Meat and Muscle Biology™

Color Evaluation and Sensory Analyses of Beef Subprimals Following Extended Frozen Storage

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Abstract: Thirty-six strip loins and top sirloin butts were collected from USDA Low Choice, "A" maturity carcasses and were assigned to freezing treatment. Subprimals were fabricated into *Gluteus medius* (GM) and *Longissimus lumborum* (LL) steaks. Beef steaks were evaluated during a 28-d retail display following freezing as a subprimal (SNGL), subprimal and steak (DBL), or never frozen (CON). Steaks were evaluated in 2 packaging films: standard/traditional rollstock packaging (ROLL) and sodium nitrite-embedded (NIT). Steak color was evaluated every 12 h instrumentally and visually every 24 h. Spoilage organisms, lipid oxidation, and purge loss analyses were conducted on days 0, 14, and 28. Trained sensory analyses were conducted on steaks without display. Color data and purge loss were analyzed using a generalized mixed linear model, while other analyses utilized a mixed model with freezing, packaging, and display as fixed effects. Redness scores of GM and LL were greatest in DBL-NIT steaks on day 0 ($P < 0.05$); LL in NIT had increased ($P < 0.05$) redness values compared with ROLL on day 28. The a^* values of LL and GM were increased ($P < 0.05$) in NIT on day 0 and 14 compared with ROLL. Aerobic counts, Enterobacteriaceae, and lactic acid bacteria of GM and LL were increased ($P < 0.05$) on day 28 compared with 0. In LL steaks, there was a packaging × display interaction with increased ($P < 0.05$) MDA in ROLL on day 28 compared with the NIT across all timepoints. Initial juiciness was reduced in GM from SNGL compared with CON and DBL ($P < 0.05$). Juiciness was reduced in SNGL and DBL compared with CON of LL ($P < 0.05$). Freezing beef subprimals had minimal influences on descriptive sensory attributes. Markers of beef color varied little due to freezing regimen after the first day of display, while NIT improved color performance.

Key words: beef, color, freezing, nitrite-embedded packaging
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Introduction

Consumers largely use meat color to determine freshness, quality, and their willingness to purchase meat products (Carpenter et al., 2001; Holman et al., 2016). It is well established that consumers gravitate towards bright-cherry red steaks that are associated with the formation of oxymyoglobin (Killinger et al., 2004). Yet as producers and consumers desire packaging systems that extend the shelf-life and freshness of meat products, there is a challenge of maintaining the optimum meat color in these systems. While vacuum-packaged steaks have improved color stability and decreased lipid oxidation during long-term retail display (Reyes et al., 2022), producing desirable red meat color becomes more challenging when utilizing a zero- or low-oxygen environment. Vacuum packaging drives myoglobin to a deoxymyoglobin state (Mancini and Hunt, 2005), which is associated with more of a deep red purple color rather than the desired cherry red color. Thus, there has been further emphasis on packaging technologies that will fix or improve meat color so that it is presented as more suitable to consumers. With this emphasis, there has been an increase in the use of nitrite-embedded vacuum packaging that will increase the red color of meat and extend shelf-life to

19 d of fresh beef during retail display and 39 d of frozen retail display (Claus and Du, 2013).

To further extend the shelf-life of steaks and beef subprimals, the occurrence of freezing meat has increased in recent years across packers, retailers, and consumers (Muela et al., 2016; Wang et al., 2020; Qian et al., 2021; Curry et al., 2023). During the freezing process, there is formation of ice crystals, which may lead to disruption in the structure of proteins and muscle ultrastructure (Xia et al., 2010; Arsiccio et al., 2020; Hu and Xie, 2021). The disruption of these proteins may lead to impaired quality and color stability following the thawing process. With repeated freeze-thaw cycles, there is a reduction in color values and water holding capacity (WHC) (Rahman et al., 2014; Cheng et al., 2019). Extended periods of freezing can increase the formation of lipid oxidation products (Vieira et al., 2009), and the thawing process may accelerate the formation of these products (Rahman et al., 2015). Thus, there must be a balance between the freezing of meat products for further profit or convenience and the potential for decreases in product quality and consumer acceptability.

While the impact of freezing individual steaks and the effect on color stability and lipid oxidation has been well-stablished across the literature (Soyer et al., 2010; Hergenreder et al., 2013; Setyabrata and Kim, 2019), there is little research on the performance of steaks that were fabricated from frozen subprimals and refrozen on the same parameters. The current study aims to evaluate the influence of subprimal freezing, a refrozen steak, and fresh product on the color stability and meat quality parameters in steaks following a 28-d display in retail cases in two different vacuum packaging films.

Materials and Methods

Animal care and use approval was not required through the Texas Tech University's Institutional Animal Care and Use Committee as all samples were collected from a USDA-inspected packing plant following harvest.

Product selection and subprimal fabrication

A total of 72 beef subprimals, 36 strip loins (Institutional Meat Purchasing Specifications #180), and 36 top sirloin butts (Institutional Meat Purchasing Specifications #184) were collected from 36 sides of individual USDA Low Choice, "A" maturity beef from a commercial processing facility equally across 3 collection replicates. Data representing all USDA yield and quality grade carcass characteristics, including back fat thickness, ribeye area, marbling scores, and lean maturity, were recorded at the time of collection. Strip loins and sirloin top butts were identified, tagged, and collected from a single side of a beef carcass, then vacuum packaged and transported to the Gordon W. Davis Meat Laboratory at Texas Tech University. Subprimals were then aged under vacuum at 4 °C until 12 d postmortem. After the 12-d aging period, each side of beef was randomly assigned to one of 3 freezing treatments: (1) frozen as a subprimal for 6 mo (SNGL; $n = 12$); (2) frozen as a subprimal for 3 mo, thawed and fabricated into steaks then frozen for an additional 3 mo (DBL; $n = 12$); and (3) a fresh group that was never frozen (CON; $n = 12$). Subprimals from the CON group were collected 12 d prior to steaks being placed into the retail case to ensure equal fresh postmortem aging. Each subprimal was fabricated to remove accessory muscles and excessive connective tissue while isolating the *M. gluteus medius* (GM) and *M. Longissimus lumborum* (LL). Each muscle was split into a cranial and caudal section that was randomly allocated to either a control rollstock vacuum packaging (ROLL) or a sodium nitrite-embedded rollstock vacuum packaging (NIT). Within each cranial and caudal section, the muscle was further subset into 3 locations for retail display timepoints of 0, 14, or 28 d where 2 steaks would be sliced, the first for sensory analysis and the second for spoilage organism and other analyses. Steaks were cut into 2.54-cm steaks using a table-top meat slicer (X13E-PLUS, Van Berkel USA Inc., Doral, FL) moving from the anterior to the caudal portion of each muscular subsection. Steaks were individually vacuum packaged using a thermoformer packaging system (F100, MULTIVAC, Kansas City, MO) with a non-forming web of 406 mm × 609 m (T6240B, Sealed Air Corp., Charlotte, NC) and the respective forming web of 422 mm × 457 m (T7080B, Sealed Air Corp., Charlotte, NC) for CON and the nitrite-embedded forming web of 422 mm × 457 m (23072FL, Sealed Air Corp., Charlotte, NC) for NIT. Steaks were allowed to color cycle and thaw in their respective packaging environment for 3 d prior to being placed into the retail display case. Steaks were placed in coffin style retail cases (M1-GEA, Hussmann, Bridgeton, MO, USA) under continuous fluorescent lighting (1495 ± 376 lux) for 28 d. Temperature was monitored and recorded every 12 h (3.1 ± 2.0 °C)

Color evaluation

Panelists were trained to evaluate steaks for redness and percent discoloration according to the

AMSA color guidelines (King et al., 2023). All attributes were scored on a 100-point scale. For redness, 0 represented dark brown, 50 represented slightly dark red/purple, and 100 represented very bright red. A discoloration score of 0 represented no discoloration, while a discoloration score of 100 represented complete discoloration. Steaks were evaluated by a minimum of 6 trained panelists ($n = 6$) every 24 h for 28 d using a ballot on a tablet (Qualtrics Surveys, Provo, UT; iPad, Apple Inc., Cupertino, CA).

Concurrently with the trained visual observations, a Hunterlab Miniscan EZ 4500 (Hunter Associates Laboratory Inc., Reston, VA) spectrophotometer using A illuminant with a 10° observer angle and 3.175 cm aperture size was used to evaluate the color of the steaks every 12 h for the 28-d display period. At each timepoint, 3 scans were taken of each steak to achieve an accurate representation of the sample and then averaged for values used in the analyses. The values of interests were L^* as a marker of blackness to whiteness, a^* as a marker of greenness to redness, and b^* as a marker of blueness to yellowness. Absorbances of 470, 530, 570, and 700 nm wavelengths were used to calculate the state of myoglobin. Metmyoglobin (MMb) was calculated by dividing the absorbance at 470 nm by the absorbance at 530 nm, and deoxymyoglobin (DMb) was calculated by dividing the absorbance at 570 nm by the absorbance at 530 nm.

Spoilage organism

At 0, 14, and 28 d of retail display, beef steaks were evaluated for spoilage organisms. Pre-hydrated 25 mL swabs were taken with a 50 cm^2 template on the steaks surface. Swabs were homogenized for 1 min at 230 RPM using an automated stomacher (Steward Laboratory Systems, Davie, FL) to ensure complete homogenization of the sample. Beef steaks were evaluated for aerobic plate counts (AC), *Enterobacteriaceae* (EB), and lactic acid bacteria (LAB) using the TEMPO enumeration system. The TEMPO cards were incubated at 35 °C for 24 h. Psychrotropic aerobic plate counts (APC-P) were performed using petrifilms, and they were incubated at 7 °C for 10 d. The APC-P were done using the 3M Petrifilm Plate Reader and checked in a standard colony counter.

Purge loss

Steaks were weighed in their packages prior to being placed into retail display on day 0. On each pull day, 0, 14, and 28, the packages were weighed after removal from the case. The packages were then cut open, and steaks were patted dried with a paper towel and weighed. The film was dried using a paper towel. Purge weight was calculated as the difference between the package weight and the weight of the steak and film. The purge was then calculated as a percent relative to weight to normalize between steaks of different sizes.

