, and A4F0T samples. This could be due to the release of oxidative enzymes and the deterioration of antioxidant substances with cellular changes during aging. Moreover, structural changes in the muscle membrane could also be another reason as the integrity of the muscle cell membrane weakens and loss of functionality (as a barrier) with aging (Cheng and Ockerman, 2003; Mungure et al., 2016). In this study, TBARS values increased with an increase in aging time and freezing duration and their combined effect was evident in aged and one-time and repeated frozen/thawed samples, indicating adverse impacts of aging/freezing/thawing on lipid oxidation of meat samples.

Carbonyl content

The formation of carbonyl compounds is one of the most noticeable changes in protein oxidation in the meat system (Estévez et al., 2011). Previous research has provided that the carbonylation of meat could be increased by the aging and freeze/thawing cycle (Estévez et al., 2011). The result of this study was also in accordance with the previous reports, showing increase in carbonyl content of loin samples with the increase in aging time and the number of freeze/thawing cycles, resulting in the least value on A0F0T and the greatest on A4F1T × 2 (Figure 7). The increase in carbonyl contents during aging can be attributed to biochemical changes, including the release of free-catalytic iron and oxidizing enzymes, as well as the propagation of lipid oxidation reactions (Lindahl et al., 2010; Liu et al., 2024). These processes result in the oxidation of proteins, leading to the formation of carbonyl groups on amino acid residues. Regarding the effect of freezing, the freezing-induced cryo-damage on muscle protein and disrupted protein structures made the proteins susceptible to oxidation. Amino acids, such as lysine, cysteine, methionine, histidine, and tryptophan, are known to be the most susceptible to oxidation and converted to carbonyl compounds (Park and Xiong, 2007). In this study, the combined effect of aging and freezing on meat was evident, as protein oxidation and carbonyl content increased with both aging time and freezing duration with the greatest value measured for A4F1T × 2 samples.

WBSF

The non-aged and never-frozen control samples (A0F0T) showed the greatest shear force value among all aging/freezing combinations (Figure 8; $P < 0.05$); however, without aging, the freeze/thaw samples had decreased WBSF values of beef steaks, where A0F5T × 2 had significantly lower WBSF values compared to A0F0T counterparts. The shear force value of A0F5T × 2 was not different from A5F0T samples ($P = 0.74$), indicating that the freeze/thawing process induces a substantial decrease in instrumental tenderness of beef loins. This suggests that repeated freezing of meat samples, even in the absence of aging, could yield shear force values comparable to those achieved after a 5-week aging period. The noted result could be attributed to cryo-damage induced by freezing, impacting the integrity of muscle fibers. This phenomenon aligns with findings by Biglia et al. (2022) reported that frozen samples exhibited significantly lower shear force

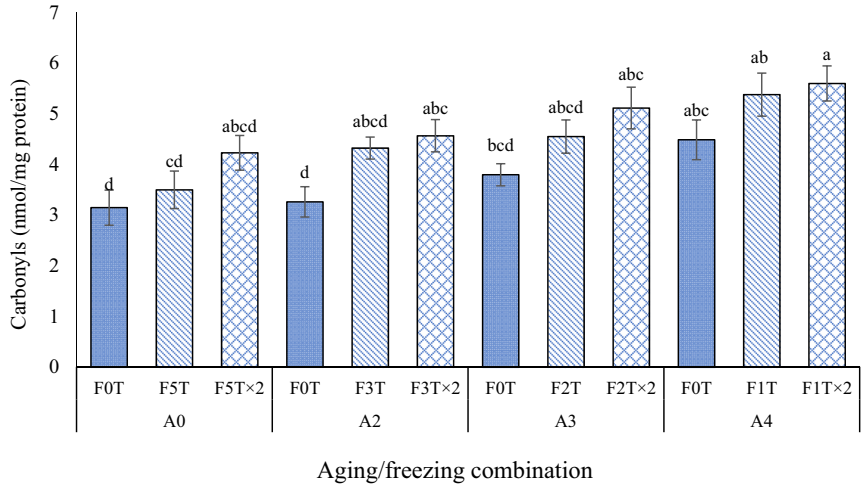

Figure 7. Influence of aging and freezing combinations on carbonyl content in beef strip loins. Distinct letters (a–c) indicate significant differences ($P < 0.05$) among the aging/freezing combinations and error bars indicate standard error. A0–A4 specifies the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

