Meat and Muscle Biology™

Effects of Chilling Duration on Marbling Score of Beef Carcasses

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Abstract: Two hundred and nine beef carcasses (body weight of 361 ± 53 kg) from crossbred, grain-finished cattle were harvested in a commercial abattoir and subjected to continuous chilling for 96 h at 0°C to 3°C in a commercial hot box with a wind speed of 3.1 m/s and 153 lux of fluorescent light. At 24, 48, 72, and 96 h, the carcasses were analyzed for fatty acid composition, marbling score, pH, shrinkage, color, and aerobic plate count ($n = 50$). Carcasses reached 3°C after 12 to 16 h of chilling. There were minimal changes in shrinkage among time point (−0.4% to 1.2%; $P \leq 0.002$), pH (5.56 to 5.69; $P \leq 0.001$), and aerobic plate count (0.1 to 0.7 log; $P < 0.001$). Initial 24-h grading by a USDA grader revealed a grade composition of 21.1% Slight ($n = 44$), 34.0% Small ($n = 71$), 17.2% Modest ($n = 37$), 17.7% Moderate ($n = 36$), and 10.1% Slightly Abundant ($n = 21$). With a marbling score in numeric values between 200 (Practically Devoid^{00}) and 1100 (Abundant^{00}), carcasses that had Small or greater marbling score at 24 h experienced a decrease of 34 to 60 points after 96 h of chilling ($P \leq 0.042$). Comparatively, the marbling scores of the Slight carcasses increased from 442 points at 24 h to 469 points at 96 h. Moreover, Slight carcasses had a greater percentage of polyunsaturated fatty acids ($P < 0.001$). Results indicate that chilling for 96 h increases the marbling score of USDA Select but has minimal impacts on the marbling score of greater USDA quality grades.

Key words: beef, marbling score, chilling
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Introduction

The beef grading system in the United States is used to classify fresh beef products based on the factors that most impact product quality and lean meat yield (Murphey et al., 1960; Smith et al., 1982; Schroeder et al., 2013; Steiner, 2014). USDA quality grade is based on animal age and the marbling content of the *longissimus* muscle at the interface of 12th and 13th ribs, which has been identified as an indicator of flavor, juiciness, and tenderness (Jeremiah et al., 1970; Smith et al., 1985; Shackelford et al., 1999; Schönfeldt and Strydom, 2011; Bonny et al., 2016). Consumer acceptability and purchasing decisions of beef greatly depend on the aforementioned sensory attributes and lean color (Huffman et al., 1996; Smith et al., 2000; Platter et al., 2003). They will choose steaks with greater marbling and brighter lean color even though they have to pay more for these products (Killinger et al., 2004). The Choice–Select spread is an important indication of the strength of demand and relative supply of these 2 beef quality grades (McCully, 2018), which are the most prevalent quality grades produced in the United States. With a spread such as $31/kg in October 2020, an increase in the marbling score of carcasses graded at the border of USDA Select and USDA Choice will have a positive economic impact on the beef industry.

Many antemortem factors—genotype, age, sex, nutrition, and stress—and postmortem factors—chilling, aging, and pH—influence marbling content and meat color (Fergusen et al., 1998; Lawrie and

Ledward, 2006; O'Neill et al., 2006; Loredo-Osti et al., 2019). Beef carcasses are normally chilled for 24 to 48 h after slaughter to achieve an internal temperature of 7°C or less (Savell, 2012). The 2 most common methods of chilling are blast chilling and spray chilling. Blast chilling involves placing beef carcasses in a "hot box" under circulated chilled air (Savell, 2012). This type of chilling may cause cold shortening, resulting in less tender meat (Locker and Hagyard, 1963), darker lean, and decreased marbling visibility (Van Moeseke et al., 2001). Spray chilling uses water with or without antimicrobial agents to decrease the shrinkage that is caused by moisture evaporation and maintain the freshness and bloom of the meat (Savell et al., 2005). Compared with the chilled air of blast chilling, the cold water used in spray chilling has greater heat conductivity and more effectively decreases carcass temperature through moisture evaporation. In many beef packing plants, it has been speculated that the USDA quality grade increases if cattle are harvested on Friday and graded on Monday, which allows beef carcasses to be chilled 48 h longer than normal. Preliminary data, collected by the authors of the current study from 10 USDA Choice carcasses evaluated by the same USDA grader, revealed a 20-point increase in marbling score after 96-h chilling (Practically Devoid $[PD]^{00} = 200$ points; Abundant $[AB]^{00} = 1{,}000$ points). However, as beef carcasses are chilled from 24 to 96 h, the carcass pH, lean color, and microbial growth may be influenced by the chilling duration. Depending on the saturation index of the fatty acid composition of the intramuscular fat, chilling duration may also influence marbling visibility on the background of lean color (Page et al., 2001; Wood et al., 2004). This measurement is important to the grader when estimating the amount and distribution of intramuscular fat (Tume, 2004). Therefore, the objective of the current study was to determine the fatty acid composition and the effects of the chilling duration on marbling score, carcass shrinkage, pH, aerobic plate count (APC), and lean color of beef carcasses of differentiated marbling score categories.

Materials and Methods

Experimental design and USDA grading

Two hundred and nine beef carcasses (body weight of 361 ± 53 kg) were processed at a commercial facility over 6 nonconsecutive weeks from November of 2019 to February of 2020 (32, 41, 42, 35, 25, and 34 carcasses, respectively). For each week, a group of Angus crossbred beef cattle from a commercial breeding and feeding facility in the Southeastern United States were selected for processing. These cattle were housed in mono-slope barns and fed a diet of 48% corn, 20% corn silage, 10% sorghum silage, 7% wet corn gluten, 6% minerals, 5% dried distillers' grain, and 4% hay for 180 to 210 d. Cattle were selected solely by the packer's live weight requirement of 589 to 635 kg. The carcasses were only spray-chilled for the first 14 h in a hot box at 0°C to 3°C with a wind speed of 3.1 m/s under 153 lux of fluorescent light. The spray chilling was conducted for 30 s every 5 min with cold water that contained 80 ppm of hypobromous acid. The spray chilling stopped after 14 h, and the entire chilling duration up to 96 h was only blast (air) chilling. At 24 h, the carcasses were ribbed between the 12th and 13th ribs to expose the ribeye surface for a repeated evaluation at 24, 48, 72, and 96 h. The same USDA grader evaluated the yield grade, quality grade, and marbling score at each point. During each evaluation, the carcasses were randomized on the rail to prevent the grader's bias. The ribeye surface was additionally refaced by approximately 3 mm with a boning knife at 48, 72, and 96 h to present a fresh surface to the USDA grader. The samples from resurfacing at 48 h were used for fatty acid analysis. The ribeye surface was allowed to bloom for 45 min before grading. The marbling scores were converted into a numerical value as follows: $PD^{00} = 200$, Trace $(TR)^{00} = 300$, Slight $(SL)^{00} = 400$, Small $(SM)^{00} = 500$, Modest $(MT)^{00} = 600$, Moderate $(MD)^{00} = 700$, Slightly Abundant $(SA)^{00} = 800$, Moderately Abundant $(MA)^{00} = 900$, and $AB^{00} = 1{,}000$ (Casas et al., 2004). Only 24-h marbling score categories of SL, SM, MT, MD, and SA had sufficient sample size (10 head or more as determined by the preliminary study) and were thus used to classify the carcasses for statistical analysis. The USDA quality and yield grades at 96 h postmortem were recorded as the official grades for purposes of sale.

