



Harvest Method Influences Color Stability of Longissimus Lumborum Steaks from *Bos indicus* Cattle

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Abstract: Brazil is a major beef exporter to countries where religious slaughter without stunning is mandatory. Fresh beef color influences consumers' purchase decisions, and harvest method influences beef quality attributes, such as color, water holding capacity, pH, and lipid oxidation. Beef color is a breed-specific trait, and the excitable temperament of *Bos indicus* cattle can affect fresh meat color. Nonetheless, the effects of harvest method on color and oxidative stabilities of beef from *Bos indicus* cattle have not been investigated yet. Therefore, our objective was to evaluate the impact of harvest method on color and oxidative stabilities of longissimus lumborum (LL) steaks from *Bos indicus* beef cattle. The LL muscles (24 h post-mortem) were obtained from 12 Nellore bull carcasses, harvested by either captive bolt stunning (CBP; $n = 6$) or without stunning (NST; $n = 6$). The muscles were fabricated into 2.54-cm steaks, aerobically packaged, and stored for 9 d at 4°C in darkness. Myoglobin concentration was analyzed on d 0, whereas pH, instrumental color, lipid oxidation, and water holding capacity were evaluated on d 0, 3, 6, and 9. While CBP and NST steaks exhibited similar ($P > 0.05$) myoglobin concentration (4.84 mg/g in CBP; 4.84 mg/g in NST), CBP steaks exhibited greater ($P < 0.05$) surface redness and color stability than their NST counterparts throughout the storage. On the other hand, NST steaks exhibited greater ($P < 0.05$) pH, yellowness, and water holding capacity than CBP steaks. Overall, the lightness and lipid oxidation were greater ($P < 0.05$) in NST steaks than their CBP counterparts. These results indicated that harvest method influences surface discoloration and oxidative stability of fresh LL steaks from *Bos indicus* cattle.

Keywords: beef color, lipid oxidation, Nellore, oxidative stability, stunning

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Introduction

Brazil is a major beef producer in the world, and the majority of Brazilian beef cattle are *Bos indicus* animals (Ferraz and Felício, 2010). Brazilian beef is exported to several countries (USDA, 2018), where religious slaughter without stunning is mandatory (USDA, 2001; Farouk, 2013; Farouk et al., 2014; Rovinsky and Cohen, 2018). Although the global trade of meat harvested without stunning is increasing (Farouk, 2013), the practice of slaughter without stunning is known to affect beef quality traits such as color (Önenç and Kaya, 2004; D'Agata et al., 2009), water holding ca-

capacity (D'Agata et al., 2009; Sabow et al., 2015b), pH (Vergara et al., 2005; D'Agata et al., 2009), and lipid oxidation (Linares et al., 2007a). Fresh meat color is the most important attribute influencing consumers' purchase decisions at the point-of-sale (Mancini and Hunt, 2005; Suman et al., 2014) and is influenced by a multitude of intrinsic and extrinsic factors (Neethling et al., 2017). Noticeably, beef color is breed-specific (Miguel et al., 2014); Nellore (*Bos indicus*) cattle are characterized by their excitable temperament, which could possibly influence antemortem handling practices as well as fresh meat color (Francisco et al., 2015).

Previous research on the influence of harvest method and stunning on fresh beef color reported conflicting results. Önenç and Kaya (2004) reported greater redness (a^*) and lightness (L^*) in longissimus muscles from *Bos taurus* cattle stunned (electrically and mechanically) than in the muscles from animals subjected to no stunning. Moreover, D'Agata et al. (2009) documented greater a^* values in longissimus muscle from non-stunned *Bos taurus* cattle than in the muscle from animals harvested after stunning. In contrast, Sazili et al. (2013) documented no effect of harvest in redness of longissimus lumborum (LL) from *Bos taurus* × *Bos indicus* cattle.

While the influence of stunning and harvest method on color of beef from *Bos taurus* animals has been documented, the effect of harvest on color and oxidative stabilities of beef from *Bos indicus* cattle is yet to be investigated. Therefore, the objective of the present study was to examine the color and oxidative stabilities of LL muscle from *Bos indicus* cattle harvested with stunning and without stunning, during refrigerated storage.

Materials and Methods

Cattle were slaughtered at a commercial facility under the Brazilian federal meat inspection, and the LL muscles were purchased from the facility and transported to Universidade Federal Fluminense (Niteroi, Rio de Janeiro, Brazil). Therefore, institutional animal care and use committee approval was not obtained.

Experimental design and beef fabrication

Twelve Nellore (*Bos indicus*) bull carcasses (24 h post mortem) were used in this experiment. The Nellore bulls (24 to 36 mo age) were pasture-fed and raised under similar conditions on a farm in Nanuque, Minas Gerais, Brazil. The age of the bulls was not different ($P > 0.05$) between the harvest methods; the average age of the bulls harvested by captive bolt pistol stunning (CBP) was 28.5

± 1.31 mo, whereas that of the bulls harvested without stunning (NST) was 29.5 ± 1.57 mo. The animals were harvested under Brazilian federal meat inspection at a commercial facility (Nanuque, Minas Gerais, Brazil). Six ($n = 6$) animals were harvested after stunning with percussive captive bolt pistol (CBP), whereas the remaining 6 ($n = 6$) cattle were harvested without stunning (NST). During the slaughter, the cattle were held in standing position and with the head restrained, to allow exposing the neck for bleeding. The carcasses were chilled for 24 h, at which the average cold carcass weight of CBP was 282.48 kg, whereas that of NST was 281.15 kg. The pH of LL muscle in the carcasses at 24 h was 5.46 in CBP and 5.61 in NST. After measurement of the pH, the LL muscles were excised from the right side of the carcasses (24 h post-mortem), individually vacuum packaged, and shipped under refrigeration to the meat laboratory at Universidade Federal Fluminense (Niteroi, Rio de Janeiro, Brazil).

