



Bone and Subcutaneous Fat Influence on Yield, Physicochemical Traits, and Color Stability of Dry-Aged Loin From Grass-Fed Nellore Bulls

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Abstract: The objective of the current study was to evaluate the influence of bone and subcutaneous fat on yield, physicochemical traits, and color stability of dry-aged beef from grass-fed Nellore bulls. Paired bone-in loins (from the 10th thoracic vertebra to 6th lumbar vertebra) from 8 carcasses were collected and cut in half, and the sections from each carcass were assigned to 4 treatments ($n = 8$): bone-in with subcutaneous fat, bone-in without subcutaneous fat, boneless with subcutaneous fat, and boneless without subcutaneous fat. Loin sections were dry-aged for 21 d, at 2°C and 70% relative humidity. After dry aging, the half-loins were weighed, trimmed, and weighed again to determine the evaporation loss, trimming loss, and yield. Additionally, water activity, pH, thiobarbituric acid-reactive substances, moisture content, cooking loss, pressed juice percentage, Warner-Bratzler shear force, and color stability (during 9 d of display) were analyzed. No interactions ($P > 0.05$) between bone and subcutaneous fat were found for evaporation and trimming loss, yield, and physicochemical traits. The treatments did not affect Warner-Bratzler shear force, pressed juice percentage, thiobarbituric acid-reactive substances, and pH values ($P > 0.05$). Regarding color stability, there was a bone-by-time interaction ($P < 0.05$) for a^* and b^* parameters. Boneless treatments showed higher a^* and b^* values ($P < 0.05$) than bone-in treatments, after 6 and 7 d of display, respectively. Bone-in treatments and treatments with subcutaneous fat had lower evaporation and trimming loss and higher yield compared to boneless treatments and treatments without subcutaneous fat, respectively ($P < 0.05$). Therefore, although bone-in treatments showed lower color stability, bone and subcutaneous fat were considered important factors to the dry-aging process, as both resulted in a greater yield over dry-aged product that had bone and/or fat removed, without compromising other physicochemical traits of dry-aged beef from grass-fed Nellore bulls.

Key words: dry-aged beef, bone-in, boneless, subcutaneous fat, yield

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Introduction

Brazil is a major exporter and the second largest producer of beef in the world, with 2.2 million metric tons (carcass weight equivalent) exported and 10.2 million tons (carcass weight equivalent) produced in 2019 (USDA, 2019a). The Brazilian cattle herd has about 214 million head (MAPA, 2019). Approximately 80% of the Brazilian herd consists of *Bos taurus indicus* cattle (Ferraz and Felício, 2010; Aroeira et al.,

2016), of which 90% belongs to the Nellore breed (Aroeira et al., 2016). In addition, most Brazilian beef cattle are grass fed (Lobato et al., 2014), and only 10% are feedlot finished (USDA, 2019b). Compared to grain-fed cattle, beef of grass-fed cattle has less marbling and thinner subcutaneous fat (Maughan et al., 2012). Additionally, the influence of *Bos taurus indicus* genes affects beef tenderness due to features like muscle structure, physiology, and enzymatic activity (Lawrie, 2005; Lobato et al., 2014).

Tenderness is the most important sensorial attribute for beef consumption (Morgan et al., 1991; Koohmaraie, 1996; Enfalt et al., 1997; Koohmaraie et al., 2002; Platter et al., 2003; Koohmaraie and Geesink, 2006) and could be improved by aging. Meat is aged by wet-aging (vacuum packaged) or dry-aging (without packaging) processes. Savell (2008) defined dry aging as a process of storing unpackaged carcasses, primals, and/or subprimals, at refrigeration temperatures for 1 to 5 wk, allowing enzymatic and biochemical processes to improve tenderness and develop the unique “dry-aged beef” flavor.