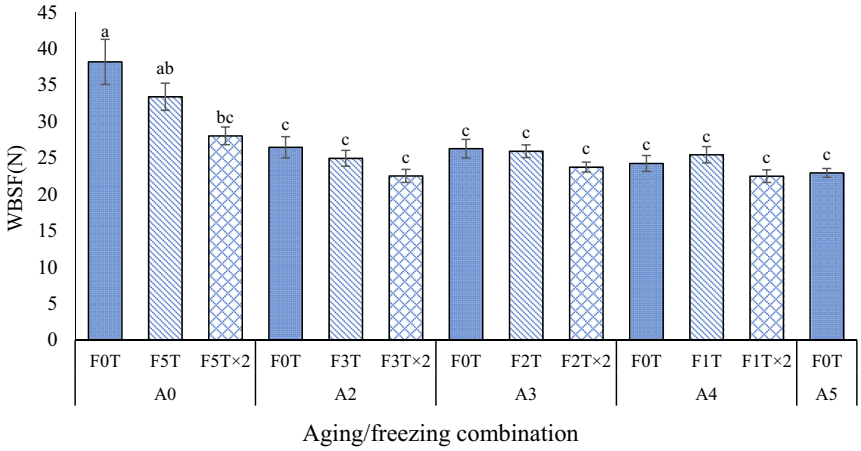

Figure 8. Impact of aging and freezing combinations on Warner-Bratzler shear force (*N*) in beef strip loins. Distinct letters (a–c) indicate significant differences ($P < 0.05$) among the aging/freezing combinations and error bars indicate standard error. A0–A5 specifies the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

compared to unfrozen ones, suggesting the possibility of using strategic freezing to expedite or simulate the tenderizing effects of conventional aging.

While aging decreased the WBSF values of the beef loins from A0F0T to A2F0T ($P < 0.001$), there were no further differences between all aged samples, regardless of freeze/thaw cycles ($P > 0.05$). This result is in agreement with the findings from Stolowski et al. (2006), where USDA Choice beef loins underwent aging for periods of 2, 14, 28, and 48 d, with a noted plateau in the WBSF value following 14 d of aging. This could potentially explain the current results where freeze/thaw cycles did not lead to a significant change in the shear force value for the loins of aforementioned aging durations.

MFI

The MFI, which shows the fragmentation of muscle structural proteins, increased with aging and freeze/thawing cycles (Figure 9; $P < 0.05$). A0F0T had the least MFI value among the aging/freezing combinations, while A4F0T had the greatest values ($P = 0.046$) indicating the increase in the extent of myofibrillar fragmentation with aging. Regardless of the aging status, freeze/thawing did not change MFI values significantly. Previously, it was reported that the MFI value could be increased by freezing coupled with aging (Feng et al., 2020; Kim et al., 2020) and (Aroeira et al., 2016; Sales et al., 2020). Although a numerical increase in MFI values was found, statistical analysis did not find these changes significant.

It has been known that the MFI values are highly correlated with the shear force (Aroeira et al., 2016). In the current study, MFI values of aged frozen/thawed beef samples were not different corroborating the outcomes of WBSF values of corresponding beef samples. For non-aged samples, no differences in MFI values between freeze/thawing cycles were aligned to WBSF values of corresponding beef samples. Freezing can cause physical damage to muscle fibers, leading to protein fragmentation; however, this effect was not determined through the MFI analysis in the current study. Therefore, further investigations including myofibrillar protein assays including western blotting are necessary to elucidate how cryo-damage in non-aged samples may have influenced muscle protein fragmentation.

Western blotting

The western blot result for desmin was in line with the WBSF results in part (Figure 10; Table 1). Freezing of non-aged samples (A0F5T) significantly decreased the extent of intact desmin, which was not different from the double-frozen beef samples (A0F5T × 2; $P = 0.99$). While aging increased the extent of desmin degradation when comparing A0F0T and A4F0T ($P < 0.01$), freeze/thawing resulted in greater degradation of intact desmin of beef samples when compared with non-aged counterparts (A0F5T and A0F5T × 2; $P < 0.05$). Desmin is one of the intermediate filament proteins located around the Z-disk, connecting the adjacent myofibrils longitudinally (Robson et al., 2004). Considering the effect of both aging and freeze/thawing on meat tenderization, there could be 2 possible explanations that may support the combined effects of aging/freezing on losing the myofibrillar structural integrity. Firstly, the ice crystal