Lipid oxidation

The thiobarbituric acid reactive substances (TBARS) procedure was used to determine lipid oxidation of samples. The method was modified from Buege (1978) and Luque et al. (2011); in brief, a solution of 3M 1,1,3,3-tetra-ethoxypropane was used to create a standard and was combined with trichloroacetic and thiobarbituric acid (TCA/TBA). A butylated hydroxyanisole (BHA) solution was also made by combining 10 g and then bringing to volume with 90% ethanol in a 100 mL volumetric flask. On 0, 14, and 28 d of retail display, samples were flash frozen in liquid nitrogen and then homogenized into a powder using a blender (nutribullet 900 Pro, nutribullet LLC, Los Angeles, CA). Samples were weighed out (5 g ± 0.1), and then 15 mL of deionized water was added to the sample. Samples were vortexed and then centrifuged for 10 min at 1850 × g. After centrifugation, 2 mL of the clear supernatant was transferred into a new tube; then, 4 mL of the TCA/TBA solution and 100 µL of the BHA solution was added to each tube. The sample was vortexed for 1 min and then placed into a water bath at 100 °C for 15 min. After the hot water bath, the samples were immediately placed into an ice bath for 10 min. Samples were centrifuged for 10 min at 1850 × g. Each sample was pipetted in duplicate placing 200 µL into each well of a 96-well plate. Once all samples and the standard curve were loaded onto the plate, the sample absorbance was analyzed at 531 nm using a microplate spectrophotometer (Agilent BioTek Epoch Microplate Spectrophotometer, BioTek Instruments Inc., Winooski, VT). Using the standard curve, the absorbance of each sample was then used to calculate the amount of malondialdehyde (MDA) in each sample on a mg/kg basis.

Descriptive sensory analysis

Steaks never subjected to retail display, day 0, were thawed at 4 °C for 24 h prior to cooking in a combi-oven (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) at 204 °C with 0% relative humidity to reach an internal temperature of 71 °C. Cooking doneness was validated by inserting a thermocouple into the geometric center of the steak. Raw and cooked

weight in addition to peak temperature of all steaks was recorded.

Panelists were trained for 20 h in accordance with the AMSA sensory guideline (AMSA, 2015) to conduct sensory analysis. Panelists were trained to identify and quantify 14 unique attributes (Table 1) from Adhikari et al. (2011) and AMSA (AMSA, 2015). All attributes were rated on a 100-point scale, with 0 representing an undetectable attribute and 100 representing an extremely intense flavor. During each panel, a minimum of 6 panelists ($n = 6$) evaluated 12 steak samples. Prior to serving, steaks were trimmed of excess fat and connective tissue. After trimming, steaks were cut into 1.27 cm^3 cubes (1/2 Sensory Box, Tallgrass Solutions Inc., Manhattan, KS). Before receiving each sample, panelists were instructed to cleanse their palate with apple juice, saltless soda crackers, and distilled water. Each panelist was given at least 2 cubes per sample for evaluation under red gel lights, and flavor attributes were recorded using a digital survey on a tablet (Qualtrics Surveys, Provo, UT; iPad, Apple Inc., Cupertino, CA). During each panel, all panelists were provided an expectorant cup, napkin, and toothpick.

Statistical analyses

All data were analyzed using the statistical software SAS version 9.4 (Cary, North Carolina, USA). For all measures, data were split by muscle, with fixed effects of freezing treatment, packaging film, and days of retail display and their respective interactions. Alpha was set at $P \leq 0.05$ for significance for all fixed effects. Covariates were tested and removed from the model in a stepwise manner where $P > 0.10$. Pairwise comparisons of significant effects were found using the PDIFF option within the LSMEANS statement.

A generalized mixed linear model was used to analyze both instrumental and trained color data as well as purge loss. Covariates included replicate of the study and panelist where applicable. Carcass was included as a random effect. The Kenward-Rogers adjustment was used to estimate the denominator degrees of freedom.

Table 1. Targeted beef flavor descriptors and anchors adapted from Adhikari et al. (2011) and AMSA (King et al., 2023).

Attribute	Definition	Reference
Beef Identity	Amount of beef flavor in a sample	Swanson Beef Broth = 31 80% Lean Ground Beef = 44 Beef Brisket = 69
Bitter	Fundamental taste factor associated with a caffeine solution	0.01% Caffeine Solution = 13 0.02% Caffeine Solution = 22
Bloody/Serumy	The aromatics associated with blood in cooked meat products. Closely related to metallic	USDA Choice Strip Steak 60 °C = 34 Beef Brisket 60 °C = 38
Brown/Roasted	Round, full aromatic associated with beef suet that has been broiled	Beef Suet = 50 80% Lean Ground Beef = 63
Cardboard	Aromatic associated with slightly oxidized fats and oils, reminiscent of wet carboard packaging	Dry Cardboard = 31 Wet Cardboard = 44
Fat-Like	Aromatics associated with cooked animal fat	Hillshire Farm Lit'l Smokies = 44 Beef Suet = 75
Juiciness	Perceived amount of moisture from sample	Eye of Round 85 °C = 25 USDA Choice Strip Steak 70 °C = 50
Liver-Like	Aromatics associated with cooked organ meat/liver	Beef Liver = 47
Metallic	Impression of slightly oxidized metal, such as iron, copper, and silver spoons	0.010% KCl Solution = 9 USDA Choice Strip Steak 60 °C = 38 Dole Canned Pineapple Juice = 38
Refrigerator Stale	Aromatics associated with products left in the refrigerator for an extended period of time	80% Lean Ground Beef Cooked and Chilled Overnight = 38
Sour	Fundamental taste factor associated with citric acid	0.015% Citric Acid Solution = 9 0.050% Citric Acid Solution = 22
Tenderness	Perceived force needed to chew meat	Eye of Round Steak 70 °C = 30 USDA Choice Strip Steak 70 °C = 63 Tenderloin Steak 65 °C = 88
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids, and other nucleotides	0.035% MSG Solution = 47

A mixed model was used to analyze measures of lipid oxidation, spoilage organisms, and descriptive sensory data. Fixed effects of freezing treatment, packaging film, and retail display were included for lipid oxidation and spoilage organisms, while only fixed effects of freezing treatment and packaging film were used for descriptive sensory analysis. Covariates of replicate and plate were included for lipid oxidation with a random effect of carcass. Replicate was included as a covariate for spoilage organisms. Descriptive sensory analyses included location of steak, side of subprimal, trial replicate, cook order, peak internal temperature, panel, and panelists as covariates.

Results

Color evaluation

Gluteus medius. Within visual evaluation of redness, there was a freezing treatment × packaging film × days of display interaction ($P < 0.001$; Figure 1) within the GM. Nitrite-embedded film yielded greater ($P < 0.05$) redness scores across all freezing treatments between 0 and 16 d of display. The DBL-NIT packaging increased redness ($P < 0.05$) compared with SNGL-NIT and CON-NIT between 0 and 19 d. Conversely, GM steaks in DBL-ROLL had reduced ($P < 0.05$) redness values compared with SNGL-ROLL and CON-ROLL on day 0, and there were no differences ($P > 0.05$) between freezing treatments in traditional rollstock between 1 and 16 d. Yet on day 28, there were no differences ($P > 0.05$) between redness values among all treatment combinations. There was a freezing treatment × packaging film × days of display interaction ($P = 0.022$; Figure 2) for percent discoloration of the GM. On day 0, discoloration was greatest ($P < 0.05$) in the DBL-ROLL steaks. On day 28, discoloration was greatest in the DBL-NIT steaks ($P < 0.05$), and there were no differences ($P > 0.05$) between the other treatment combinations.

There was a packaging film × days of display interaction ($P < 0.001$; Figure 3) for L^* values of GM steaks, wherein there were no differences ($P > 0.05$) between ROLL and NIT, but at 28 d ROLL had greater ($P < 0.05$) L^* values compared with NIT. There was a freezing treatment × packaging film × days of retail display interaction ($P < 0.001$; Figure 4) for a^* of GM steaks. At day 0, all NIT freezing treatments were similar ($P > 0.05$) and greater ($P < 0.05$) than ROLL across all freezing treatments. Also at day 0, GM from CON-ROLL and SNGL-ROLL groups had greater ($P < 0.05$) a^* values than DBL-ROLL. All NIT GM steaks regardless of freezing treatment had greater ($P < 0.05$) a^* than ROLL for the first 13 d of retail display. By the end of the 28-d retail display, there were no

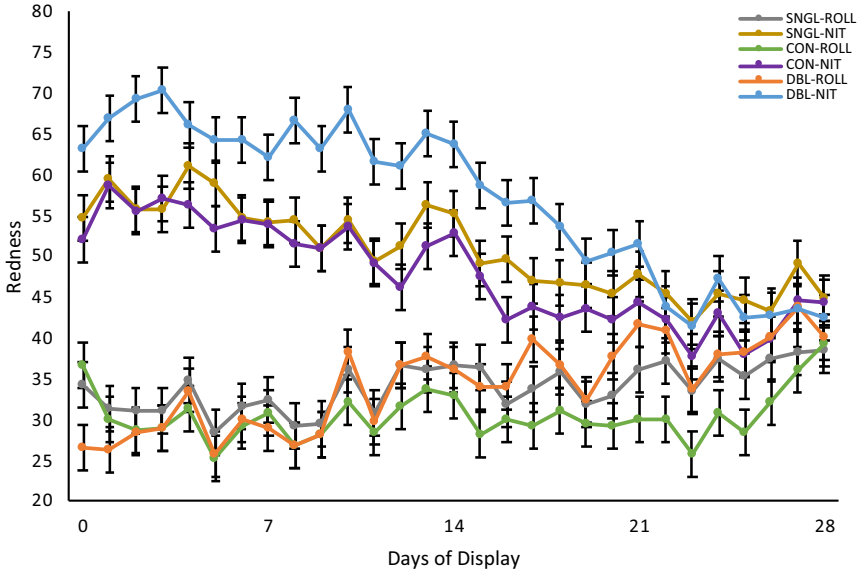

Figure 1. Interaction of freezing treatment, packaging film, and days of retail display ($P < 0.001$) within beef *Gluteus medius* steaks of visual redness score. Redness was evaluated on a scale of 0 to 100 every 24 h for a 28-d retail display period, wherein 0 represented dark brown, 50 represented slightly dark red/purple, and 100 represented very bright red. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

