

Intramuscular variation in mitochondrial functionality of beef *semimembranosus*

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Abstract

Intramuscular color stability variations in beef *semimembranosus* have been reported previously. Mitochondria remain biochemically active in postmortem muscle and can influence fresh beef color stability. However, the role of mitochondrial functionality in intramuscular color variations in beef *semimembranosus* is yet to be examined. We examined the functionality of mitochondria isolated from outside (OSM) and inside (ISM) regions of beef *semimembranosus*. *Semimembranosus* muscles (n = 5) were collected from inside rounds of beef carcasses 48 h post-mortem and were separated to OSM and ISM steaks. Color attributes were evaluated instrumentally and biochemically on days 0 and 4 of retail display, whereas mitochondrial oxygen consumption rate (OCR) was measured on day 0 using succinate as substrate using steaks frozen during fabrication. Mitochondrial OCR was significantly greater in OSM than in ISM on day 0. The ISM steaks exhibited significantly greater redness (a^* value) than OSM steaks on day 0, but OSM steaks had significantly greater redness than the ISM counterparts on day 4. During retail display, ISM steaks exhibited greater lightness (L^* value) than OSM steaks. However, OSM demonstrated significantly greater color stability and metmyoglobin reducing activity than ISM throughout the display. The observed differences in mitochondrial OCR between ISM and OSM steaks indicated that mitochondrial biochemistry possibly contributes to the intramuscular color variations in beef *semimembranosus*.

Keywords: Beef color, color stability, mitochondria, *semimembranosus*

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Introduction

Fresh meat color critically influences consumer purchase decisions (Suman *et al.*, 2014), and the annual revenue loss to the United States beef industry due to discoloration is estimated to be more than 1 billion USD (Smith *et al.*, 2000). Beef color and color stability are muscle-specific traits (McKenna *et al.*, 2005; Von Seggern *et al.*, 2005). Large beef muscles such as *semimembranosus* (Sammel *et al.*, 2002a) and *biceps femoris* (Pastsart *et al.*, 2013) exhibit intramuscular variations in color stability. Beef *semimembranosus* can be separated into color-stable outside (OSM) and color-labile inside (ISM) regions based on the location within the carcass. The variation in color has been partly attributed to differences in temperature and pH decline during carcass chilling (Sammel *et al.*, 2002a). Further research indicated that differential abundance of sarcoplasmic proteome could also result in rapid pH fall and compromise color stability of ISM (Nair *et al.*, 2016).

Myoglobin is the sarcoplasmic heme protein responsible for meat color, and the interactions between myoglobin and cellular components govern color biochemistry (Suman & Joseph, 2013; Suman & Nair, 2017). Mitochondria remain biochemically active in postmortem muscle and may influence the bloom development and color stability by competing with myoglobin for available oxygen and by providing reducing equivalents for metmyoglobin reduction (Ashmore *et al.*, 1972; Tang *et al.*, 2005a, 2005b). Furthermore, Belskie *et al.* (2015a) reported muscle-specificity in beef mitochondrial biochemistry, with color-labile *psaos major* demonstrating rapid decrease in mitochondrial oxygen consumption rate (OCR) compared to color-stable *longissimus dorsi*. However, intramuscular variation in functionality of mitochondria and its relationship with color biochemistry are

yet to be examined. Therefore, the objective of the present study was to examine the functionality of mitochondria isolated from beef OSM and ISM.

Materials and Methods

Carcasses obtained from beef cattle of unknown backgrounds were utilized in this study in an attempt to effectively simulate commercial market scenario in the United States beef industry. Beef inside rounds (USDA Select grade; IMPS # 168) from five carcasses ($n = 5$) were obtained from a commercial packing plant 48 h post-mortem. The cuts were vacuum packaged in the plant and transported under refrigeration to the USDA-inspected meat laboratory at University of Kentucky, where the *semimembranosus* muscle was separated and fabricated into 2.54 cm OSM and ISM steaks. Each muscle was fabricated to seven OSM and seven ISM steaks. One steak was vacuum packaged and frozen immediately at $-80\text{ }^{\circ}\text{C}$ for mitochondria isolation, whereas the six other steaks were individually placed on Styrofoam trays and aerobically overwrapped with oxygen-permeable polyvinyl chloride film ($15,500 - 16,275\text{ cm}^3/\text{m}^2/24\text{ h}$ oxygen transmission rate at $23\text{ }^{\circ}\text{C}$). The steaks were assigned randomly for refrigerated retail display ($2\text{ }^{\circ}\text{C}$) under constant, cool white fluorescent lighting (1300 lx) for 0 or 4 days and were utilized for evaluation of instrumental color and biochemical traits.