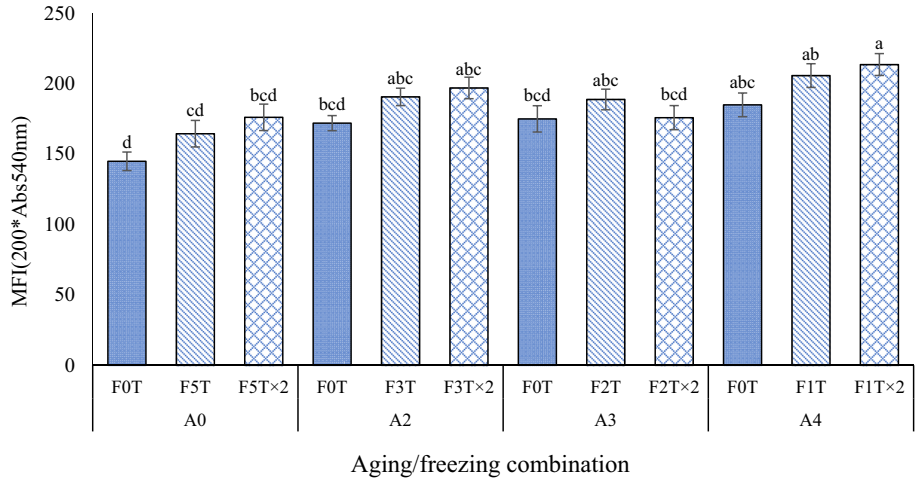

Figure 9. Impact of aging and freezing combinations on myofibrillar fragmentation index (MFI) in beef strip loins. Distinct letters (a–d) indicate significant differences ($P < 0.05$) among the aging/freezing combinations and error bars indicate standard error. A0–A4 specifies the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

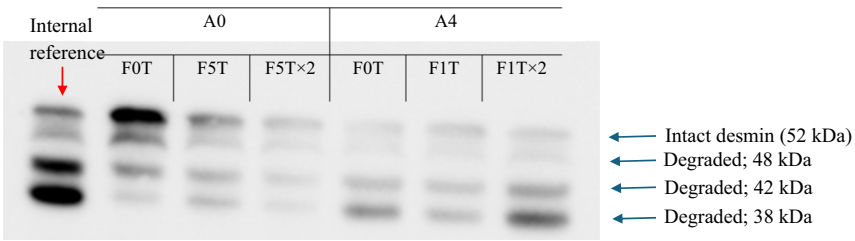

Figure 10. Representative image showing the influence of aging and freezing and repeated freezing on desmin content relative abundance. A0 and A4 specify the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

Table 1. Effect of different aging/freezing combinations on the relative abundance of desmin and troponin-T proteins

Aging/freezing combination		Intact desmin (52 kDa)	Degraded desmin (48 kDa)	Degraded desmin (42kDa)	Degraded desmin (38 kDa)	Intact troponin-T (35 kDa)	Degraded troponin-T (30 kDa)
A0	**F0T**	3.35^{a}	1.13	0.37^{b}	0.12^{b}	0.84	0.65^{b}
	F5T	2.24^{bc}	1.24	0.45^{ab}	0.20^{b}	0.90	0.67^{b}
	F5T × 2	2.40^{b}	1.32	0.52^{ab}	0.24^{b}	0.93	0.68^{b}
A4	**F0T**	1.42^{bcd}	0.87	0.67^{a}	0.53^{a}	0.92	0.89^{a}
	F1T	1.08^{d}	0.82	0.52^{ab}	0.73^{a}	1.03	0.90^{a}
	F1T × 2	1.33^{cd}	0.73	0.62^{a}	0.70^{a}	0.97	1.05^{a}
	SEM[1]	0.25	0.15	0.06	0.07	0.09	0.06
	***P* value**	<0.0001	0.06	0.01	<0.0001	0.48	<0.0001

A0 and A4: Specifies the number of weeks the samples underwent aging. F0–F5: Specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

[1]SEM, Standard error of means.

[a-d]Different superscripts within a column show differences among aging/freezing combinations ($P < 0.05$)

An internal reference consisting of 4-week-aged beef samples was used for band intensity normalization.

formation during the freezing could result in the fragmentation of intermediate filaments by damaging the muscle structures. Secondly, freezing could possibly decrease the activity of calpastatin, an inhibitor of calpains (Setyabrata and Kim, 2019). Thus, it may result in more proteolytic activity of calpain for structural protein degradation. These postulations would warrant further confirmation.

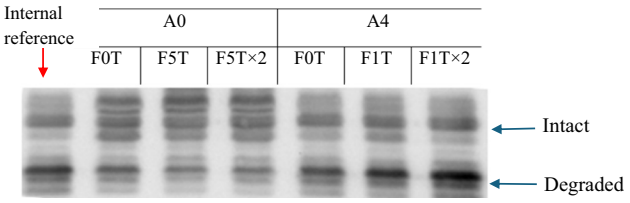

Figure 11. Representative image showing the influence of aging and freezing and repeated freezing on troponin-T protein intact and degraded content relative abundance. A0 and A4 specify the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

The degradation of desmin on non-aged samples is in agreement with the previous explanation. It is worth noting that in the 4-week-aged samples, regardless of their freezing status, no significant differences were observed (Figure 10). This may be attributed to the proteolytic degradation of desmin during the extended aging process. In contrast, desmin in non-aged frozen samples was significantly degraded compared to their never-frozen counterpart (Figure 10), suggesting a cryo-induced degradation, of intermediate filament proteins, in particular, upon freezing/thawing.

