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Color Stability, Reducing Activity, and Cytochrome *c* Oxidase Activity of Five Bovine Muscles

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The objective was to characterize the beef *psoas major* (PM), *longissimus lumborum* (LL), superficial *semimembranosus* (SSM), deep *semimembranosus* (DSM), and *semitendinosus* (ST) muscles for differences in instrumental and visual color, metmyoglobin-reducing activity (MRA), total reducing activity (TRA), and cytochrome *c* oxidase activity. The LL and ST had the most color stability and MRA ($p < 0.05$), the DSM and PM had the least ($p < 0.05$), and values for the SSM were intermediate. Visual color ($r = -0.66$) and a^* and chroma ($r = 0.68$) were more correlated with MRA than with TRA ($r < 0.14$ for all measures). This research supports previous reports that color stability among muscles is variable and that MRA is more useful than TRA for explaining the role of reducing activity in muscle-color stability.

KEYWORDS: Beef; color; color stability; cytochrome *c* oxidase; reducing activity

INTRODUCTION

Meat color forms the fundamental basis for consumer purchasing decisions (*1*). Meat with a bright cherry red appearance is associated with superior quality and freshness and is preferred to meat with a brownish-tan (metmyoglobin) appearance. Numerous antemortem and postmortem factors interact to influence meat color (*1*). Mancini and Hunt (*2*) recently provided a current update of research regarding many of these factors.

Different muscles have unique purposes in living animals, resulting in variations in fiber type (*3*) and metabolic function (*4*). As a result, meat exhibits a wide range of postmortem oxygen consumption rates (OCR) and subsequent color stability, depending on its muscle source (*5*−*7*), due to an inverse relationship between rate of discoloration and OCR (*8*, *9*).

Meat OCR and pigment reduction are regulated by mitochondrial enzymes, particularly cytochrome *c* oxidase, which continue to consume oxygen postmortem (*10*). In meat, mitochondria use and reduce the amount of oxygen available to bind to myoglobin, which leads to deoxymyoglobin, rather than oxymyoglobin, formation (*10*). Deoxymyoglobin is more susceptible to oxidation than is oxymyoglobin (*11*). Although many studies have characterized the histochemical and biochemical properties of muscle (*12*), no studies have directly related mitochondria content or enzyme activity within different bovine muscles to color stability.

Metmyoglobin (MMb) may be reduced nonenzymatically (*13*) or enzymatically (*14*, *15*) by enzymes located within muscle mitochondria (*16*) such as cytochome *c* oxidase. Reduction of MMb occurs in the presence of mitochondria and succinate in vitro (*10*, *17*). Thus, mitochondria as a source of NADH, a key component of metmyoglobin-reducing activity (MRA), should provide for MRA (*18*). It is not known, however, how cytochome *c* oxidase activity within muscles may relate to MRA.

Another measurement of reducing activity in muscle is total reducing activity (TRA). This assay is not specific for MRA-related enzymes; rather, it is indicative of the muscle's overall reductive state. It correlates well with visual color during display (*19*), but does not reflect color differences between muscles (*20*). The relationship of TRA to mitochondria concentration and color stability for various muscles is unclear.

The objectives of this study were to explore the relationships among color, color stability, metmyoglobin-reducing activity, total reducing activity, and cytochrome *c* oxidase activity for four muscles differing in color stability.

MATERIALS AND METHODS

Raw Materials and Preparation. Seven beef carcass sides representing different animals (steers of *Bos taurus* influence, 340−390 kg, USDA yield grade 2, USDA Select, A^{50} to A^{100} maturity, exposed *longissimus* with normal color and absent of quality defects) were selected randomly 2 d postmortem at a commercial abattoir. Carcass sides had been stimulated electrically (48 V continuously for 30 s approximately 30 min after exsanguination). From each carcass side, *psoas major* (PM), *longissimus lumborum* (LL), *semimembranosus* (SM), and *semitendinosus* (ST) muscles were obtained and stored in

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vacuum until 11 days postmortem at 1.1 ± 0.5 °C. From each muscle, three 2.54-cm-thick steaks were prepared and designated for chemical analysis on days 0, 4, or 7 of retail display.