Fatty acid analysis

The muscle samples that were collected at 48 h from the ribeye surfaces were placed in Whirlpak® bags, vacuum packaged, and immediately frozen at −20°C. The samples were transported to Mississippi State University on dry ice and thawed overnight. They were then trimmed of all external fat, connective tissues, and accessory muscles, leaving only the *longissimus* muscle. The *longissimus* muscle was cut into

small pieces, frozen in liquid nitrogen, and pulverized into powder. One gram of a homogenized sample was weighed into a 20-mL flat-bottom glass vial with a Teflon®-lined screw cap, and 1.5 mg of methyl tridecanoate was added as an internal standard. Fatty acids were methylated by direct transesterification (O'Fallon et al., 2007) in which they were saponified in the presence of potassium hydroxide and methanol then subsequently transesterified in the presence of sulfuric acid into fatty acid methyl esters (FAME), which were extracted in hexane. Fatty acid methyl esters were identified by a gas chromatography system (Agilent Technologies, Santa Clara, CA) equipped with an HP-88 capillary column (30 m × 0.25 mm i.d. × 0.2 μm film thickness; Supelco Inc., Bellefonte, PA) and an Agilent 5975C inert XL MSD with triple-axis mass detector. Peaks were identified by FAME standards in Supelco® 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO), FAME #21 Mix (AOCS #6; Restek, Bellefonte, PA), a customized 17-component FAME mix (Nu-Chek-Prep, Elysian, MN), and various individual FAME standards. All fatty acids were monitored by their target and quantitative ions in the selected ion monitoring mode. Fatty acid concentration (micrograms per gram of sample) was quantified by an internal standard calibration method. The fatty acid percentage was subsequently calculated as grams per 100 g of total fatty acids. The saturation index was calculated by the ratio of saturated fatty acids (SFA) to the sum of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The P/S was the ratio of PUFA to SFA. The iodine value (IV; grams of iodine per 100 g of sample) was calculated as IV = [16:1] × 0.95 + [18:1] × 0.86 + [18:2] × 1.732 + [18:3] × 2.616 + [20:1] × 0.785 + [22:1] × 0.723 (IV; AOCS, 1989).

Temperature, shrinkage, pH, and aerobic plate count

Temperature data were recorded continuously for 96 h by inserting 2 portable temperature probes (Stainless Steel Pro Logger probe, ThermoWorks, American Fork, UT) into the *longissimus* muscle on the left (not ribbed) and right (ribbed) sides and between the 12th and 13th ribs of an additional carcass chilled under the same conditions. The hot box cooler was also monitored for temperature, wind speed, and light during the entire study. Hot carcass weight (HCW) and daily cold carcass weight (CCW) were recorded by a Vande Berg® static scale (ST-MONO-1000, Vande Berg Scales, Sioux Center, IA). Shrinkage was determined by subtracting the CCW from the HCW, dividing by the HCW, and multiplying by 100. At 24, 48, 72, and 96 h, the pH was measured by laterally inserting—approximately 1.3 cm deep into the *longissimus* muscle—a temperature compensated pH probe that was connected to a pH meter (model #HI99163, Hanna Instruments, Inc., Woonsocket, RI). The pH meter was calibrated using pH standards of 4, 7, and 10. Fifty total carcasses were randomly selected from weeks 2 through 6 (10 carcasses per week) to determine the APC. A sterile sponge wetted with buffered peptone water (3M Health Care, St. Paul, MN) was used to swab a 12.5-cm × 12.5-cm area on the round and chuck of the carcass at 24, 48, 72, and 96 h. Sponges were transported in sterile Whirlpak® bags to a commercial laboratory (IEH Laboratories and Consulting Group, Lakeland, FL) for analysis. The APC was reported as the log of colony-forming units per square centimeter (log CFU/cm^2 or log).

Instrumental color

The lean color was measured at 24, 48, 72, and 96 h using a HunterLab MiniScan 4500L Spectrophotometer (Hunter Associates Inc, Reston, VA) on the ribeye surface, which had bloomed for 45 min. The CIE L^*, a^*, b^* values were analyzed in triplicate with an illuminant A, 10° observer's angle, and 25-mm aperture size.

Statistical analysis

The temperature graph was printed by the instrument (data export not available), and no statistical analysis was conducted. Marbling score category at 24 h, animal, and postmortem time served as the main factor, main plot, and split-plot (in time) factor, respectively, in a split-plot design. Marbling score, shrinkage, pH, and L^*, a^*, and b^* data were acquired at 24, 48, 72, and 96 h and analyzed using a generalized linear mixed model. Marbling score category, time, and their interaction were defined as fixed effects, and "animal within a marbling score category" was defined as a random effect. The selection of the appropriate covariance structure for the repeated measurement was based on 3 default Information Criteria that were calculated by SAS (SAS Institute Inc., Cary, NC) in the smaller-is-better format (Akaike's Information Criteria, Akaike's Information Criteria Corrected, and Bayesian Information Criteria) (Kincaid, 2005), resulting in the use of a compound symmetry structure. Fatty acid composition and APC were analyzed in a similar statistical model with only marbling category (fatty acid composition) or time (APC) as fixed effects and animal as

a random effect. The analysis of variance was performed by the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc.). When means were different, they were separated using a protected *t* test using the LSMEANS/PDIFF/SLICEDIFF statement of the GLIMMIX procedure. Actual probability values were reported.