Troponin-T is a regulatory muscle protein essential for muscle contraction and relaxation by controlling the formation of cross-bridges between actin and myosin filaments. The extent of troponin-T degradation has been used as an indication of meat tenderization (Huff-Lonergan and Lonergan, 2005). Our study shows no significant change in troponin-T degradation within the same aging period, regardless of freezing frequency (Figure 11; Table 1); however, with extended aging of loin muscle, a significant increase in troponin-T degradation was observed. This aligns with the results from Setyabrata and Kim (2019), who reported no effect of freezing but a notable effect of aging on troponin-T degradation. Conversely, some studies have indicated that long-term freezing can reduce the intensity of intact troponin-T bands, suggesting the need for further investigation into ice-crystallization-induced degradation of myofibrillar proteins (Lu et al., 2020; Setyabrata et al., 2019).

Consumer panel sensory evaluation

Detailed demographic survey results including the consumer panelists' preferences and beef consumption traits are presented in Supplementary Table S2. Briefly, a majority of panelists (87.8%) consume beef twice or more per week. The panelists rated tenderness (43.3%) and flavor (42.2%) as the most important palatability traits. In the present study, the sensory evaluation revealed no differences in eating quality attributes ($P > 0.05$), regarding liking of tenderness, flavor, juiciness, and overall liking among the different aging/freezing combinations (Table 2). The lack of

Table 2. Effect of different aging/freezing combinations on consumer sensory panel responses (n = 90) regarding likeness, acceptability and overall quality perception

	Aging/freezing combination						
Aging time	A0	A2	A3	A4	A5		
Freezing cycle	F5T	F3T	F2T	F1T	F0T	SEM[1]	*P* value
Likeness (0–100)							
Tenderness	66.27	71.88	71.98	72.17	69.62	2.32	0.18
Flavor	66.77	68.23	70.37	72.09	68.07	2.15	0.26
Juiciness	67.33	68.54	65.47	69.73	65.89	2.43	0.56
Overall quality	67.11	70.49	69.60	72.51	68.71	2.41	0.35
Acceptability (%)							
Tenderness acceptability	83.33	91.11	92.22	94.44	88.89	0.19	0.14
Flavor acceptability	86.67	86.67	92.22	94.44	87.78	0.19	0.26
Juiciness acceptability	87.78	85.56	81.11	91.11	86.67	0.23	0.42
Overall acceptability	85.56	85.56	87.78	92.22	88.89	0.22	0.68
Perceived quality (%)							
Unsatisfactory	11.11	13.33	7.78	7.78	10.00	0.22	0.78
Everyday quality	51.11	33.33	43.33	43.33	47.78	0.31	0.16
Better than everyday quality	25.56	41.11	37.78	36.67	34.44	0.33	0.22
Premium quality	11.11	12.22	11.11	12.22	7.78	0.20	0.85

A0-A5 specifies the number of weeks the samples underwent aging. F0–F5 specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing.

[1]SEM, Standard error of means.

significant differences in eating quality attributes, especially tenderness, between the non-aged control (A0F5T) and the other aged samples was somewhat unexpected; however, it has to be noted that the WBSF values of A0F5T were below 40 *N*, which can be considered tender (Destefanis et al., 2008; Miller et al., 2001). Destefanis et al. (2008) reported that the consumer panel found it difficult to differentiate between 2 extreme categories, such as "very tough" (>62.59 *N*) and "tough" (52.78–62.59 *N*) or "very tender" (<32.96 *N*) and "tender" (42.77–32.96 N). Thus, the observed differences (23 *N* to 33 *N*) in instrumental tenderness (WBSF) between aging/freezing samples were not reflected in consumer sensory results in the present study, likely due to the limited discriminating ability of the panel within too close tenderness classifications.

While not significant, the A0F5T samples had numerically lower values in tenderness ($P = 0.18$) and tenderness acceptability ($P = 0.14$) compared with other aged/frozen samples (Table 2). The results also showed no differences in sensory attributes of beef loins subjected to aged/frozen/thawed and aged-only (never-frozen) controls ($P > 0.05$). This observation is partially in agreement with instrumental tenderness results, where no differences in WBSF were found among the aged/frozen/thawed beef loins (A2F3T, A3F2T, A4F1T) and long-term aged-only (never-frozen; A5F0T) control ($P > 0.05$). This indicates that the sensory quality of meat subjected to freezing/thawing remains comparable to fresh meat without freezing.

It is generally perceived that freezing can lower meat quality traits, including sensory characteristics (Curry et al., 2023; Fevold et al., 2024; Hati et al., 2021); however, several studies have reported either no negative effects (Correa et al., 2024; Tindel et al., 2018) or even improvements in the sensory quality of frozen meat (Beyer et al., 2024; Vieira et al., 2009), which is in agreement with our findings.

