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Effect of freezing prior to aging on myoglobin redox forms and CIE color of beef from Nellore and Aberdeen Angus cattle

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ABSTRACT

The objective of this study was to evaluate the effect of freezing prior to wet aging on the color of Nellore and Aberdeen Angus cattle meat. Samples of the *Longissimus thoracis* muscle were subjected to two treatments: conventional aging (0, 7, 14 and 21 days); and freezing (−20 °C for 40 days) followed by thawing and aging. Freezing promoted ($P < 0.05$) formation of metmyoglobin during aging, especially in Nellore beef. Frozen meats showed ($P < 0.05$) lower lightness (L^*) values and higher redness (a^*), chroma (C^*) and hue angle (h^*) values at the first day of storage, deteriorating quickly with aging time. The color of the Nellore meat was less ($P < 0.05$) stable to freezing, being lighter, yellower and less red than Angus meat. The results suggest that color stability in vacuum-packed beef is reduced by freezing prior to aging and that reduction depends on the animal breed.

1. Introduction

Vacuum packaging (or wet aging) is the most commonly used aging system by the meat industry to improve tenderness, which results in a more homogeneous and acceptable product for the consumer (Vitale, Pérez-Juan, Lloret, Arnau, & Realini, 2014). The tenderness improvement is due to the prolonged activity of enzymes naturally present in meats, which lead to proteolysis of specific myofibrillar proteins. The influence of the calpain system and its specific inhibitor, calpastatin, on proteolysis *post mortem*, has been characterized as a decisive factor for meat tenderness (Huff Lonergan, Zhang, & Lonergan, 2010). In this context, some authors (Crouse & Koohmaraie, 1990; Grayson, King, Shackelford, Koohmaraie, & Wheeler, 2014; Aroeira et al., 2016) have suggested that the freezing process performed before meat aging can be an option to improving beef tenderness, since calpastatin has greater susceptibility to the inactivation when submitted to freezing temperatures, while the calpain activity remain relatively stable (Koohmaraie, 1990). However, although freezing is traditionally used as a way to prolong meat storage time providing similar nutritional quality as fresh meat, some studies (Ben Abdallah, Marchello, & Ahmad, 1999; Jeong, Kim, Yang, & Joo, 2011; Lanari, Bevilacqua, & Zaritzky, 1990) indicate that freezing and thawing of meat contribute to rapid color deterioration, which significantly reduces retail life.

In fresh meat, color is the most important attribute that consumers use as purchase criterion (Mancini & Hunt, 2005), and it is dependent on the content of heme pigments and muscle structure. However, the color of meat is mainly determined by the relative concentrations of purplish-red deoxymyoglobin (DMb), bright cherry-red oxymyoglobin (OMb) and brown metmyoglobin (MMb) (Ramos & Gomide, 2007). Aging conditions influence the cellular mechanisms governing myoglobin redox chemistry and thus can affect color stability when aged beef is subsequently displayed in retail (Suman, Hunt, Nair, & Rentfrow, 2014). Furthermore, although vacuum-packing is commonly used in the conditioning and commercialization of primal and subprimal cuts of fresh meat, it is not usually used in other display of meat in retail due to the unattractive purplish-red color of DMb that results from the lack of oxygen (Lagerstedt, Lundström, & Lindahl, 2011). However, upon exposure to oxygen, the color of fresh meat turns to the consumer desirable bright cherry-red, and this process referred to as blooming is critical to fresh beef retailing (Suman et al., 2014). Therefore, it is common for Brazilian supermarkets to remove the original vacuum-package after aging and repackage the meat in high-oxygen-permeable systems, ensuring an attractive bright cherry-red for the consumer at the time of purchase. In this situation, the meat's blooming capacity after removal from vacuum-packing becomes very important.

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Since it has been suggested that freezing/thawing/aging process could be a strategy to overcome toughness that is not resolved during normal *post mortem* aging, this process may be used to address Brazilian beef tenderness problems. Brazilian herd consists mostly (80%) of Zebu breeds, with Nellore maintaining 90% of this share, but the productive chain of Brazilian livestock is experiencing an increase in the introduction of taurine breeds, such as Aberdeen Angus. Therefore, it is important to understand how freezing prior to aging affect the color of the packaged beef from different breeds to maintain color at a desirable level of a retail meat distribution system to function properly. Thus, the objective of this study was to evaluate the effects of meat aging after frozen storage on the color of the *Longissimus thoracis* muscle of Aberdeen Angus and Nellore cattle.