Results and Discussion

Distribution of 24-h marbling score and quality grade and fatty acid composition

The marbling score distribution (Figure 1) was 21% SL ($n = 44$), 34% SM ($n = 71$), 17% MT ($n = 37$), 18% MD ($n = 36$), and 10% SA ($n = 21$), resulting in a quality grade distribution of 0% Standard, 21% Select, 69% Choice, and 10% Prime. The same commercial processing facility reported that within 7,574 steers and heifers harvested in 2020, there were no Standard, 17% USDA Select, 72% USDA Choice, and 8% USDA Prime carcasses. Compared with the National Beef Quality Audit (NBQA) of 9,106 fed steer and heifer carcasses (Boykin et al., 2017), carcasses in the current study had greater USDA marbling scores than the NBQA distribution of 5.6% Standard and lower, 23% Select, 67% Choice, and 4% Prime. Marbling score distribution in the 2016 NBQA audit had 0.8% TR or less, 23.6% SL, 39.6% SM, 23.5% MT, 7.6% MD, and 0.9% SA or greater. The TR and PD marbling scores were rare in the cattle selected for the current study. Based on the structure of the marbling scores used to assign USDA quality grades, USDA Choice and Prime grades were spread within a wider range of marbling scores than the USDA Select.

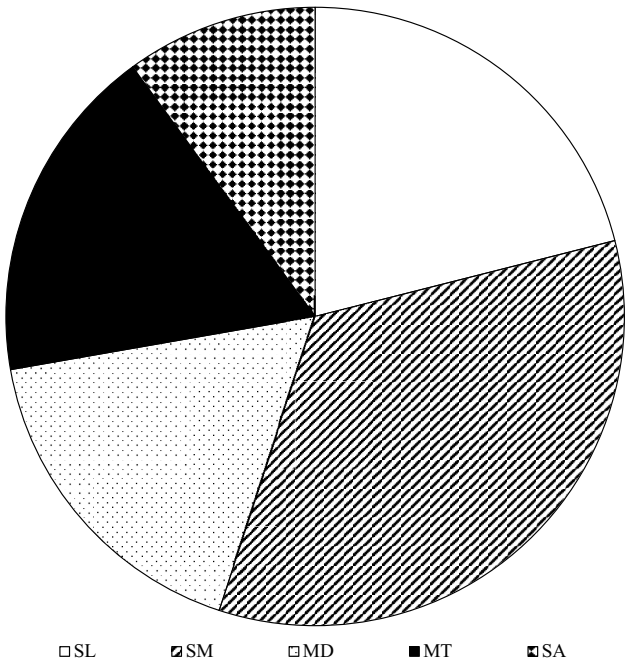

Figure 1. USDA marbling score composition for 209 beef carcasses at 24 h postmortem, including Slightly Abundant (SA; $n = 21$), Moderate (MD; $n = 36$), Modest (MT; $n = 37$), Small (SM; $n = 71$), and Slight (SL; $n = 44$), used to determine the effects of prolonged chilling on marbling score.

The total fatty acids of the *longissimus* muscle differed among SL, SM, MT, MD, and SA carcasses, at 39.19, 53.36, 69.36, 84.68, and 101.05 mg/g of muscle ($P < 0.001$). The fatty acid composition (Table 1) was typical of beef marbling with predominant fatty acids being 16:0 (27% to 32%), 18:1 cis9 (25% to 31%), 18:0 (13% to 18%), and 18:2 cis 9,12 (4% to 5%). There were differences in fatty acid composition among marbling categories, with SL carcasses consistently having less SFA and MUFA and greater PUFA ($P \leq 0.009$). Although the saturation index, P/S ratio, and IV were similar among marbling categories ($P \geq 0.269$), the SL carcasses had 2.5%, 3.2%, 3.9%, and 4.0% more PUFA than SM, MT, MD, and SA carcasses, respectively ($P < 0.001$). Moreover, there were approximately 6.4% to 8.4% branch-chained fatty acids, which has not been reported previously in beef intramuscular fat. The USDA FoodData Central reports that USDA Prime, Choice, and Select strip steaks have approximately 45% to 46% SFA, 49% MUFA, and 4% to 6% PUFA, respectively. Litwi czuk et al. (2015) also reported 49% to 56% SFA, 38% to 49% MUFA, and 2% to 7% PUFA in intramuscular fat of Friesian Holstein heifers, cows, young bulls, and calves. These results were similar to values from the current study. Dinh et al. (2010) reported 3% to 8% PUFA for Angus, Brahman, and Romosinuano cattle, with leaner carcasses having greater PUFA percentage in addition to phospholipids being more concentrated in leaner carcasses than in fatter carcasses. Legako et al. (2015) reported smaller percentages of PUFA in the neutral lipid fraction (triglycerides) in comparison to that in the polar lipid fraction. As ruminant animals deposit fat, more SFA and MUFA are accumulated because of biohydrogenation in the rumen (Vahmani et al., 2015) along with the elongation and the Δ^9 desaturase activity in adipose and muscle tissues (Smith et al., 2006). Although saturation index, P/S, and IV values did not differ among marbling categories, the greater PUFA percentage could delay the solidification of marbling in the SL carcasses.

Table 1. Fatty acid composition (percentage of total fatty acids) of the *longissimus* muscle from beef carcasses having marbling score of Slightly Abundant (SA; $n = 21$), Moderate (MD; $n = 36$), Modest (MT; $n = 37$), Small (SM; $n = 71$), and Slight (SL; $n = 44$)