Conclusion

The results of the current study demonstrated the aging improved WHC of frozen/thawed as well as double-frozen meat, as evidenced by reduced freeze/thaw and display losses. Myofibrillar fragmentation index and desmin analyses showed structural changes in muscle fibers, with increased protein fragmentation due to aging and freezing-induced cryo-damage. Thus, aging can provide protective effects against moisture loss during freezing and thawing processes, possibly by fragmenting and disrupting organized cytoskeletal structural proteins in muscle fibers. Instrumental tenderness exhibited significant improvements with aging; however, freeze/thawing resulted in a substantial decrease in instrumental tenderness of beef loins at early postmortem. The study also found that repeatedly frozen/thawed samples were comparable to 5-week-aged, never-frozen controls, suggesting a potential avenue for enhancing tenderness through a strategic stepwise aging/freezing process. Further, sensory evaluation results indicated no significant differences in juiciness, tenderness, and flavor between aged/frozen/thawed loins and aged-only (never-frozen) controls. In conclusion, the findings of the present study suggest that stepwise aging/freezing can be a viable approach for the meat industry to consistently supply frozen beef with quality equivalent to fresh (never-frozen) meat.

Conflict Of Interest

The authors declare no conflicts of interest regarding the content of this manuscript.

Acknowledgments

Research funded by National Cattlemen's Beef Association, a contractor to the Beef Checkoff.

Author Contribution

S. Rehman contributed to data curation, methodology, data analysis, writing, and original draft preparation. J. K. Seo contributed to data curation, methodology, writing, reviewing, and editing. M. Romanyk contributed to data curation and methodology. D.J. Shin contributed to data curation, writing, reviewing, and editing, Y. H. B. Kim contributed to conceptualization, investigation, supervision, and editing, and funding acquisition

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Supplementary data:

Table S1. Effect of aging and freezing combinations on color stability of beef samples across 7 d of display period