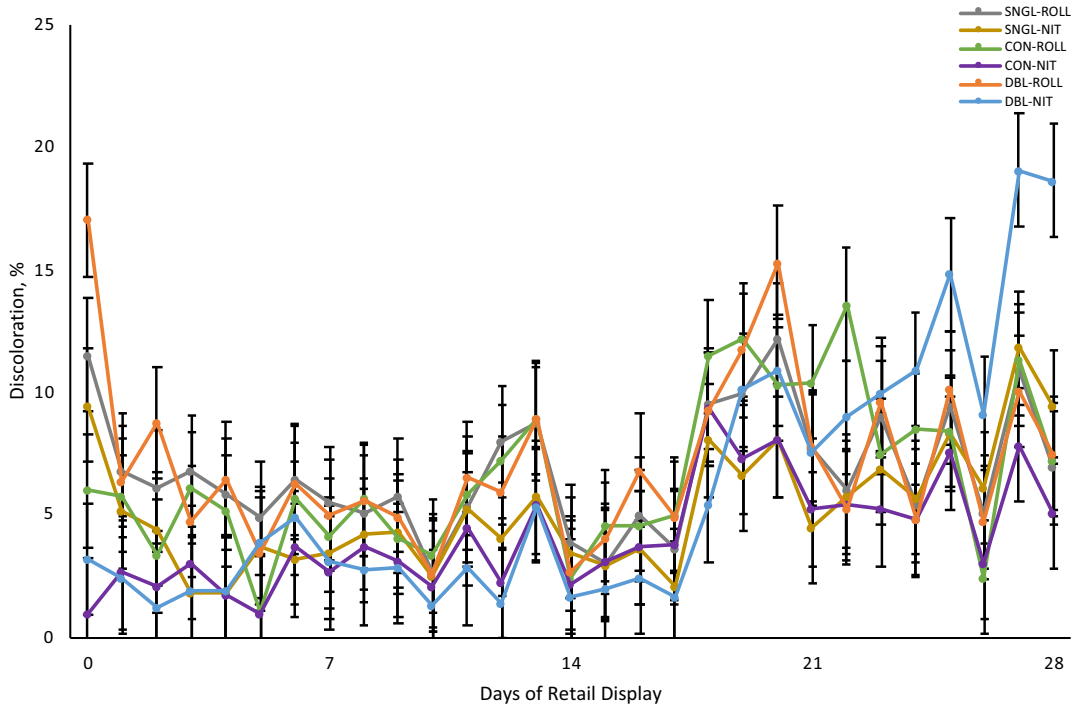

Figure 2. Interaction of freezing treatment, packaging film, and days of retail display ($P = 0.002$) within beef *Gluteus medius* steaks of visual discoloration on a percent basis. Every 24 h for a 28-d retail display period, steaks were evaluated for percent visual surface discoloration. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

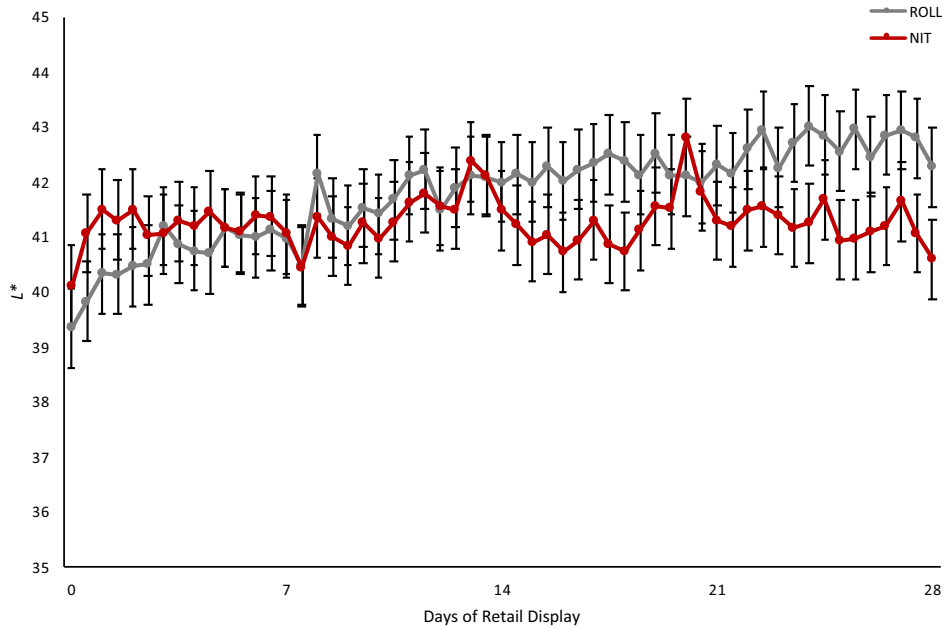

Figure 3. Interaction of packaging film and days of retail display ($P < 0.001$) on L^* values of beef *Gluteus medius* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Error bars are representative of the largest SEM. Standard rollstock packaging (ROLL) is represented by the grey line, and nitrite-embedded packaging (NIT) is represented by the red line.

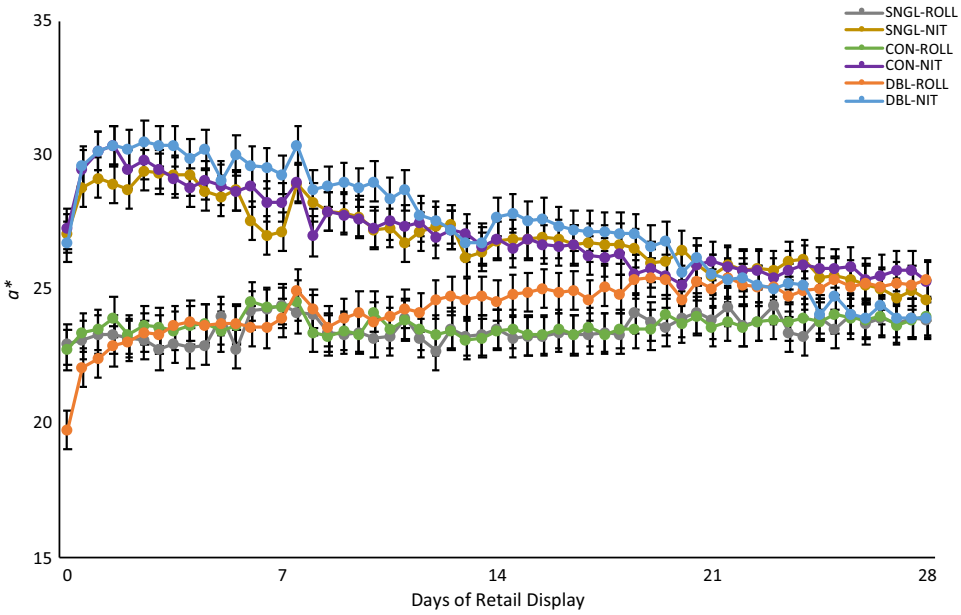

Figure 4. Interaction of freezing treatment, packaging film, and days of retail display ($P < 0.001$) on a^* values of *Gluteus medius* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

differences ($P > 0.05$) between any freezing treatment or packaging film. There is a freezing treatment × days of retail display interaction ($P < 0.001$; Figure 5) for b^* where SNGL had decreased ($P < 0.05$) values for the first 2 d. By day 21 of retail display, there were no differences ($P > 0.05$) between b^* of any freezing treatment. There was a packaging film × days of retail display interaction ($P < 0.001$; Figure 6) in which b^* values in NIT were greater ($P < 0.05$) than ROLL for the duration of the display. However, b^* values of NIT packages decreased ($P < 0.05$) over time, while ROLL b^* values remained mostly stagnant ($P > 0.05$). For MMb percent, there was a freezing treatment × packaging film × days of retail display interaction ($P = 0.008$; Figure 7). On day 0, MMb percent was greatest in CON-NIT, DBL-NIT, DBL-ROLL, and SNGL-NIT ($P < 0.05$). From 0.5 to 17 d in retail display, NIT packaging had increased ($P < 0.05$) MMb percent compared with ROLL. There was a freezing treatment × packaging film × days of retail display interaction ($P < 0.001$; Figure 8) for DMb percent. Nitrite-embedded packaging had reduced ($P < 0.05$) DMb percent relative to ROLL at all timepoints except for 18.5 and 20 d. On day 28, all freezing treatments in ROLL were similar ($P > 0.05$) in DMb percent, whereas CON-NIT was greater ($P < 0.05$) than SNGL-NIT and DBL-NIT, and SNGL-NIT was greater ($P < 0.05$) than DBL-NIT.

Longissimus lumborum. There was a freezing treatment × packaging film × days of retail display interaction ($P < 0.001$; Figure 9) for visual redness score of LL steaks. From 0 to 25 d, all steaks in NIT had increased ($P < 0.05$) redness scores compared with those in ROLL. On day 0, DBL-NIT had greater ($P < 0.05$) redness scores than SNGL-NIT and CON-NIT, while visual redness of SNGL-NIT was greater ($P < 0.05$) than CON-NIT. From 0 to 18 d of retail display, DBL-NIT LL steaks had the greatest ($P < 0.05$) redness scores. On day 28, DBL-NIT steaks had greater ($P < 0.05$) redness scores relative to all treatment combinations except SNGL-NIT. The SNGL-NIT LL steaks had greater ($P < 0.05$) redness scores compared with all ROLL steaks at day 28. There were no differences ($P > 0.05$) between CON-NIT and DBL-ROLL on day 28, but both groups had increased redness scores compared with SNGL-ROLL and CON-ROLL. Finally, the CON-ROLL LL steaks had the smallest ($P < 0.05$) visual redness score on day 28 of retail display. There was a packaging film × days of retail display interaction ($P < 0.001$; Figure 10) for percent discoloration of LL steaks. On day 0, there were no differences ($P > 0.05$) between ROLL and NIT, while at day 28 NIT packages had less ($P < 0.05$) discoloration compared with steaks in ROLL packaging. There was a freezing treatment × days of retail display interaction ($P = 0.006$; Figure 11) for L^* wherein DBL

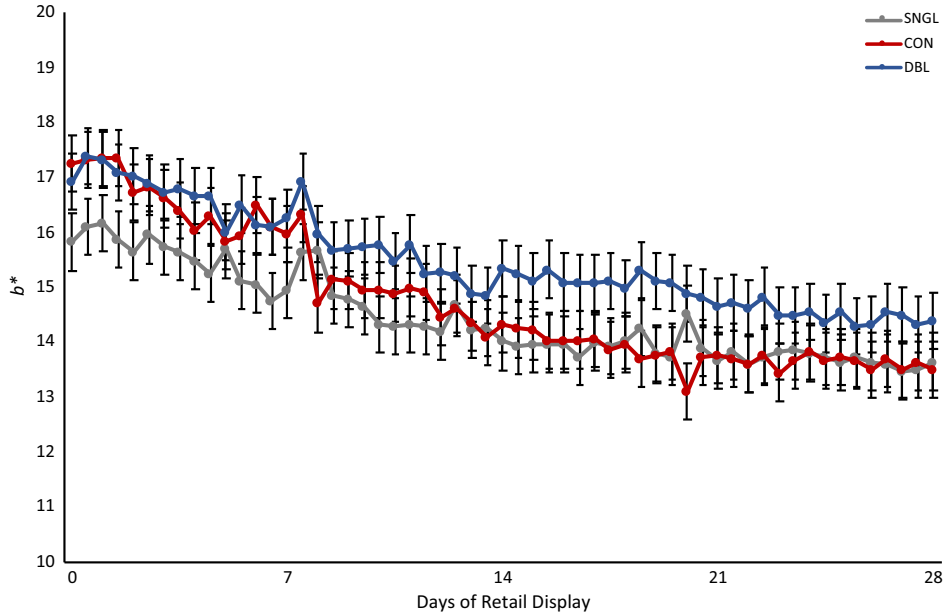

Figure 5. Interaction of freezing treatment and days of retail display ($P < 0.001$) on b^* values of beef *Gluteus medius* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Error bars are representative of the largest SEM. Single frozen steaks (SNGL) are represented by the grey line, fresh never frozen steaks (CON) are represented by the red line, and double frozen steaks (DBL) are represented by the blue line.