Fatty Acids	SA	MD	MT	SM	SL	SE	*P* Value
SFA	49.63	51.23	49.48	49.59	48.42	1.80	0.486
10:0	0.07	0.07	0.11	0.06	0.05	0.02	0.192
12:0	0.08	0.08	0.09	0.07	0.06	0.01	0.060
14:0	3.01	3.30	3.36	2.85	2.35	0.26	< 0.001
15:0	0.37	0.37	0.35	0.31	0.35	0.03	0.069
16:0	31.72	31.38	30.66	29.46	26.69	1.01	< 0.001
17:0	0.93	0.88	0.79	0.70	0.70	0.06	< 0.001
18:0	13.34	15.01	13.99	15.98	18.05	1.13	< 0.001
19:0	0.05	0.05	0.05	0.06	0.08	0.01	< 0.001
20:0	0.07	0.08	0.08	0.09	0.12	0.01	< 0.001
22:0	0.01	0.01	0.00	0.01	0.02	0.01	0.314
MUFA	38.96	37.50	38.14	37.64	35.32	1.19	0.010
14:1 cis9	0.79	0.83	0.84	0.67	0.51	0.11	< 0.001
15:1 cis9	0.10	0.14	0.16	0.23	0.33	0.03	< 0.001
16:1 cis6	0.04	0.03	0.03	0.04	0.89	0.74	0.454
16:1 cis9	3.75	3.50	3.58	3.16	2.46	0.25	< 0.001
16:1 cis7	0.11	0.11	0.10	0.09	0.16	0.06	0.833
17:1 cis10	0.79	0.70	0.63	0.52	0.44	0.05	< 0.001
18:1 trans11	1.46	1.19	1.83	2.30	3.56	0.68	< 0.001
18:1 cis9	30.47	29.38	29.26	28.94	25.71	1.41	0.002
18:1 cis11	0.74	0.87	1.05	1.06	1.96	0.90	0.504
18:1 cis12	0.01	0.07	0.04	0.10	0.16	0.05	0.036
18:1 cis13	0.38	0.44	0.34	0.30	0.24	0.05	< 0.001
19:1 cis10	0.07	0.07	0.06	0.06	0.04	0.01	0.004
19:1 cis	0.03	0.02	0.02	0.01	0.01	0.00	< 0.001
20:1 cis11	0.23	0.15	0.17	0.16	0.11	0.03	0.002
PUFA	4.78	4.87	5.53	6.21	8.71	1.02	< 0.001
18:2 trans9,12	0.15	0.14	0.14	0.18	0.20	0.02	< 0.001
18:2 cis9,12	3.85	3.69	4.25	4.45	5.13	0.42	< 0.001
18:2 cis12,15	0.02	0.02	0.03	0.04	0.16	0.12	0.562
18:3 γ cis6,9,12	0.02	0.03	0.03	0.05	0.06	0.01	< 0.001
18:3 cis9,12,15	0.09	0.11	0.11	0.12	0.16	0.01	< 0.001
20:2 cis11,14	0.05	0.04	0.04	0.05	0.05	0.01	0.227
20:2 cis9,12	0.00	0.02	0.02	0.03	0.06	0.01	< 0.001
20:3 cis5,8,11	0.01	0.02	0.02	0.04	0.07	0.01	< 0.001
20:3 cis8,11,14	0.20	0.24	0.27	0.37	0.46	0.05	< 0.001
20:4 cis5,8,11,14	0.24	0.32	0.37	0.53	0.77	0.07	< 0.001
20:5 cis5,8,11,14,17	0.00	0.02	0.02	0.04	0.09	0.02	< 0.001
22:4 cis7,10,13,16	0.10	0.11	0.12	0.15	0.20	0.02	< 0.001
22:5 cis7,10,13,16,19	0.05	0.10	0.10	0.15	0.22	0.02	< 0.001
22:6 cis4,7,10,13,16,19	0.00	0.00	0.00	0.01	0.02	0.01	0.012
BCFA	6.63	6.40	6.85	6.56	8.42	1.02	0.068
14:0 13-methyl	0.05	0.07	0.09	0.07	0.14	0.06	0.308
15:0 14-methyl	0.09	0.09	0.09	0.10	0.12	0.01	0.001
16:0 15-methyl	2.56	2.58	3.01	2.95	3.52	0.41	0.057
16:0 14-methyl	3.75	3.50	3.58	3.16	2.46	0.16	0.041
P/S ratio	0.10	0.10	0.11	0.13	0.35	0.17	0.276
Saturation index	1.14	1.22	1.15	1.15	1.35	0.16	0.351
Iodine value	38.17	36.95	37.98	37.80	35.35	1.82	0.269

BCFA = branched-chain fatty acid; MUFA = monounsaturated fatty acid; *P* = probability value; P/S ratio = ratio of PUFA to SFA; PUFA = polyunsaturated fatty acid; SE = pooled standard error; SFA = saturated fatty acid.

Temperature, shrinkage, pH, and aerobic plate count

Regardless of whether a beef side was ribbed, the temperature of the *longissimus* muscle started at 37.5°C to 39°C and reached 3°C after approximately 16 h (Figure 2). The temperature did not change any further during the remaining 96 h. There was a two-way marbling category × time interaction for shrinkage (Table 2; $P = 0.002$). Carcasses in SA, MD, MT, and SM categories had similar shrinkage at 24 and 96 h ($P \geq 0.083$). At 48 h, MD, MT, and SM shrinkage was 0.5% to 0.7% ($P \leq 0.007$), which increased to 0.9% to 1.2% at 72 h ($P \leq 0.001$). Although SL shrinkage was similar to that of other categories at 48 and 72 h ($P \leq 0.192$), shrinkage of SL differed slightly from other categories at 96 h (−0.4%, $P = 0.031$). There was a two-way marbling category × time interaction for pH (Table 2; $P \leq 0.001$). The MT and SL carcasses reached an average ultimate pH of 5.56 at 24 h, whereas the pH of the *longissimus* muscle did not change during cold storage ($P \geq 0.079$). However, for SA, the pH declined from 5.64 at 24 h to 5.57 at 48 h ($P = 0.034$) but increased back to 5.64 and 5.69 at 72 and 96 h ($P = 0.025$ and < 0.001, respectively). For MD, the pH reached 5.57 at 24 h and increased to a final value of 5.65 to 5.68 at subsequent time points ($P \leq 0.001$). For SM, the pH slightly dropped to 5.57 at 72 h ($P = 0.010$) but increased back to 5.62 at 96 h ($P = 0.011$), which was similar to that at 24 h and 48 h ($P = 0.124$ and 0.981, respectively). At 24 h, the APC value was 0.1 log, which gradually increased to 0.7 log by 96 h ($P < 0.001$; Figure 3).

Postmortem chilling of beef carcasses is to ensure food safety, maximize shelf life, and reduce shrinkage with little emphasis on beef quality attributes (Savell et al., 2005). Aalhus et al. (2001) reported that conventionally chilled carcasses reached 4°C at 24 h, similar to the current study. However, Sørheim et al. (2001) used similar chilling conditions to those in the current study and reported that carcasses reached 4°C to 5°C at 10 h postmortem when cut at the 10th and 11th thoracic vertebrae and behind the 5th sacral vertebrae, through the shaft of the *ilium* of the pelvic bone at approximately 2 cm cranial to the hip bowl. The data in the current study indicate that regardless of whether a carcass is ribbed, the pattern of temperature decrease remains the same during spray chilling, with the final internal temperature being reached before 24 h. Therefore, the findings of fat solidification and marbling score in the current study apply to normal production settings, in which carcasses remain intact until a specific time when they are ribbed for grading.