External fat was removed, and the muscles were fabricated into ten 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; 15,500 – 16,275 cm³/m² per 24 h oxygen transmission rate at 23°C), and were assigned randomly for 0, 3, 6, and 9 d at 4°C in darkness. On d 0, 4 steaks were assigned for analyses of myoglobin concentration, pH, instrumental color, lipid oxidation and water holding capacity. The remaining six steaks were utilized for evaluation of instrumental color and biochemical attributes on d 3, 6, and 9 (2 steaks/d; 1 each for color and biochemical analyses). All the analyses were performed in triplicate.

Myoglobin concentration

Myoglobin (Mb) concentration was estimated according to the method described by 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 using Whatman No. 1 paper, followed by an additional filtering through a 22-µm membrane filter. The absorbance of the filtrate at 525 nm (A525) was recorded using a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with sodium phosphate buffer as a blank. The Mb concentration was calculated using the following equation:

$$\text{Myoglobin (mg/g muscle tissue)} = [A525 / (7.6 \text{ mM}^{-1} \text{ cm}^{-1} \times 1 \text{ cm})] \times [17,000 / 1000] \times 10$$

Where: 7.6 mM⁻¹ cm⁻¹ = millimolar extinction 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.

Meat pH

The pH of LL steaks was measured directly utilizing a portable pH meter (Hanna Instruments, Woonsocket, RI) equipped with an insertion type probe (González-Fuentes et al., 2014).

Instrumental color evaluation

The surface L^* (lightness), a^* (redness), and b^* (yellowness) values were measured using a portable spectrophotometer CM-600D (Konica Minolta Sensing Inc., Osaka, Japan) equipped with illuminant A, 8 mm aperture, and 10° standard observer (American Meat Science Association, 2012). Color was measured at three random locations on the steak surfaces. In addition, color stability was indirectly estimated through the ratio of reflectance at 630 nm and 580 nm (R630/580) according to American Meat Science Association (2012).

Lipid oxidation

Lipid oxidation was evaluated using the method described by Sinnhuber and Yu (1958) and Buege and Aust (1978). Samples (5 g) were homogenized with 22.5 mL trichloroacetic acid solution (TCA; 11%) and centrifuged ($11,000 \times g$ at 4°C for 15 min). One milliliter of the supernatant 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).

Water holding capacity (WHC)

The water holding capacity was evaluated using the method described by Quéguiner et al. (1989) with modifications proposed by Verbeken et al. (2005). Samples (10 g) were centrifuged at $12,000 \times g$ for 30 min (4°C) and the results of WHC were estimated as a percentage of retained-water, using the following equation:

$$\text{WHC} = (W2/W1) \times 100$$

Where: W1 = weight sample prior centrifugation; W2 = sample weight after centrifugation.

Statistical analysis

Twelve beef carcasses were utilized in this study. The experimental design was a split-plot with randomized block design with 6 replicates ($n = 6$) in CBP and NST treatments. The muscles from each carcass served as the experimental unit and whole-plot, whereas the steaks were the sub-plots within the experimental unit. The effects of harvest (CBP and NST) and storage (0, 3, 6, and 9 d) were analyzed using the PROC MIXED procedures of SAS Version 9.4 (SAS Inst. Inc., Cary, NC), and the differences among means were detected using the least significance difference (LSD) at a 5% level. The data of Mb concentration were analyzed only for the effect of harvest method. In addition, PROC CORR procedure was used to determine the Pearson's correlation coefficients between the color parameters and biochemical attributes.

Results and Discussion

Myoglobin concentration

Harvest method did not influence ($P > 0.05$) the Mb concentration of steaks (4.84 ± 0.17 mg/g in CBP; 4.84 ± 0.06 mg/g in NST). Supporting our results, Agbeniga (2011) reported that harvest method (slaughter without stunning vs. stunning with captive bolt pistol) did not influence the myoglobin concentration in longissimus muscle from cross-bred *Bos indicus* × *Bos taurus* cattle. In addition, Sabow et al. (2015a) documented similar myoglobin concentration in LL muscles from goats harvested without stunning or with minimal anesthesia prior to slaughter.

Meat pH

There was no harvest × storage interaction ($P = 0.8436$; Table 1) for pH. However, there was an effect of harvest ($P < 0.0001$) and storage ($P < 0.0001$) on pH. NST steaks exhibited greater ($P < 0.05$) pH values than their CBP counterparts throughout the storage (Table 1). The observed differences in pH between CBP and NST steaks could be possibly attributed to animal stress (Ferguson and Warner, 2008). The harvest without stunning can cause stress in animals (D'Agata et al., 2009; Gregory et al., 2010) leading to the release of catecholamines (Muchenje et al., 2009a), which in turn promotes muscle glycogen depletion. Lack of muscle glycogen consequently results in a minimal pH decline in post-mortem muscles and a greater meat pH (D'Agata et al., 2009).