2. Material and methods

Experimental procedures were approved by the Ethics and Animal Welfare Committee of the Universidade Federal de Lavras (UFLA). The animals were raised in the outbuildings of the Department of Animal Science, and the experiment was conducted in the Laboratory of Meat Technology (LabCarnes) of the Department of Food Science.

2.1. Animals and sample preparation

The animals and sampling used in the present work were the same as described in a previous report (Aroeira et al., 2016). Briefly, 17 Nellore (*Bos indicus*) and 17 Aberdeen Angus (*Bos taurus*) young bulls (20 months age), originated from different beef cattle farms in Minas Gerais and Rio Grande do Sul states, were finished in feedlot system for 112 days up to a live weight of 492 ± 61 kg. The animals were slaughtered, without applying electric stimulation, and the carcasses were chilled and hung by the foramen pelvis for 24 h (4 °C). The right *Longissimus thoracis* muscle was removed from the 6th thoracic vertebra (average pH of 5.65 ± 0.09) and 12 steaks (2.54-cm thick) were obtained (from the caudal end) and sequentially identified for the treatments in the following manner: steaks # 1, 4, 7 and 10 were subjected for the conventional aging treatment; and steaks # 3, 6, 9 and 12 used for the treatment with freezing prior to aging. Steaks # 2, 5, 8 and 11 were used in the previous work.

The steaks of each treatment were individually packaged under vacuum (Packer model BS420, R. Baião, Brazil) in nylon-polyethylene packages and randomly distributed with respect to aging time. The samples for conventional aging (control) were stored under refrigeration (1 ± 0.5 °C) in an environmental chamber (Model EL202, EletroLab, Brazil), for 0 (24 hours *post mortem*), 7, 14 and 21 days. The samples subjected to freezing prior to aging (frozen-thawed) were frozen and stored in a conventional freezer (−20 °C) for a 40-day period. After frozen storage, the samples were thawed (4 °C) for 24 h and aged for the same durations used for the control treatment.

2.2. Instrumental-color evaluation

The myoglobin chemical forms and the instrumental color parameters (CIELAB) of the samples at each aging time were determined using a CM-700 spectrophotometric colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), with 8 mm aperture, illuminant A, and 10° observer angle. Both specular component included (SCI) and specular component excluded (SCE) modes were used. The color measurements were taken after removing each sample from the vacuum package and exposing it to atmospheric air for 30 min for blooming. Data were recorded using an average of five consecutive measurements representing the entire surface of each sample.

The proportions of the myoglobin chemical forms were estimated by the Krzywicki (1979) mathematical method. Intermediate reflectance values (473, 525 and 572 nm) obtained on the SCI mode were determined by linear interpolation and the relative content of the heme pigments, expressed as percentage of oxymyoglobin (OMb), deoxymyoglobin (DMb) and metmyoglobin (MMb), were calculated.

Based on the readings taken on the SCE mode, the lightness (L^*), redness (a^*) and yellowness (b^*) values were obtained. Chroma (C^*) and hue angle (h^*) were also determined as: $C^* = (a^{*2} + b^{*2})^{1/2}$; and $h^* = \tan^{-1}(b^*/a^*)$.

2.3. Statistical analysis

The treatments were arranged in a split-plot design, with breed (Aberdeen Angus × Nellore) and treatment (Control × Frozen) as whole plots, and aging times (0, 7, 14 and 21 days) as subplots. Analysis of variance, Tukey's test and Pearson's correlation were evaluated on SAS® System for Windows™ software, version 9.0 (SAS Institute Inc., Cary, SC) at a significance level of 5%.

3. Results and discussion

3.1. Chemical forms of myoglobin

The breed × treatment × aging time interaction was observed ($P < 0.05$) for all myoglobin chemical forms on the meat surfaces. In the conventional aging system (control) OMb increased similarly in Angus and Nellore beef (Fig. 1). These initial increase in OMb may be explained by a reduction in the oxygen consumption rate (OCR), which is related to the *post mortem* residual mitochondrial respiration (McKenna et al., 2005). This respiratory system competes with myoglobin by the atmospheric oxygen which diffuses into the meat (Lanari & Cassens, 1991). The net result of oxygen diffusion and consumption by the mitochondria determines the concentration gradient of oxygen from the air to the interior of the meat cut (O'Keeffe & Hood, 1982). Thus, the meat at time zero (24 h *post mortem*) shows a high OCR, which results in a lower penetration of the oxygen into the meat, causing the OMb formation at a shallow depth. With increased aging time, OCR was decreased due to the depletion of substrates and coenzymes and due to the degradation of the enzymes involved in mitochondrial respiration, including succinate dehydrogenase and electron transport chain enzymes (Lanari & Cassens, 1991; O'Keeffe & Hood, 1982; Tang et al., 2005), which promotes oxygen penetration. This explains the greater OMb formation in the control samples from the seventh aging day.