On day 0 of retail display, the instrumental color attributes were evaluated after allowing the steaks to bloom for 2 h at 2°C after fabrication, whereas on day 4 of display the measurements were taken on the light-exposed steak surfaces. A HunterLab LabScan XE colorimeter (Hunter Associates Laboratory, Reston, VA, USA) with 2.54 cm diameter aperture, illuminant A, and 10° standard observer was used to measure CIE lightness (L^*), redness (a^*), yellowness (b^*), hue (trueness of red), and chroma (saturation index) values on from three random locations on the steak surfaces (AMSA, 2012). Standard black and white plates were used for calibrating the colorimeter. In addition, the ratio of reflectance at 630 nm and 580 nm ($R_{630/580}$) was calculated to estimate the surface color stability of steaks; greater ratio indicates lesser amount of metmyoglobin and thus greater color stability (AMSA, 2012).

The pH of steaks was determined according to the method of Strange *et al.* (1977) using an Accumet AR25 pH-meter (Fisher Scientific, Pittsburgh, PA, USA). Duplicate 5 g samples were homogenized in 30 mL distilled deionized water before measuring the pH.

Thiobarbituric acid assay was employed for measuring the lipid oxidation (Yin *et al.*, 1993). Briefly, duplicate 5 g samples were homogenized with 11% trichloroacetic acid in a blender. The homogenate was then filtered using Whatman No. 1 filter paper. One mL of filtrate was mixed with 1 mL of aqueous thiobarbituric acid (20 mM) and incubated at 25°C for 20 h. The absorbance of samples at 532 nm measured spectrophotometrically (UV-2401 spectrophotometer, Shimadzu Inc., Columbia, MD, USA) were reported as thiobarbituric acid reactive substances (TBARS).

Metmyoglobin reducing activity (MRA) was evaluated according to the method described by Sammel *et al.* (2002b). Cubes ($2.5 \times 2.5 \times 2.5\text{ cm}^3$) of meat were removed from the light-exposed surfaces. Metmyoglobin formation was induced in the samples by submerging them in a solution of 0.3% sodium nitrite for 20 min at room temperature. After 20 min, samples were removed from the solution, blotted dry, vacuum packaged. The reflectance spectra from 700 to 400 nm were recorded on the light-exposed surface using a HunterLab LabScan XE colorimeter. The vacuum-packaged samples were then incubated at $30\text{ }^{\circ}\text{C}$ for 2 h to induce reduction of metmyoglobin. The reflectance spectra from 700 to 400 nm were recorded again at the end of incubation. Percentage of pre- and post- incubation surface metmyoglobin was calculated based on K/S ratios and according to established formulas (AMSA, 2012). MRA was calculated using the following equation:

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

Mitochondria were isolated from OSM and ISM steaks frozen during fabrication (on day 0) according to Lanari & Cassens (1991), with minor modifications. Finely minced meat (35 g) was suspended in 70 mL mitochondria suspension buffer (250mM Sucrose, 10 mM HEPES, pH 7.2). The suspension was stirred slowly and hydrolyzed with trypsin (1 mg/g of tissue) for 15 min. After proteolytic digestion, the suspension was diluted to 350 mL with mitochondria isolation buffer (67 mM sucrose, 50 mM Tris-HCl, 50 mM KCl, 10 mM EDTA, and 0.2% BSA, pH 7.2) and homogenized using a Kontes Duall grinder (Vineland, NJ) followed by a Wheaton Potter-Elvehjem grinder (Millville, NJ). The homogenate was centrifuged at $900 \times g$ for 15 min with a Sorvall refrigerated RC-5C plus centrifuge (Thermo Fisher Scientific, Newtown, CT), and the resulting supernatant was again centrifuged at $14000 \times g$ for 15 min. Mitochondrial pellets obtained were washed twice and suspended in

mitochondria suspension buffer. All steps were performed at 0 - 4 °C. Mitochondrial protein content was determined using a bicinchoninic acid protein assay.