Compared to wet aging, dry aging is more expensive (Miller et al., 1985; Smith et al., 2008; DeGeer et al., 2009), and during the dry-aging process, beef loses weight due to moisture evaporation from the lean. Producing dry-aged beef requires strict control of cooling conditions and larger spaces in chambers (Smith et al., 2014). Also, the dry-aging process has significant costs related to weight loss and required trimming due to its dried surface (Kim et al., 2017).

It is well known that the lean tissue expresses greater evaporation loss compared to fat tissue (Johnson et al., 1988). During carcass chilling, subcutaneous fat reduces carcass shrinkage and prevents evaporation from lean meat (Savell et al., 2005). According to Pascoal et al. (2011), the thicker the subcutaneous fat, the lower the carcass weight loss during chilling. However, it is still unclear the amount of evaporation from the lean that the presence of bone and subcutaneous fat prevents during dry aging. In addition, some studies showed that bone-in beef had less weight loss during dry aging compared to boneless beef (Laster et al., 2008; DeGeer et al., 2009; Lepper-Blilie et al., 2016).

Most beef produced in Brazil is from grass-fed Nellore cattle with thin subcutaneous fat; therefore, knowledge of the impact of both subcutaneous fat and bone on dry-aged beef is important to increase yield and achieve a consistent and less costly product. In this sense, to evaluate the feasibility of dry-aged beef produced from grass-fed Nellore bulls, this work assessed the combined effects of bone (bone-in and boneless) and subcutaneous fat (with or without fat) on yield and physicochemical traits of dry-aged beef from grass-fed Nellore bulls.

Materials and Methods

Samples preparation and treatments

Paired bone-in loins (from the 10th thoracic vertebra to 6th lumbar vertebra) from 8 grass-fed Nellore

bulls (approximately 30 mo old; on average 290 ± 36 kg of carcass weight; 6.0 ± 0.4 mm of fat thickness, measured at the 9th/10th rib interface at three-fourths of the length of the ribeye from its chine-bone end; marbling score: traces) were collected at a commercial beef plant at 2 d postmortem. The loins were packed in plastic bags (not vacuumed or sealed), placed in a portable cooler with ice, and transported to the meat lab at the University of Campinas.

At the laboratory, each pair of bone-in loins was cut in half, providing 4 half-loin sections per carcass (2 anterior and 2 posterior sections). Then, the half-loin sections from each carcass were balance assigned into the 4 treatments in a predetermined design to avoid an effect of anatomical position. The treatments were bone-in with subcutaneous fat, bone-in without subcutaneous fat, boneless with subcutaneous fat, and boneless without subcutaneous fat. The sections assigned to boneless treatments were deboned (bones were weighed), and sections for treatments without subcutaneous fat had the fat removed (fat was not weighed, since the aim was to simulate carcasses with little or no fat), following the natural connective tissue seam.

After fabrication, the loin sections were aged for another 21 d in an aging chamber (VN50R model, Metalfrio 2010 ©, Brazil) adapted with a humidifier to control the relative humidity of the chamber. The aging condition was 2°C , $70\% \pm 5\%$ relative humidity, and 2.5 m/s of air speed.

Evaporation loss, trimming loss, yield, and sampling

For the bone-in treatments, either with or without subcutaneous fat, each loin section was weighed (initial weight) and then dry-aged for 21 d. After aging, the bone-in loin sections were reweighed (post-aging weight), and the evaporation loss was calculated according to following equation: $[(\text{initial weight} - \text{post-aging weight}) / \text{initial weight}] \times 100$. Then, the bone-in loin sections were deboned, and the dried crust was trimmed. Afterward, bone (bone weight), trimmings (trimming weight), and the loin section (final weight) were weighed. The trimming loss was determined by $(\text{trimming weight} / \text{initial weight}) \times 100$. Furthermore, the yield was calculated by $(\text{final weight} / \text{initial weight}) \times 100$.