Packaging and Sample Location. All steaks were placed individually on a foam tray (17S; McCune Paper Company, Salina, KS) with a Dri-Loc soaker pad (AC-50; Sealed Air Corp., Duncan, SC) and overwrapped with polyvinyl chloride film (MAPAC L, 21700 cm^3 oxygen/m^2/24 h; Borden Packaging and Industrial Products, North Andover, MA).

For ST steaks, analysis was conducted in the superficial region where muscle fibers are predominantly α-white (*21*). The SM steaks were assessed at both deep (DSM; inner region of muscle closest to the femur) and superficial (SSM; region of muscle adjacent to subcutaneous fat) locations because these regions had contrasting quality resulting from differing postmortem temperature and pH declines (*20*, *22*). Samples from LL and PM were taken from the center of each steak.

pH. Samples from day 0, visually devoid of intermuscular fat and connective tissue, were frozen in liquid nitrogen and blended in a Waring table-top blender (Dynamics Corp. of America, New Hartford, CT). To determine pH, 10 g of pulverized sample was combined with 100 mL of deionized water and mixed for 30 s, and the pH values were obtained by using an Accumet combination glass electrode connected to an Accumet 50 pH meter (Fisher Scientific, Fairlawn, NJ).

Retail Display. Steaks were displayed under continuous fluorescent lighting (Bulb F32T8/ADV830, 3000 K, CRI = 86; Phillips, Bloomfield, NJ) of 2150 ± 50 lux in open-top display cases (DMF8; Tyler Refrigeration Corp., Niles, MI) that defrosted every 12 h. Case temperatures, 1.7 ± 3.2 °C, were monitored at the meat level with temperature loggers (RD-TEMP-XT; Omega Engineering, Inc., Stamford, CT). All packages were rotated daily to minimize case location effects.

Instrumental and Visual Color. Steaks designated for day 7 chemical analysis were analyzed daily from day 0 to day 7 for instrumental color by using a HunterLab MiniScan XE Plus Spectrophotometer (45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). Three scans from each steak or region were obtained and averaged for statistical analysis. CIE L^*, a^*, and b^* values (Illuminant A) were used to calculate chroma ($(a^{*2} + b^{*2})^{1/2}$), an indicator of saturation or vividness of color.

A trained panel ($n = 6$) conducted visual-color evaluations on the same steaks. All panelists passed the Farnsworth Munsell 100-hue test (Macbeth, Newsburgh, NY). Initial beef color was evaluated on day 0 to the nearest 0.5 according to an 8-point scale in which 1 = bleached red, 2 = slightly cherry red, 3 = moderately light cherry red, 4 = cherry red, 5 = slightly dark red, 6 = moderately dark red, 7 = dark red, and 8 = very dark red. Visual color was assessed daily according to a 5-point scale, to the nearest 0.5, in which 1 = very bright cherry red, 2 = bright cherry red, 3 = slightly dark red to tannish red, 3.5 = borderline panelist acceptable, 4 = moderately grayish tan to brown, 5 = tan to brown. Using a 7-point scale, panelists determined discoloration, defined as the percentage of surface MMb, each day to the nearest 1.0 where 1 = none (0%), 2 = slight (1−19%), 3 = small (20−39%), 4 = modest (40−59%), 5 = moderate (60−79%), 6 = extensive (80−99%), 7 = total (100%).