Aging/freezing combination	Display time	CIE L^*	CIE a^*	CIE b^*	Chrome value	Hue-angle
A0F0T	**1**	35.61 $^{X, B}$	21.04 $^{XY, AB}$	19.44 $^{Y, A}$	28.69 $^{Y, A}$	42.95 ^{C}
	2	32.93 $^{Y, C}$	23.65 $^{X, A}$	24.20 $^{X, A}$	33.86 $^{X, A}$	45.88 ^{B}
	3	32.95 $^{Y, D}$	23.91 $^{X, A}$	24.88 $^{X, A}$	34.55 $^{X, A}$	46.32 ^{B}
	4	35.35 $^{X, A}$	20.60 $^{XY, B}$	20.43 $^{Y, B}$	29.04 $^{Y, B}$	44.85 ^{D}
	5	35.54 $^{X, ABC}$	19.39 $^{Y, B}$	19.67 $^{Y, B}$	27.66 $^{Y, B}$	45.52 ^{E}
	6	30.23 $^{Z, B}$	24.11 $^{X, A}$	24.48 $^{X, A}$	34.38 $^{X, A}$	45.62 ^{F}
	7	32.55 $^{Y, D}$	24.84 $^{X, A}$	25.98 $^{X, A}$	35.98 $^{X, A}$	46.51 ^{F}
A0F5T	**1**	36.08 ^{AB}	21.50 $^{X, A}$	20.33 $^{X, A}$	29.62 $^{X, A}$	43.56 $^{Y, BC}$
	2	36.65 ^{AB}	20.20 $^{XY, B}$	20.64 $^{X, B}$	28.90 $^{X, BC}$	45.76 $^{Y, B}$
	3	34.79 ^{BCD}	17.49 $^{Y, CD}$	18.84 $^{Y, B}$	25.73 $^{XY, CD}$	47.18 $^{XY, B}$
	4	35.15 ^{A}	16.67 $^{Y, D}$	18.05 $^{Y, CDE}$	24.59 $^{Y, D}$	47.35 $^{XY, BC}$
	5	35.19 ^{BC}	15.64 $^{YZ, D}$	18.24 $^{Y, BCD}$	24.05 $^{YZ, CD}$	49.53 $^{X, CD}$
	6	35.40 ^{A}	14.96 $^{YZ, CD}$	17.92 $^{Y, CD}$	23.38 $^{YZ, CD}$	50.38 $^{X, DE}$
	7	35.64 ^{BCD}	12.99 $^{Z, C}$	16.65 $^{Y, C}$	21.17 $^{Z, CD}$	52.44 $^{X, CDE}$
A0F5T × 2	**1**	38.11 ^{AB}	20.64 $^{X, AB}$	20.82 $^{X, A}$	29.35 $^{X, A}$	45.42 $^{Z, AB}$
	2	37.99 ^{A}	16.92 $^{Y, C}$	18.56 $^{XY, D}$	25.14 $^{XY, E}$	47.76 $^{YZ, A}$
	3	36.92 ^{ABC}	15.85 $^{Y, E}$	18.73 $^{XY, B}$	24.56 $^{XY, CD}$	49.90 $^{Y, A}$
	4	38.51 ^{A}	14.87 $^{Y, DE}$	18.71 $^{XY, CDE}$	23.92 $^{XY, D}$	51.67 $^{Y, A}$
	5	38.92 ^{AB}	12.94 $^{YZ, FG}$	16.99 $^{Y, CD}$	21.38 $^{Y, EF}$	52.93 $^{XY, AB}$
	6	38.67 ^{A}	12.39 $^{YZ, CDEF}$	16.95 $^{Y, CD}$	21.03 $^{Y, DE}$	54.13 $^{XY, ABC}$
	7	40.11 ^{A}	11.25 $^{Z, C}$	16.42 $^{Y, D}$	19.94 $^{Z, D}$	55.93 $^{X, ABCD}$
A2F0T	**1**	39.56 ^{A}	21.42 $^{X, A}$	20.44 $^{X, A}$	29.62 $^{X, A}$	43.69 $^{Y, BC}$
	2	39.54 ^{A}	20.37 $^{X, B}$	19.72 $^{X, BCD}$	28.36 $^{X, BCD}$	44.09 $^{Y, B}$
	3	39.41 ^{A}	19.52 $^{X, B}$	19.57 $^{X, B}$	27.65 $^{X, B}$	45.09 $^{XY, B}$
	4	37.24 ^{A}	18.67 $^{X, C}$	19.24 $^{X, C}$	26.82 $^{X, C}$	45.93 $^{XY, CD}$
	5	39.12 ^{A}	17.51 $^{XY, C}$	18.60 $^{X, BC}$	25.57 $^{X, C}$	46.87 $^{XY, DE}$
	6	38.45 ^{A}	16.87 $^{XY, B}$	18.39 $^{X, C}$	25.00 $^{XY, C}$	47.76 $^{XY, E}$
	7	36.91 ^{B}	15.42 $^{Y, B}$	17.45 $^{Y, C}$	23.35 $^{Y, B}$	49.00 $^{X, F}$
A2F3T	**1**	37.54 ^{AB}	21.59 $^{X, A}$	20.00 $^{X, A}$	29.44 $^{X, A}$	42.89 $^{Z, BC}$
	2	36.59 ^{AB}	20.42 $^{XY, B}$	20.36 $^{X, BC}$	28.85 $^{X, BC}$	44.92 $^{Z, B}$
	3	33.79 ^{BCD}	17.73 $^{Y, CD}$	18.29 $^{XY, B}$	25.48 $^{Y, CD}$	45.84 $^{YZ, B}$
	4	35.30 ^{A}	16.06 $^{Y, D}$	17.25 $^{Y, DE}$	23.59 $^{Y, D}$	47.02 $^{YZ, BC}$
	5	34.36 ^{C}	14.97 $^{YZ, DE}$	17.15 $^{Y, CD}$	22.79 $^{Y, DEF}$	48.96 $^{Y, CD}$
	6	34.92 ^{A}	13.99 $^{YZ, CDE}$	16.76 $^{Y, CD}$	21.87 $^{YZ, DE}$	50.39 $^{XY, CDE}$
	7	35.09 ^{BCD}	11.98 $^{Z, C}$	15.66 $^{Y, C}$	19.78 $^{YZ, D}$	53.07 $^{X, BCD}$
A2F3T × 2	**1**	37.50 ^{AB}	21.03 $^{X, AB}$	20.76 $^{X, A}$	29.57 $^{X, A}$	44.57 $^{Z, AB}$
	2	38.02 ^{AB}	17.74 $^{Y, C}$	19.70 $^{X, BCD}$	26.53 $^{XY, DE}$	48.06 $^{Y, A}$
	3	36.10 ^{ABCD}	16.46 $^{Y, DE}$	19.28 $^{XY, B}$	25.37 $^{Y, CD}$	49.54 $^{Y, A}$
	4	36.38 ^{A}	15.37 $^{YZ, DE}$	18.99 $^{XY, C}$	24.45 $^{Y, D}$	51.10 $^{XY, A}$
	5	36.77 ^{ABC}	13.33 $^{Z, EFG}$	17.36 $^{Y, CD}$	21.92 $^{YZ, DEF}$	52.64 $^{XY, AB}$
	6	38.12 ^{A}	12.09 $^{Z, EFG}$	16.90 $^{Y, CD}$	20.83 $^{Z, E}$	54.71 $^{X, AB}$
	7	37.10 ^{B}	11.19 $^{Z, C}$	16.14 $^{Y, C}$	19.70 $^{Z, D}$	55.66 $^{X, ABCD}$
A3F0T	**1**	37.43 ^{AB}	21.14 $^{X, AB}$	20.12 $^{X, A}$	29.20 $^{X, A}$	43.67 $^{Y, BC}$
	2	35.82 ^{BC}	20.20 $^{X, B}$	20.01 $^{X, BCD}$	28.44 $^{X, BCD}$	44.75 $^{Y, B}$
	3	36.36 ^{BCD}	18.61 $^{XY, BC}$	18.81 $^{XY, B}$	26.47 $^{XY, BC}$	45.37 $^{Y, B}$
	4	36.60 ^{A}	16.49 $^{Y, D}$	17.17 $^{Y, E}$	23.82 $^{Y, D}$	46.16 $^{Y, CD}$
	5	37.13 ^{ABC}	15.45 $^{Y, D}$	16.63 $^{Y, D}$	22.71 $^{Y, DEF}$	47.19 $^{XY, DE}$
	6	37.48 ^{A}	14.42 $^{Y, CD}$	16.19 $^{Y, D}$	21.70 $^{Y, DE}$	48.48 $^{XY, E}$
	7	37.37 ^{B}	12.81 $^{Y, D}$	15.89 $^{Y, C}$	20.44 $^{Y, CD}$	51.48 $^{X, DE}$