Similarly, evaporation, trimming loss, and yield of the boneless treatments, either with or without subcutaneous fat, were determined according to the following steps. First, each loin section assigned to the boneless treatment was deboned. Bone (bone weight)

and loin sections (initial weight) were weighed. Subsequently, the boneless loin sections were dry-aged for 21 d. After aging, boneless loin sections were reweighed (post-aging weight), and the evaporation loss was calculated by $[(\text{initial weight} - \text{post-aging weight}) / \text{initial weight}] \times 100$. Then, boneless loin sections were trimmed. The trimmings (trimming weight) and loin sections (final weight) were weighed. The trimming loss was determined by $[(\text{trimming weight} / \text{initial weight}) \times 100]$. The yield was calculated by $[(\text{final weight} / [\text{initial weight} + \text{weight of bone}]) \times 100]$.

Following the fabrication process, the loin sections were cut into steaks, without trimming any of the subcutaneous fat. The steaks were sequentially assigned to the analyses, following the respective order (anterior to posterior): pH and moisture content (2.0 cm thick), thiobarbituric acid-reactive substances (TBARS; 1.0 cm thick), Warner-Bratzler shear force and pressed juice percentage (2.5 cm thick), and instrumental color (1.5 cm thick).

Water activity and pH

The water activity (a_w) was measured on the surface of dry-aged samples. A 2-mm-thick sample was cut out from the dried surface of each loin section and placed in a container for a_w analysis. Then, a_w was determined using a water activity meter (Decagon, Brazil, Aqualab 4TE). The pH was determined in non-aged and aged steaks. To determine the pH, the probe (Mettler Toledo, Brazil, MP125 pH meter) was first calibrated with buffer solutions of 4.01 and 7.00 pH, at 5°C. Then, the pH probe previously calibrated was introduced directly into each steak in 2 different positions, measuring the pH in duplicate.

TBARS and moisture content

The moisture content was measured in non-aged and aged samples. Each steak assigned to the moisture content analysis had the fat removed, and the internal lean beef was ground. Approximately 10 g of each ground steak was dried in a forced air convection oven at 105°C for 20 h, in triplicate, according to AOAC (1990) procedures. TBARS were measured, prior to color display, on samples of lean beef after aging, in quadruplicate, following Bruna et al. (2001) methodology modified by changing the 15 mL of 0.38 M HClO₄ for 20 mL of 5% trichloroacetic acid.

Cooking loss, pressed juice percentage, and Warner-Bratzler shear force

The pressed juice percentage and Warner-Bratzler shear force were both analyzed using the same steaks. Immediately after aging and fabrication, the steaks were prepared for cooking. Steaks (2.5 cm thick) were weighed and cooked in an electric convection oven (Fritomaq, Brazil) at 170°C, until the internal temperature reached 71°C. After cooking, the steaks were reweighed to determine the cooking loss.

The pressed juice percentage was determined according to Lucherik et al. (2017) methodology. Immediately after cooking, a slice (1.00 cm thick) was cut from the center of each steak. Then, 3 cubes (1.0 cm width) were removed from each slice (1.00 cm thick). Each cube was weighed between 2 sheets of filter paper (Unifil Filter Paper 501.011, 80 g, 11 cm; Unifil, Brazil) previously stored in a desiccator. Samples were compressed for 30 s at 78.45 N using a cylindrical compression probe (model P/36R, Texture Technologies Corp./Stable Micro Systems, UK) coupled to a texture analyzer (model TA-XT Plus; Texture Technologies Corp./Stable Micro Systems, UK). After compression, the sample was removed from the filter paper and the filter paper was reweighed. The results were expressed as the percentage of fluid loss during compression, according to the following equation: $(\text{fluid released during compression} / \text{cube weight}) \times 100$.

