**Color attributes and oxidative stability of Longissimus lumborum and Psoas
major muscles from Nellore bulls**

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Abstract

The influence of muscle source on color stability of fresh beef from purebred *Bos indicus* cattle was investigated. Longissimus lumborum (LL) and psoas major (PM) muscles obtained from twelve (n = 12) Nellore bull carcasses (24 h post-mortem) were fabricated into 2.54-cm steaks, aerobically packaged, and stored at 4°C for nine days. Steaks were analyzed on day 0 for proximate composition and myoglobin concentration, whereas pH, instrumental color, metmyoglobin reducing activity (MRA), lipid oxidation, and protein oxidation were evaluated on days 0, 3, 6, and 9. LL steaks exhibited greater ($P < 0.05$) redness, color stability, and MRA than PM counterparts. On the other hand, PM steaks demonstrated greater ($P < 0.05$) myoglobin content, lipid oxidation, and protein oxidation than LL steaks. These results indicated the critical influence of muscle source on discoloration of fresh beef from *Bos indicus* animals and suggested the necessity to engineer muscle-specific strategies to improve color stability and marketability of beef from *Bos indicus* cattle.

Keywords: *Bos indicus*; Beef color stability; Muscle-specificity; Oxidation; Longissimus lumborum; Psoas major

1. Introduction

Brazil is a major producer and exporter of beef (Ferraz & Felício, 2010; USDA, 2015), and beef production in this country is primarily a pasture-based system (Ferraz & Felício, 2010; Lobato et al., 2014). The Brazilian beef herd consists of 209 million head (Lobato et al., 2014), and more than three-quarter of these beef cattle are *Bos indicus* animals, primarily the Nellore breed (Ferraz & Felício, 2010; Lobato et al., 2014).

Color is the main quality trait critical to fresh beef purchase decisions (Mancini & Hunt, 2005; Suman, Hunt, Nair, & Rentfrow, 2014). Fresh beef color is governed primarily by myoglobin (Mb) content, its redox state, and interactions with several intrinsic and extrinsic factors (Faustman & Cassens, 1990; Renner, 1990; Suman & Joseph, 2013). The bright cherry-red color attractive to consumer has been often associated with freshness of meat, whereas a brownish discoloration is perceived as indicative of spoilage leading to product rejection (Faustman & Cassens, 1990; Suman et al., 2014). Meat discoloration results in an annual sales loss of more than \$1 billion to the US beef industry (Smith, Belk, Sofos, Tatum, & Williams, 2000).

Among several intrinsic factors influencing beef color, muscle source received significant attention, and several studies documented that beef color stability is a highly muscle-specific trait (Jeong et al., 2009; Joseph, Suman, Rentfrow, Li, & Beach, 2012; McKenna et al., 2005; O'Keeffe & Hood, 1982; Seyfert et al., 2006; Seyfert, Mancini, Hunt, Tang, & Faustman, 2007). Psoas major (PM) is a color-labile beef muscle, whereas longissimus thoracis et lumborum is color-stable. These two muscles exhibit differences in surface color, metmyoglobin reducing activity, oxygen consumption rate, and lipid oxidation (Joseph et al., 2012; McKenna et al., 2005;

O’Keeffe & Hood, 1982; Seyfert et al., 2006; Seyfert et al., 2007). Notably, all these studies were undertaken in beef breeds with predominantly *Bos taurus* influence.

In addition to the muscle source, breed and species of beef animals influence the color attributes (King et al., 2010; Miguel et al., 2014; Norman, 1982; Page, Wulf, & Schwotzer, 2001; Renerre, 1990; Rotta et al., 2009). Miguel et al. (2014) reported that longissimus thoracis steaks from Nellore animals (*Bos indicus*) exhibited greater redness (a^* value) and lightness (L^* values) than those from crossbred (50% Nellore and 50% Aberdeen Angus; *Bos indicus* × *Bos taurus*) cattle. Moreover, Page et al. (2001) reported that longissimus thoracis et lumborum muscle from dairy breeds demonstrated lower a^* values than European (*Bos taurus*) and Brahman (*Bos indicus*) breeds. Further, Rotta et al. (2009) reported that longissimus thoracis et lumborum muscle from Nellore were darker than those from the two other *Bos taurus* breeds (Caracu and Holstein Friesian).

While muscle-specific beef color stability in beef breeds with predominantly *Bos taurus* influence has been extensively studied (Hwang, Kim, Jeong, Hur, & Joo, 2010; Jeong et al., 2009; Joseph et al., 2012; McKenna et al., 2005; O’Keeffe & Hood, 1982; Seyfert et al., 2006; Seyfert et al., 2007), the influence of muscle source on color stability of fresh steaks from *Bos indicus* animals **has not been fully investigated**. Therefore, the objective of the present study was to examine the color stability of longissimus lumborum (LL) and psoas major (PM) steaks from *Bos indicus* (purebred Nellore) animals during refrigerated storage under aerobic conditions.