Metmyoglobin-Reducing Activity. Metmyoglobin-reducing activity (MRA) was determined by using a 2.54-cm cube from each steak or region within a steak on days 0, 4, and 7, according to a procedure described by Sammel et al. (*19*). Samples were submerged for 20 min in a 0.3% solution of sodium nitrite to facilitate nitric oxide metmyoglobin (MMb) formation, then removed, blotted dry, and vacuum-packaged (3-mil, standard-barrier nylon/polyethylene, 0.6 cm^3/645.16 cm^2/24 h at 0 °C oxygen transmission rate; Koch Supplies, Inc., Kansas City, MO). On the light-exposed display surface, samples were scanned twice with a HunterLab MiniScan XE Plus Spectrophotometer (D/8-S, 14.3-mm diameter aperture; Hunter Associates Laboratory, Inc., Reston, VA) to obtain 400−700 nm reflectance data.

Samples were incubated at 30 °C for 2 h (Thelco model 4; Precision Scientific, Chicago, IL) to induce nitric oxide MMb reduction to deoxymyoglobin (DMb). Upon removal from the incubator, samples were rescanned twice immediately to determine the percentage of remaining surface MMb, using K/S ratios and equations from AMSA (*23*). The following equation was used to calculate MRA: (Δ % surface MMb/preincubation % surface MMb) × 100.

Total Reducing Activity. Total reducing activity was measured according to the procedure of Lee et al. (*24*). In duplicate, 2-g samples from the steak surface, devoid of any visible fat or connective tissue, were homogenized (PowerGen 35; Fisher Scientific, Fairlawn, NJ) with 10 mL of 25 mM PIPES (piperazine-*n*, *n*-bis-(2-ethane-sulfonic acid)) buffer. After homogenization, 5 mL of homogenate was added to 2 mL of 5 mM potassium ferricyanide, vortexed, and stored in the dark at 2 °C for 1 h, with intermittent vortexing every 10 min. Then, 0.1 mL of 0.04 M ammonium sulfamate and 0.2 mL of 0.5 M lead acetate were added, and the sample was vortexed and held at 25 °C for 5 min. Finally, 2.5 mL of 20% trichloroacetic acid was added, and the solution was brought to volume (10 mL) with deionized water. After 5 min, the solution was filtered by using a syringe (3 mL Luer-Lok; Becton Dickinson and Co., Franklin Lakes, NJ) with a 0.45-μm filter attached (Nalgene surfactant-free cellulose acetate membrane; Nalge Nunc International Corp., Rochester, NY), and the filtered sample was dispensed into a 1.5 mL polystyrene microcuvette. Solution absorbance was measured at 420 nm (UV-2010; Hitachi Instruments, Inc., San Jose, CA), with 1 mM ferricyanide solution as a standard. Total reducing activity was expressed as a unitless quantity: A_{420} of standard − A_{420} of sample filtrate.

Cytochrome *c* Oxidase Activity. Cytochrome *c* oxidase (CCO) activity was determined by using a colorimetric-assay kit (Sigma, St. Louis, MO). Samples for CCO were removed on day 0 from each steak, frozen instantly in liquid nitrogen, vacuum-packaged, and stored at −80 °C until analysis. Mitochondria were isolated from steak samples according to the following procedure.

Five grams of muscle, devoid of fat and connective tissue, was homogenized in 50 mL of isolation buffer 1 (0.1 M KCl, 50 mM Tris HCl, 5 mM $MgCl_2$, 1 mM EDTA, and 1 mM ATP, pH 7.5) by using a homogenizer (Kinematica Polytron benchtop Model PT 3100; Brinkmann, Lucerne, Switzerland). After homogenization, 25 g of Proteinase K was added and the sample was incubated for 5 min at 0 °C before 100 mL of isolation buffer 1 was added. Further homogenization at 0 °C was accomplished with a Kontes Duall grinder (Vineland, NJ) and then with a Wheaton Potter-Elvehjem grinder (Millville, NJ). All Teflon grinding pestles rotated at 1400 rpm. Each sample was centrifuged at 600*g* at 0 °C for 10 min (Sorvall RC-5B, Newtown, CT) and filtered through two layers of cheesecloth. The supernatant was again centrifuged at 14000*g* at 0 °C for 10 min, and the resulting pellet was vortexed with 5 mL of isolation buffer 2 (0.1 M KCl, 50 mM Tris HCl, 1 mM $MgCl_2$, 0.2 mM EDTA, and 1% BSA, pH 7.5). Samples were again centrifuged at 7000*g* at 0 °C for 10 min and the resulting pellet was vortexed with isolation buffer 2. Final centrifugation was accomplished at 3500*g* at 0 °C for 10 min, and the isolated mitochondria pellet was vortexed with 0.25 M sucrose.