After cutting the samples for the pressed juice percentage method, the samples were saved at room temperature for approximately 30 min to cool and then they were overwrapped in polyvinyl chloride film and kept at 4°C overnight before proceeding with the Warner-Bratzler shear force method, following the American Meat Science Association (2015) cookery guidelines. Shear force was determined using 6 round cores (1.27 cm diameter), cut following the muscle fibers from each steak using a handheld coring device. Shear force was measured with a texture analyzer (TA-XT Plus, Texture Technologies Corp./Stable Micro Systems, UK) by shearing each core in the center, using a Warner-Bratzler blade attached to the Texture Analyzer. A crosshead speed of 250 mm/min was used (American Meat Science Association, 2015).

Color stability during storage

Color stability during storage was evaluated using a colorimeter (CM 508-d, Hunter MiniScan TMXE, Hunter Associates Laboratory, Inc., Reston, VA). Each steak assigned to color display was placed in

a polystyrene tray, then wrapped with a polyvinyl chloride film and kept in a refrigerator at 4°C, with no lights, for 9 d. Color was determined every day following the American Meat Science Association (2012) protocol. The color was evaluated using a colorimeter attached to a moisture protector accessory and previously calibrated using white and black tile standards. The color was measured in 3 different positions of the steak surface (triplicate) by Commission Internationale de l'Éclairage (CIE; “International Commission on Illumination”) L^* , a^* , and b^* values using the standard observer of 10°, illuminant D65 source and aperture size 25 mm.

Data analyses

Sixteen bone-in loins, from 8 beef carcasses, were used in this experiment. The bone-in loins were cut in half and then balanced across to the treatments (8 half-loins per treatment, $n = 8$). The experimental design was a 2×2 factorial with 2 bone effects (bone-in and boneless) and 2 subcutaneous fat effects (with and without fat). The data obtained were statistically analyzed using Statistica version 10.0 (StatSoft, 2010; StatSoft, Hamburg, Germany) by two-way analysis of variance, using the GLM procedure. The degrees of freedom were calculated by Kenward-Roger approximation (DDFM = KR2). Data from the color analysis were analyzed by SAS version 9.2 (SAS Institute Inc., Cary, North Carolina). The PROC GLIMMIX procedure was used with bone, fat, and time as a fixed factor and carcasses as a random factor. When significance ($P < 0.05$) was indicated by analysis of variance, the LSMEANS and DIFF functions were used to separate the least-squares means.

Results and Discussion

Evaporation loss, trimming loss, and yield

No interactions ($P > 0.05$; Table 1) between bone and subcutaneous fat were found for evaporation, trimming loss, and yield.

Boneless samples showed higher evaporation and trimming loss and consequently lower yield compared to bone-in samples ($P < 0.05$; Table 1). As expected, bone had a protective effect over the lean tissue, reducing losses in the dry-aging process and increasing yield. Other studies also reported higher evaporation and trimming loss for boneless compared to bone-in dry-aged beef (Lepper-Bililie et al., 2016; DeGeer et al., 2009). Similarly, samples without subcutaneous fat had higher evaporation and trimming loss and lower yield compared to samples with subcutaneous fat ($P < 0.05$; Table 1). These results indicate that subcutaneous fat also protects the lean tissue, reducing moisture evaporation and increasing dry-aging yield. Although the literature does not report the effects of subcutaneous fat on dry-aged beef, it is known that subcutaneous fat acts as a barrier during carcass chilling, reducing carcass shrinkage and evaporation loss from lean tissue (Savell et al., 2005; Smith and Carpenter, 1973).

Thus, bone and subcutaneous fat were considered important factors during the dry-aging process, reducing evaporation and trimming loss and increasing yield.

pH, a_w , moisture content, and TBARS

No interactions ($P > 0.05$; Table 2) between bone and subcutaneous fat were found for the pH, surface a_w , moisture content, and TBARS.