The Meat and Poultry Inspection Program regulations sections 301.2(c)(8) and 318.4(d), FSIS Directive 6340.1, and FSIS Directive 8830.1 state that CCW may not increase by more than 2% of the HCW if spray chilling is used. In the current study, carcass shrinkage ranged from 0.5% to 1.5% over a 96-h period with a rare −0.4% for SL carcasses at 96 h. This finding was consistent with reports from Jones and Robertson (1988), Strydom and Buys (1995), and Schweihofer (2011), who observed 0.8% to 2.0% shrinkage in North American carcasses in commercial operations that used spray chilling. During 48-h spray chilling,

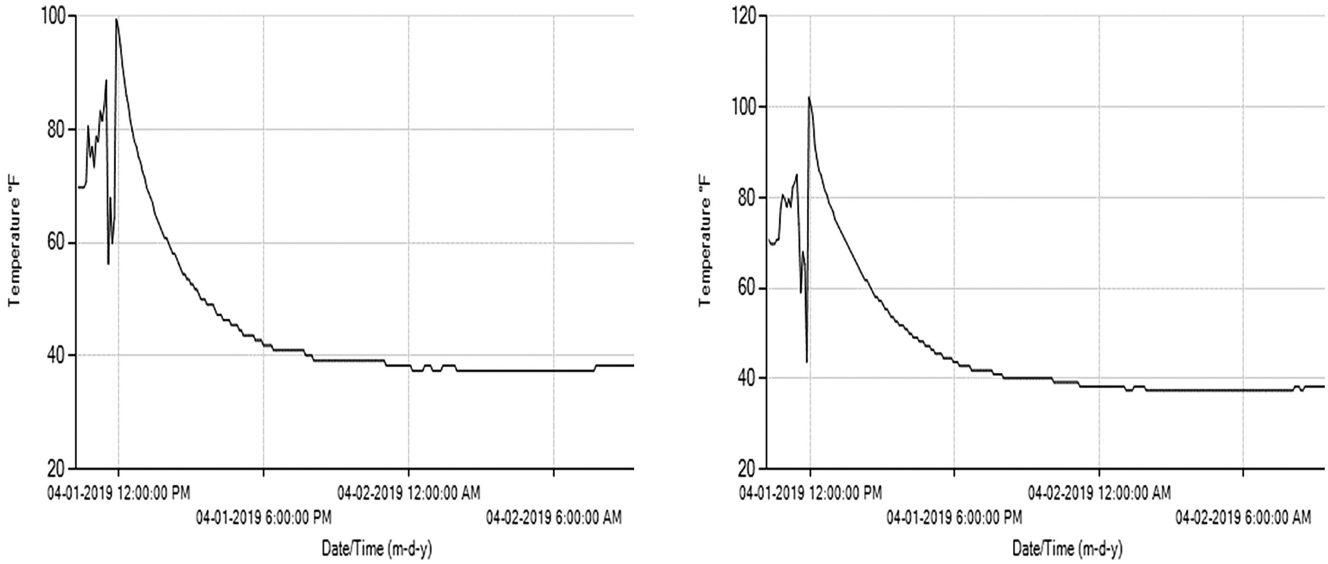

Figure 2. Core temperature of beef carcasses during chilling for 96 h at 0 to 3°C with a wind speed of 3.1 m/s under 153 lux of fluorescent light in a commercial hot box.

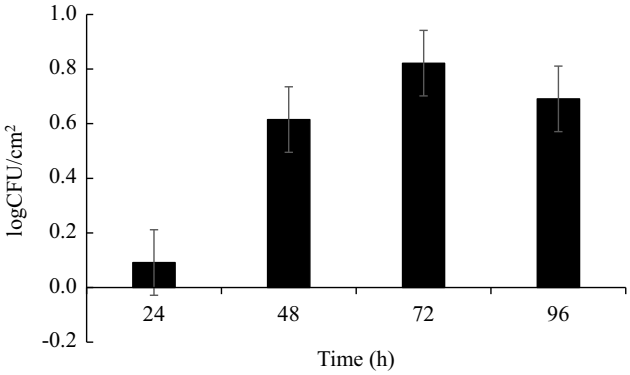

Figure 3. Aerobic plate count of 50 carcasses from data collection #2 to #6 from a 12.5-cm × 12.5-cm area on the round and chuck of the carcass at 0°C to 3°C with a wind speed of 3.1 m/s under 153 lux of fluorescent light in a commercial hot box at 24, 48, 72, and 96 h of chilling. Only value at 24 h differed from others ($P < 0.001$).

Kinsella et al. (2006) found that carcass shrinkage was 1.6 % of HCW, slightly more than what was observed in the current study. Greer and Jones (1997) found a linear relationship between the duration of spray chilling and carcass weight loss and concluded that carcass shrinkage only decreased by less than 0.1% for every hour of spray chilling. It is well known that the chilling system is successful at preventing 1% to 2% weight loss by evaporation, depending on how chilling cycles are arranged. However, weight gain may occur because of water absorption (Prado and Felicio, 2010). These findings assure that if prolonged chilling is needed to increase marbling score, there will be minimal impacts on CCW.

The normal pH for beef carcasses is 5.40 to 5.59, which allows for the development of desirable meat quality traits (Page et al., 2001; Viljoen et al., 2002). Van Moeseke et al. (2001) measured the pH of beef carcasses at 1, 5, and 24 h postmortem at a commercial facility and reported pH values of 5.45 to 5.71. These findings were similar to the values reported in the current study, although Reid et al. (2017) documented a slightly higher 24-h pH of 6.17 that decreased

Table 2. Carcass shrinkage, pH, and lean color (lightness [L^*], redness [a^*] yellowness [b^*], chroma, and hue angle) of beef carcasses with initial marbling score of Slightly Abundant (SA; $n = 21$), Moderate (MD; $n = 36$), Modest (MT; $n = 37$), Small (SM; $n = 71$), and Slight (SL; $n = 44$) at 24, 48, 72, and 96 h of chilling at 0°C to 3°C with a wind speed of 3.1 m/s under 153 lux of fluorescent light in a commercial hot box