Mitochondrial oxygen consumption rate (OCR) was measured using a Clark electrode attached to a Rank Brothers digital model 20 oxygen controller (Cambridge, England). Oxygen consumption was recorded over time at by suspending mitochondria (2 mg/mL) in incubation buffer (250 mM sucrose, 5 mM KH₂PO₄, 5 mM MgCl₂, 0.1 mM EDTA, 0.1% BSA, and 20 mM maleic acid) in the Clark oxygen electrode with the addition of succinate (40 mM) at pH 5.6 and 25 °C. The chamber was stirred with a 10 mm magnetic bar at 600 rpm. Following the addition of substrate, oxygen consumption per mg of mitochondria from each muscle was calculated based on the method of Estabrook (1967).

The experimental design was a randomized complete block design, where each carcass (n = 5) served as a block. The effects of muscle location (ISM and OSM), storage (0 and 4 days), and their interactions on color and other biochemical parameters were analyzed using the Mixed Procedure of SAS. Least square means for protected F-tests ($P < 0.05$) were separated by using least significant differences and were considered significant at $P < 0.05$.

Results and Discussion

The results of instrumental color and biochemical attributes are presented in Table 1.

Table 1 Instrumental color, biochemical attributes, and mitochondrial oxygen consumption rate (OCR) of beef inside (ISM) and outside (OSM) *semimembranosus* steaks (n = 5) during refrigerated retail display (2 °C) for 4 days under aerobic packaging (Mean ± SE)

Attribute	Muscle	Retail display days	
		0	4
L* value	ISM	49.51 ^{ax} ± 1.36	46.93 ^{ax} ± 1.50
	OSM	39.81 ^{ay} ± 1.41	39.86 ^{ay} ± 0.68
a* value	ISM	35.74 ^{ax} ± 0.34	22.83 ^{by} ± 0.85
	OSM	33.10 ^{ay} ± 0.37	26.69 ^{bx} ± 0.50
b* value	ISM	27.75 ^{ax} ± 0.37	19.71 ^{bx} ± 0.28
	OSM	24.94 ^{ay} ± 0.50	20.73 ^{bx} ± 0.43
Chroma	ISM	45.25 ^{ax} ± 0.49	29.84 ^{by} ± 0.71
	OSM	41.45 ^{ay} ± 0.57	33.57 ^{bx} ± 0.47
Hue	ISM	37.82 ^{ax} ± 0.14	30.71 ^{bx} ± 0.79
	OSM	36.99 ^{ax} ± 0.35	33.80 ^{by} ± 0.65
R630/580	ISM	5.87 ^{ay} ± 0.24	2.63 ^{by} ± 0.17
	OSM	6.88 ^{ax} ± 0.36	3.93 ^{bx} ± 0.16
MRA	ISM	27.17 ^{ay} ± 1.86	4.96 ^{by} ± 1.95
	OSM	62.61 ^{ax} ± 1.55	34.13 ^{bx} ± 2.10
pH	ISM	5.38 ^{bx} ± 0.01	5.42 ^{ax} ± 0.01
	OSM	5.36 ^{bx} ± 0.01	5.40 ^{ax} ± 0.01
TBARS	ISM	0.016 ^{bx} ± 0.002	0.108 ^{ax} ± 0.034
	OSM	0.016 ^{bx} ± 0.001	0.113 ^{ax} ± 0.022
Mitochondrial OCR ^ψ	ISM	16.89 ^y ± 2.13	NA
	OSM	41.18 ^x ± 8.22	NA

^{a-b} 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 ($P < 0.05$).

^ψ OCR measured as nanomoles of oxygen consumed per mg mitochondria per minute

NA = not available

ISM exhibited greater ($P < 0.05$) redness (a^* value) than OSM after 2 h of blooming on day 0. However, by day 4 of display, this trend reversed, and the OSM steaks had greater ($P < 0.05$) redness than their ISM counterparts, indicating that OSM is more color stable compared to ISM. Our results are in agreement with previous studies which also reported greater a^* values for ISM than OSM on day 0 of display, and a similar reversal of redness with OSM having greater redness for rest of the display days (Sammel *et al.*, 2002b, Nair *et al.*, 2016). As anticipated, the redness of both ISM and OSM steaks decreased ($P < 0.05$) during retail display.