In the frozen samples, however, the OMb at time zero was higher ($P < 0.05$) than in the control samples, possibly due to inactivation of the enzymes involved in mitochondrial respiration, which would reduce the OCR and consequently promote the penetration of oxygen. Phung, Sælid, Egelandsdal, Volden, and Slinde (2011) reported that the OCR of mitochondria isolated from pig muscles was reduced upon freezing and thawing when pH values were lower than 6.0. A likely decrease in OCR in the frozen samples at the beginning of aging is reinforced by the lower DMb values observed in these samples as compared with the control samples (Fig. 1).

From the seventh aging day and thereafter, the OMb and DMb levels in the samples subjected to conventional aging remained constant. The same behavior was observed by Vitale et al. (2014), who reported an increase in OMb level in the bovine *L. dorsi* muscle during the first five vacuum-aging days (4 °C), with subsequent maintenance through 25 days of storage. However, in our experiment, although the initial OMb levels in the frozen samples (70.70 ± 7.57%) were greater ($P < 0.05$) than in the control samples (56.50 ± 4.78%), they did not remain so throughout all the aging time. In these samples, OMb levels dropped from the seventh aging day on which is consistent with the concurrent accumulation of MMb observed during the same period (Fig. 1). This corroborates observations from other studies (Ben Abdallah et al., 1999; Chu, Huffman, Trout, & Egbert, 1987; Jeong et al., 2011; Lanari et al., 1990), in which freezing of beef caused an increase in MMb content, contributing to a rapid deterioration of color.