Table 1. Meat pH, surface yellowness (b^* values), and water holding capacity of longissimus lumborum steaks from *Bos indicus* bulls harvested with captive bolt stunning (CBP) or without stunning (NST) during aerobic storage at 4°C for 9 d¹

Parameter	Harvest method	Days of storage			
		0	3	6	9
Meat pH	CBP	5.47 ± 0.03 ^{cy}	5.59 ± 0.02 ^{by}	5.63 ± 0.01 ^{by}	5.76 ± 0.05 ^{ay}
	NST	5.61 ± 0.01 ^{cx}	5.70 ± 0.08 ^{bx}	5.72 ± 0.01 ^{bx}	5.85 ± 0.03 ^{ax}
b^* value	CBP	13.86 ± 0.13 ^{ay}	12.52 ± 0.07 ^{by}	9.79 ± 0.39 ^{cy}	8.05 ± 0.35 ^{dy}
	NST	14.06 ± 0.33 ^{ax}	13.26 ± 0.28 ^{ax}	10.27 ± 0.39 ^{bx}	9.17 ± 0.31 ^{cx}
Water holding capacity	CBP	26.89 ± 1.63 ^{by}	27.43 ± 1.49 ^{by}	32.09 ± 2.22 ^{by}	35.94 ± 1.78 ^{ay}
	NST	33.69 ± 1.24 ^{bx}	36.76 ± 1.08 ^{bx}	38.07 ± 1.19 ^{abx}	44.03 ± 2.28 ^{ax}

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

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

¹Results expressed as mean ± standard error of the mean (SEM).

In agreement with our results, D'Agata et al. (2009) documented greater pH in longissimus muscle from *Bos taurus* cattle harvested without stunning (pH values of 5.62, 5.67, and 5.80 on d 0, 3, and 6 of storage respectively) than the muscles from their stunned counterparts (pH values of 5.55, 5.61, and 5.58 on d 0, 3, and 6 of storage respectively). Vergara et al. (2005) reported greater pH values in longissimus muscle from lambs harvested without stunning than the muscles from their stunned counterparts on d 0 of storage. On the contrary, Sabow et al. (2017) documented similar pH values in LL muscle from goats harvested without stunning and electrically stunned during 7 d of storage. Önenç and Kaya (2004) also reported similar pH in longissimus muscle from *Bos taurus* cattle harvested without stunning and stunned with captive bolt pistol on d 0 of storage.

Both CBP and NST steaks demonstrated an increase ($P < 0.05$) in pH values during storage (Table 1). The observed increase in pH could be due to the post-mortem proteolysis and generation of basic metabolites, such as amines, both of which contribute to the increase of alkalinity during storage (Muchenje et al., 2009b). In agreement with our results, D'Agata et al. (2009) documented an increase of pH in longissimus muscle from *Bos taurus* cattle harvested without stunning and harvested with stunning during 7 d of storage. In addition, Sabow et al. (2017) documented an increase of pH values in LL muscle from goats harvested without stunning and electrically stunned during 7 d of storage. In contrast, Vergara et al. (2005) reported no changes in the pH values of longissimus muscles from lambs harvested without stunning and with electrically stunning during 7 d of storage.

Lightness (L^* value)

There was a harvest × storage interaction ($P = 0.0098$; Table 2) for L^* value. NST steaks exhibited

greater ($P < 0.05$) lightness (L^* values) than CBP counterparts on d 6 and 9 (Table 2), whereas CBP and NST steaks exhibited similar ($P > 0.05$) L^* values on d 0 and 3 of storage. The observed difference in L^* values between NST and CBP steaks on d 6 and 9 may be attributed to pre-slaughter stress and possible increase of catecholamine levels (Muchenje et al., 2009a). Muchenje et al. (2009a) evaluated the correlation between pre-slaughter stress and catecholamine levels in *Bos taurus* cattle and observed that animals that suffered greater pre-slaughter stress exhibited more pronounced increase of catecholamine and had meat with greater surface L^* values than their counterparts exposed to lower stress.

In agreement with our results, Önenç and Kaya (2004) documented no differences in L^* values of longissimus muscles from *Bos taurus* cattle harvested without stunning and with captive bolt pistol stunning on d 0 and 3 of storage. In partial agreement, Sazili et al. (2013) reported similar L^* values in LL muscle from crossbred (*Bos taurus* × *Bos indicus*) cattle harvested without stunning and stunned with high power non-penetrative pistol on d 0 and 7 of storage. In addition, D'Agata et al. (2009) reported similar L^* values in longissimus muscle from *Bos taurus* cattle harvested with stunning and without stunning on d 2 of storage. On contrary, Agbeniga (2011) documented greater L^* values in longissimus muscle from crossbred (*Bos taurus* × *Bos indicus*) cattle harvested without stunning than their stunned counterparts on d 0 of storage.

While storage did not ($P > 0.05$) affect the L^* values of NST steaks, CBP steaks demonstrated a decrease ($P < 0.05$) in L^* values from d 3. In partial agreement, several studies (Önenç and Kaya, 2004; D'Agata et al., 2009) reported that storage did not affect the L^* values of longissimus muscle from *Bos taurus* cattle harvested with stunning or without stunning during 14 and 6 d

Table 2. Surface lightness (L^* values) and lipid oxidation of longissimus lumborum steaks from *Bos indicus* bulls harvested with captive bolt stunning (CBP) or without stunning (NST) during aerobic storage at 4°C for 9 d¹

Parameter	Harvest method	Days of storage			
		0	3	6	9
L^* value	CBP	37.99 ± 0.41 ^a	37.75 ± 0.54 ^{ab}	36.05 ± 0.30 ^c	37.09 ± 0.31 ^b
	NST	39.00 ± 0.49 ^a	38.23 ± 0.74 ^a	38.98 ± 0.46 ^a	38.34 ± 0.35 ^a
Lipid oxidation ²	CBP	0.003 ± 0.000 ^f	0.004 ± 0.000 ^e	0.011 ± 0.000 ^c	0.019 ± 0.001 ^a
	NST	0.004 ± 0.000 ^e	0.006 ± 0.000 ^d	0.014 ± 0.000 ^b	0.020 ± 0.001 ^a

^{a-e}Means without common superscripts within an attribute are different ($P < 0.05$).