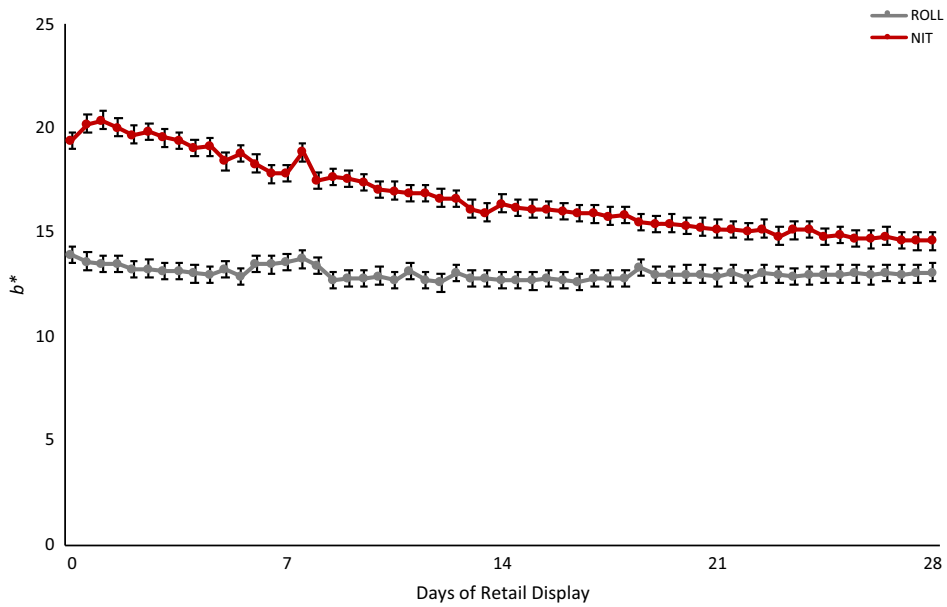

Figure 6. Interaction of packaging film and days of retail display ($P < 0.001$) on b^* values of beef *Gluteus medius* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Error bars are representative of the largest SEM. Standard rollstock packaging (ROLL) is represented by the grey line, and nitrite-embedded packaging (NIT) is represented by the red line.

steaks had increased ($P < 0.05$) values compared with CON at 0 d, but no differences ($P > 0.05$) between freezing treatments at 28 d. There was also packaging film × days of retail display interaction ($P < 0.001$; Figure 12) for L^* values. Steaks in the NIT group had greater ($P < 0.05$) L^* values from 0 to 4.5 d, and then at 28 d ROLL had increased ($P < 0.05$) L^* values compared with NIT. There was a packaging film × days of retail display interaction ($P < 0.001$; Figure 12) for a^* wherein NIT steaks had greater ($P < 0.05$) values compared with ROLL for the duration of the 28-d retail display. There was a freezing treatment × days of retail display interaction ($P = 0.008$; Figure 13) for b^* values. On day 0, b^* values were greater ($P < 0.05$) in

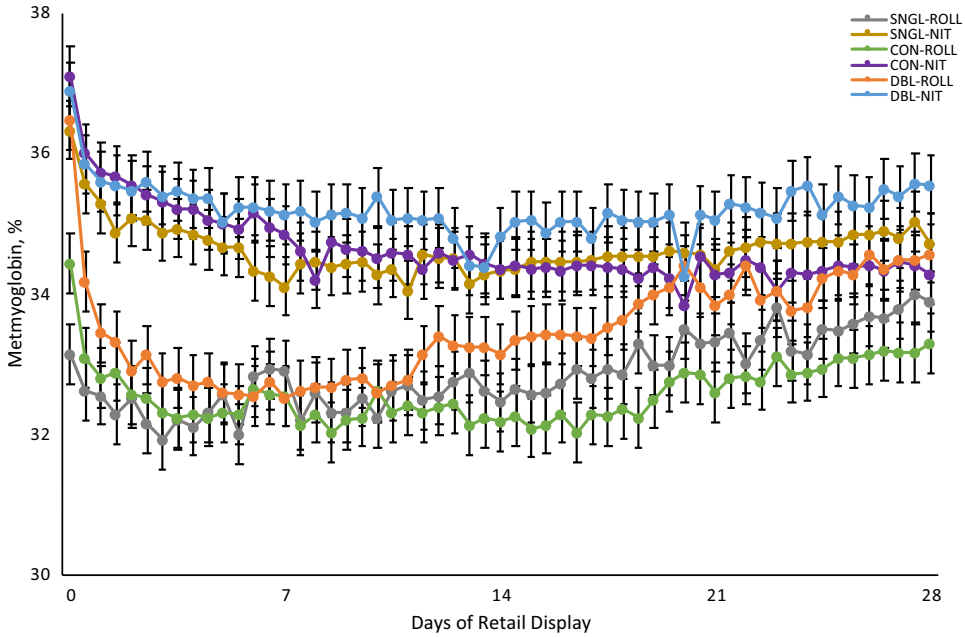

Figure 7. Interaction of freezing treatment, packaging film, and days of retail display ($P = 0.008$) on metmyoglobin percent of beef *Gluteus medius* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Surface metmyoglobin was calculated by the ratio of 470 nm:530 nm. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

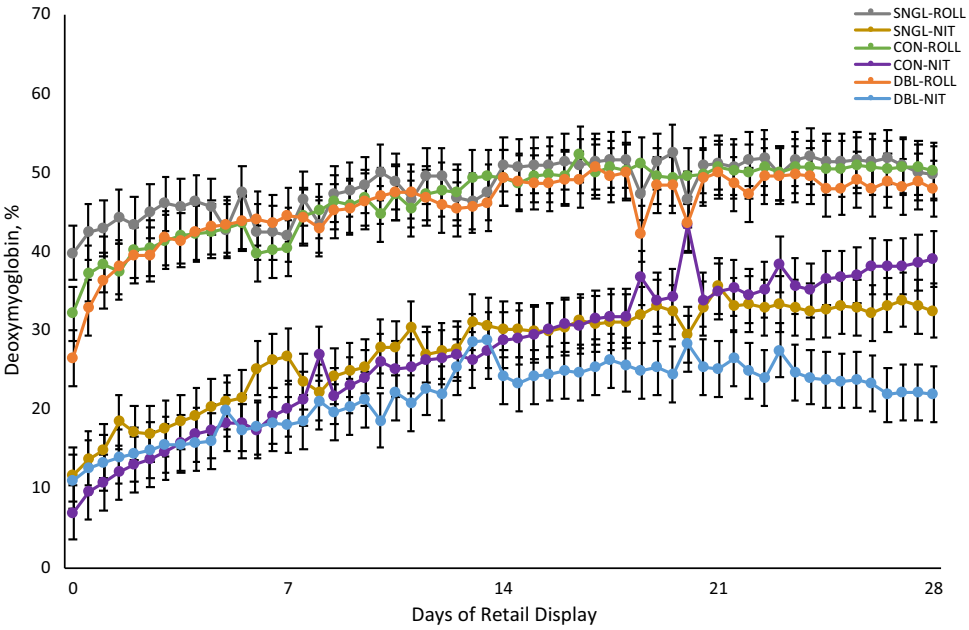

Figure 8. Interaction of freezing treatment, packaging film, and days of retail display ($P < 0.001$) on deoxymyoglobin percent of beef *Gluteus medius* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Surface deoxymyoglobin was calculated by the ratio of 570 nm:530 nm. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

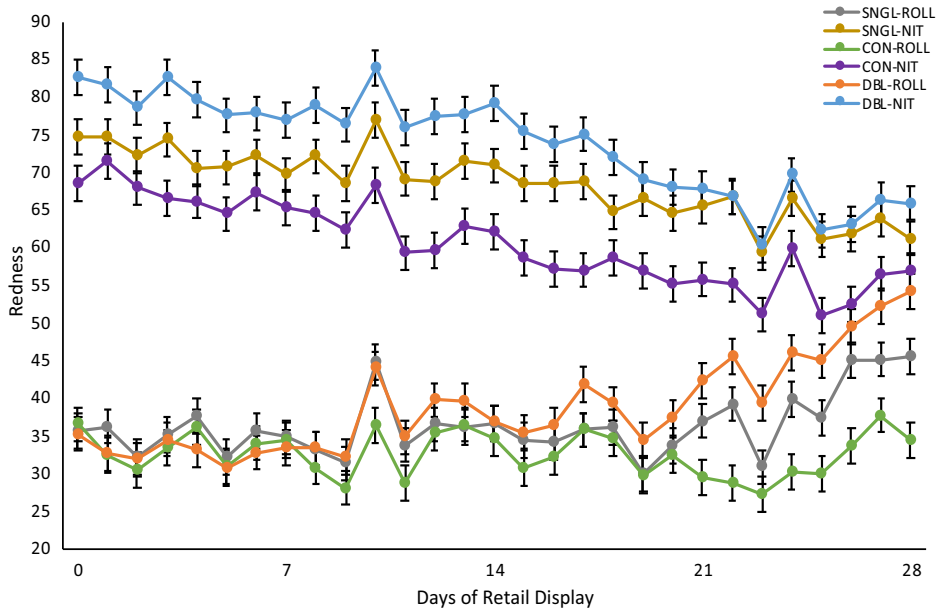

Figure 9. Interaction of freezing treatment, packaging film, and days of retail display ($P < 0.001$) of visual redness scores in beef *Longissimus lumborum* steaks. Redness was evaluated on a scale of 0 to 100 every 24 h for a 28-d retail display period, wherein 0 represented dark brown, 50 represented slightly dark red/purple, and 100 represented very bright red. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