The presence or absence of bone did not affect the pH or TBARS values ($P > 0.05$; Table 2). Boneless samples had lower values of moisture and surface a_w than bone-in samples ($P < 0.05$; Table 2). These results were expected as boneless loins had more exposed muscle surface, which increased evaporation from the lean. Thus, bone influenced only traits related to water content and had no impact on the pH and lipid oxidation. DeGeer et al. (2009) reported similar results in an assessment of bone-in and boneless loin-cut, dry-aged for 21 and 28 d at 50% relative humidity. The authors found no differences in

Table 1. Effects (mean \pm SEM) of bone (bone-in and boneless treatments) and subcutaneous fat (with and without subcutaneous fat treatments) on evaporation loss, trimming loss, and yield ($n = 32$)

| Trait | Treatments | | | | P Value | | |
|---------------------|------------------|------------------|------------------|------------------|---------|---------|-------------------|
| | Bone | | Subcutaneous Fat | | Bone | Fat | Bone \times Fat |
| | Bone-in | Boneless | With | Without | | | |
| Evaporation Loss, % | 15.63 \pm 0.76 | 22.76 \pm 0.96 | 16.58 \pm 0.88 | 21.81 \pm 1.23 | <0.0001 | <0.0001 | 0.249 |
| Trimming Loss, % | 8.16 \pm 0.55 | 18.18 \pm 0.65 | 11.70 \pm 1.21 | 14.64 \pm 1.53 | <0.0001 | <0.0001 | 0.127 |
| Yield, % | 49.77 \pm 1.15 | 40.79 \pm 1.42 | 48.92 \pm 1.46 | 41.64 \pm 1.46 | <0.0001 | <0.0001 | 0.749 |

Table 2. Effects (mean \pm SEM) of bone (bone-in and boneless treatments) and subcutaneous fat (with and without subcutaneous fat treatments) on pH, TBARS, moisture content, surface a_w , cooking loss, pressed juice percentage, and WBSF ($n = 32$)

| Trait | Treatments | | | | P Value | | |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------|-------|-------------------|
| | Bone | | Subcutaneous Fat | | Bone | Fat | Bone \times Fat |
| | Bone-in | Boneless | With | Without | | | |
| pH | 5.44 \pm 0.01 | 5.45 \pm 0.01 | 5.46 \pm 0.01 | 5.43 \pm 0.01 | 0.326 | 0.069 | 0.912 |
| TBARS, mg MDA/kg | 0.19 \pm 0.02 | 0.20 \pm 0.02 | 0.21 \pm 0.02 | 0.19 \pm 0.02 | 0.691 | 0.588 | 0.325 |
| Moisture Content, % | 74.06 \pm 0.23 | 72.97 \pm 0.21 | 73.63 \pm 0.26 | 73.40 \pm 0.26 | <0.05 | 0.469 | 0.091 |
| Surface a_w | 0.9409 \pm 0.0020 | 0.9324 \pm 0.0021 | 0.9396 \pm 0.0020 | 0.9336 \pm 0.0024 | <0.05 | <0.05 | 0.177 |
| Cooking Loss, % | 16.66 \pm 0.58 | 14.06 \pm 0.56 | 16.49 \pm 0.39 | 14.23 \pm 0.74 | <0.05 | <0.05 | 0.322 |
| Pressed Juice, % | 33.19 \pm 0.67 | 32.67 \pm 0.87 | 33.06 \pm 0.72 | 32.79 \pm 0.83 | 0.645 | 0.811 | 0.292 |
| WBSF, N | 36.03 \pm 1.98 | 36.46 \pm 3.08 | 37.97 \pm 3.14 | 34.52 \pm 1.78 | 0.905 | 0.342 | 0.113 |

a_w , water activity; MDA, malondialdehyde; TBARS, thiobarbituric acid-reactive substances; WBSF, Warner-Bratzler shear force.

the pH and TBARS values between bone-in and boneless samples; however, they found lower moisture content for boneless compared to bone-in samples.