¹Results expressed as mean ± standard error of the mean (SEM).

²Result expressed as absorbance at 532 nm.

of storage, respectively. In addition, Sazili et al. (2013) documented stable L^* values in LL muscle from *Bos taurus* × *Bos indicus* cattle harvested without stunning or with stunning using high power non-penetrative percussive pistol, during 14 d of storage. In contrast, Sabow et al. (2017) documented an increase in L^* values in LL from goats harvested without stunning and after electrically stunning, during 14 d of storage.

Redness (a^* value)

There was no harvest × storage interaction ($P = 0.6863$; Fig. 1) for a^* value. However, there was an effect of harvest ($P = 0.0002$) and storage ($P < 0.0001$) on redness. CBP steaks exhibited greater ($P < 0.05$) redness (a^* values) than NST counterparts throughout the storage (Fig. 1). The differences in a^* values could be attributed to the differences in the pH (Agbeniga, 2011). Harvest without stunning can negatively affect the glycogen content in muscle (D'Agata et al., 2009) leading to an increase of post-mortem muscle pH (Linares et al., 2007b). This in turn favors the maintenance of mitochondrial metabolism (Ashmore et al., 1973) preventing blooming and decreasing surface redness (O'Keeffe and Hood, 1982; D'Agata et al., 2009). In agreement, Agbeniga (2011) documented greater redness (a^* values) in longissimus muscle from crossbred *Bos indicus* × *Bos taurus* cattle harvested with stunning than those from un-stunned animals on d 0. In contrast, Önenç and Kaya (2004) and Sazili et al. (2013) documented no differences in a^* values of longissimus muscle from *Bos taurus* cattle harvested without stunning and with stunning. In addition, Sabow et al. (2017) reported similar redness (a^* values) in LL muscle from goats harvested without stunning or after electrically stunning, during 14 d of storage.

CBP and NST steaks exhibited a decrease in a^* values ($P < 0.05$) from d 3 of storage (Fig. 1). The observed decline in a^* value could be related to the dif-

ferences in meat pH and lipid oxidation. The increase in pH during storage (as observed in CBP and NST) favors mitochondrial activity leading to a decline in oxymyoglobin content (Bendall and Taylor, 1972) and a concomitant decrease in redness (Abril et al., 2001; D'Agata et al., 2009). In addition, the increase in TBARS (observed in CBP and NST; Table 2) accelerates metmyoglobin formation, contributing to meat discoloration and decrease of a^* values (Faustman et al., 2010). A negative correlation was observed between a^* value and pH ($r = -0.81$, $P < 0.05$) and TBARS ($r = -0.85$, $P < 0.05$), which further reiterates the relationship between surface redness, pH, and lipid oxidation.

In agreement, D'Agata et al. (2009) documented a decrease of redness in longissimus muscle from *Bos taurus* cattle harvested without stunning and with stunning from d 2 to 6 of storage. In addition, Sabow et al. (2017) reported a decrease in a^* values of LL muscle from goats harvested without stunning or their electrically stunned counterparts during 14 d of storage.

Yellowness (b^* value)

There was no harvest × storage interaction ($P = 0.4830$; Table 1) for b^* value. However, there was an effect of harvest ($P < 0.0053$) and storage ($P < 0.0001$) on yellowness. The NST steaks exhibited greater ($P < 0.05$) yellowness (b^* values) than CBP steaks throughout the storage (Table 1). In contrast with our results, D'Agata et al. (2009) documented similar yellowness in longissimus muscle from *Bos taurus* cattle harvested with and without stunning on d 0 and 6 of storage. In addition, Vergara et al. (2005) reported similar b^* values in longissimus muscles from lambs harvested without stunning and their counterparts from electrically stunned lambs during 7 d of storage. Both CBP and NST steaks demonstrated a decrease ($P < 0.05$) in yellowness during storage (Table 1). In agreement, D'Agata et al. (2009) documented a decrease in b^* values in

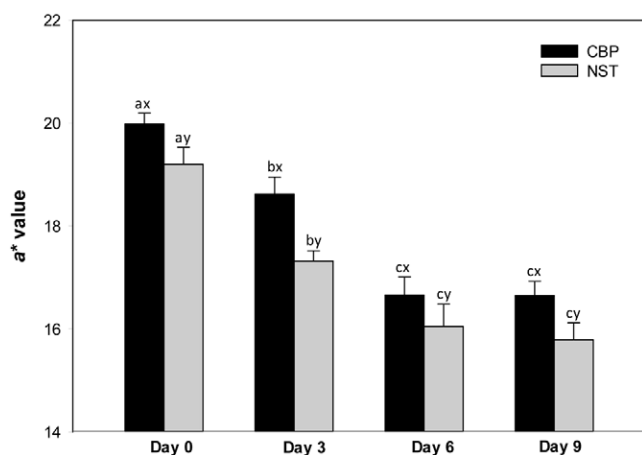


Figure 1. Surface redness (a^* value) of longissimus lumborum steaks from *Bos indicus* bulls harvested with captive bolt stunning (CBP) or without stunning (NST) during aerobic storage at 4°C for 9 d. Standard error bars are indicated. ^{a-c}Means within a harvest method without common superscripts are different ($P < 0.05$). ^{x,y}Means within a day of storage without common superscripts are different ($P < 0.05$).

longissimus muscle from *Bos taurus* cattle harvested without stunning and with stunning from d 2 to 6 of storage. In partial agreement, Önenç and Kaya (2004) reported a decrease in b^* values of longissimus muscle from *Bos taurus* cattle harvested without stunning from d 3 to 9, whereas their counterparts from stunned animals exhibited steady b^* values during storage. On contrary, several previous investigations documented an increase in yellowness in longissimus muscle from lambs (Vergara et al., 2005) and goats (Sabow et al., 2017) harvested without stunning and with electrically stunning during 7 and 14 d of storage, respectively.