Throughout the display, ISM demonstrated greater ($P < 0.05$) lightness (L^* value) compared to OSM, and it is one of the most visually appreciable differences in color between ISM and OSM. ISM had greater ($P < 0.05$) yellowness (b^* value) than OSM on day 0, whereas ISM and OSM had similar ($P > 0.05$) b^* value on day 4. Similar to a^* value, chroma was greater ($P < 0.05$) in ISM on day 0, and in OSM on day 4 of display. In the present study, hue angle (trueness of red) was similar ($P > 0.05$) between ISM and OSM on day 0, and OSM had greater ($P < 0.05$) hue angle by end of display, as documented in earlier studies (Sammel *et al.*, 2002a, Nair *et al.*, 2016). The OSM steaks in the current study demonstrated greater ($P < 0.05$) color stability (R630/580) and MRA than their ISM counterparts throughout display days, which indicated that OSM is color-stable muscle whereas ISM is color-labile. In support, Sammel *et al.* (2002b) and Nair *et al.* (2016) also reported a similar trend with color stability and MRA in OSM and ISM. In partial agreement, Pastsart *et al.* (2013) reported intramuscular variation in color stability of *biceps femoris* from double-muscled Belgian Blue cattle. Lipid oxidation and pH were similar ($P > 0.05$) for OSM and ISM and is in agreement with that of Nair *et al.* (2016). In general, our results on color parameters are in agreement with previous research (Sammel *et al.*, 2002a; Nair *et al.*, 2016) and confirm the intramuscular variation in color and color stability of beef *semimembranosus*.

Mitochondrial OCR, indicative of mitochondrial functionality, was lower ($P < 0.05$) in ISM than OSM. In support, Sammel *et al.* (2002b) reported lower oxygen consumption in ISM when measured directly from muscle samples and suggested that the lower oxygen consumption of ISM allowed more oxygen penetration into the muscle resulting in improved bloom. Mitochondrial functionality and myoglobin chemistry are interrelated (Wittenberg & Wittenberg, 2007). Active mitochondria could out-compete myoglobin for the available oxygen leading to low oxygen availability for formation of cherry-red oxymyoglobin (i.e. bloom development). The lower mitochondrial OCR in ISM could facilitate greater oxygen availability for myoglobin oxygenation resulting in greater blooming and initial redness in ISM, as observed in the present study.

Active mitochondria can also provide reducing equivalents for metmyoglobin reduction, a process critical in maintaining color stability during retail display (Arihara *et al.*, 1995; Tang *et al.*, 2005a). Moreover, mitochondria can regenerate NADH, which can be utilized for metmyoglobin reduction through electron transport-mediated and enzymatic pathways (Belskie *et al.*, 2015b). OSM, which demonstrated greater ($P < 0.05$) mitochondrial OCR than ISM, had greater ($P < 0.05$) redness on day 4 as well as greater ($P < 0.05$) color stability (R630/580) and MRA throughout the display. Furthermore, Belskie *et al.* (2015a) reported that mitochondria from color-stable *longissimus dorsi* steaks demonstrated greater activity than those from color-labile *psoas major* on days 5 and 7 of display resulting in improved color stability for *longissimus dorsi* steaks. These results, along with the observations in the present study, highlight the critical role of mitochondrial functionality in beef color stability.

Conclusion

The results of the present study indicated the existence of intramuscular variation in mitochondrial functionality in beef *semimembranosus*. ISM exhibited lower mitochondrial OCR and greater initial redness, whereas OSM demonstrated greater ($P < 0.05$) redness at the end of retail display as well as greater mitochondrial OCR and color stability throughout the display. These results suggested that the variations in mitochondrial biochemistry (between ISM and OSM) could be partially responsible for the intramuscular color variations in beef *semimembranosus*.

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Authors' Contributions

Conception and design – MNN & SPS; Data collection and analyses – MNN, RR, & GR; Drafting of paper – MNN; Critical revision and final approval of version to be published – SPS.

Conflict of Interest Declaration

The authors declare that they have no affiliations with any organization or entity with any financial or non-financial interest that could bias the subject matter and outcomes discussed in this manuscript.

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