Marbling Category		Shrinkage	pH	L^*	a^*	b^*	Chroma	Hue Angle
SA	24 h	0.0^{b}	5.64^{a}	43.47^{b}	28.53^{ab}	21.27^{b}	35.63^{b}	36.59^{a}
	48 h	0.7^{a}	5.57^{b}	44.40^{ab}	29.81^{a}	22.18^{b}	37.16^{a}	36.63^{a}
	72 h	0.4^{b}	5.64^{a}	45.41^{a}	29.82^{a}	22.64^{a}	37.45^{a}	37.21^{a}
	96 h	0.1^{b}	5.69^{a}	45.34^{a}	30.28^{a}	23.56^{a}	38.39^{a}	37.86^{a}
MD	24 h	0.0^{b}	5.57^{b}	42.23^{c}	29.55^{a}	21.54^{b}	36.58^{a}	36.09^{a}
	48 h	0.7^{a}	5.68^{a}	44.33^{b}	30.00^{a}	22.53^{a}	37.53^{a}	36.88^{a}
	72 h	1.0^{a}	5.64^{a}	44.72^{b}	30.27^{a}	23.01^{a}	38.03^{a}	37.20^{a}
	96 h	0.3^{b}	5.65^{a}	46.15^{a}	29.69^{a}	22.66^{a}	37.36^{a}	37.31^{a}
MT	24 h	0.0^{b}	5.60^{a}	41.77^{b}	30.32^{a}	22.13^{a}	37.55^{a}	36.06^{a}
	48 h	0.5^{c}	5.65^{a}	44.25^{a}	30.70^{a}	22.44^{a}	38.07^{a}	36.28^{a}
	72 h	0.9^{a}	5.61^{a}	44.15^{a}	30.90^{a}	23.09^{a}	38.60^{a}	36.85^{a}
	96 h	0.0^{b}	5.62^{a}	45.52^{a}	29.85^{a}	22.49^{a}	37.38^{a}	36.96^{a}
SM	24 h	0.0^{c}	5.59^{ab}	41.63^{c}	29.51^{a}	21.53^{b}	36.54^{b}	36.11^{a}
	48 h	0.6^{b}	5.62^{a}	43.09^{b}	29.54^{a}	22.27^{a}	37.04^{ab}	36.93^{a}
	72 h	1.2^{a}	5.57^{b}	43.33^{b}	29.99^{a}	22.87^{a}	37.76^{a}	37.25^{a}
	96 h	-0.2^{c}	5.62^{a}	45.05^{a}	29.65^{a}	22.54^{a}	37.32^{ab}	37.15^{a}
SL	24 h	0.0^{c}	5.59^{a}	41.20^{a}	28.85^{a}	21.78^{a}	36.18^{a}	37.04^{a}
	48 h	0.5^{b}	5.58^{a}	42.05^{a}	28.80^{a}	20.64^{b}	35.47^{ab}	35.75^{a}
	72 h	1.0^{a}	5.56^{a}	41.54^{a}	29.26^{a}	21.91^{a}	36.65^{a}	36.55^{a}
	96 h	-0.4^{d}	5.56^{a}	41.62^{a}	27.77^{b}	20.22^{b}	34.37^{b}	36.02^{a}
SE		0.521	5.690	2.251	1.592	1.529	2.131	1.321
$P_{marbling}$		0.509	< 0.001	< 0.001	0.002	0.003	< 0.001	0.175
P_{time}		< 0.001	0.673	< 0.001	0.043	0.089	0.084	0.501
$P_{interaction}$		0.002	< 0.001	0.007	0.363	0.005	0.046	0.082

[a–c]Within a row, means with different letters differ ($P \leq 0.05$).

P = probability value; SE = pooled standard error.

to 5.57 by 96 h. The pH values observed in the current study are comparable to what has been reported in the literature (Savell et al., 2005; Emerson et al., 2013; Zhang et al., 2018). Meat pH affects quality attributes such as color and water-holding capacity (Jacob and Hopkins 2014; Kim et al., 2014). Meat with high pH due to the depletion of glycogen antemortem fails to develop bright cherry red lean color and has greater water-holding capacity but shorter shelf life (Holman et al., 2016; Zhang et al., 2018). Water-holding capacity affects the reflectance of light on the surface of meat and the visibility of marbling (Swatland, 2013; Hughes et al., 2014a). Ijaz et al. (2020) observed an increase in pH for dark, firm, and dry (DFD) carcasses and an increase in lightness, redness, and chroma after day 3. However, Purchas et al. (1999) reported a decline in L^*, a^*, and b^* values in DFD carcasses during storage because of high pH (> 6.0). The pH in the current study was not as high as what was reported for DFD beef, and although there was a fluctuation of pH in some marbling categories, such changes were not large enough to impact lean color.

The APC value observed in the current study was less than what has been reported for beef carcasses. Ahmad et al. (2013) sampled 100 cm^2 of the forequarter and hindquarter of the beef carcasses and reported an APC value of 2.8 log. Hauge et al. (2015) reported 4.3 to 4.5 log after 24-h chilling. However, these authors did not use spray chilling with antimicrobials. Reyes et al. (2018) used 1,3-dibromo-5,5-dimethylhydantoin in spray chilling and reported a reduction from 3.0 to less than 1.2 log CFU/cm^2 at 24 h. When other antimicrobials such as chlorine dioxide and peroxyacetic acid were used, there was a similar reduction of 2.6 to 4.0 log from 24 to 72 h (Kocharunchitt et al., 2020). Spray chilling is combined with antimicrobial interventions to control bacterial growth on wet surfaces of carcasses not only during chilling but also before fabrication (Okuro et al., 2013). The findings in the current study indicated that prolonged chilling up to 96 h did not have any significant impact on microbial growth on the surface of beef carcasses.

Lean color

There was a two-way marbling category × time interaction for lightness (L^*, $P = 0.007$). The SA, MD, MT, and SM categories increased in lightness from 24 h (41.6 to 43.5) to 96 h (45.1 to 46.2; $P \leq 0.037$). The SL category had similar lightness from 24 h to 96 h ($P > 0.170$). There were overall marbling and time effects on redness (a^*; $P = 0.002$ and 0.042, respectively; Figure 4). Carcasses with SA, MD, MT, and SM marbling scores all had similar redness values from 29.1 to 30.4 ($P \geq 0.057$). Carcasses with SL marbling scores had the lowest redness value of 28.7 ($P \leq 0.001$). Carcasses at 48 and 72 h (30.0 and 29.9, respectively) had greater redness than at 24 and 96 h (29.6 and 29.1, respectively; $P \leq 0.001$).

There was a two-way marbling × time interaction for yellowness (b^*; $P = 0.005$). Carcasses from SA, MD, MT, and SM all had similar yellowness from 24 to 96 h (28.5 to 30.3, 21.5 to 22.7, 22.1 to 23.1, and 21.5 to 22.9, respectively, $P < 0.071$). The SL carcasses decreased in yellowness by 1 to 1.5 units from 24 to 48 h and again from 72 h to 96 h ($P \leq 0.021$). In addition, the SA, MD, MT, and SM had similar chroma values of 37.2 to 38.5 ($P = 0.820$). The SL carcasses