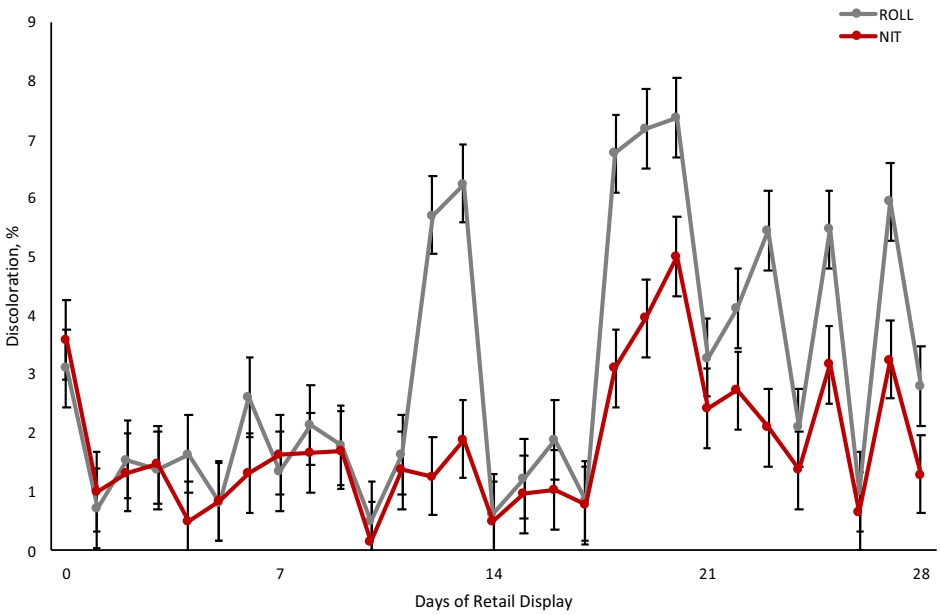

Figure 10. Interaction of packaging film and days of retail display ($P < 0.001$) of visual percent discoloration in beef *Longissimus lumborum* steaks. Every 24 h for a 28-d retail display period, steaks were evaluated for percent visual surface discoloration. Error bars are representative of the largest SEM. Standard rollstock packaging (ROLL) is represented by the grey line, and nitrite-embedded packaging (NIT) is represented by the red line.

DBL than CON and SNGL, whereas on day 28 b^* values were greater ($P < 0.05$) in DBL LL steaks compared with CON. For b^* values, there was also a packaging film × days of retail display interaction ($P < 0.001$; Figure 12) wherein NIT LL steaks had greater b^* values compared with ROLL for the duration of the retail display period. For MMB percent, there was a freezing treatment × days of retail display

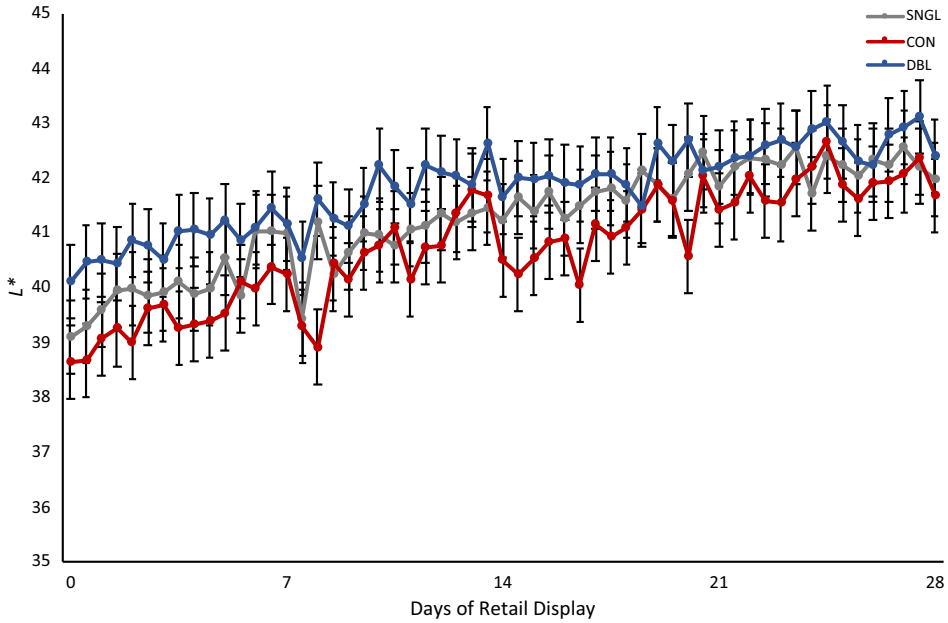

Figure 11. Interaction of freezing treatment and days of retail display ($P = 0.006$) on L^* values of beef *Longissimus lumborum* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Error bars are representative of the largest SEM. Single frozen steaks (SNGL) are represented by the grey line, fresh never frozen steaks (CON) are represented by the red line, and double frozen steaks (DBL) are represented by the blue line.

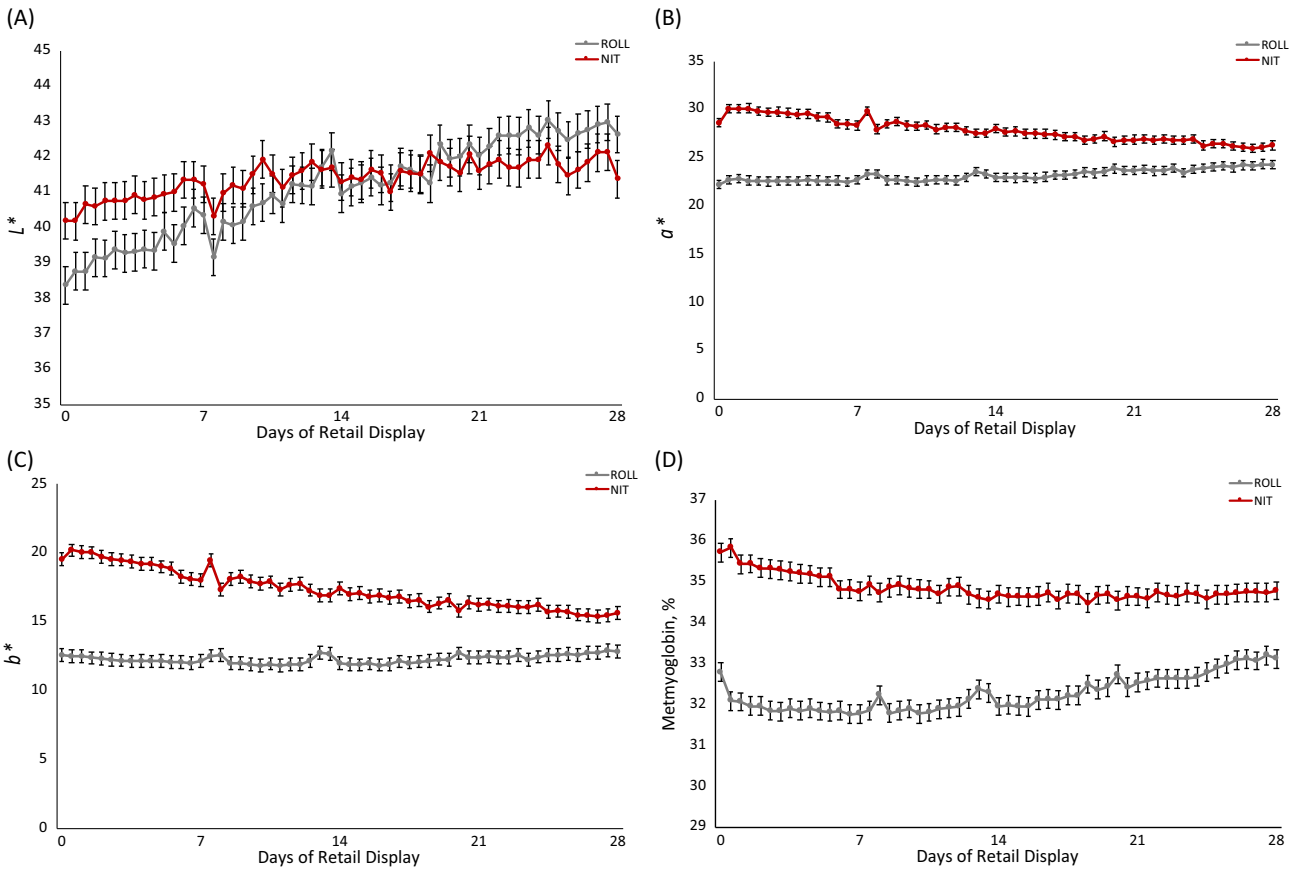

Figure 12. Interaction of packaging film and days of retail display on (A) L^* values ($P < 0.001$), (B) a^* values ($P < 0.001$), (C) b^* values ($P < 0.001$), and (D) metmyoglobin percent ($P < 0.001$) of beef *Longissimus lumborum* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Surface metmyoglobin was calculated by the ratio of 470 nm:530 nm. Error bars are representative of the largest SEM. Standard rollstock packaging (ROLL) is represented by the grey line, and nitrite-embedded packaging (NIT) is represented by the red line.

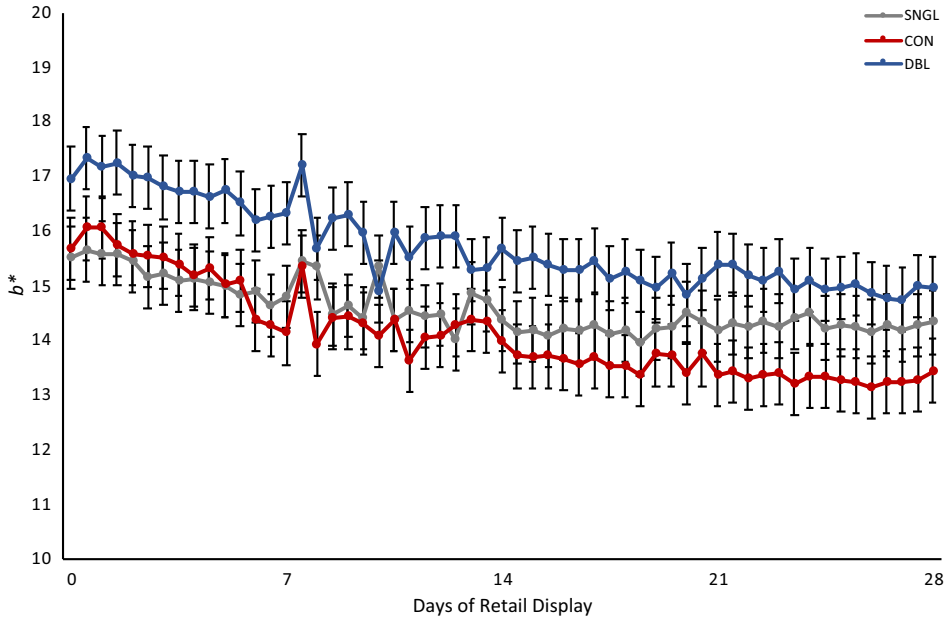

Figure 13. Interaction of freezing treatment and days of retail display ($P = 0.008$) on b^* values of beef *Longissimus lumborum* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Error bars are representative of the largest SEM. Single frozen steaks (SNGL) are represented by the grey line, fresh never frozen steaks (CON) are represented by the red line, and double frozen steaks (DBL) are represented by the blue line.

interaction ($P < 0.001$; Figure 14) wherein there were no differences ($P > 0.05$) on day 0, but on day 28 DBL LL steaks had greater ($P < 0.05$) MMB percent compared with CON. There was also a packaging film × days of retail display interaction ($P < 0.001$; Figure 12) for MMB percent wherein there was greater ($P < 0.05$) MMB formation in NIT compared with ROLL for the entirety of retail display. For DMb percent within the LL, there was a freezing treatment × packaging film × days of retail display interaction ($P < 0.001$; Figure 15). Within the interaction all ROLL steaks had a greater ($P < 0.05$) DMb percent than NIT packaging for all of the display period. From day 19 to 28 of retail display, DBL-NIT steaks had a reduced ($P < 0.05$) DMb percent compared with CON-NIT and SNGL-NIT steaks.