(*Continued*)

Table S1. (*Continued*)

Aging/freezing combination	Display time	CIE L^*	CIE a^*	CIE b^*	Chrome value	Hue-angle
A3F2T	**1**	38.15 $^{X, AB}$	21.90 $^{X, A}$	20.25 $^{X, A}$	29.85 $^{X, A}$	42.68 $^{Z, C}$
	2	36.53 $^{X, AB}$	19.97 $^{X, B}$	20.10 $^{X, BCD}$	28.34 $^{X, BCD}$	45.10 $^{YZ, B}$
	3	33.93 $^{Y, CD}$	17.34 $^{XY, CDEF}$	18.35 $^{XY, B}$	25.25 $^{XY, CD}$	46.55 $^{Y, B}$
	4	35.19 $^{XY, A}$	15.66 ± 0.43 $^{Y, DE}$	17.16 $^{Y, E}$	23.24 $^{Y, D}$	47.53 $^{Y, BC}$
	5	34.55 $^{Y, C}$	14.52 $^{Y, DEF}$	17.38 $^{Y, CD}$	22.66 $^{Y, DEF}$	50.11 $^{XY, C}$
	6	35.26 $^{XY, A}$	13.59 $^{Y, CDEF}$	17.32 $^{Y, CD}$	22.04 $^{Y, DE}$	52.04 $^{XY, BCD}$
	7	33.93 $^{Y, CD}$	11.59 $^{YZ, C}$	15.98 $^{Y, C}$	19.78 $^{YZ, D}$	54.22 $^{X, ABCD}$
A3F2T × 2	**1**	38.40 ^{AB}	20.52 $^{X, AB}$	20.66 $^{X, A}$	29.12 $^{X, A}$	45.22 $^{Z, AB}$
	2	37.97 ^{AB}	17.83 $^{X, C}$	19.92 $^{X, BCD}$	26.74 $^{XY, BCDE}$	48.20 $^{Y, A}$
	3	37.01 ^{ABCD}	15.52 $^{XY, E}$	18.85 $^{X, B}$	24.42 $^{Y, CD}$	50.52 $^{Y, A}$
	4	36.85 ^{A}	14.87 $^{XY, DE}$	18.93 $^{X, CD}$	24.07 $^{Y, D}$	51.90 $^{XY, A}$
	5	37.07 ^{ABC}	12.61 $^{Y, G}$	16.63 $^{Y, D}$	20.88 $^{Z, F}$	52.80 $^{XY, AB}$
	6	38.36 ^{A}	11.77 $^{Y, EF}$	16.99 $^{Y, CD}$	20.69 $^{Z, E}$	55.29 $^{X, AB}$
	7	36.41 ^{BCD}	10.60 $^{Y, C}$	15.74 $^{Y, C}$	19.00 $^{Z, D}$	56.18 $^{X, ABC}$
A4F0T	**1**	38.82 $^{X, A}$	19.09 $^{Y, B}$	17.86 $^{Y, B}$	26.16 $^{XY, B}$	43.10 $^{Z, BC}$
	2	38.67 $^{X, A}$	19.05 $^{Y, B}$	18.75 $^{Y, CD}$	26.73 $^{XY, BCDE}$	44.51 $^{Z, B}$
	3	37.97 $^{X, A}$	17.58 $^{Y, CD}$	18.23 $^{Y, B}$	25.32 $^{XY, CD}$	46.00 $^{YZ, B}$
	4	34.39 $^{Y, B}$	32.01 $^{X, A}$	28.57 $^{X, A}$	42.98 $^{X, A}$	41.85 $^{Z, E}$
	5	35.95 $^{Y, B}$	29.75 $^{X, A}$	28.06 $^{X, A}$	40.96 $^{X, A}$	43.44 $^{Z, F}$
	6	36.09 $^{X, AB}$	17.17 $^{Y, B}$	20.70 $^{XY, B}$	26.94 $^{XY, B}$	50.71 $^{Y, CDE}$
	7	35.87 $^{X, B}$	12.01 $^{Z, C}$	18.83 $^{Y, B}$	22.41 $^{Y, BC}$	57.99 $^{X, A}$
A4F1T	**1**	38.06 $^{X, AB}$	21.67 $^{X, A}$	20.30 $^{X, A}$	29.70 $^{X, A}$	43.14 $^{Z, BC}$
	2	37.38 $^{Y, AB}$	20.10 $^{XY, B}$	20.92 $^{X, B}$	29.00 $^{X, B}$	46.11 $^{Z, B}$
	3	34.94 $^{Y, BCD}$	17.30 $^{Y, CD}$	18.79 $^{XY, B}$	25.55 $^{Y, CD}$	47.33 $^{YZ, B}$
	4	36.28 $^{X, A}$	15.87 $^{Y, D}$	17.95 $^{Y, CDE}$	23.97 $^{Y, D}$	48.50 $^{YZ, B}$
	5	35.25 $^{X, BC}$	15.01 $^{YZ, DE}$	18.32 $^{Y, BCD}$	23.70 $^{Y, CDE}$	50.72 $^{Y, BC}$
	6	35.21 $^{Z, A}$	13.61 $^{Z, CDEF}$	17.65 $^{Y, CD}$	22.34 $^{YZ, DE}$	52.55 $^{XY, ABCD}$
	7	34.80 $^{Y, BCD}$	10.31 $^{Z, C}$	16.02 $^{Y, C}$	19.12 $^{Z, D}$	57.35 $^{X, AB}$
A4F1T × 2	**1**	39.92 ^{AB}	19.19 $^{X, B}$	20.24 $^{X, A}$	27.91 $^{X, A}$	46.57 $^{Z, A}$
	2	38.63 ^{AB}	16.67 $^{XY, C}$	19.25 $^{X, BCD}$	25.48 $^{XY, E}$	49.07 $^{Z, A}$
	3	36.68 ^{ABCD}	15.32 $^{Y, E}$	18.73 $^{X, B}$	24.21 $^{Y, D}$	50.76 $^{YZ, A}$
	4	38.09 ^{A}	14.01 $^{Y, E}$	18.36 $^{X, CDE}$	23.11 $^{Y, D}$	52.74 $^{Y, A}$
	5	37.21 ^{ABC}	12.27 $^{YZ, G}$	16.62 $^{XY, D}$	20.68 $^{Z, F}$	53.63 $^{XY, A}$
	6	37.98 ^{A}	11.45 $^{YZ, F}$	16.85 $^{XY, CD}$	20.41 $^{Z, E}$	55.94 $^{X, A}$
	7	38.25 ^{B}	10.37 $^{Z, C}$	16.04 $^{Y, C}$	19.13 $^{Z, D}$	57.28 $^{X, AB}$
SEM[1]		0.78	0.49	0.40	0.56	0.68
***P* value**		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