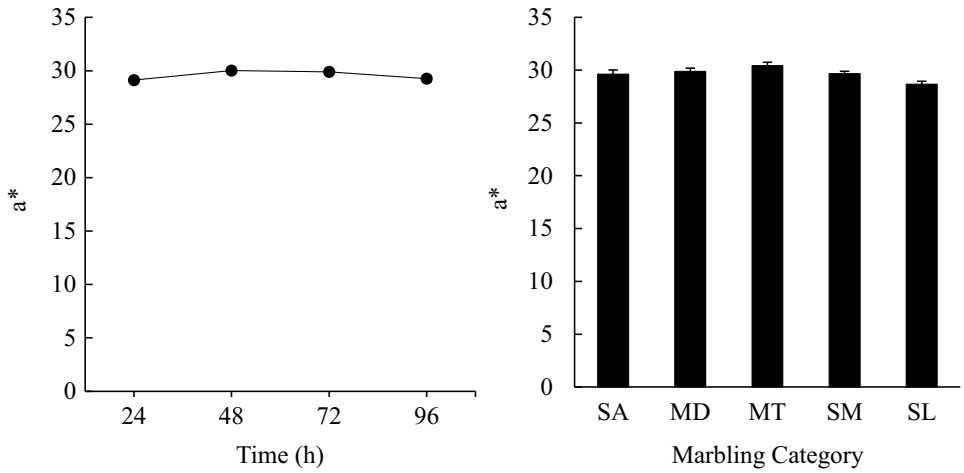

Figure 4. Redness (a^*) of beef *longissimus* muscles of differentiated initial marbling scores, chilled in a commercial hot box under a wind speed of 3.1 m/s and 153 lux of fluorescent light, averaged across 24, 48, 72, and 96 h (lowest at 24 and 96 h; $P < 0.001$) and across USDA quality grades of Slightly Abundant (SA; $n = 21$), Moderate (MD; $n = 36$), Modest (MT; $n = 37$), Small (SM; $n = 71$), and Slight (SL; $n = 44$; lowest redness; $P < 0.001$).

had the lowest chroma value of 35.6 ($P \leq 0.001$). No differences existed in hue angle ($P = 0.082$).

Using illuminant D65, 8-mm aperture, and 10° observer angle, Gagaoua et al. (2018) reported 24-h L^*, a^*, b^*, and chroma values of 32.4, 18.9, 18.7, and 26.6, respectively, under similar environmental conditions to those in the current study. The D65 illuminant simulates natural daylight and highlights blueish tones while subduing green and red tones (Salueña et al., 2019), yielding slightly less redness in comparison with the A10 illuminant. Using the same instrumental settings as those in the current study to measure the lean color of USDA Choice *longissimus* muscle under the simulated retail display, King et al. (2012) reported that L^* decreased from 41 units at 24 h to 39 units at 96 h and that a^* decreased from 34 units at 24 h to 30 units at 96 h. Chroma value likewise decreased from 24 to 96 h in increments similar to those found in the current study, although in too small of a magnitude to be detectable by consumers. Though myoglobin oxidation may affect surface color, the effects of this oxidation on lean color were minimalized in the current study by refacing the ribeye surfaces before grading and color measurement. Kirchofer et al. (2002) evaluated the impact of chilling time on a^* and b^* in carcasses at 2 commercial facilities. These authors reported an a^* value of 32.5 at 24 h and 33.9 to 35.0 at 48 h, which are similar to those in the current study. However, King et al. (2011) documented a decrease in redness as holding time increased. Because this decrease was caused by oxidation, such a phenomenon was unlikely to occur in the current study owing to the refacing of evaluated ribeye surfaces.

The pH decline postmortem also affects color, as it generally increases lightness (Hughes et al., 2014b). However, changes in pH from 24 to 96 h in the current study were minimal. Stiffler et al. (1984) reported that L^*, a^*, and b^* values did not differ between 24- and 48-h chilling in electrically stimulated beef carcasses; however, in carcasses that had not been electrically stimulated, the L^* and b^* values were higher and a^* values were lower at 48 h compared with 24 h. These findings were reflected in the current study's results of the SL carcasses because they were not electrically stimulated. Lastly, Vierck et al. (2018) reported that Choice steaks with more marbling were darker in color than Select steaks at 24 h. In contrast, the current study found that chilling duration up to 96 h induced minimal changes in lean color; However, such minimal changes in SL carcasses compared with carcasses in other marbling categories might influence the grader's perception of the lean color background, upon which marbling content was scored.

Effects of chilling time on marbling score

There was a two-way marbling × time interaction ($P < 0.001$; Figure 5) for the marbling score. For SA, the marbling score was 840 at 24 h, which decreased to 796 at 48 h and 797 at 96 h ($P = 0.004$). The MD was 743 at 24 h, which continued to decrease to 685 at 48 h, 694 at 72 h, and 683 at 96 h ($P \leq 0.001$). At 24 h, the MT was 635, which decreased to 597 at 48 h ($P < 0.001$), increased back to 621 at 72 h ($P = 0.032$), and then continued to decrease to 590 at 96 h ($P \leq 0.001$). For SM, the marbling score was 539 at 24 h, which decreased to 523 at 48 h ($P = 0.042$) and finished at 505 at 96 h ($P < 0.001$). The SL started at 442 at 24 h, increased to 450 at 48 and 72 h, and continued to increase to 469 at 96 h ($P < 0.001$).

Acheson et al. (2018) found 6 anatomical locations in the *longissimus* dorsi muscle with marbling varying from 504 to 565. Early studies (Blumer et al., 1962; Cook et al., 1964; Cross et al., 1975) indicated that variation in marbling located throughout the rib and loin sections of beef carcasses exists; however, the pattern has not been determined. Although the ribeye surface was refaced for each grading time, only 3 mm of lean was removed. Acheson et al. (2018) reported that marbling particles extended approximately 8 to 9 mm throughout the muscle. Thus, refacing the ribeye surface by approximately 9 mm over 96-h grading should not impact the marbling pattern of the graded surface because marbling particles are distributed evenly throughout the *longissimus* muscle. Although dorsal marbling distribution in the beef *longissimus* muscle has not been researched, such distribution was reported to be even until the end of the thoracic vertebrae in pork *longissimus* muscle (Faucitano et al., 2004). The overall hypothesis for the current study was that carcasses would continue to be chilled; thus, fat would continue to solidify and become more visible. As stated earlier, the internal temperature of the *longissimus* muscle was reached within 16 h, at 3°C, and remained constant after that. Historical temperature recording of carcass temperature in the processing facility (data not shown) indicated that final internal temperature was reached within 12 to 16 h postmortem and was not decreased further. Bowling et al. (1987) observed a greater increase in marbling score and a greater decrease in lightness in rapidly chilled carcass compared with conventionally chilled carcasses. Carcasses reaching −2 to 0°C in 5 h (rapid chilling) had more visible intramuscular fat, graded at MT^{95}, than those reaching the same temperature within 10 to 12 h (conventional chilling), graded at MT^{50}. Janz et al. (2004) used a similar