Spoilage organisms

In GM steaks, a freezing effect ($P = 0.025$; Table 2) for AC counts was observed wherein CON and SNGL had greater ($P < 0.05$) counts compared with DBL. There was also a days of retail display effect ($P < 0.001$; Table 2) for AC counts, with there being increased ($P < 0.05$) counts on day 14 and 28 compared with day 0. For EB and LAB counts within the GM, there was a days of retail display effect ($P < 0.001$; Table 2), with counts being increased ($P < 0.05$) at 28 d relative to 0 and 14 d. Psychrotropic counts from GM steaks had a days of retail display effect ($P < 0.001$; Table 2) where counts were greatest ($P < 0.05$) on day 14, and counts on day 0 were greater ($P < 0.05$) than counts on day 28. For AC counts of LL steaks, there was a freezing treatment effect ($P = 0.003$; Table 2) wherein CON had greater ($P < 0.05$) counts compared with DBL. Additionally for AC and LAB counts of LL steaks, there was a days of retail display effect ($P < 0.001$; Table 2) wherein counts were increased ($P < 0.05$) on days 14 and 28 compared with day 0. Abundance of EB in LL had an effect of freezing treatment ($P = 0.015$) in the LL compared with increased ($P < 0.05$) counts in SNGL compared with DBL. There was also a day effect ($P < 0.001$; Table 2) for EB counts from LL steaks wherein abundance increased ($P < 0.05$) at each sampling timepoint. There was a freezing treatment × sampling day interaction ($P = 0.017$; Table 3) for psychrotrophs from LL steaks. Abundance of psychrotrophs was greatest ($P < 0.05$) on day 14 across all freezing treatments. There were no differences ($P > 0.05$) between freezing treatments on day 0, and on day 28 CON had increased ($P < 0.05$) psychrotrophs relative to SNGL and DBL.

Purge loss

There was a freezing treatment × days of retail display interaction ($P < 0.001$; Table 3) for purge loss from GM steaks. Across all freezing treatments, purge

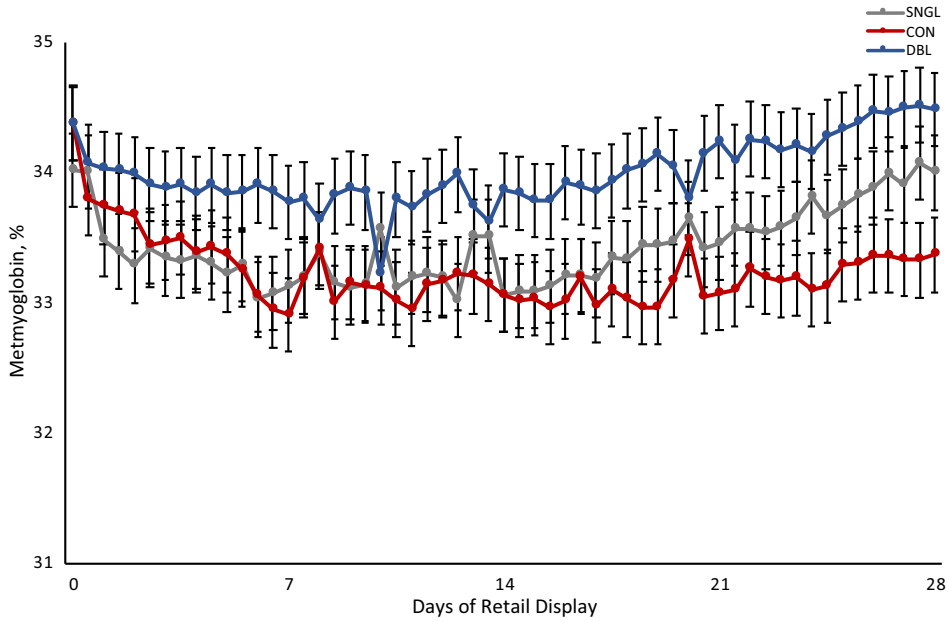

Figure 14. Interaction of freezing treatment and days of retail display ($P < 0.001$) on metmyoglobin percent of *Longissimus lumborum* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Surface metmyoglobin was calculated by the ratio of 470 nm:530 nm. Error bars are representative of the largest SEM. Single frozen steaks (SNGL) are represented by the grey line, fresh never frozen steaks (CON) are represented by the red line, and double frozen steaks (DBL) are represented by the blue line.

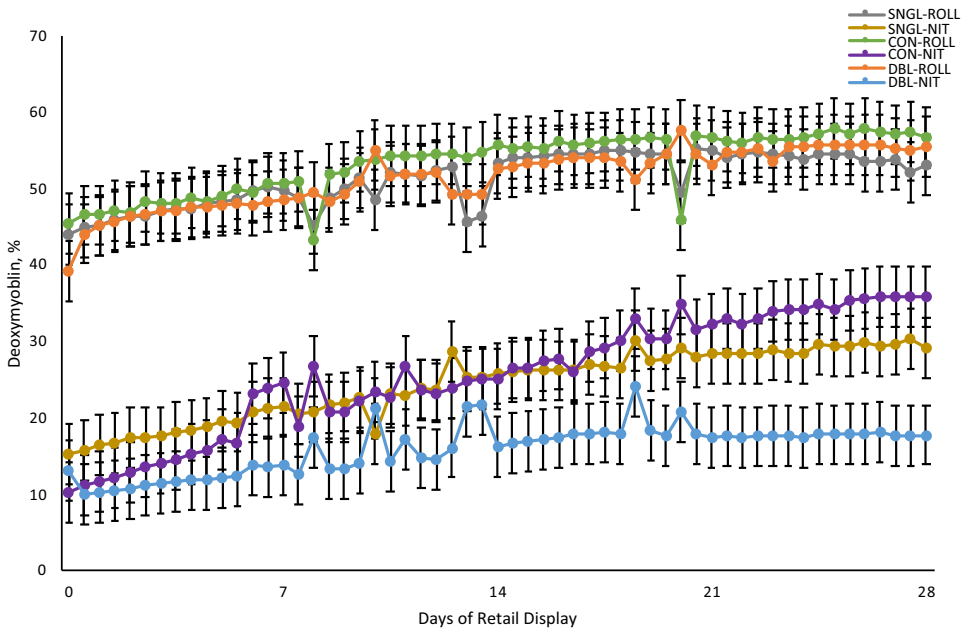

Figure 15. Interaction of freezing treatment, packaging film, and days of retail display ($P < 0.001$) on deoxymyoglobin percent in beef *Longissimus lumborum* steaks. Every 12 h for a 28-d display period, instrumental color data were evaluated using a spectrophotometer. Surface deoxymyoglobin was calculated by the ratio of 570 nm:530 nm. Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL) are represented by the grey line, single frozen steaks in nitrite-embedded packaging (SNGL-NIT) are represented by the mustard line, fresh never frozen steaks in rollstock packaging (CON-ROLL) are represented by the green line, fresh never frozen steaks in nitrite-embedded packaging (CON-NIT) are represented by the purple line, double frozen steaks in rollstock packaging (DBL-ROLL) are represented by the orange line, and double frozen steaks in nitrite-embedded packaging (DBL-NIT) are represented by the blue line.

loss was least ($P < 0.05$) on day 0. Additionally, steaks that underwent any freezing, SNGL and DBL, had increased ($P < 0.05$) purge loss compared to fresh, never-frozen beef, CON. Within LL steaks there was a freezing treatment effect ($P < 0.001$; Table 4), with purge loss being increased ($P < 0.05$) in SNGL and

Table 2. Least-squares means of significant ($P \leq 0.05$) main effects of freezing treatment, packaging film type, and retail display period of spoilage organisms[1] within *Gluteus medius* and *Longissimus lumborum* steaks.

	Freezing Treatment[2]					Packaging[3]				Days of Display				
Parameter	Single	Control	Double	SEM[4]	*P* Value[5]	Rollstock	Nitrite	SEM[4]	*P* Value[5]	0	14	28	SEM[4]	*P* Value[5]
Gluteus medius														
AC, log[CFU]/cm^2	3.46^a	3.53^a	3.15^b	0.11	0.025	3.45	3.30	0.09	0.212	1.63^b	4.3^a	4.17^a	0.11	<0.001
EB, log[CFU]/cm^2	1.06	1.22	0.80	0.20	0.200	1.07	0.98	0.18	0.626	0.22^b	2.51^a	2.44^a	0.36	<0.001
LAB, log[CFU]/cm^2	2.42	2.64	2.39	0.12	0.211	2.5	2.47	0.09	0.826	1.09^b	3.17^a	3.19^a	0.12	<0.001
Psychrotrophs, log[CFU]/cm^2	1.20	1.20	1.23	0.04	0.860	1.18	1.24	0.03	0.237	1.18^b	1.41^a	1.03^c	0.04	<0.001
Longissimus lumborum														
AC, log[CFU]/cm^2	3.60^{ab}	3.90^a	3.37^b	0.11	0.003	3.60	3.64	0.09	0.708	1.90^b	4.49^a	4.47^a	0.11	<0.001
EB, log[CFU]/cm^2	1.53^a	1.34^{ab}	0.97^b	0.17	0.015	1.28	1.28	0.14	0.972	<1.00^c	2.17^b	2.52^a	0.26	<0.001
LAB, log[CFU]/cm^2	2.42	2.58	2.55	0.15	0.690	2.48	2.55	0.10	0.597	1.22^b	3.18^a	3.15^a	0.13	<0.001
Psychrotrophs, log[CFU]/cm^2						1.27	1.28	0.03	0.706					

[1]Aerobic plate counts (AC), Enterobacteriaceae (EB), and lactic acid bacteria (LAB).

[2]Single = Subprimals frozen for 180 d; Control = Never frozen steaks; Double = Subprimals frozen for 90 d, fabricated into steaks, and frozen an additional 90 d.