Determination of cytochrome *c* oxidase activity was based on a colorimetric assay that quantifies oxidation of ferrocytochrome *c* to ferricytochrome *c* via cytochrome *c* oxidase, a reaction that results in a decrease in absorbance at 550 nm (Sigma cytochrome *c* oxidase technical bulletin). The decrease in absorbance at 550 nm was monitored by using a spectrophometer (UV 2101-PC: Shimadzu, Kyoto, Japan) combined with a temperature-regulated (25 °C) 6-cell position chamber (CPS 260, Shimadzu, Kyoto, Japan). The spectrophotometer was calibrated to zero by using assay buffer (10 mM Tris-HCl and 120 mM KCl, pH 7.0, 25 °C).

In a cuvette, 0.95 mL of assay buffer was combined with 50 μL of enzyme buffer (10 mM Tris-HCl and 250 mM sucrose, pH 7.0, 4 °C) and 50 μL of isolated mitochondria. The reaction was initiated by the addition of 50 μL of ferrocytochrome *c* (reduced with 0.1 M dithiothreitol), and the decrease in absorbance at 550 nm was measured every second for 1 min by using a kinetics program. Activity was calculated based on the following equation. Units/mL = [(ΔAbs_{550}/min for the sample − ΔAbs_{550}/min for the blank) × dilution factor (*6*) × total reaction volume (1.1 mL)]/[mitochondria isolate volume (0.05 mL) × the difference in extinction coefficients between ferro- and

Table 1. pH and Initial Beef Color of Five Bovine Muscles on Day 0 of Display[a]

trait	muscles[b] PM	LL	SSM	DSM	ST	SE[c]
pH	5.7 x	5.5 z	5.5 yz	5.6 y	5.6 y	0.03
initial beef color[d]	3.8 y	4.1 xy	4.7 x	2.8 z	2.9 z	0.35

[a] x,y,z = means in a row with a different letter differ ($p < 0.05$). [b] PM = *psoas major*, LL = *longissimus lumborum*, SSM = superficial *semimembranosus*, DSM = deep *semimembranosus*, ST = *semitendinosus*. [c] SE = standard error. [d] 1 = bleached red to 8 = very dark red.

ferricytochrome *c* at 550 nm (21.84)]. One unit will oxidize 1 μM reduced cytochrome *c* per min at pH 7.0 and 25 °C.

Statistical Analysis. The experimental design for determining muscle effects on visual and instrumental color was a randomized complete block with repeated measures. Beef carcass sides ($n = 7$ animals) served as blocks. Time of color evaluation (day 0 to 7) was a repeated measure determined on steaks within a muscle (*25*). Type-3 tests of fixed effects for muscle, display time, and their interaction were performed by using the MIXED procedure of SAS (*26*). Beef carcass sides (animal) were considered a random effect. To account for visual- and instrumental-color repeated measures, F-test denominator degrees of freedom were estimated by using the Kenward-Rogers adjustment. Covariance−variance structure for repeated measurements taken on steaks within a muscle was modeled by using the repeated option of MIXED. The most appropriate structure for the repeated measure was selected using Akaike's information criterion. Least-squares means for protected *F*-tests ($p < 0.05$) were separated by using the diff option, and pairwise comparisons were considered significant at $p < 0.05$.