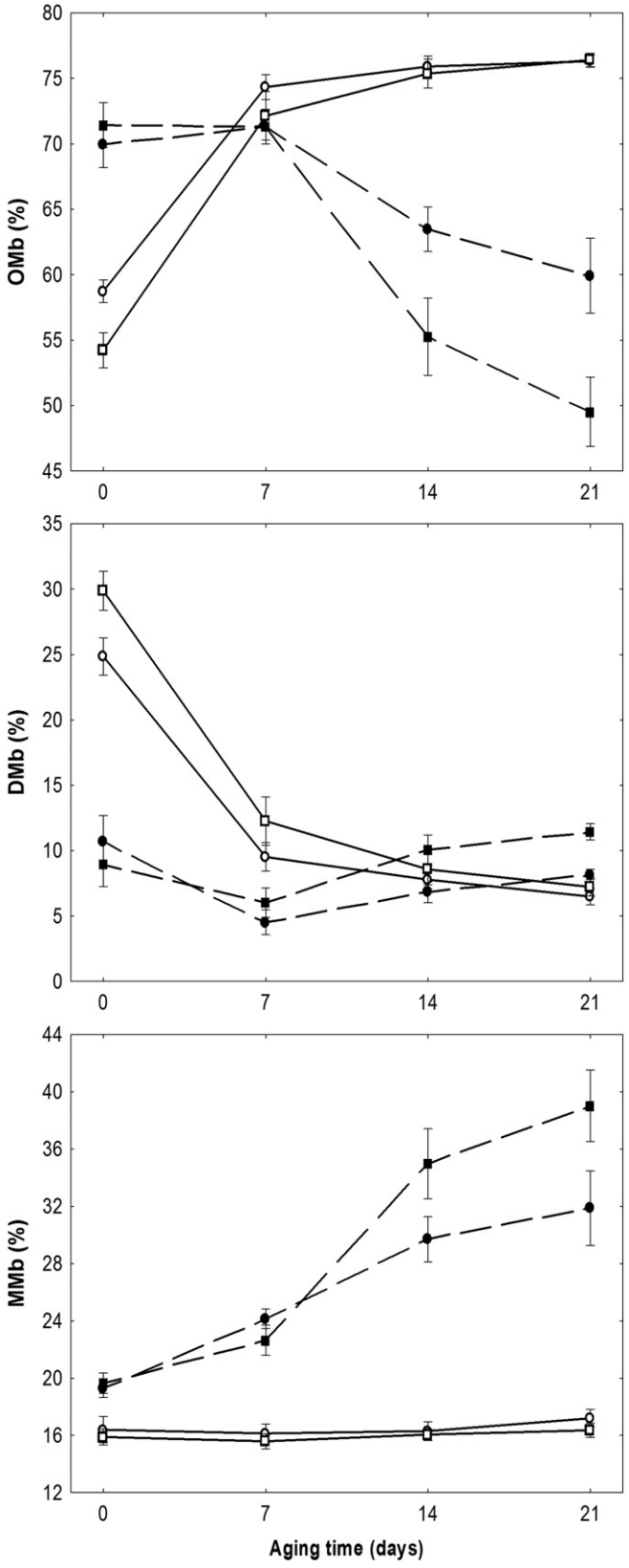

Fig. 1. Proportion of oxymyoglobin (OMb), deoxymyoglobin (DMb) and metmyoglobin (MMb) in beef *Longissimus thoracis* samples for the interaction aging time × breed × treatment. (○) Angus and (□) Nellore control = fresh samples aged at 1 °C; and (●) Angus and (■) Nellore frozen-thawed = samples frozen (−20 °C) for 40 days, thawed (24 h at 4 °C) and aged at 1 °C. Bars represent a standard error.

It has been reported that large fluctuation in temperature during the freezing and thawing processes stimulate lipid oxidation and accelerate the meat discoloration (Akamittath, Brekke, & Schanus, 1990; Moore & Young, 1991). Chu et al. (1987) suggested that the rapid MMb increase in samples stored under freezing can be explained by the formation of ice crystals during the transition phase between freezing and thawing of meat at −2 °C. According to these authors, it is possible that damage of cells and intracellular compartments suffered from the physical rupture caused by the ice crystals provide greater contact of myoglobin and lipids with oxidation-catalytic substances. There is an interrelationship between myoglobin and lipid oxidation (Mancini & Hunt, 2005), wherein the instability of the meat color has been attributed to the decreased stability of the myoglobin redox forms caused by secondary products (α and β aldehydes) of lipid oxidation (Lynch & Faustman, 2000).

In addition to the possibility of greater interaction with pro-oxidative compounds, myoglobin oxidation also can be attributed to denaturation of the myoglobin molecule due to an increase in the intracellular solute concentration, caused by the meat freezing/thawing process. According to Ben Abdallah et al. (1999), denaturation of globin moiety from the myoglobin molecule that occurs during the meat thawing process leads to an increased susceptibility of myoglobin to auto-oxidation, which explains the rapid discoloration observed in thawed meats. These authors also suggest that freezing reduces the MMb reductase activity (MRA) of the meat, thereby contributing to an increased MMb accumulation on the meat surface. It is believed that the MRA in meat occurs mainly due to the activity of a system of NADH-dependent reducing enzymes present in the muscle, which are capable of reducing the ferric form of MMb to the ferrous form of DMb (Mancini & Hunt, 2005). The maintenance of the ferrous form is extremely important for blooming to occur when meat is exposed to oxygen. In the present experiment, an increase in MMb was only observed in the frozen samples, and the MMb levels in the control samples remained low (~16%) and constant ($P > 0.05$) during all aging time.

Among the breeds, the main observation was the lower stability of Nellore meat when it was frozen, with reduced OMb and accumulating greater MMb at the final aging time. Considering that consumers reject the meat when the MMb reaches values close to 40% of the myoglobin chemical forms on the surface (Greene, Hsin, & Zipser, 1971), the frozen Nellore meats on the 21st aging day probably would be rejected by it's color. This lower stability in the color of Nellore meat may be associated with greater susceptibility to lipid oxidation, due to the greater proportion of polyunsaturated fatty acids (PUFA) observed in its intramuscular fat content as compared with *Bos taurus* animals reared in the same conditions (Bressan, Rossato, et al., 2011). Higher degree of lipid unsaturation increases its oxidation susceptibility (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998), which, coupled with cell damage during the freezing/thawing process, probably promoted myoglobin oxidation during storage, as previously discussed.