Subcutaneous fat had no effect on the pH, TBARS, or moisture content values ($P > 0.05$; Table 2). Samples without subcutaneous fat showed lower surface a_w values than samples with subcutaneous fat

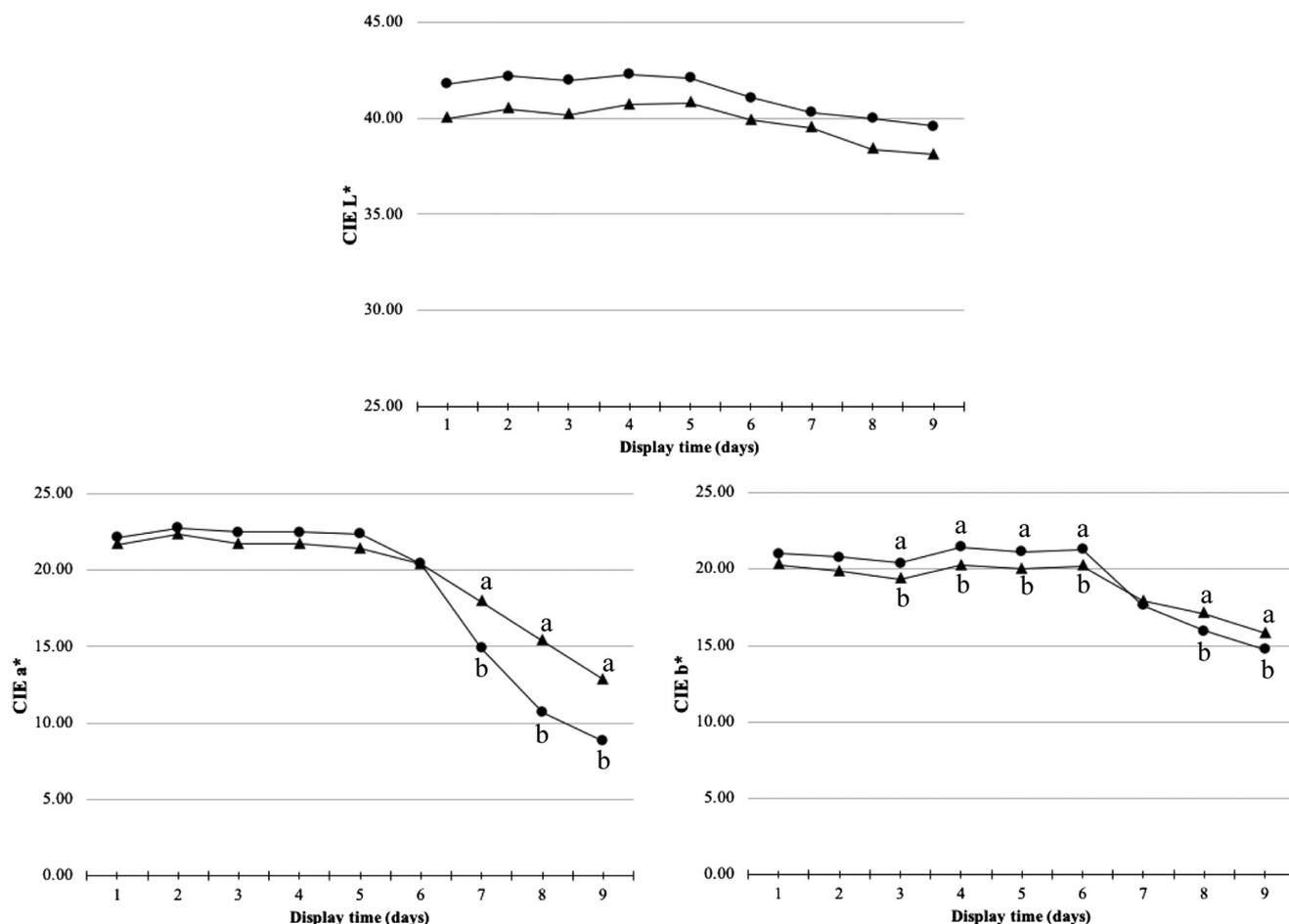


Figure 1. Effects of bone and time interaction on CIE L^* , CIE a^* , and CIE b^* color parameters during 9 d of display. Standard error: 1.073 (CIE L^*), 0.588 (CIE a^*), and 0.331 (CIE b^*). “a” and “b” letters between bone-in and boneless treatments within each day of display are significantly different ($P < 0.05$). (—●—) Bone-in treatments; (—▲—) Boneless treatments. CIE, Commission Internationale de l’Eclairage (CIE; “International Commission on Illumination”).