2. Materials and methods

2.1. Experimental design and beef fabrication

Muscles were obtained from 12 purebred Nellore (*Bos indicus*) bull carcasses, 24 h post mortem. The Nellore bulls were pasture-fed and raised under similar conditions on a farm in Aimores, Minas Gerais, Brazil. The bulls were 30-32 months-of-age at slaughter and were processed at a commercial facility 50 km from the farm in Colatina, Espirito Santo, Brazil. The carcasses had an average cold carcass weight of 288.6 kg. Longissimus lumborum (LL) and psoas major (PM) muscles were excised from the left side of the carcass, individually vacuum-packed, and shipped under refrigeration to the laboratory at Universidade Federal Fluminense (Niteroi, Rio de Janeiro, Brazil).

External fat was excised, and the muscles were fabricated into twelve 2.54-cm thick steaks. The steaks were individually packaged on polystyrene trays with soaker pads, over-wrapped with oxygen-permeable polyvinyl chloride (PVC) film (0.014 mm thickness; Orleplast Ind. e Com de Plásticos Ltda, Orleans, Santa Catarina, Brazil), and stored for 9 days at 4°C. Proximate composition and myoglobin concentration were analyzed on day 0, whereas meat pH, instrumental color, metmyoglobin reducing activity (MRA), lipid oxidation, and protein oxidation were evaluated on four different time points (0, 3, 6, and 9 days). All the analyses were performed in triplicate.

2.2. Proximate composition

Moisture, protein, and ash contents in the steaks were determined according to AOAC (2012). The total lipid content was evaluated utilizing a cold extraction method according to Bligh and Dyer (1959).

2.3. Myoglobin concentration

The myoglobin concentration was estimated according to method of Faustman and Phillips (2001). Samples (5 g) were homogenized with 45 mL of ice cold sodium phosphate buffer (40 mM, pH 6.8) and filtered through Whatman no. 1 paper. The absorbance of the filtrate was measured at 525 nm (A_{525}) utilizing a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and using 40 mM sodium phosphate buffer as a blank. The myoglobin concentration was calculated using the following equation:

$$\text{Myoglobin (mg/g)} = (A_{525} / (7.6 \text{ mM}^{-1} \text{ cm}^{-1} \times 1 \text{ cm})) \times (17,000/1000) \times 10$$

Where: $7.6 \text{ mM}^{-1} \text{ cm}^{-1}$ = millimolar absorptivity coefficient of myoglobin at 525 nm; 1 cm = light path length of cuvette; 17,000 Da = average molecular weight of myoglobin; and 10 = dilution factor.

2.4. Meat pH

The pH of LL and PM steaks was measured directly utilizing a portable pH meter (Hanna Instruments, Woonsocket, RI, USA) equipped with an insertion type probe (Samuel & Trabelsi, 2012).

2.5. Instrumental color evaluation

The CIE L^* (lightness), a^* (redness) and b^* (yellowness) values were measured employing a portable spectrophotometer CM-600D (Konica Minolta Sensing Inc., Osaka, Japan) equipped with illuminant A, 8 mm aperture, and 10° standard observer (AMSA, 2012). Color values were recorded at three random locations on steak surface. In addition, surface color stability during refrigerated storage period was indirectly estimated through the ratio of

reflectance at 630 nm and 580 nm evaluation (R630/580), in which a greater ratio indicates a lower accumulation of metmyoglobin on beef surface, thus greater color stability (AMSA, 2012).

2.6. *Metmyoglobin reducing activity (MRA)*

MRA determination was performed according to method proposed by Sammel, Hunt, Kropf, Hachmeister, and Johnson (2002). Briefly, three cubes ($2.5 \times 2.5 \times 2.5 \text{ cm}^3$) were sliced from each steak and individually submerged in sodium nitrite solution (0.3%) for 20 min to induce metmyoglobin formation. The cubes were arranged with their initially light-exposed side facing up in order to ensure contact with the nitrite solution. The cubes were blot dried, vacuum packed, and the reflectance values (400 to 700 nm) were evaluated utilizing a portable spectrophotometer CM-600D (Konica Minolta Sensing Inc., Osaka, Japan). After incubation at 30°C for 2 h allowing for metmyoglobin reduction, the previously evaluated surfaces were rescanned. The formation of metmyoglobin on surface was calculated utilizing the K/S ratios and formulas proposed by AMSA (2012), and the MRA was estimated using the following equation:

$$\text{MRA} = ((\text{pre-incubation \% surface metmyoglobin} - \text{post-incubation \% surface metmyoglobin}) / \text{pre-incubation \% surface metmyoglobin}) \times 100$$

2.7. *Lipid oxidation*

Lipid oxidation was evaluated using the method described by Yin, Faustman, Riesen, and Williams (1993). Samples (5 g) were homogenized with 22.5 mL of 11% trichloroacetic acid solution (TCA) and filtered through Whatman no. 1 paper. One mL filtrate was mixed with 1 mL of aqueous solution of thiobarbituric acid (20 mM), and incubated at 25°C for 20 h. The absorbance values at 532 nm were measured utilizing a UV-1800 spectrophotometer (Shimadzu

Corporation, Kyoto, Japan), and were presented as thiobarbituric acid reactive substances (TBARS).