Color stability (R630/580)

There was no harvest \times storage interaction ($P = 0.7006$; Fig. 2) for R630/580. However, there was an effect of harvest ($P < 0.0013$) and storage ($P < 0.0001$) on surface color stability. CBP steaks demonstrated greater ($P < 0.05$) surface color stability (R630/580) than NST steaks throughout the storage (Fig. 2). The R630/580 estimates surface discoloration; greater ratio indicates lower surface metmyoglobin accumulation and consequently greater redness and color stability (American Meat Science Association, 2012). The differences in R630/580 could be attributed to the differences in meat pH (Abril et al., 2001; McKeith et al., 2016). Lower ultimate meat pH (as observed in CBP steaks) decreases mitochondria respiration, thereby increasing the availability of oxygen to bind to myoglobin, and consequently increases oxymyoglobin levels

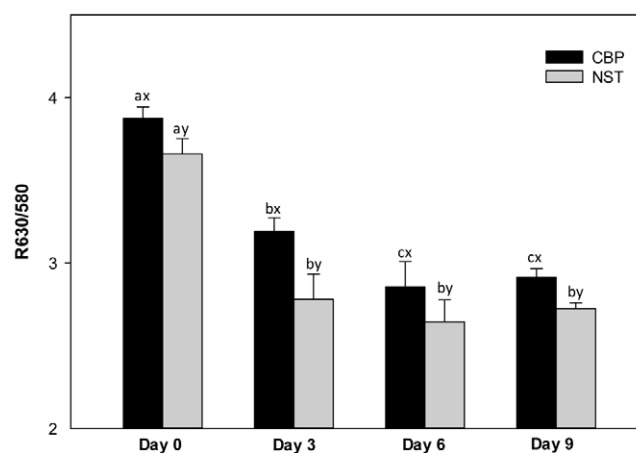


Figure 2. Surface color stability (R630/580) of longissimus lumborum steaks from *Bos indicus* bulls harvested with captive bolt stunning (CBP) or without stunning (NST) during aerobic storage at 4°C for 9 d. Standard error bars are indicated. ^{a-c}Means within a harvest method without common superscripts are different ($P < 0.05$). ^{x,y}Means within a day of storage without common superscripts are different ($P < 0.05$).

on meat surface (McKeith et al., 2016) and R630/580 (Abril et al., 2001). Contrasting our results, D'Agata et al. (2009) measured the percentage of surface oxymyoglobin (employing K/S 610 and K/S 525 values) and documented similar oxymyoglobin content in longissimus muscles from *Bos taurus* cattle harvested without stunning and after stunning on d 0, 2, and 6 of storage.

CBP and NST exhibited a decrease ($P < 0.05$) on surface color stability during storage (Fig. 2). The decline in R630/580 may associated with the differences in meat pH and lipid oxidation. The pH increase enhances mitochondrial oxygen consumption leading to a decrease in surface oxymyoglobin (Bendall and Taylor, 1972) and a decline in R630/580 (Abril et al., 2001; D'Agata et al., 2009). In addition, the increase in TBARS (Table 2) favors myoglobin oxidation, contributing to surface browning and a decrease in R630/580 values (Faustman et al., 2010). A negative correlation was observed in the present study between R630/580 and pH ($r = -0.72$, $P < 0.05$), and lipid oxidation ($r = -0.68$, $P < 0.05$), which further reiterates the relationship between surface color stability, pH, and lipid oxidation. In contrast with our results, D'Agata et al. (2009) reported steady oxymyoglobin content in longissimus muscle from *Bos taurus* cattle harvested without stunning and after stunning during 6 d of storage.

Lipid oxidation (TBARS)

There was a harvest \times storage interaction ($P = 0.0454$; Table 2) for lipid oxidation. NST steaks dem-

onstrated greater ($P < 0.05$) TBARS than CBP counterparts on d 0, 3, and 6 (Table 2), whereas both treatments exhibited similar ($P > 0.05$) TBARS on d 9. The differences in TBARS between CBP and NST may be attributed to the differences in pre-slaughter stress (Linares et al., 2007a), which is known to increase the susceptibility of post-mortem muscles to lipid oxidation. Pre-slaughter stress increases the levels of catecholamine (Sabow et al., 2016) and cortisol (Wernicki et al., 2006), which promote lipolysis and mobilization of free fatty acids in post-mortem muscles (Warriss, 1990; Wernicki et al., 2006). Furthermore, the release of cortisol leads to the generation of reactive oxygen species, which initiate the process of lipid oxidation (Wernicki et al., 2006; Linares et al., 2007a).

In partial agreement, Linares et al. (2007a) documented greater TBARS in longissimus muscle from lambs harvested without stunning than in their counterparts from electrically stunned lambs on d 7 of storage. Sabow et al. (2015a) reported similar TBARS in LL muscle from goats harvested without stunning and harvested with minimal anesthesia prior on d 0 and 7 of storage. On the contrary, Sazili et al. (2013) reported greater TBARS in LL muscle from crossbred *Bos taurus* × *Bos indicus* cattle stunned with non-penetrative pistol than in their counterparts from non-stunned animals during 14 d of storage.