[3]Rollstock = control vacuum packaging forming film; Nitrite = sodium nitrite-embedded forming film.

[4]Largest standard error of least-squares means.

[5]Observed significance level.

[a–c]Least-squares means in the same row without a common superscript differ ($P < 0.05$).

Table 3. Least-squares means the interaction of freezing treatment × retail days of display on purge loss and psychrotrophs within *Gluteus medius* and *Longissimus lumborum* steaks.

	Single[1]			Control[1]			Double[1]				
Parameter	0	14	28	0	14	28	0	14	28	SEM[2]	*P* Value[3]
Gluteus medius											
Purge loss, %	2.94^d	4.64^c	6.39^{ab}	1.38^e	3.13^d	3.05^d	5.07^c	7.00^a	6.07^b	0.32	<0.001
Longissimus dorsi											
Psychrotrophs, log[CFU]/cm^2	1.12^{bc}	1.60^a	1.04^c	1.09^{bc}	1.49^a	1.24^c	1.22^b	1.65^a	1.04^c	0.07	0.017

[1]Single = Subprimals frozen for 180 d; Control = Never frozen steaks; Double = Subprimals frozen for 90 d, fabricated into steaks, and frozen an additional 90 d.

[2]Largest standard error of least-squares means.

[3]Observed significance level.

[a–c]Least-squares means in the same row without a common superscript differ ($P < 0.05$).

DBL compared with CON. There was also a days of retail display effect ($P < 0.001$; Table 4) for purge loss of LL steaks, with increased ($P < 0.05$) purge loss on days 14 and 28 compared with day 0.

Lipid oxidation

In GM steaks, there was a freezing treatment × packaging film interaction ($P = 0.031$; Figure 16). Within ROLL packaging, there were no differences ($P > 0.05$) in MDA concentration between freezing groups; however, DBL-NIT had increased ($P < 0.05$) MDA compared with SNGL-NIT. Furthermore, SNGL-NIT had reduced ($P < 0.05$) MDA concentrations compared with CON-ROLL, SNGL-ROLL, and DBL-NIT. In LL steaks, there was a packaging film × days of retail display interaction ($P = 0.031$; Figure 17). There were no differences ($P > 0.05$) within each packaging film across all timepoints; however, on day 28 ROLL had increased ($P < 0.05$) MDA concentration compared with NIT.

Descriptive sensory

Gluteus medius. There were no effects ($P \geq 0.051$; Table 5) for beef, fat-like, sour, bitter, or liver flavors among freezing treatments or packaging film. There was a freezing treatment × packaging film interaction ($P = 0.024$; Figure 6) for tenderness, where tenderness was greater ($P < 0.05$) in DBL-NIT compared with

Table 4. Least-squares means of significant ($P \leq 0.05$) main effects of freezing treatment, packaging film type, and retail display period of purge loss and thiobarbituric acid reactive species[1] from *Gluteus medius* and *Longissimus lumborum* steaks.

	Freezing Treatment[2]					Packaging[3]				Days of Display				
Parameter	Single	Control	Double	SEM[4]	*P* Value[5]	Rollstock	Nitrite	SEM[4]	*P* Value[5]	0	14	28	SEM[4]	*P* Value[5]
Gluteus medius														
Purge loss, %						4.58	4.23	0.15	0.105					
MDA[6], mg/kg										0.0472	0.0472	0.0470	0.0002	0.629
Longissimus lumborum														
Purge loss, %	4.77[a]	1.82[b]	4.64[a]	0.22	<0.001	3.66	3.82	0.17	0.421	2.61[b]	4.30[a]	4.33[a]	0.19	<0.001
MDA[6], mg/kg	0.0462	0.0464	0.0462	0.0002	0.517									

[1]Thiobarbituric reactive species measured using concentration of malondialdehyde.

[2]Single = Subprimals frozen for 180 d; Control = Never frozen steaks; Double = Subprimals frozen for 90 d, fabricated into steaks, and frozen an additional 90 d.

[3]Rollstock = control vacuum packaging forming film; Nitrite = sodium nitrite-embedded forming film.

[4]Largest standard error of least-squares means.

[5]Observed significance level.

[6]MDA = malondialdehyde.

[a–c]Least-squares means in the same row without a common superscript differ ($P < 0.05$).

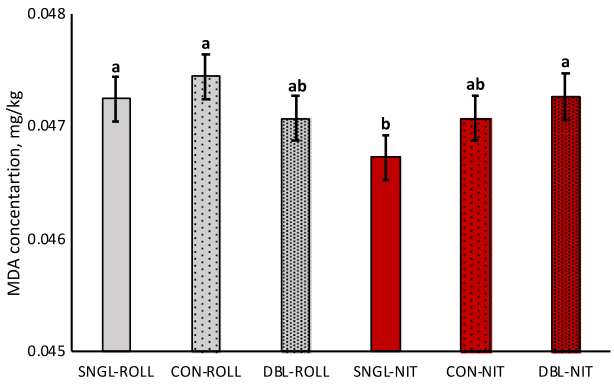

Figure 16. Interaction of freezing treatment and packaging film ($P = 0.031$) within beef *Gluteus medius* steaks for thiobarbituric acid reactive substance values (malondialdehyde [MDA], mg/kg). Error bars are representative of the largest SEM. Single frozen steaks in rollstock packaging (SNGL-ROLL), single frozen steaks in nitrite-embedded packaging (SNGL-NIT), fresh never frozen steaks in rollstock packaging (CON-ROLL), fresh never frozen steaks in nitrite-embedded packaging (CON-NIT), double frozen steaks in rollstock packaging (DBL-ROLL), and double frozen steaks in nitrite-embedded packaging (DBL-NIT).

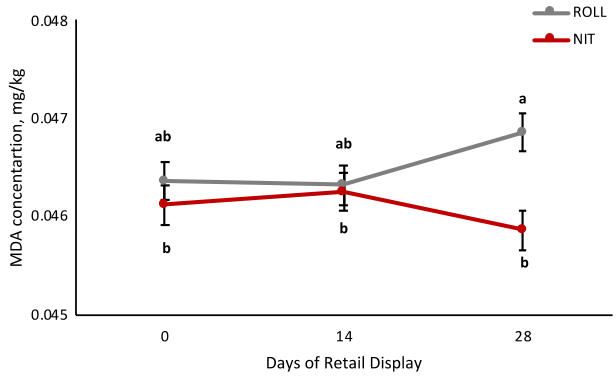

Figure 17. Interaction of packaging film and retail display period ($P = 0.001$) within beef *Longissimus lumborum* steaks for thiobarbituric acid reactive substance values (malondialdehyde [MDA], mg/kg). Error bars are representative of the largest SEM. Standard rollstock packaging (ROLL) is represented by the grey line, and nitrite-embedded packaging (NIT) is represented by the red line.

CON-NIT, yet there were no differences ($P > 0.05$) between any other groups. There was a main effect of freezing treatment ($P = 0.001$; Table 5) wherein CON had increased ($P < 0.05$) juiciness compared with SNGL and DBL. There was also a main effect of packaging film ($P = 0.004$; Table 5) for initial juiciness in which NIT had increased ($P < 0.05$) values compared with ROLL. Sustained juiciness was influenced by freezing treatment ($P = 0.027$; Table 5), with CON having increased ($P < 0.05$) sustained juiciness compared with SNGL. There was a freezing treatment × packaging film interaction ($P = 0.011$; Table 6) for brown/roasted flavor. Steaks in NIT packaging had greater ($P < 0.05$) browned/roasted flavor compared with ROLL steaks from the same freezing treatment. Additionally, there were no differences ($P > 0.05$) in brown/roasted flavor intensity between freezing treatments within packaging film type. Bloody/serumy was influenced by freezing treatment ($P = 0.010$; Table 5), with CON having increased ($P < 0.05$) values compared with SNGL and DBL. Metallic flavor varied with packaging film ($P = 0.007$; Table 5), where ROLL had increased ($P < 0.05$) metallic flavor compared with NIT. There was

Table 5. Least-squares means of significant ($P \leq 0.05$) main effects of freezing treatment and packaging on descriptive sensory attributes from *Gluteus medius* steaks.

	Freezing Treatment[1]					Packaging[2]			
Parameter	Single	Control	Double	SEM[3]	*P* Value[4]	Rollstock	Nitrite	SEM[3]	*P* Value[4]
Initial Juice	46.71[b]	50.00[a]	47.66[b]	0.61	0.001	47.16[b]	49.09[a]	0.48	0.004
Sustained Juice	39.93[b]	42.55[a]	40.98[ab]	0.71	0.027	41.45	40.96	0.55	0.528
Beef Identity	53.39	53.72	53.15	0.32	0.456	53.25	53.58	0.26	0.365
Fat	11.78	12.02	12.09	0.34	0.765	11.61	12.32	0.26	0.051
Bloody/Serumy	5.60[b]	7.29[a]	5.20[b]	0.49	0.010	5.76	6.30	0.38	0.313
Metallic	3.37	3.59	4.72	0.44	0.067	4.58[a]	3.20[b]	0.37	0.007
Refrigerator Stale	3.67	4.15	4.05	0.39	0.657	4.08	3.83	0.32	0.581
Sour	3.35	3.46	3.33	0.41	0.973	3.22	3.54	0.33	0.483
Bitter	2.91	2.87	2.93	0.32	0.991	2.95	2.86	0.26	0.809
Liver	2.12	2.51	3.11	0.36	0.152	2.49	2.67	0.30	0.663

[1]Single = Subprimals frozen for 180 d; Control = Never frozen steaks; Double = Subprimals frozen for 90 d, fabricated into steaks, and frozen an additional 90 d.

[2]Rollstock = control vacuum packaging forming film; Nitrite = sodium nitrite-embedded forming film.

[3]Largest standard error of least-squares means.

[4]Observed significance level.

[a,b]Least-squares means in the same row without a common superscript differ ($P < 0.05$).

Table 6. Interaction of freezing treatment and packaging film on tenderness, brown/roasted, umami, and carboard flavor attributes of *Gluteus medius* steaks.

	Single[1]		Control[1]		Double[1]			
Parameter	CON	NIT	CON	NIT	CON	NIT	SEM[2]	*P* Value[3]
Tenderness	50.84[ab]	53.06[ab]	52.91[ab]	50.68[b]	51.66[ab]	53.27[a]	0.99	0.024
Brown/Roast	49.97[c]	53.48[ab]	51.49[bc]	54.65[a]	46.95[c]	55.05[a]	1.02	0.011
Umami	13.05[bc]	13.55[abc]	14.22[ab]	14.17[ab]	12.21[c]	14.42[a]	0.50	0.040
Cardboard	4.05[ab]	3.19[abc]	3.01[bc]	2.00[c]	2.81[bc]	4.38[a]	0.48	0.012

[1]Single = Subprimals frozen for 180 d; Control = Never frozen steaks; Double = Subprimals frozen for 90 d, fabricated into steaks, and frozen an additional 90 d.