2.8. Protein oxidation

The total carbonyl content was estimated utilizing a 2,4-Dinitrophenylhydrazine (DNPH) derivitization assay based on the methods proposed by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987) with modifications (Armenteros, Heinonen, Ollilainen, Toldra, & Estevez, 2009; Mercier, Gatellier, Viau, Remignon, & Renerre, 1998). Briefly, samples (3 g) were homogenized with 30 mL of 0.15 M KCl (pH 7.4) using an Ultra Turrax T18 basic (IKA, Wilmington, NC, USA) for 90 s. The homogenates were divided into two equal volumes (0.1 mL), and individually homogenized with 1 mL of 10% TCA on a bench vortex and centrifuged at $5,000 \times g$ for 5 min (4°C) to promote protein precipitation. The supernatants were discarded and each pellet was homogenized with either 1 mL of 2 N HCl or 1 mL of 10 mM DNPH in 2 N HCl in order to estimate protein and carbonyl contents, respectively. The homogenates were incubated for 1 h at 25°C with intermittent brief vortexing every 15 min following a final precipitation with 1 mL of 10% TCA, and centrifugation at $11,000 \times g$ for 10 min (4°C). The precipitate was washed three times with 1 mL of 1:1 ethanol/ethyl acetate (v/v) solution, and centrifuged at $15,000 \times g$ for 10 min (4°C). The pellets were dissolved in 1.5 mL of buffer consisting of 20 mM sodium phosphate (pH 6.5) and 6 M guanidine hydrochloride. The homogenates were centrifuged at $11,000 \times g$ for 10 minutes to remove insoluble particles. The protein content was estimated utilizing the absorbance value at 280 nm against a bovine serum albumin standard curve, whereas carbonyls content was calculated utilizing absorption at 370 nm and an absorptivity coefficient

for the protein hydrazones of $21.0 \text{ mM}^{-1} \text{ cm}^{-1}$. The results were expressed as nmol of carbonyl per mg of protein.

2.9. Statistical analysis

Twelve ($n = 12$) beef carcasses were utilized in this study, and the experimental design was a completely randomized design with repeated measures. The effects of muscle source (LL and PM), storage (0, 3, 6 and, 9 days), and their interactions were analyzed using the PROC MIXED option (SAS, 2013) with the repeated option to assess covariance resulting from the repeated measures. The differences among means were detected using the least significant difference (LSD) at a 5% level.

3. Results and discussion

3.1. Proximate composition

The muscle source influenced ($P < 0.05$) the moisture and lipid contents of Nellore beef steaks; LL steaks demonstrated greater ($P < 0.05$) moisture and lower ($P < 0.05$) lipid contents than PM counterparts (Table 1). Highfill, Esquivel-Font, Dikeman, and Kropf (2012) compared the proximate composition from ten different muscles from *Bos taurus* and F1 *Bos indicus* $\times$ *Bos taurus* crossbred cattle and observed that LL was leaner (lower lipid content) than PM in both genetic groups. In addition, Suman et al. (2004) documented that lean beef patties from LL muscle exhibited greater moisture and lower fat contents than patties prepared from PM muscle. In partial agreement to our results, Hunt and Hedrick (1977) documented numerically greater lipid content in PM than in longissimus thoracis et lumborum muscle from *Bos taurus* cattle, whereas no difference was observed in moisture content between the two muscles. Furthermore,

these authors documented that oxidative muscles such as PM usually demonstrate greater lipid content than the glycolytic muscles like LL, and in the muscles studied (longissimus thoracis et lumborum, psoas major, gluteus medius, semimembranosus, and semitendinosus) the moisture content was inversely related to lipid content. In contrast to our results, Hwang et al. (2010) documented that PM muscle from Hanwoo (*Bos taurus* × *Bos indicus*) steers exhibited greater moisture content and lower fat content than its longissimus thoracis et lumborum counterpart.

In the present study, muscle source did not affect ($P > 0.05$) protein or ash content (Table 1). In support, Mitterer-Dalton et al. (2012) reported that generally protein content exhibits low variability in beef muscles. In agreement with our results, Kim, Kim, Lee, and Baik (2000) evaluated the quality of meat from Hanwoo bulls and reported similar values for protein and ash contents in PM and longissimus thoracis et lumborum muscles. Moreover, Suman et al. (2004) also reported similar protein values for ground beef patties from LL and PM muscles. In partial agreement with our results Mohan, Hunt, Muthukrishnan, Barstow, and Houser (2010) documented no difference on the ash content between *Bos taurus* LL and PM muscles, whereas LL muscle exhibited greater protein content than PM counterparts. Contrary to our findings, Hunt and Hendrick (1977) reported greater protein content in longissimus thoracis et lumborum than in PM samples.