The experimental design for determining muscle effects on cytochrome *c* oxidase activity was a randomized complete block. Type-3 tests of fixed effects for muscle were performed by using the MIXED procedure of SAS (*26*), and beef carcass sides ($n = 7$ animals) were considered random blocking effects.

The experimental designs for determining muscle effects (whole-plot fixed effect) on metmyoglobin-reducing and total reducing activity were split plots. The whole plot consisted of a randomized complete block in which beef carcass sides ($n = 7$ animals) served as blocks. Time of chemical analysis (subplot treatment) was assigned to one of the three steaks (subplot unit) from each muscle (105 total units; 7 animals × 5 muscle treatments × 3 display times). Type-3 tests of fixed effects for muscle, display time, and their interaction were performed by using the MIXED procedure of SAS (*26*). Beef carcass sides (animals) were considered random effects for the randomized complete-block portion of the whole plot, and muscle*animal was used to determine whole-plot error. Least-squares means were separated as described for color analysis.

RESULTS

pH and Initial Beef Color. The PM had the highest pH ($p < 0.05$), but all muscles were between 5.5 and 5.7 (**Table 1**). The DSM and ST had the lowest ($p < 0.05$) initial-beef-color scores, indicating that they were the lightest and palest muscles, only moderately light cherry red (**Table 1**). Scores of the LL and PM were similar ($p > 0.05$), with a more typical cherry-red appearance. The highest score (slightly dark red) was given to the SSM, which differed from all muscles ($p < 0.05$) except the LL.

Instrumental Color. Instrumental-color data showed many differences among the muscles throughout display (**Table 2**).

Table 2. Instrumental Color of Five Bovine Muscles during 7 Days of Display[a]

trait	display day	muscles[b] PM	LL	SSM	DSM	ST	SE[c]
*L**	0	46.3 my	41.3 mz	40.7 mz	51.9 mx	47.6 oy	
	1	43.7 ny	40.5 mnz	40.0 mnz	49.3 opx	47.9 nox	
	2	43.2 noy	41.1 myz	40.0 mnz	48.9 px	48.7 mnx	
	3	42.0 py	40.6 myz	39.1 noz	49.0 px	48.3 nox	0.48 to 1.34
	4	41.8 pz	40.8 mz	39.4 noz	48.8 py	48.3 noy	
	5	41.6 py	39.8 nyz	39.2 noz	49.7 nopx	48.8 mnx	
	6	42.3 opy	39.3 oz	38.8 oz	50.1 nox	49.4 mx	
	7	42.3 opy	39.1 oz	39.3 noz	50.4 nx	48.5 nox	
*a**	0	33.4 myz	34.4 my	33.6 myz	35.1 my	32.2 mz	
	1	26.6 nz	33.3 mnw	32.6 mwx	29.7 ny	31.7 mx	
	2	22.4 oz	32.7 nw	29.8 nx	24.7 oy	30.1 nx	
	3	20.2 pz	32.4 nw	28.0 oy	21.6 pz	30.2 nx	0.61 to 2.40
	4	18.2 qz	31.3 ox	25.9 py	19.7 qz	29.5 nx	
	5	17.0 rz	30.1 px	24.1 qy	17.8 rz	28.0 ox	
	6	15.9 sz	28.8 qx	22.8 ry	16.6 sz	26.1 pxy	
	7	15.2 sz	27.2 rx	20.6 sy	15.9 sz	25.0 qx	
*b**	0	25.7 mz	27.1 mz	27.4 mz	29.5 my	27.1 mnz	
	1	21.6 nz	26.3 mny	26.6 my	26.1 ny	27.4 my	
	2	20.2 oz	26.0 mnx	24.7 nxy	23.6 oy	26.2 nx	
	3	19.4 pz	25.5 nw	23.9 ox	21.6 py	26.6 nw	0.28 to 1.07
	4	18.8 qz	24.6 ox	22.4 py	21.0 qy	26.2 nx	
	5	18.8 qz	24.1 ox	21.5 qy	20.0 ryz	25.3 ox	
	6	18.4 qz	23.2 pwx	21.3 qxy	19.4 syz	24.0 pw	
	7	18.0 qz	22.0 qxy	20.1 ryz	19.2 sz	23.8 px	
chroma[d]	0	42.1 mz	43.8 myz	43.4 myz	45.8 my	42.1 mz	
	1	34.3 nz	42.4 mnx	42.1 mx	39.6 ny	41.9 mx	
	2	30.1 oz	41.8 mnw	38.7 nx	34.1 oy	39.9 mnwx	
	3	28.1 pz	41.2 nw	36.9 ox	30.6 py	40.3 nw	0.82 to 2.53
	4	26.2 qz	39.9 ox	34.2 py	28.9 qz	39.4 nx	
	5	25.4 qz	38.6 px	32.3 qy	26.8 rz	37.8 ox	
	6	24.4 rz	37.0 qx	31.2 ry	25.6 sz	35.5 pxy	
	7	23.7 rz	35.0 rx	28.9 sy	25.0 syz	34.6 qx	