($P < 0.05$; Table 2), which suggested that the removal of subcutaneous fat increased evaporation from the lean, resulting in higher surface dehydration.

Cooking loss, pressed juice percentage, and Warner-Bratzler shear force

No interactions ($P > 0.05$; Table 2) between bone and subcutaneous fat were found for cooking loss, WBSF, and pressed juice percentage.

Bone-in samples had higher cooking loss values compared to boneless ($P < 0.05$; Table 2), attributed to higher moisture content in bone-in samples. The WBSF and pressed juice percentage were not affected by bone-in and boneless treatments ($P > 0.05$; Table 2). Other studies also indicated no differences of WBSF values in bone-in and boneless samples (DeGeer et al., 2009; Lepper-Blilie et al., 2016). Similarly, samples with subcutaneous fat showed higher cooking loss than those without subcutaneous fat ($P < 0.05$; Table 2), and no differences were observed in WBSF and pressed juice percentage values ($P > 0.05$; Table 2).

Regardless of the presence of bone and subcutaneous fat, the treatments were considered very tender, as the WBSF values were lower than 38.2 N (ASTM, 2011). Furthermore, after cooking, all treatments had similar levels of pressed juice percentage.

Color stability during storage

No interaction ($P > 0.05$) between bone, fat, and time were found for L^* values (lightness), and no differences were observed between the treatments ($P > 0.05$). However, there was a time effect ($P < 0.05$) for L^* , reducing values from 40.92 to 38.86 (standard error: 0.536; data not shown in tabular form), comparing the first to the last day of display, respectively.

Additionally, there was a bone-by-time interaction ($P < 0.05$; Figure 1) for a^* (redness) and b^* (yellowness) parameters. Both bone-in and boneless treatments showed a decrease in a^* values during display. However, after 6 d of display, bone-in treatments had a greater decrease in a^* values compared to boneless ($P < 0.05$; Figure 1). Decrease in a^* values indicated discoloration during display (Hui et al., 2005;