Genetic variation in proximate composition has been reported earlier, and *Bos taurus* muscles (LL, PM, gluteus medius, semimembranosus, semitendinosus, biceps femoris, deep pectoral, triceps brachii, infraspinatus, and supraspinatus) exhibited greater fat content than muscles from cattle with *Bos indicus* genetic influence (Highfill et al., 2012). In addition, Cole, Ramsey, Hobbs, and Temple (1964) reported greater moisture and lower fat contents in beef muscle from Zebu cattle than in British cattle. Moreover, Mitterer-Dalton et al. (2012)

investigated the influence of genetics on chemical composition of six beef muscles (semimembranosus, biceps femoris, semitendinosus, gluteus medius, and longissimus thoracis et lumborum) and in general the majority of the muscles from Crioulo Lageano (a *Bos taurus* cattle breed from Portugal) demonstrated greater lipid and lower moisture contents than Nellore. In contrast, Wheeler, Cundiff, Koch, and Crouse (1996) compared the proximate composition of Longissimus thoracis from different *Bos taurus* breeds and crossbred (*Bos taurus* × *Bos indicus*) animals and observed no difference in protein content.

3.2. Myoglobin concentration

Myoglobin (Mb) concentration in PM steaks was greater ($P < 0.05$) than in their LL counterparts (Table 1). Similar to the muscle-specificity observed in the present study, previous investigation (Hwang et al., 2010; Jeong et al., 2009) also reported greater Mb content in color-labile PM muscle than in color-stable longissimus thoracis et lumborum from Hanwoo cattle. In partial agreement, King, Shackelford, and Wheeler (2011) examined Mb concentration from five different muscles (longissimus lumborum, semimembranosus, biceps femoris, gluteus medius, and triceps brachii) in a *Bos taurus* cattle and observed that LL muscle had the lowest Mb concentration. In contrast to the present results, previous studies documented similar Mb concentration in longissimus thoracis et lumborum and PM muscles from Nellore (Norman, 1982) and European (Hunt & Hedrick, 1977) beef breeds.

Oxidative muscles such as PM exhibit greater Mb content (Hwang et al., 2010; Jeong et al., 2009; King, et al., 2011; Klont, Brocks, & Eikelenboom, 1998) and mitochondrial concentration (Jeong et al., 2009; King et al., 2010; Klont et al., 1998; Mohan et al., 2010) than glycolytic muscles such as longissimus thoracis et lumborum. In post-mortem oxidative muscles,

mitochondrial metabolism surpasses myoglobin's ability to bind oxygen leading to accelerated metmyoglobin formation and surface discoloration (Jeong et al., 2009; King et al., 2010; Klont et al., 1998; Mohan et al., 2010). PM contains greater proportion of fiber type I than longissimus thoracis et lumborum muscle (Hwang et al., 2010; Totland & Kryvi, 1991), which is positively correlated to Mb content (Hwang et al., 2010; Klont et al., 1998). Furthermore, an increased level of Mb in muscles has been previously reported to be associated with low color stability (Jeong et al., 2009; King et al., 2011; McKenna et al., 2005).

3.3. Meat pH

The pH values of LL and PM steaks during refrigerated storage are presented in Table 2. Except on day 0, PM steaks consistently demonstrated greater ($P < 0.05$) pH than their LL counterparts (Table 2). In agreement with our results, several previous investigations (Kim, Keeton, Smith, Berghman, & Savell, 2009; McKenna et al., 2005; Mohan et al., 2010) reported that LL and PM steaks from *Bos taurus* cattle exhibited similar pH on first day of display. In partial agreement, Joseph et al. (2012) reported greater pH in PM steaks compared to the LL counterparts during 9-day display. On the other hand, Hwang et al. (2010) reported greater pH for longissimus thoracis et lumborum than PM in Hanwoo cattle. The observed difference in pH between LL and PM steaks in the present study during the refrigerated storage could be attributed to the difference in fiber type composition of the muscles (Anderson et al., 2012; Savell, Mueller, & Baird, 2005). Glycolytic muscles such as LL usually exhibit greater glycogen levels favoring increased post-mortem accumulation of lactic acid than oxidative muscles such as PM (Lefaucheur, 2010).

While storage did not influence ($P > 0.05$) pH of LL steaks, PM steaks on day 9 exhibited greater ($P < 0.05$) pH than their day 0 counterparts. Previous research on pH of beef LL and PM steaks from *Bos taurus* cattle during storage documented conflicting results. In partial agreement with our findings, McKenna et al. (2005) reported that a 5-day retail display exerted negligible effect on pH in beef muscles. Kim et al. (2009) also reported that storage had no effect on pH of LL supporting our results, whereas, these authors documented that in PM, the pH on day 7 of display was lower than that on the initial days. On the other hand, an increase in the pH of both LL and PM muscles during retail display has been previously reported in beef (Joseph et al., 2012; Wu et al., 2015a).

3.4. Instrumental color

The muscle source influenced ($P < 0.05$) the surface lightness (L^* value; Table 2), redness (a^* values; Figure 1), yellowness (b^* value; Table 2), and color stability (R630/580; Figure 2) during refrigerated storage.