3.2. Instrumental color

Freezing prior to aging affected ($P < 0.05$) the L^* values, with thawed samples being darker (45.15 ± 2.49) than control samples (49.51 ± 1.23) at day zero of aging. Lamb (Kim, Frandsen, & Rosenvold, 2011; Moore & Young, 1991) and cattle (Vieira, Diaz, Martínez, & García-Cachán, 2009) meat subjected to freezing/thawing have also been described as darker than unfrozen meats. The formation of ice crystals in the meat during freezing leads to a series of alterations that may affect meat quality, especially its color. During slow freezing process, a gradual migration of water starts from intracellular to extracellular spaces, increasing the concentration of solutes in the intracellular medium (Zaritzky, 2000). It is likely that the large concentration of solutes, especially heme pigments, in the intracellular medium caused by freezing contributed to a greater absorption of light, and is thus responsible for the reduced lightness observed in the frozen meats.

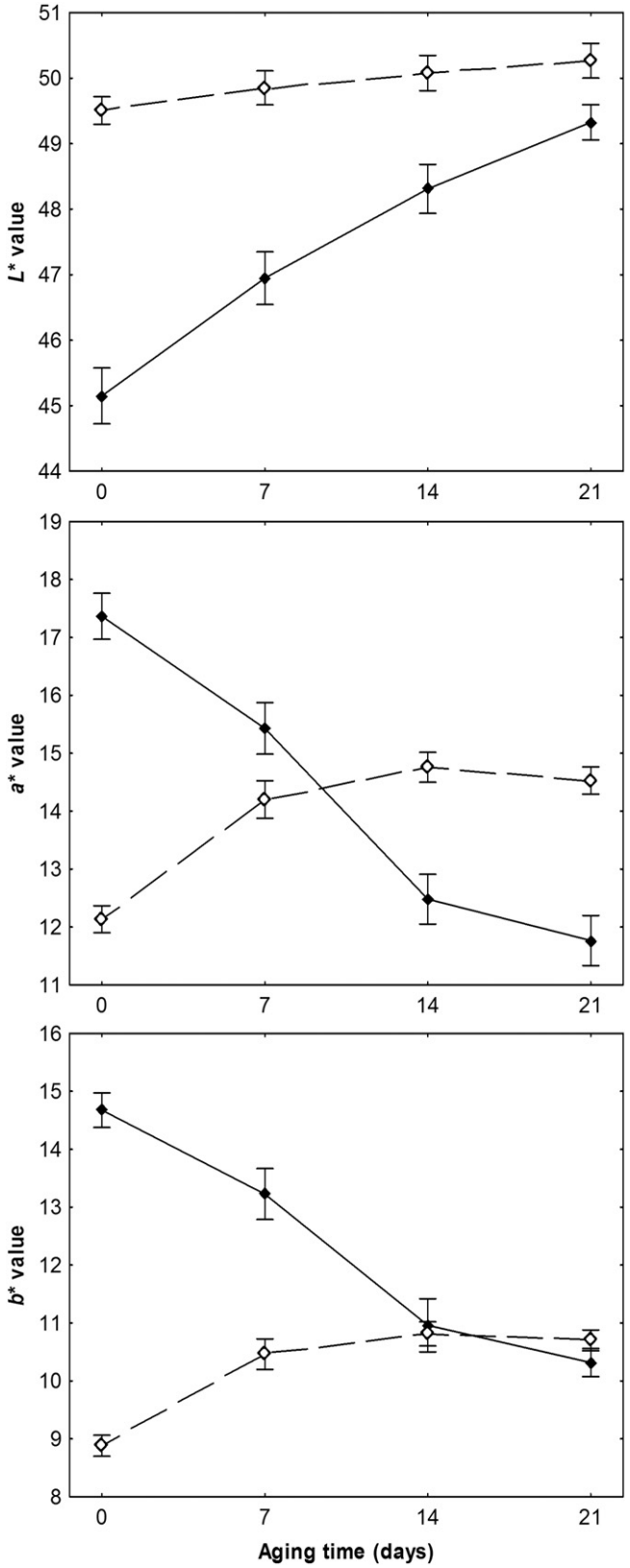

Fig. 2. Lightness (L^* value), redness (a^* value) and yellowness (b^* value) of beef *Longissimus thoracis* samples for the interaction aging time × treatment. (◊) Fresh samples aged at 1 °C; and (♦) samples frozen (−20 °C) for 40 days, thawed (24 h at 4 °C) and aged at 1 °C. Bars represent a standard error.