Both CBP and NST steaks demonstrated an increase ($P < 0.05$) in TBARS during storage (Table 1). The observed differences in TBARS during storage could be related to the decrease of redox capacity of meat and generation of free radicals during storage (Min and Ahn, 2005). These biomolecular imbalances interact with the fatty acids, triggering the chain reaction of lipid oxidation and subsequently increasing TBARS (Min and Ahn, 2005). In agreement, Linares et al. (2007a) documented an increase on TBARS in longissimus muscle from lambs harvested without stunning and in their counterparts from electrically stunned animals during 7 d of storage. In addition, Sazili et al. (2013) reported an increase on TBARS in LL muscle from crossbred *Bos taurus* × *Bos indicus* cattle harvested without stunning and with stunning using a non-penetrative pistol during 14 d storage.

Water holding capacity (WHC)

There was no harvest × storage interaction ($P = 0.7615$; Table 1) for water holding capacity. However, there was an effect of harvest ($P < 0.0001$) and storage ($P < 0.0001$) on water holding capacity. The NST steaks exhibited greater ($P < 0.05$) WHC than their

CBP counterparts throughout the storage (Table 1). The observed differences in WHC between CBP and NST steaks could be attributed to the differences in meat pH (Önenç and Kaya, 2004; Sabow et al., 2015b). The decrease of muscle pH promotes conformational changes of cytoskeletal proteins, increasing their hydrophobicity and aggregation, which in turn favors the release of bound water (Huff-Lonergan and Lonergan, 2005) decreasing the WHC in CBP steaks (Önenç and Kaya, 2004; Sabow et al., 2015b).

Supporting our results, Önenç and Kaya (2004) documented greater WHC in longissimus muscle from *Bos taurus* cattle harvested without stunning than in their counterparts from animals stunned with captive bolt pistol during 7 d of storage. Additionally, Sabow et al. (2015b) reported greater WHC in LL muscle from goats harvested without stunning than in the muscles from animals harvested with pre-slaughter anesthesia during 7 d of storage. In contrast, Sazili et al. (2013) reported similar WHC in LL muscle from *Bos taurus* × *Bos indicus* cattle harvested without stunning and with stunning. In addition, Vergara et al. (2005) reported similar WHC in longissimus muscles from lambs harvested without stunning and after electrical stunning.

The CBP and NST steaks demonstrated an increase ($P < 0.05$) in WHC from d 6 of storage (Table 1). The observed increase in WHC during storage could be attributed to the muscle proteolysis and degradation of the cytoskeletal proteins, which in turn minimize the shrinkage of myofibrils and thereby decrease the release of bound water, increasing the WHC (Kristensen and Purslow, 2001). A positive correlation was observed in the present study between pH and WHC ($r = 0.69$, $P < 0.05$), which further reiterates the relationship between pH and WHC.

In partial agreement, Önenç and Kaya (2004) documented an increase of WHC during 7 d storage in longissimus muscle from *Bos taurus* cattle harvested without stunning, but not in their counterparts from animals harvested after stunning. On the contrary, Sabow et al. (2015b) reported a decrease of WHC in LL muscle from goats harvested without stunning and harvested with pre-slaughter anesthesia during 7 d of storage.

Instrumental color and their correlation with biochemical attributes

Several variables were strongly ($r > 0.6$; $P < 0.05$) correlated: a^* value was positively correlated with b^* value ($r = 0.76$; $P < 0.05$) and R630/580 ($r = 0.80$; $P < 0.05$). In addition, pH exhibited a positively correlation

with WHC ($r = 0.69$; $P < 0.05$), and lipid oxidation ($r = 0.74$; $P < 0.05$). Furthermore, WHC demonstrated a positive correlation with lipid oxidation ($r = 0.72$; $P < 0.05$). In contrast, a^* value exhibited a negative correlation with pH ($r = -0.81$; $P < 0.05$), WHC ($r = -0.75$; $P < 0.05$), and lipid oxidation ($r = -0.85$; $P < 0.05$). Yellowness (b^* value) demonstrated a negative correlation with lipid oxidation ($r = 0.87$; $P < 0.05$). In addition, R630/580 was negatively correlated with pH ($r = -0.72$; $P < 0.05$) and lipid oxidation ($r = -0.68$; $P < 0.05$).

Conclusions

The findings of the present study indicate that the harvest method influenced the quality attributes of LL steaks from *Bos indicus* (Nellore) cattle. CBP steaks exhibited greater color and lipid stabilities than NST steaks. Nevertheless, NST steaks exhibited greater pH and WHC than their CBP counterparts. These results suggested the necessity to develop suitable processing strategies to improve the color stability of beef from *Bos indicus* cattle harvested without stunning.