3.4.1. Lightness (L^* value)

LL steaks exhibited greater ($P < 0.05$) L^* values than PM counterparts on days 0 and 6 of storage (Table 2), whereas both muscles demonstrated similar ($P > 0.05$) values on days 3 and 9. Nonetheless, the L^* values for LL steaks were numerically greater than PM on days 3 and 9. The observed difference in L^* values between LL and PM could be attributed to the greater myoglobin content in PM than in LL as myoglobin concentration is negatively correlated to surface lightness (L^* values) in beef (Onyango, Izumimoto, & Kutima, 1998). In support to our results, Hwang et al. (2010) reported greater L^* values on longissimus thoracis et lumborum than

on PM steaks in Hanwoo cattle. In partial agreement with our results, Jeong et al. (2009) also documented greater lightness on longissimus thoracis et lumborum than in PM on days 3 and 7 of storage under refrigeration. On contrary, Joseph et al. (2012) and Kim et al. (2009) reported that, in *Bos Taurus* carcasses, muscle source (LL and PM) did not affect L^* values during 9-day and 7-day retail display, respectively.

The storage did not affect ($P > 0.05$) L^* values of LL steaks, whereas in PM steaks the L^* values at day 9 was greater ($P < 0.05$) than days 0, 3, and 6. Jeong et al. (2009) and McKenna et al. (2005) reported that the variation of lightness during storage was poorly associated with beef color stability. In partial agreement, previous investigations (Jeong et al., 2009; Norman, 1982) reported that storage did not affect L^* values of longissimus thoracis et lumborum and PM from *Bos indicus* and crossbred (*Bos indicus* $\times$ *Bos taurus*) cattle. Furthermore, Joseph et al. (2012) documented stable L^* values during storage of LL and PM steaks from *Bos taurus* animals.

3.4.2. Redness (a^* value)

The muscle source influenced ($P < 0.05$) the surface redness (a^* values; Figure 1) during storage, and LL steaks demonstrated greater ($P < 0.05$) a^* values than PM steaks throughout the storage (Figure 1). In support, previous investigations on fresh beef products from *Bos taurus* cattle reported that steaks (Kim et al., 2009; Mancini, Suman, Konda, & Ramanathan, 2009; Suman, Mancini, Ramanathan, & Konda, 2009) and ground beef patties (Suman et al., 2004) prepared from LL exhibited greater a^* values than their counterparts from PM, during refrigerated storage under aerobic conditions. In partial agreement with our results, Joseph et al. (2012) and Seyfert et al. (2006) also documented greater a^* values in LL steaks than in PM counterparts during storage, except on day 0. Similarly, Jeong et al. (2009) documented greater

a^* values on days 5 and 7 of storage in longissimus thoracis et lumborum than PM muscles from Hanwoo cattle. On contrary, Hwang et al. (2010) reported similar a^* values in longissimus thoracis et lumborum and PM steaks from Hanwoo cattle.

In agreement with our findings, Page et al. (2001) reported a negative correlation between a^* values and pH, which can partially explain the greater a^* values in LL steaks than in PM steaks. Furthermore, the greater a^* values observed in LL than in PM steaks may be attributed to differences on the fiber type (Hwang et al., 2010; Seyfert et al., 2006; Totland & Kryvi, 1991). PM demonstrates high proportion of type I fiber and consequently a predominant oxidative metabolism (Hwang et al., 2010; Seyfert et al., 2006; Totland & Kryvi, 1991), which is characterized by high concentration of mitochondria and myoglobin (Hwang et al., 2010; Jeong et al. 2009; King et al., 2010; King et al., 2011; Mohan et al., 2010). This in turn results in greater metmyoglobin accumulation and surface discoloration in oxidative muscles than in predominantly glycolytic muscles such as longissimus thoracis et lumborum (Jeong et al. 2009; Joseph et al., 2012; Seyfert et al., 2006).

During storage, although both muscles (LL and PM) demonstrated a decrease in surface redness ($P < 0.05$), the decline was more pronounced in PM steaks than in LL ones (Figure 1). In agreement with our results, previous studies (Joseph et al., 2012; Seyfert et al., 2006; Wu et al., 2015a) also documented a decrease in a^* values of LL and PM muscles from *Bos taurus* cattle, with a greater decline in PM muscle than in LL.

3.4.3. Yellowness (b^* value)

With respect to yellowness (b^* value), LL steaks demonstrated a trend similar to the surface redness, exhibiting greater ($P < 0.05$) values than PM steaks throughout the storage

(Table 2). In agreement with our results, Kim et al. (2009) documented greater b^* values for LL steaks than PM counterparts throughout the seven days of display. In addition, McKenna et al. (2005) reported that in general the color-stable muscles (e.g. LL) had greater b^* values than muscles considered either low or very low in color-stability (e.g. PM). Seyfert et al. (2006) reported lower yellowness in PM steaks than in LL steaks during 7-day storage, except on day 0, and this finding partially agree with the present study. Furthermore, Jeong et al. (2009) reported that PM muscle from Hanwoo cattle exhibited lower b^* values than the longissimus thoracis et lumborum counterpart on days 3, 5, and 7 of storage. In contrast with our results, Joseph et al. (2012) demonstrated similar b^* values between LL and PM muscles during retail display. Likewise, Hwang et al. (2010) documented similar b^* values in longissimus thoracis et lumborum and PM steaks from Hanwoo cattle.