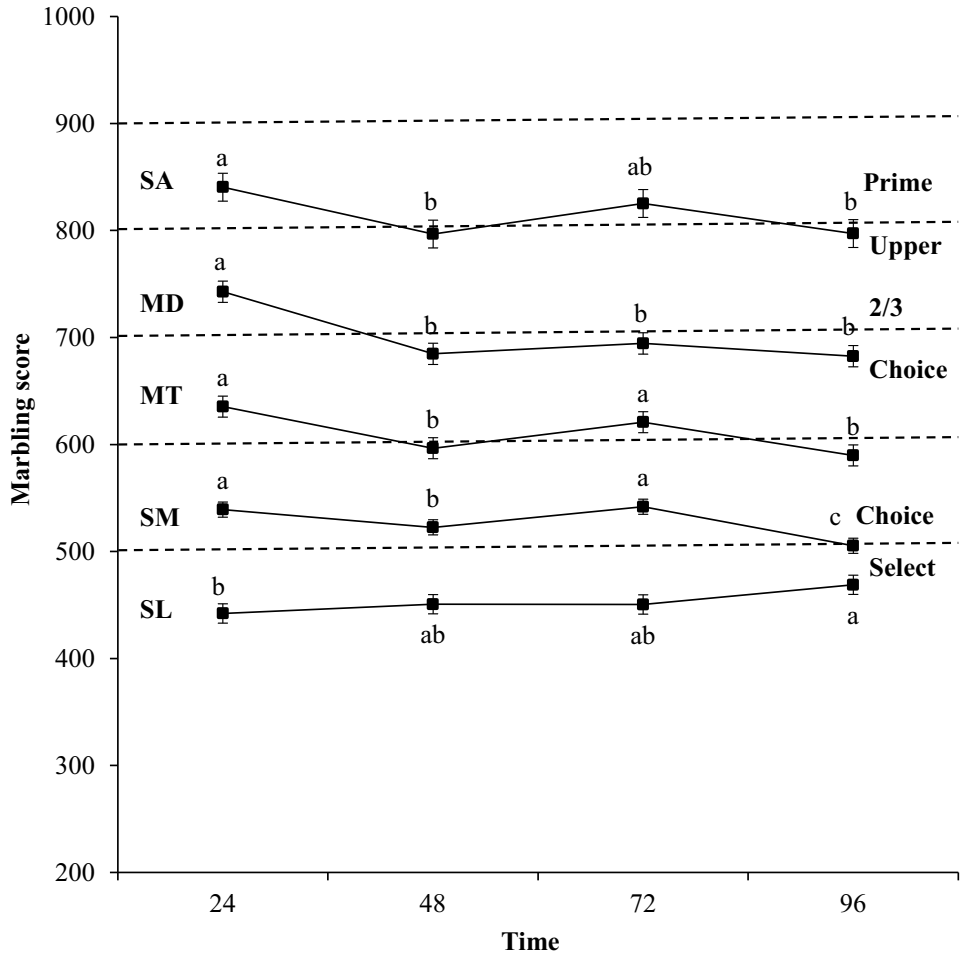

Figure 5. USDA marbling scores of beef carcasses graded Slightly Abundant (SA; $n = 21$), Moderate (MD; $n = 36$), Modest (MT; $n = 37$), Small (SM; $n = 71$), and Slight (SL; $n = 44$) at 24, 48, 72, and 96 h of chilling. Within a line, means with different letters differ ($P \leq 0.05$).

chilling method to the one in the current study and reported a temperature of 5°C at 24 h postmortem. These authors observed an increase in marbling score of 10 to 110 points in comparison with the score of 527 at 48 h under modified chilling conditions of 5°C at 24 h and 0°C to 2°C at 48 h. These authors also reported that carcasses of SM or lower marbling scores had a greater increase in marbling score than higher marbling score categories, which was similar to the findings for the SL category in the current study. The authors attributed such changes to an increase in chilling time; however, they could not conclude whether the longer holding time or the modified chilling method increased the solidification and visibility of the intramuscular fat.

In the current study, the decrease in marbling score in SA, MD, MT, and SM and the increase in marbling score in SL might be attributed to the slight changes in lightness and saturation (chroma) of the lean color background, as well as leaner carcasses having less saturation of fatty acid composition. Page et al. (2001) and Wulf et al. (1994) observed that darker lean color is negatively correlated with the L^* value. Bak et al. (2012) stated that the chroma and hue angle can be used to reflect the saturation and the shade (perceived color) of lean meat color. In the current study, there was no change in the perceived color (hue angle), which indicated that the ribeye surface would be perceived in a similar shade of redness. However, the redness saturation was decreased in the SL category, indicating that redness was being diluted. Malau-Aduli et al. (2000) also reported less color saturation with lower marbling scores in Angus and crossbred steers and heifers, which is reflected in the current study's findings for SL carcasses.

As discussed with fatty acid composition, leaner carcasses have a greater proportion of PUFA because phospholipids of lean tissues are more predominant (Legako et al., 2015), whereas, in fattier carcasses, more SFA and MUFA are being deposited in adipose tissues (Dinh et al., 2010). The proportions of SFA, MUFA, and PUFA greatly influence the melting point of animal fats and thus their solidification under

refrigeration. Saturated fatty acids solidify at 20°C to 22°C, whereas unsaturated fatty acids become solid below 20°C (Moorthy, 2018). Yang et al. (1999) found that subcutaneous fat in carcasses starts to solidify between 8°C and 15°C, with many carcasses having fats transition from liquid to solid at 10°C for 18 to 20 h. The rate of fatty acid solidification depends on the length of the carbon chain and the existence of double bonds (Wood et al., 2004). The fatty acid analysis in the current study revealed a greater proportion of PUFA in SL carcasses. Although saturation index, P/S ratio, and IV indicated that most beef carcasses have their marbling solidified within 24 h of chilling at the current chilling rate, the more unsaturated marbling in SL carcasses might continue to solidify up to 96 h of chilling. This might explain the increase in marbling score in the SL carcasses in the current study over a 96-h duration.

Conclusions and Implications

Prolonged chilling had minimal effects on marbling score and other carcass quality measurements because carcasses reached a final internal temperature of 3°C by 16 h postmortem and arrived at the final pH within 24 h, with minimal fluctuation until 96 h. Our results differ from industry-standard chilling times (36 to 48 h) and recent audits by allowing carcasses an extra 48 h of chilling duration (96 h) and greater solidification of unsaturated fatty acids, potentially affecting marbling visibility and score. Like Janz et al. (2004), carcasses with a marbling score of USDA Select have the potential to increase in quality grade. Unlike Janz et al. (2004), carcasses with greater marbling scores may decrease in quality grade after prolonged spray chilling up to 96 h. The main reason for these result differences may be the fact that fats in carcasses with greater quality grades no longer solidified because their fatty acid composition was more saturated. Moreover, the grader's perception of higher quality grades might be different from that of the lower ones. The results suggest that the increase in quality grades experienced in the industry may be influenced by not only the chilling duration but also the initial marbling visibility. Further research is needed to determine the frequency by which $SL^{60–100}$ carcasses reach SM marbling score after prolonged chilling and whether the cost is worth the increase in cooler value. Such research should be conducted under instrumental grading with verification of USDA graders.

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