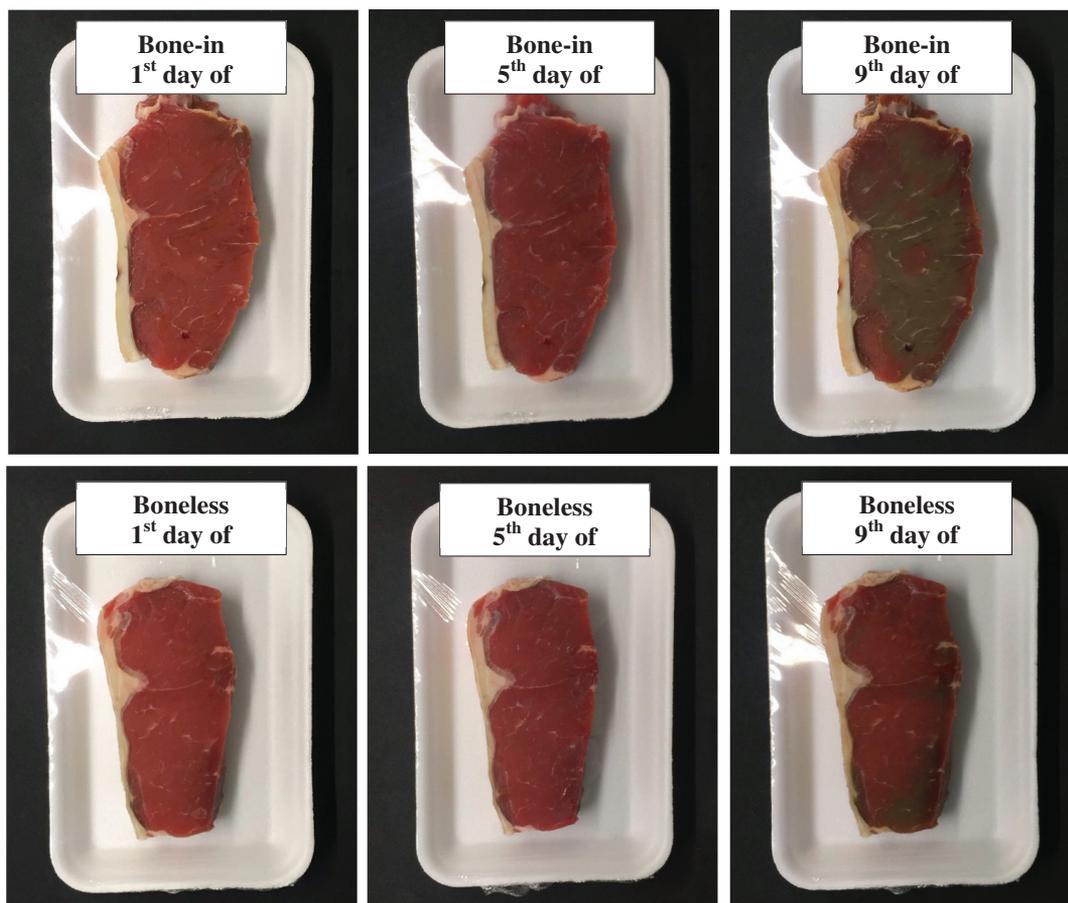


Figure 2. Pictures illustrating the development of color discoloration on bone-in and boneless steaks during display.

Kim et al., 2017). The b^* values were higher for bone-in treatments from the third to the sixth day of display compared to boneless ($P < 0.05$; Figure 1). No difference was found on the seventh day of display ($P > 0.05$; Figure 1), and after 8 d of display, boneless showed higher b^* values than bone-in treatments ($P < 0.05$; Figure 1). Thus, the results of instrumental color suggested that boneless treatments had slight, but significant, greater color stability than bone-in (Figure 2), as boneless showed higher a^* and b^* values after 6 and 7 d of display, respectively.

Meat discoloration is influenced by many biochemical mechanisms, including metmyoglobin reducing ability and oxygen consumption (English et al., 2016). Both mechanisms are controlled by enzyme activity, and the concentration of enzymes and coenzymes, such as nicotinamide adenine dinucleotide, could change meat color stability. Therefore, further studies are suggested to evaluate these biochemical and enzymatic mechanisms on color stability during the dry-aging process.

In this study, the steaks assigned for the color analysis were refrigerated at 4°C without light exposure. In a supermarket display, discoloration could occur faster, due to the temperature fluctuations and light exposure. Lower temperature could increase display color life; however, retail display temperature is frequently up to 7°C (Hui et al., 2005). Furthermore, exposure to light promotes the formation of metmyoglobin through photochemical autoxidation (Hui et al., 2005).

Conclusions

The data from the current study indicate that dry-aged beef from grass-fed Nellore bulls resulted in a very tender product. However, further sensory studies should be performed to investigate consumers' acceptance of dry-aged beef from grass-fed Nellore bulls in the Brazilian market. Furthermore, the results show that both bone and subcutaneous fat had a similar protective effect on lean beef, reducing evaporation loss and increasing yield of dry-aged beef. Therefore, considering these results and taking into account that dry-aged beef is an expensive product mainly due to the weight lost during its production, the use of bone-in loins with thicker subcutaneous fat to produce dry-aged beef is highly recommended.

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