Literature Cited

- Abril, M., M. M. Campo, A. Onenç, C. Sanudo, P. Alberti, and A. I. Negueruela. 2001. Beef colour evolution as a function of ultimate pH. *Meat Sci.* 58:69–78. doi:10.1016/S0309-1740(00)00133-9
- Agbeniga, B. 2011. Influence of conventional and kosher slaughter techniques in cattle on carcass and meat quality. M.S. thesis, University of Pretoria, Pretoria. <https://repository.up.ac.za/handle/2263/26243/>. (accessed 17 April 2018).
- American Meat Science Association. 2012. Meat color measurement guidelines. 2nd ed. Am. Meat Sci. Assoc., Champaign, IL.
- Ashmore, C. R., F. Carroll, L. Doerr, G. Tompkins, H. Stokes, and W. Parker. 1973. Experimental prevention of dark-cutting meat. *J. Anim. Sci.* 36:33–36. doi:10.2527/jas1973.36133x
- Bendall, J. R., and A. A. Taylor. 1972. Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. *J. Sci. Food Agric.* 23:707–719. doi:10.1002/jsfa.2740230606
- Buege, J. A., and S. D. Aust. 1978. Microsomal lipid peroxidation. In: S. Flesischer and L. Packer, editors, *Methods in Enzymology*. Academic Press, New York, p. 302–310.
- D'Agata, M., C. Russo, and G. Prezioso. 2009. Effect of Islamic ritual slaughter on beef quality. *Ital. J. Anim. Sci.* 8:489–491. doi:10.4081/ijas.2009.s2.489
- Farouk, M. M. 2013. Advances in the industrial production of halal and kosher red meat. *Meat Sci.* 95:805–820. doi:10.1016/j.meatsci.2013.04.028
- Farouk, M. M., H. M. Al-Mazeedi, A. B. Sabow, A. E. Bekhit, K. D. Adeyemi, A. Q. Sazili, and A. Ghani. 2014. Halal and kosher slaughter methods and meat quality: A review. *Meat Sci.* 98:505–519. doi:10.1016/j.meatsci.2014.05.021
- Faustman, C., and A. L. Phillips. 2001. Measurement of discoloration in fresh meat. In: R. E. Wrolstad, editor, *Current Protocols in Food Analytical Chemistry*. Wiley & Sons, New York. p. F3.3.1-F3.3.13. doi:10.1002/0471142913.faf0303s00
- Faustman, C., Q. Sun, R. Mancini, and S. P. Suman. 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Sci.* 86:86–94. doi:10.1016/j.meatsci.2010.04.025
- Ferguson, D. M., and R. D. Warner. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Sci.* 80:12–19. doi:10.1016/j.meatsci.2008.05.004
- Ferraz, J. B. S., and P. E. Felício. 2010. Production systems—An example from Brazil. *Meat Sci.* 84:238–243. doi:10.1016/j.meatsci.2009.06.006
- Francisco, C. L., F. D. Resende, J. M. B. Benatti, A. M. Castilhos, R. F. Cooke, and A. M. Jorge. 2015. Impacts of temperament on Nellore cattle: Physiological responses, feedlot performance, and carcass characteristics. *J. Anim. Sci.* 93:5419–5429. doi:10.2527/jas.2015-9411
- González-Fuentes, H., A. Hamedy, E. von Borell, E. Luecker, and K. Riehn. 2014. Tenacity of *Alaria alata* mesocercariae in home-made German meat products. *Int. J. Food Microbiol.* 176:9–14. doi:10.1016/j.ijfoodmicro.2014.01.020
- Gregory, N. G., H. R. Fielding, M. von Wenzlawowicz, and K. von Holleben. 2010. Time to collapse following slaughter without stunning in cattle. *Meat Sci.* 85:66–69. doi:10.1016/j.meatsci.2009.12.005
- Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* 71:194–204. doi:10.1016/j.meatsci.2005.04.022
- Kristensen, L., and P. P. Purslow. 2001. The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. *Meat Sci.* 58:17–23. doi:10.1016/S0309-1740(00)00125-X
- Linares, M. B., M. I. Berruga, R. Bornez, and H. Vergara. 2007a. Lipid oxidation in lamb meat: Effect of the weight, handling previous slaughter and modified atmospheres. *Meat Sci.* 76:715–720. doi:10.1016/j.meatsci.2007.02.012
- Linares, M. B., R. Bornez, and H. Vergara. 2007b. Effect of different stunning systems on meat quality of light lamb. *Meat Sci.* 76:675–681. doi:10.1016/j.meatsci.2007.02.007
- Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. *Meat Sci.* 71:100–121. doi:10.1016/j.meatsci.2005.03.003
- McKeith, R. O., D. A. King, A. L. Grayson, S. D. Shackelford, K. B. Gehring, J. W. Savell, and T. L. Wheeler. 2016. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. *Meat Sci.* 116:165–173. doi:10.1016/j.meatsci.2016.01.016
- Miguel, G. Z., M. H. Faria, R. O. Roça, C. T. Santos, S. P. Suman, A. B. G. Faitarone, N. L. C. Delbem, L. V. C. Girao, J. M. Homem, E. K. Barbosa, L. S. Su, F. D. Resende, G. R. Siqueira, A. D. Moreira, and T. V. Savian. 2014. Immunocastration improves carcass traits and beef color attributes in Nellore and Nellore × Aberdeen Angus crossbred animals finished in feedlot. *Meat Sci.* 96:884–891. doi:10.1016/j.meatsci.2013.08.030
- Min, B., and D. U. Ahn. 2005. Mechanism of lipid peroxidation in meat and meat products—a review. *Food Sci. Biotechnol.* 14:152–163. https://www.researchgate.net/publication/228577245_Mechanism_of_lipid_peroxidation_in_meat_and_meat_products_-_A_review. (accessed 17 September 2018).