During aging time, a slight increase in L^* values was observed for control samples, while thawed samples had a higher and significant increase (Fig. 2). With refrigerated storage, part of the extracellular water may have reallocated in the intracellular medium and the effects of the pigments concentration may have been gradually overcome by the protein denaturation and lipid oxidation effects induced by freezing. These factors promote an increase in light scattering (Farouk & Wieliczko, 2003) and contribute to the auto-oxidation of DMb to MMb (Renerre, 1990), which is consistent with the observed increase in the proportion of MMb in the frozen samples (Fig. 1). Although a low very weak negative correlation was observed ($r = -0.17$; $P = 0.0063$) between the L^* values and MMb, a weak positive correlation ($r = 0.32$; $P < 0.0001$) was found when only frozen samples were evaluated.

A slight increase in L^* values during the conventional aging has been reported in many studies (Boakye & Mittal, 1996; Lagerstedt et al., 2011; Vitale et al., 2014), although some authors (Oliete et al., 2006; Oliete et al., 2005) reported that beef lightness did not vary significantly during 14 and 21 days of aging. According to Lee, Apple, Yancey, Sawyer, and Johnson (2008), the change in the chemical form of DMb to OMb during the blooming of bovine *L. dorsi* muscles results in higher L^* values. Thus, it has been suggested (Boakye & Mittal, 1996; Kim et al., 2011; Lagerstedt et al., 2011; Vitale et al., 2014) that the higher L^* values observed with increased aging time are associated with reduction of mitochondrial respiratory activity, which provides greater oxygenation of the myoglobin molecule, resulting in greater formation of OMb. Nevertheless, this is not consistent with our results for frozen samples, because the behavior of the L^* values with aging time was the inverse of that shown by OMb in these meats (Fig. 1). Moreover, although the thawed meats showed greater OMb proportions on the first aging day and equal values on the seventh aging day, L^* values were always lower than the control samples in this period. This explains the absence of correlation ($r = 0.04$; $P = 0.5191$) between the L^* and OMb in this experiment. However, when the relationship between L^* and OMb is evaluated for isolated treatments a positive weak correlation ($r = 0.39$; $P = 0.0001$) was observed for the control samples and a negative weak correlation ($r = -0.35$; $P < 0.0001$) was detected in the thawed samples.

About the differences between breeds, the L^* values were higher ($P < 0.05$) in Nellore (49.02 ± 2.54) than Aberdeen Angus meat (48.34 ± 2.38), data not shown. Bressan, Rodrigues, Rossato, Ramos, and Gama (2011) also observed higher L^* values in *Bos indicus* as compared with *Bos taurus* animals finished in feedlots. These authors suggested that this difference might be related to a greater susceptibility to lipid oxidation in the meat from zebu animals, due to its greater polyunsaturated fatty acids (PUFA) content in intramuscular fat than in taurine animals (Bressan, Rossato, et al., 2011).

Both aging time and treatment (freezing prior to aging) affected ($P < 0.05$) the a^* and b^* values on the meat surface. In the control samples, both a^* and b^* values were higher on the seventh aging day and then remained constant until the end of the aging period (Fig. 2). These changes are due to the greater oxygenation of the myoglobin molecule, since this behavior is consistent with the increased OMb (Fig. 1), being attributed to the reduced OCR during aging (O'Keeffe & Hood, 1982) as previously described. High correlations of the OMb with a^* ($r = 0.68$; $P < 0.0001$) and b^* ($r = 0.45$; $P < 0.0001$) values were observed for controls. Increase in the a^* and b^* values in the bovine *longissimus* muscle along aging time is consistent with reports from other studies (Boakye & Mittal, 1996; Vitale et al., 2014), although some investigators (Oliete et al., 2006; Oliete et al., 2005) observed an increase only in the a^* value.

Just as occurred with the myoglobin chemical forms, freezing altered a^* and b^* values during aging. At time zero, these components were higher in thawed than controls (Fig. 2), probably due to the lower OCR originated from freezing, which corroborates the higher OMb observed in the frozen-thawed samples than in the control samples. However, during refrigerated storage, a^* and b^* values were gradually reduced, probably due to auto-oxidation of DMb to MMb (Fig. 1), induced by the effects of freezing on the MRA, protein denaturation and/or lipid oxidation. This degradation of a^* and b^* values was not observed in the control samples, which is consistent with the maintenance

of the low MMb values in these samples throughout all aging time. Due to the large difference in the MMb values between the treatments, the correlation of this chemical form with a^* was relatively low ($r = -0.39$; $P < 0.0001$), whereas with b^* was not significant ($r = 0.01$; $P = 0.9714$). However, considering only the frozen samples, the correlations of MMb with a^* ($r = -0.68$; $P < 0.0001$) and b^* ($r = -0.43$; $P < 0.0001$) were stronger.