[a] m,n,o,p,q,r,s = means in a column within a trait with a different letter differ ($p < 0.05$). w,x,y,z = means in a row with a different letter differ ($p < 0.05$). [b] PM = *psoas major*, LL = *longissimus lumborum*, SSM = superficial *semimembranosus*, DSM = deep *semimembranosus*, ST = *semitendinosus*. [c] SE = standard error. [d] $(a^{*2} + b^{*2})^{1/2}$.

All muscles became darker (L^* decreased) during display ($p < 0.05$), except the ST. After day 0, the DSM and ST were lighter (greater L^*) than the other muscles ($p < 0.05$). The darkest muscles were the SSM and LL ($p < 0.05$), and the color of the PM was intermediate.

Each muscle experienced a loss of redness (decreased a^*) during display ($p < 0.05$). Compared with the SSM, PM, and DSM, LL exhibited a slow and gradual decline in a^* until day 3, and a^* of ST declined slowly until day 4; after day 4, discoloration increased for both. The PM and DSM had lower a^* values ($p < 0.05$) during display. The SSM was intermediate in a^* ($p < 0.05$) and underwent a more consistent, moderate discoloration after day 1. On day 0, DSM and LL were more red (greater a^*) than ST ($p < 0.05$), which was similar in redness to SSM and PM ($p > 0.05$). By day 1, the PM had the lowest a^* ($p < 0.05$), and the DSM had lower a^* values than the ST, SSM, and LL ($p < 0.05$). After day 2, the LL and ST had the greatest a^* values ($p < 0.05$), a^* of the SSM was intermediate, and the DSM and PM had the lowest a^* ($p < 0.05$).

As with a^*, all muscles decreased in b^* value throughout display ($p < 0.05$). The most rapid decrease ($p < 0.05$) was seen in PM and DSM, which stabilized after day 5 ($p > 0.05$). More gradual declines were seen for LL, ST, and SSM. After day 0, the PM had the lowest b^* ($p < 0.05$) until day 5, when it was similar to the DSM ($p > 0.05$). After day 2 of display, the ST and LL had the greatest b^* ($p < 0.05$) until day 6, when b^* of the SSM was similar to the b^* of both ST and LL ($p > 0.05$).

Chroma (vivid redness) decreased ($p < 0.05$) for all muscles during display. The chroma values of LL and ST were stable ($p > 0.05$) to days 2 and 3, respectively, with a consistent decrease ($p < 0.05$) each day thereafter. The PM rapidly decreased in chroma until day 4 ($p < 0.05$), and it continued a slow decline in chroma thereafter. Chroma values of both the SSM and DSM declined each day ($p < 0.05$), but the chroma value of the DSM did so more rapidly and to a greater extent. On days 1 and 2 of display, the LL, SSM, and ST had greater chroma values than the DSM and PM had ($p < 0.05$). From days 3−5, chroma values of the LL and ST were similar ($p > 0.05$), followed by the SSM ($p < 0.05$), which in turn had greater chroma values than the PM and DSM ($p < 0.05$). By day 7, the chroma value of the SSM had declined and was similar to that of DSM ($p > 0.05$).