[2]Largest standard error of least-squares means.

[3]Observed significance level.

[a–c]Least-squares means in the same row without a common superscript differ ($P < 0.05$).

a freezing treatment × packaging film interaction ($P = 0.040$; Table 6) for umami wherein DBL-NIT, CON-NIT, and CON-ROLL had greater ($P < 0.05$) umami flavor intensity than DBL-ROLL. There was a freezing treatment × packaging film interaction ($P = 0.012$; Table 6) for cardboard flavor, with DBL-NIT and SNGL-ROLL having increased ($P < 0.05$) intensity compared with CON-NIT.

Longissimus lumborum. There were no differences ($P \geq 0.184$; Table 7) in tenderness, beef identity, browned/roasted, fat-like, bloody/serumy, umami, refrigerator stale, cardboard, sour, bitter, or liver flavor attributes among freezing treatments or packaging type. Initial juiciness had a main effect of freezing treatment ($P = 0.008$; Table 7) where CON had greater ($P < 0.05$) values than SNGL and DBL, and SNGL initial juiciness was greater ($P < 0.05$) than DBL. Sustained juiciness also had a main effect of freezing treatment ($P = 0.025$; Table 7) wherein CON had greater ($P < 0.05$) sustained juiciness compared with SNGL and DBL. Metallic had a main effect of packaging film ($P = 0.025$; Table 7), where ROLL had increased ($P < 0.05$) metallic flavor intensity compared with NIT.

Discussion

Freezing can be a useful tool to extend or sustain the shelf-life of freshly packaged meat. In particular, the freezing of meat may be beneficial to packers and consumers alike dependent on economic state and the supply of fresh product available. By freezing and storing product at low temperatures for up to 90 d, there is

Table 7. Least-squares means of main effects of freezing treatment and packaging on descriptive sensory attributes from *Longissimus lumborum* steaks.

	Freezing Treatment[1]					Packaging[2]			
Parameter	Single	Control	Parameter	SEM[3]	P-value[4]	Parameter	Single	SEM[3]	P-value[4]
Tenderness	52.80	52.35	53.38	0.65	0.504	52.82	52.87	0.49	0.950
Initial Juice	48.79[b]	49.43[a]	47.14[c]	0.53	0.008	48.83	48.07	0.44	0.210
Sustained Juice	39.89[b]	41.15[a]	38.68[b]	0.74	0.025	40.11	39.70	0.56	0.594
Beef Identity	52.25	52.56	52.56	0.36	0.771	52.53	52.39	0.29	0.735
Brown/Roast	53.11	52.96	53.23	0.45	0.905	52.93	53.27	0.36	0.507
Fat	11.78	11.70	11.75	0.29	0.982	11.63	11.85	0.24	0.517
Bloody/Serumy	5.00	5.72	5.15	0.53	0.543	5.55	5.02	0.41	0.343
Metallic	2.92	2.45	2.44	0.34	0.536	3.05[a]	2.16[b]	0.28	0.025
Umami	13.65	14.23	13.58	0.46	0.544	13.47	14.17	0.37	0.184
Refrigerator Stale	3.51	3.38	3.48	0.37	0.969	3.27	3.65	0.31	0.377
Carboard	2.96	3.22	3.43	0.33	0.590	3.21	3.20	0.27	0.964
Sour	2.46	2.51	2.03	0.32	0.484	2.50	2.17	0.26	0.370
Bitter	2.06	2.12	2.32	0.30	0.820	2.19	2.14	0.24	0.892
Liver	1.70	1.23	1.23	0.25	0.312	1.52	1.25	0.21	0.354

[1]Single = Subprimals frozen for 180 d; Control = Never frozen steaks; Double = Subprimals frozen for 90 d, fabricated into steaks, and frozen an additional 90 d.

[2]Rollstock = control vacuum packaging forming film, Nitrite = sodium nitrite-embedded forming film.

[3]Largest standard error of least-squares means.

[4]Observed significance level.

[a–c]Least-squares means in the same row without a common superscript differ ($P < 0.05$).

minimal growth of spoilage organisms (Vieira et al., 2009) as many become dormant at or below −18 °C. Furthermore, storage of meat in anaerobic conditions can limit microbial growth and extend the shelf-life of meat products (Lorenzo and Gómez, 2012). The combination of the freezing treatments and vacuum packaging limited overall microbial growth during the freezing process, yet there still was an increased abundance of spoilage organisms during the 28 d in retail display. Specifically, the greatest abundance of AC across any freezing treatment and packaging combination was 4.49 log[CFU]/cm^2, which is still below spoilage threshold guidance of 7 log[CFU]/cm^2 (Rodríguez-Melcón et al., 2017). Thus, the extended freezing of steaks or subprimals does not increase microbial load leading to the development of off-flavored sensory attributes (Tables 5, 6, and 7). It must also be further noted that freezing is efficient at limiting microbial growth, because following freezing, spoilage organisms were similar or reduced compared with fresh product despite an additional 180 d of postmortem age (Table 2).

Additionally, while there were statistical differences in measures of lipid oxidation all values are under the detectable limit of 0.06 mg MDA/kg (Tarladgis et al., 1960) and well below 2.28 mg MDA/kg, at which point rancid flavors begin to overtake beef flavor (Campo et al., 2006). The limited amount of lipid oxidation can also be attributed to the meat being stored under anaerobic conditions (Lorenzo and Gómez, 2012; Maqsood et al., 2016). While not evaluated in this particular study, it has been previously noted that TBARS lipid oxidation products increases the most during the first 2 mo of frozen storage and then will continue to increase through 6 mo of frozen storage at a decreasing rate (Soyer et al., 2010). The present study had a total freeze time of approximately 6 mo, wherein there was minimal influence of freezing treatment on lipid oxidation. However, if freezing duration had been further extended, lipid oxidation measures may have begun to approach detectable limits regardless of freezing treatment as oxidation products continue to increase during frozen storage. Based on the results from the present study, beef products may be frozen for 180 d while preserving a high-quality product throughout the duration of a 28-d retail display.

The freezing of meat may greatly influence the palatability of the meat especially after repeated freeze-thaw cycles (Zhu et al., 2023). Results from the current study are in agreement with previous studies describing a decrease in juiciness values (Beyer et al., 2024) following freezing of beef steaks. Similar to results reported by Hergenreder et al. (2013), there were minimal differences in off-flavors following freezing and

no detectable differences in tenderness of LL or GM steaks. However, after extended periods of freezing, there is greater and larger formation of ice crystals within the meat which can lead to an increase in drip and cooking loss (Choi et al., 2018). Within this same study it was also noted that freezing at lower temperatures will minimize some of the negative side effects associated with freezing meat. This is in support of the current study in which purge loss was greater in steaks that had undergone any type of freezing. Further studies in pork have also shown that purge loss increased with freeze-thaw cycling and general storage (Kim et al., 2013). While previous reports from Vieira et al. (2009) showed no differences in juiciness following 90 d of frozen storage, there was still a reduction in WHC. Within the current study there was a reduction in initial and sustained juiciness when comparing frozen LL steaks to fresh LL steaks, which is expected when accompanied by the increase in purge loss. The differences between the two studies may be attributed to the additional 90 d of time in frozen storage in the present study. Furthermore, the present study only evaluated sensory attributes of steaks directly after the freezing treatment without undergoing retail display. There was a reduction in juiciness values in conjunction with increased purge loss on day 0 steaks, and the observed differences in juiciness would be expected to increase in a similar trend to purge loss in the steaks throughout retail display. Between freezing treatment and packaging film, there was also a lack of differences between other flavor attributes. This lack of differences may be attributed to the same minimal differences between the abundance of spoilage organisms (Table 2) and lipid oxidation values (Table 4; Figure 16; Figure 17), which are often attributed to the development of off-flavors.

While the influences of packaging film were limited among spoilage organisms, lipid oxidation, and descriptive sensory, there were vast differences between color measurements of steaks packaged in traditional rollstock and nitrite-embedded film. Previous studies have shown that nitrite-embedded film improves meat color in both fresh and frozen steaks (Claus and Du, 2013). These results are very similar to the present study wherein steaks packaged in nitrite film had increased subjective redness scores and instrumental a^* values. Nitrite-embedded packaging may also be used to improve a^* and chroma values of dark-cutting beef in a retail display setting compared to a traditional polyvinyl chloride (PVC) overwrap packaging (Ramanathan et al., 2018). Yet another benefit of nitrite packaging may be increasing the color stability of myoglobin forms. The use of a nitrite-embedded film decreases MMb in bison (Roberts et al., 2017) and pork (Chatkitanan and Harnkarnsujarit, 2020), indicating an increase in stability of myoglobin. However, in the present study, surface MMb percent was increased in GM steaks in NIT packaging compared with CON, and no effects were observed within packaging film for LL steaks. While nitrite-embedded film will improve the color of raw meat products, a potential downfall is the phenomenon of persistent pinking, found more commonly in processed meats after the addition of nitrates; however, the use of nitrite-embedded film in one study did increase a^* following cooking in comparison with a control packaging (Claus and Du, 2013). While measures of cooked color were not accounted for in this particular study, it is a point of interest in future research.

The majority of differences in visual and instrumental color appear to be driven by packaging type, yet freezing treatment appears to alter the color stability of both GM and LL steaks. Within the interaction of redness for LL steaks, steaks that were never frozen had reduced redness scores from day 21 to 28 of retail display. Prior research contradicts the present finding as Setyabrata and Kim (2019) found that following freezing and thawing there was a reduced lean color score from 3 to 7 d of retail display. Yet the differences between the studies are that vacuum packaging was used in the present study, while previous studies primarily utilized PVC overwrapped products. Furthermore, the freezing of meat products is known to decrease color stability and increase the oxidation of myolglobin (Hergenreder et al., 2013; Henriott et al., 2020). These claims are further supported by the present study as surface MMb percent was increased in steaks that had undergone any freezing treatment relative to steaks that were never frozen.

Conclusion

The results of this study reveal that long-term freezing of beef subprimals has minimal influence on descriptive sensory, abundance of spoilage organisms, and lipid oxidation of steaks packaged in anaerobic conditions. Additionally, this study supports the use of nitrite-embedded film to increase color stability and performance during a 28-d retail display in comparison with a traditional rollstock packaging. However, the novelty of long-term freezing steaks in nitrite-embedded film led to greater redness values at the beginning of a retail display period compared to

a more traditional vacuum package, which may provide a more appealing product to consumers.

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