Although few studies have evaluated the instrumental color of beef aged after freezing/thawing, they also have reported degradation of the color components during aging. Jeong et al. (2011) observed lower a^* values only in the *Semimembranosus* muscle samples from beef frozen (12 h at −65 °C), thawed (12 h at 4 °C) and aged for 7 days (4 °C) as compared with the 7-day aged control (not frozen) samples. These authors did not observe a difference in the b^* values between frozen and control samples after seven days aging. Bressan, Rodrigues, et al. (2011), analyzing the quality of meat (*L. dorsi*) from *B. indicus* and *B. taurus* animals reared in different finishing systems (pasture × feedlot), also observed a decrease in a^* and b^* values in samples of both genetic groups after 10 days of aging (4 °C). These authors kept the meats frozen (−20 °C) for 30 days before starting the aging process.

Yellowness values were affected ($P < 0.05$) by breed, with lower b^* values in Nellore meat (10.98 ± 2.45) than Aberdeen Angus meat (11.52 ± 2.40), data not shown. The a^* values were also affected by breed, but these effects varied according to the treatment (Fig. 3). There were no differences ($P > 0.05$) for redness between breeds in the control treatments, but the frozen/thawed Angus samples had higher ($P < 0.05$) a^* values than Nellore samples. This difference may be associated with the lower stability shown by the Nellore meat with freezing, demonstrated by the greater MMb accumulation in the final aging time (Fig. 1). According to King et al. (2010), genetics have a substantial influence on meat color and the ability to maintain color stability appears to be more important than the initial color.

Although a^* and b^* values could reflects important color changes, basing treatment effects on just one parameter may not tell the complete color history (AMSA, 2012). Color is a three-dimensional attribute better described by its hue, lightness and saturation properties.

The color differences observed between control and aged samples seem to have been only due to the blooming ability, whereas in the frozen samples they originate from myoglobin auto-oxidation. The sample's C^* values during aging had a similar behavior to the a^* and b^* values, increasing ($P < 0.05$) in the control samples and decreasing ($P < 0.05$) in the thawed samples (Fig. 4), but h^* values did not change ($P > 0.05$) over the aging period. Chroma represents the color intensity,

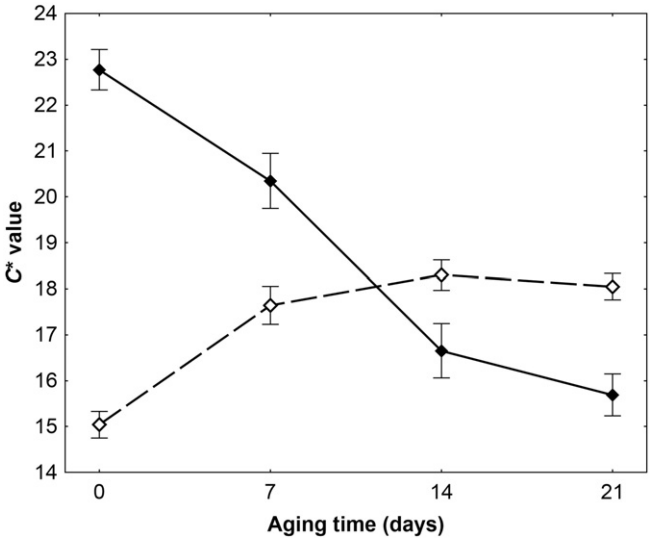

Fig. 4. Chroma (C^* value) of beef *Longissimus thoracis* samples for the interaction aging time × treatment. (◇) Fresh samples aged at 1 °C; and (♦) samples frozen (−20 °C) for 40 days, thawed (24 h at 4 °C) and aged at 1 °C. Bars represent a standard error.

describing how vivid or dull the color is (AMSA, 2012), and is a good indicator of the oxygenation of meat recently exposed to air (Ramos & Gomide, 2007). This is consistent with the higher correlation observed between C^* and OMb ($r = 0.61$; $P < 0.0001$) and a low, but significant, correlation between C^* and MMb ($r = -0.23$; $P = 0.0002$).