- Muchenje, V., K. Dzama, M. Chimonyo, P. E. Strydom, and J. G. Raats. 2009a. Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. *Meat Sci.* 81:653–657. doi:10.1016/j.meatsci.2008.11.004
- Muchenje, V., K. Dzama, M. Chimonyo, P. E. Strydom, A. Hugo, and J. G. Raats. 2009b. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chem.* 112:279–289. doi:10.1016/j.foodchem.2008.05.103
- Neethling, N. E., S. P. Suman, G. O. Sigge, L. C. Hoffman, and M. C. Hunt. 2017. Exogenous and endogenous factors influencing color of fresh meat from ungulates. *Meat and Muscle Biol.* 1:253–275. doi:10.22175/mmb2017.06.0032
- O’Keeffe, M., and D. E. Hood. 1982. Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. *Meat Sci.* 7:209–228. doi:10.1016/0309-1740(82)90087-0
- Önenç, A., and A. Kaya. 2004. The effects of electrical stunning and percussive captive bolt stunning on meat quality of cattle processed by Turkish slaughter procedures. *Meat Sci.* 66:809–815. doi:10.1016/S0309-1740(03)00191-8
- Quéguiner, C., E. Dumay, C. Cavalier, and J. C. Cheftel. 1989. Reduction of *Streptococcus thermophilus* in a whey protein isolate by low moisture extrusion cooking without loss of functional properties. *Int. J. Food Sci. Technol.* 24:601–612. doi:10.1111/j.1365-2621.1989.tb00686.x
- Rovinsky, J. A., and H. S. Cohen. 2018. The standard procedures for animal slaughtering in the industry. In: E. Ali and N. N. A. Nizar, editors, *Preparation and Processing of Religious and Cultural Foods*. Woodhead Publishing Series in Food Science, Technology and Nutrition, Duxford, UK. p. 325–348. doi:10.1016/B978-0-08-101892-7.00017-1
- Sabow, A. B., A. Q. Sazili, I. Zulkifli, Y. M. Goh, M. Z. Ab Kadir, N. R. Abdulla, K. Nakyinsige, U. Kaka, and K. D. Adeyemi. 2015a. A comparison of bleeding efficiency, microbiological quality and lipid oxidation in goats subjected to conscious halal slaughter and slaughter following minimal anesthesia. *Meat Sci.* 104:78–84. doi:10.1016/j.meatsci.2015.02.004
- Sabow, A. B., A. Q. Sazili, I. Zulkifli, Y. M. Goh, M. Z. Ab Kadir, and K. D. Adeyemi. 2015b. Physico-chemical characteristics of Longissimus lumborum muscle in goats subjected to halal slaughter and anesthesia (halothane) pre-slaughter. *Anim. Sci. J.* 86:981–991. doi:10.1111/asj.12385
- Sabow, A. B., Y. M. Goh, I. Zulkifli, A. Q. Sazili, U. Kaka, M. Z. Ab Kadi, M. Ebrahimi, K. Nakyinsige, and K. D. Adeyemi. 2016. Blood parameters and electroencephalographic responses of goats to slaughter without stunning. *Meat Sci.* 121:148–155. doi:10.1016/j.meatsci.2016.05.009
- Sabow, A. B., K. D. Adeyemi, I. Zulkifli, Y. M. Goh, M. Z. A. Ab Kadir, U. Kaka, Z. A. Aghwan, A. A. Abubakar, and A. Q. Sazili. 2017. Carcase characteristics and meat quality assessments in goats subjected to slaughter without stunning and slaughter following different methods of electrical stunning. *Ital. J. Anim. Sci.* 16:416–430. doi:10.1080/1828051X.2017.1291287
- Sazili, A. Q., B. Norbaiyah, I. Zulkifli, Y. M. Goh, M. Lotfi, and A. H. Small. 2013. Quality assessment of longissimus and semitendinosus muscles from beef cattle subjected to non-penetrative and penetrative percussive stunning methods. *Asian-australas. J. Anim. Sci.* 26(5):723–731. doi:10.5713/ajas.2012.12563
- Sinnhuber, R. O., and T. C. Yu. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. *J. Food Sci.* 23:626–634. doi:10.1111/j.1365-2621.1958.tb17614.x
- Suman, S. P., M. C. Hunt, M. N. Nair, and G. Rentfrow. 2014. Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Sci.* 98:490–504. doi:10.1016/j.meatsci.2014.06.032
- USDA. 2001. Israel. Kosher Foods. Israeli Requirements for Recognition of Kosher Slaughter in the U.S. <https://apps.fas.usda.gov/gainfiles/200107/120681187.pdf>. (accessed 5 November 2018).
- USDA. 2018. Brazil: Livestock and Products Semi-annual, 2018 Semi-Annual Livestock Report. https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Livestock%20and%20Products%20Semi-annual_Brasilia_Brazil_2-28-2018.pdf (accessed 13 May 2018).
- Verbeken, D., N. Neirinck, P. V. D. Meerem, and K. Dewettinck. 2005. Influence of k-carrageenan on the thermal gelation of salt-soluble meat proteins. *Meat Sci.* 70:161–166. doi:10.1016/j.meatsci.2004.12.007
- Vergara, H., M. B. Linares, M. I. Berruga, and L. Gallego. 2005. Meat quality in suckling lambs: Effect of pre-slaughter handling. *Meat Sci.* 69:473–478. doi:10.1016/j.meatsci.2004.09.002
- Warriss, P. D. 1990. The handling of cattle pre-slaughter and its effects on carcass and meat quality. *Appl. Anim. Behav. Sci.* 28:171–186. doi:10.1016/0168-1591(90)90052-F
- Wernicki, A., R. Urban-chmiel, M. Kankofer, P. Mikucki, A. Puchalski, and S. Tokarzewski. 2006. Evaluation of plasma cortisol and TBARS levels in calves after short-term transportation. https://www.revmedvet.com/2006/RMV157_30_34.pdf. (accessed 15 January 2018).