During storage, LL steaks exhibited a decrease on b^* value ($P < 0.05$) whereas, in PM steaks the b^* values remained stable ($P > 0.05$). In agreement with our results on LL, Joseph et al. (2012) and King et al. (2011) demonstrated a decrease on b^* values of beef LL steaks during retail display of 9 days. In contrast with our findings, McKenna et al. (2005) reported that while PM muscle exhibited a decrease in b^* values, the LL counterparts demonstrated an increase in the yellowness during retail display. Furthermore, Joseph et al. (2012) reported a decrease in b^* values on beef PM steaks during 9 days of retail display, which disagreed with our results.

3.4.4. Color stability (R630/580)

Corroborating our findings on a^* values, the muscle source also influenced ($P < 0.05$) the ratio of reflectance at 630nm and 580 nm (R630/580; Figure 2). In support, Mancini et al. (2009) and Suman et al. (2009) reported greater R630/580 values on LL steaks than PM counterparts

under refrigeration and aerobic conditions. Joseph et al. (2012) documented greater R630/580 values on LL than on PM muscles during retail display on days 5 and 9, and this observation agreed with our results.

LL and PM steaks demonstrated a decrease ($P < 0.05$) on surface color stability (R630/580) during storage (Figure 2). In agreement with our results, several previous investigations (Joseph et al., 2012; Mancini et al., 2009; Suman et al., 2009) also documented a decrease on R630/580 values for LL and PM steaks during 9 days of storage under aerobic conditions. Our results on R630/580 in *Bos indicus* indicate a lower metmyoglobin accumulation on the surface of LL steaks than on PM steaks, characterizing LL as a color-stable muscle and PM as a color-labile muscle. In support to our results, previous investigations in *Bos taurus* (Joseph et al., 2012; McKenna et al., 2005; Seyfert et al., 2006; Wu et al., 2015a) and *Bos indicus* × *Bos taurus* (Jeong et al., 2009) also reported that longissimus thoracis et lumborum muscle exhibited greater color stability than its PM counterpart.

3.5. Metmyoglobin reducing activity (MRA)

The muscle type influenced ($P < 0.05$) MRA (Figure 3). The MRA was consistently greater ($P < 0.05$) in the color-stable LL than in color-labile PM, indicating that LL steaks had greater capacity to reduce the oxidized ferric form of myoglobin. The MRA results are in congruence with the surface redness (a^* value) and color stability (R630/580) data. In support to our findings, previous investigations in beef from *Bos taurus* cattle demonstrated greater MRA in color-stable muscles such as LL than in color-labile muscles such as PM on first day of storage (Joseph et al., 2012; Kim et al., 2009; McKenna et al., 2005; Seyfert et al., 2006; Wu et al., 2015a) and during display (Joseph et al., 2012; Kim et al., 2009; McKenna et al., 2005; Seyfert et

al., 2006). In contrast with our results, Jeong et al. (2009) reported no difference in MRA between longissimus thoracis et lumborum and PM muscles from Hanwoo cattle during storage.

Both muscles (LL and PM) exhibited a decrease ($P < 0.05$) in MRA during storage (Figure 3). Previous studies (Joseph et al., 2012; Seyfert et al., 2006; Wu et al., 2015a) also documented a decrease on MRA in LL and PM from *Bos taurus* cattle during refrigerated storage. Similarly, Jeong et al. (2009) reported similar trend in MRA from longissimus thoracis et lumborum and PM from Hanwoo cattle under refrigeration condition. In partial agreement with our results, Kim et al. (2009) reported that LL steaks exhibited a decrease on MRA whereas, PM maintained similar values during the entire retail display.

The observed differences in MRA between LL and PM steaks could be attributed to difference in NADH regeneration, which in turn influences metmyoglobin reduction (Kim et al., 2009; Mohan et al., 2010). Kim et al. (2009) reported that LL muscle demonstrated greater lactate dehydrogenase-B activity than PM muscle, favoring replenishment of NADH and consequently aiding the reduction of ferric metmyoglobin to ferrous forms. The delay in metmyoglobin accumulation on meat surfaces results in improved color stability (Kim et al., 2009). The decrease in MRA observed in both muscles (LL and PM) during post-mortem storage could be due to decrease in NADH supply as a result of interruption in metmyoglobin reductase system in post-mortem skeletal muscles (Wu et al., 2015a; Wu et al., 2015b).