A0–A4: Specifies the number of weeks the samples underwent aging. F0–F5: Specifies the number of weeks the samples underwent the initial freezing. "T" is denoted for thawing and "× 2" denotes repeated freezing.

[1]SEM, Standard error of means.

The superscript symbols used in the table indicate the significance levels resulting from statistical tests conducted to compare aging/freezing combinations ($P < 0.05$).

$^{X-Z}$Highlights significant differences observed over the course of 7 d within the same samples undergoing similar aging/freezing conditions.

$^{A-G}$Denotes significant differences among the aging/freezing combinations on the same display days.

Table S2. Demographic characteristics of consumer sensory evaluation panelist ($n = 90$)

Demographic questions	Response options	Frequency (%)
Gender	Female	48.9
	Male	50.0
	Other	1.1
Household size	1 person	28.9
	2 people	35.6
	3 people	11.1
	4 people	14.4
	5 people	5.6
	>5 people	2.2
	Prefer not to disclose	2.2
Marital status	Married	53.3
	Single	45.6
	Prefer not to disclose	1.1
Age	<20 years old	2.2
	20 to 29 years old	41.1
	30 to 39 years old	27.8
	40 to 49 years old	15.6
	50 to 59 years old	10
	>60 years old	3.3
Ethnic origin	African American	4.4
	Asian	24.4
	Caucasian/white	51.1
	Hispanic	7.8
	Mixed race	3.3
	Other	7.8
Annual household income	<$25,000	27.8
	$25,000 to $34,999	13.3
	$35,000 to $49,999	5.6
	$50,000 to $74,999	15.6
	$75,000 to $99,999	6.7
	$100,000 to $149,999	13.3
	$150,000 to $199,999	5.6
	>$199,999	4.4
	Prefer not to disclose	7.8
When eating beef, what palatability trait is the most important to you?	Flavor	42.2
	Juiciness	14.4
	Tenderness	43.3
When eating beef, what degree of doneness do you prefer?	Rare	2.2
	Medium rare	32.2
	Medium	25.6
	Medium well	20.0
	Well done	20.0
How many times per week do you consume beef?	0 time/week	2.2
	1 time/week	10.0
	2 times/week	26.7
	3 times/week	18.9
	4 times/week	13.3
	5 times/week	12.2
	6–10 times/week	11.1
	> 10 times/week	5.6