Furthermore, freezing prior to aging altered the final color, leading to higher h^* values in the samples subjected to freezing (Fig. 5). This effect was also breed-dependent, with no differences ($P > 0.05$) in h^* values between control samples of Nellore and Angus animals. Hue is the color description as we communicate it in language (red, yellow, green, blue, *etc.*) (AMSA, 2012) and, for meat, hue angle describes the development of color from red to yellow: larger angles indicate a less red hue (Ramos & Gomide, 2007). The thawed Nellore meat presented ($P < 0.05$) a more yellowish hue (higher h^* values) than thawed Angus meats. Higher h^* values in the frozen samples, especially in samples from Nellore animals, indicate lower color stability and explain the high correlation observed between h^* and MMb ($r = 0.70$; $P < 0.0001$). Moreover, low color stability in Nellore meats is also verified by its

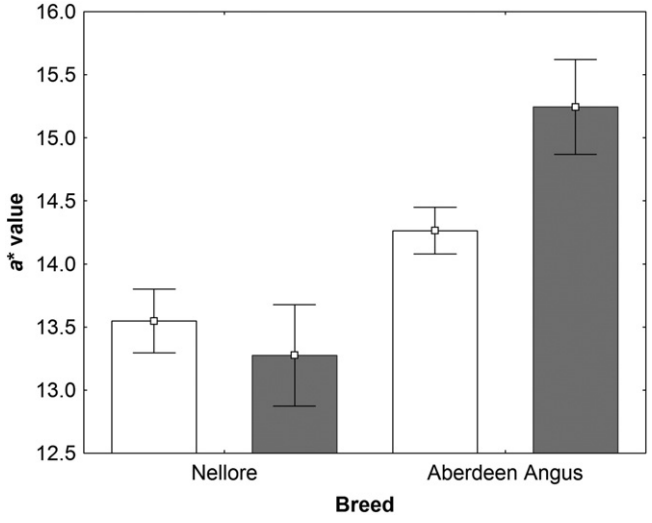

Fig. 3. Redness (a^* value) of beef *Longissimus thoracis* samples for the interaction breed × treatment. (□) Fresh sample aged at 1 °C; and (■) sample frozen (−20 °C) for 40 days, thawed (24 h at 4 °C) and aged at 1 °C. Bars represent a standard error.

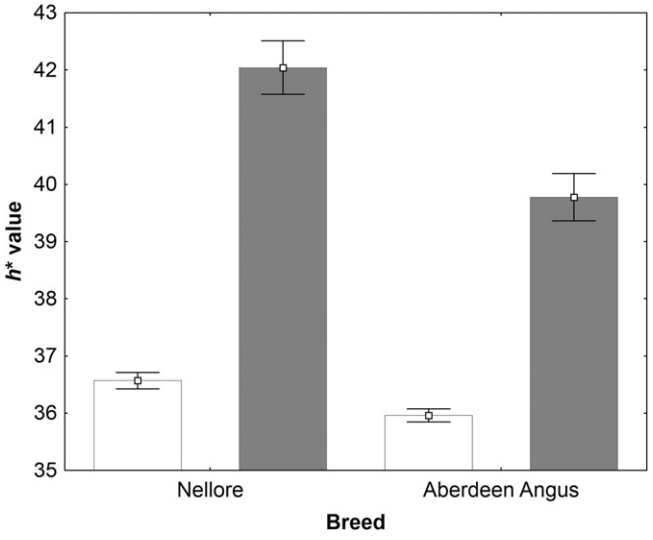

Fig. 5. Hue angle (h^* value) of beef *Longissimus thoracis* samples for the interaction breed × treatment. (□) Fresh sample aged at 1 °C; and (■) sample frozen (−20 °C) for 40 days, thawed (24 h at 4 °C) and aged at 1 °C. Bars represent a standard error.

lower ($P < 0.05$) C^* values (17.37 ± 3.50) than in the Angus meats (18.75 ± 3.31).

4. Conclusion

Freezing altered meat color in both cattle breeds, making them darker and redder during the first few days of aging. However, freezing also led to greater metmyoglobin deposition on the meat surface during aging, which contributed to faster deterioration of the color components, which was dependent on the animal breed. Although color stability was affected by freezing-thaw treatment, frozen meats aged for 21 days had similar color values as unfrozen meats not aged.

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