3.6. Lipid oxidation (TBARS)

On day 0, lipid oxidation products were not detected in LL and PM (Table 2), and this could possibly be due to TBARS values below the detection limits of the spectrophotometric techniques used. While PM steaks exhibited greater ($P < 0.05$) TBARS than LL on days 3 and 9,

similar ($P > 0.05$) values were observed in both muscles on day 6 (Table 2). Nevertheless, PM steaks demonstrated TBARS numerically greater than LL counterparts on day 6. In agreement with our results, Joseph et al. (2012) reported greater TBARS values in PM than in LL on day 9 of display in *Bos taurus* cattle. In partial agreement with our results, Kim et al. (2000) documented similar TBARS between longissimus thoracis et lumborum and PM from Hanwoo bulls at the beginning of storage (days 1 and 3) and greater TBARS values in PM than in longissimus thoracis et lumborum towards the end of display (days 14 and 21). Likewise, McKenna et al. (2005) documented that, in general, color-stable muscles such as LL exhibited lower TBARS than color-labile counterparts such as PM during 5 days of display. On contrary, Jeong et al. (2009) reported no difference in TBARS between longissimus thoracis et lumborum and PM muscles from Hanwoo bulls during entire storage of 7 days.

Lipid oxidation increased ($P < 0.05$) during storage in both PM and LL steaks (Table 2). In support of our results, previous investigations in beef muscles from *Bos taurus* cattle (Joseph et al., 2012; McKenna et al., 2005; Wu et al., 2015a) also documented an increase on TBARS in LL and PM steaks during refrigerated storage. Moreover, longissimus thoracis et lumborum and PM from Hanwoo cattle exhibited an increase in TBARS during storage at 4°C (Jeong et al., 2009; Kim et al., 2000).

Muscles predominantly constituted of oxidative fiber, such as PM, usually exhibit greater contents of lipid and myoglobin as well as greater oxygen consumption rate than muscles with high percentage of glycolytic fiber, such as longissimus thoracis et lumborum (Hwang et al., 2010; McKenna et al., 2005; O’Keeffe & Hood, 1982; Renerre & Labas, 1987). Lipid oxidation-induced myoglobin oxidation leads to accumulation of metmyoglobin and subsequent surface discoloration (Faustman, Sun, Mancini, & Suman, 2010; O’Grady, Monahan, & Brunton, 2001;

Suman, Faustman, Stamer, & Liebler, 2007). The observed greater lipid oxidation in PM than in LL could have contributed to the lower color stability of PM steaks during storage.

3.7. Protein oxidation (carbonyl content)

The carbonyl contents in LL and PM steaks are presented on Table 2. PM steaks demonstrated greater ($P < 0.05$) carbonyl content than LL steaks on days 0 and 9 of storage, whereas no difference ($P > 0.05$) was observed between the muscles on days 3 and 6. However, similar to TBARS results, PM demonstrated numerically greater protein oxidation than LL throughout the storage. These results are in congruence with lipid oxidation data suggesting the correlation between lipid and protein oxidation (Baron & Andersen, 2002; Estevez & Cava, 2004; Faustman et al., 2010; Lund, Heinonen, Baron, & Estevez, 2011; Mercier et al., 1998). In partial agreement with our findings, Estevez, Ventanas, Heinonen, and Puolanne (2011) also reported greater content of protein oxidation products in fresh pork PM muscle than in longissimus thoracis et lumborum.

Both LL and PM demonstrated an increase ($P < 0.05$) in carbonyl content during storage (Table 2). In support of our results, Cho, Kang, Seong, Park, and Kang (2015) observed an increase on protein oxidation in LL steaks during 12 days of storage in *Bos taurus* cattle. Moreover, Popova, Marinova, Vasileva, Gorinov, and Lidji (2009) also reported an increase on carbonyl content in beef longissimus thoracis et lumborum during 6 days of storage. Insani et al. (2008) documented an increase in protein oxidation on PM steaks on day 9 of storage in a crossbred (*Bos taurus* × *Bos indicus*) cattle fed with grain, partially agreeing with our results.

In general, muscles predominantly composed of oxidative fibers (PM) demonstrate greater content of prooxidants such as myoglobin (Estevez, 2011; Hwang et al., 2010; Jeong et

al., 2009; Renerre, 1990), heme iron (Utrera, Parra, & Estevez, 2014), and phospholipids (Morcuende, Estevez, Ruiz, & Cava, 2003) than muscles with greater content of glycolytic fiber such as longissimus thoracis et lumborum. The oxidation of myoglobin and lipids leads to formation of secondary products such as ferryl myoglobin and peroxy radicals, which in turn accelerate protein oxidation (Estevez, 2011).

The observed lower color stability in PM steaks than in LL can also be explained by the variations in lipid and protein oxidation between the two muscles. Insani et al. (2008) reported that beef discoloration is accelerated by the oxidation of lipids and proteins. Furthermore, several other reports documented that lipid oxidation (Estevez & Cava, 2004; Faustman et al., 2010; McKenna et al., 2005) and protein oxidation (Lund et al., 2011; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004) compromise fresh meat color stability.

4. Conclusions

The results of the present study indicate that the muscle source influenced color attributes and oxidative stability of fresh beef from *Bos indicus* (Nellore) bulls. LL steaks exhibited greater color and oxidative stability than their PM counterparts, which could be attributed to the differences in fiber type, myoglobin content, and muscle biochemistry. These findings suggested the necessity of muscle-specific processing and retailing strategies to improve color stability of fresh beef from *Bos indicus* cattle.

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Table 1. Proximate composition and myoglobin concentration in longissimus lumborum (LL) and psoas major (PM) steaks (n = 12) from *Bos indicus* (Nellore) bulls.

Parameter	Muscle	
	LL	PM
Moisture (%)	77.32 ± 0.30 ^a	75.94 ± 0.30 ^b
Protein (%)	19.39 ± 0.26	20.21 ± 0.36
Ash (%)	1.08 ± 0.01	1.10 ± 0.02
Lipid (%)	1.32 ± 0.08 ^b	2.48 ± 0.14 ^a
Myoglobin concentration (mg/g)	4.96 ± 0.13 ^b	5.54 ± 0.14 ^a

Results are expressed as mean ± standard error.

^{a-b} Means without common superscripts in a row are different ($P < 0.05$).

Table 2. Meat pH, instrumental color, lipid oxidation, and protein oxidation of longissimus lumborum (LL) and psoas major (PM) steaks (n = 12) from *Bos indicus* (Nellore) bulls during 9 days of storage at 4°C under aerobic conditions.

Parameter	Muscle	Days of storage			
		0	3	6	9
Meat pH	LL	5.35 ± 0.01 ^{ax}	5.35 ± 0.02 ^{ay}	5.32 ± 0.01 ^{ay}	5.36 ± 0.01 ^{ay}
	PM	5.37 ± 0.01 ^{cx}	5.43 ± 0.01 ^{abx}	5.40 ± 0.01 ^{bcx}	5.44 ± 0.01 ^{ax}
<i>L</i> * value	LL	43.63 ± 0.45 ^{abx}	42.72 ± 0.40 ^{bx}	43.46 ± 0.47 ^{abx}	44.60 ± 0.55 ^{ax}
	PM	41.78 ± 0.30 ^{by}	41.64 ± 0.32 ^{bx}	41.45 ± 0.45 ^{by}	43.82 ± 0.62 ^{ax}
<i>b</i> * value	LL	20.60 ± 0.33 ^{ax}	18.03 ± 0.24 ^{bcx}	17.91 ± 0.17 ^{cx}	18.61 ± 0.27 ^{bx}
	PM	16.11 ± 0.16 ^{ay}	15.91 ± 0.28 ^{ay}	16.28 ± 0.27 ^{ay}	16.50 ± 0.17 ^{ay}
Lipid oxidation ^Ψ	LL	ND	0.004 ± 0.001 ^{cy}	0.010 ± 0.001 ^{bx}	0.020 ± 0.001 ^{ay}
	PM	ND	0.007 ± 0.001 ^{cx}	0.011 ± 0.001 ^{bx}	0.034 ± 0.002 ^{ax}
Protein oxidation [†]	LL	1.53 ± 0.08 ^{by}	1.92 ± 0.14 ^{bx}	2.48 ± 0.21 ^{ax}	2.54 ± 0.09 ^{ay}
	PM	2.23 ± 0.12 ^{bx}	2.28 ± 0.16 ^{bx}	2.86 ± 0.18 ^{ax}	3.04 ± 0.20 ^{ax}

Results are expressed as mean ± standard error.

^{a-c} Means without common superscripts in a row are different ($P < 0.05$).

^{x-y} Means without common superscripts in a column within a parameter are different ($P < 0.05$).

ND = Not detected

^Ψ Expressed as absorbance at 532 nm.

[†] Expressed as nmol of carbonyl per mg of protein.

Figure 1. Surface redness (a^* value) of longissimus lumborum (LL) and psoas major (PM) steaks (n = 12) from *Bos indicus* (Nellore) bulls during 9 days of storage at 4°C under aerobic condition.

Standard error bars are indicated.

^{a-c} Means within a muscle without common superscripts are different ($P < 0.05$).

^{x-y} Means within a day of storage without common superscripts are different ($P < 0.05$).

Figure 2. Surface color stability (R630/580) of longissimus lumborum (LL) and psoas major (PM) steaks (n = 12) from *Bos indicus* (Nellore) bulls during 9 days of storage at 4°C under aerobic condition.

Standard error bars are indicated.

^{a-c} Means within a muscle without common superscripts are different ($P < 0.05$).

^{x-y} Means within a day of storage without common superscripts are different ($P < 0.05$).

Figure 3. Metmyoglobin reducing activity (MRA) of longissimus lumborum (LL) and psoas major (PM) steaks (n = 12) from *Bos indicus* (Nellore) bulls during 9 days of storage at 4°C under aerobic condition.

Standard error bars are indicated

^{a-d} Means within a muscle without common superscripts are different ($P < 0.05$).

^{x-y} Means within a day of storage without common superscripts are different ($P < 0.05$).