Visual Color. Visual color and discoloration were correlated ($p < 0.0001$) with a^* and chroma ($r > -0.93$). Each muscle increased in visual-color score (lost redness) and became more discolored (increased surface MMb) during the 7-day display ($p < 0.05$; **Table 3**). The DSM and PM lost the most redness and discolored the most ($p < 0.05$), ST and LL were the most color stable visually ($p < 0.05$), and color stability of the SSM was intermediate.

On day 0, DSM had a lower visual score ($p < 0.05$) than SSM, PM, and LL did, indicating that it was brighter cherry red in color. After day 2, the DSM and PM were least red ($p < 0.05$), and the score of SSM was intermediate to the reddest muscles, LL and ST ($p < 0.05$). Visual discoloration was equal ($p > 0.05$) among all muscles on days 0 and 1. Beyond day 1, PM and DSM had more discoloration than LL, SSM, and ST did ($p < 0.05$). By day 5, the SSM was more discolored than ST ($p < 0.05$), which had greater discoloration than the LL ($p < 0.05$).

Metmyoglobin-Reducing Activity. All muscles decreased in metmyoglobin-reducing activity (MRA) each day ($p < 0.05$; **Figure 1**). The SSM, ST, and LL had greater MRA than did DSM and PM ($p < 0.05$) on day 0. By day 4, the LL and ST had more MRA than SSM had ($p < 0.05$); PM and DSM still had the least ($p < 0.05$). At the end of display, the LL had the most MRA ($p < 0.05$), and PM and DSM had less than ST ($p < 0.05$). The MRA of SSM was intermediate and similar to ST, PM, and DSM ($p > 0.05$). Visual color and discoloration ($r = -0.66$ and -0.70) and instrumental a^* and chroma measurements ($r = 0.68$) were highly correlated ($p < 0.0001$) with MRA.

Total Reducing Activity. Total reducing activity (TRA) data were not as conclusive as those for MRA (**Table 4**). Only LL decreased ($p < 0.05$) in TRA from day 0 to day 7, TRA of ST actually increased ($p < 0.05$), and the TRA of SSM, DSM, and PM did not change ($p > 0.05$). Neither day nor muscle showed a clear, consistent trend as being the greatest or least in TRA. Visual color, a^*, and chroma were not significantly correlated ($p > 0.41$) with TRA ($r < 0.14$ for all correlations).

Cytochrome *c* Oxidase Activity. No significant differences ($p = 0.46$) were detected among the muscles for cytochome *c* oxidase activity (PM = 1.22 units/mL mitochondria; LL = 1.31

Table 3. Visual Color and Discoloration of Five Bovine Muscles during 7 Days of Display[a]

trait	display day	muscles[b] PM	LL	SSM	DSM	ST	SE[c]
visual color[d]	0	1.9 rxy	2.1 pxy	2.2 rx	1.4 rz	1.8 qyz	0.19
	1	2.8 qy	2.2 pz	2.5 qyz	2.4 qz	2.3 pz	
	2	3.4 px	2.3 pz	2.7 py	2.7 py	2.4 opyz	
	3	3.7 ox	2.6 oz	3.1 oy	3.6 ox	2.6 oz	
	4	4.2 nx	3.0 nz	3.5 ny	4.1 nx	2.8 nz	
	5	4.5 mx	3.0 nz	3.6 ny	4.3 nx	2.9 nz	
	6	4.6 mx	3.3 mz	3.8 my	4.5 mx	3.5 myz	
	7	4.7 mx	3.4 mz	3.9 my	4.5 mx	3.5 mz	
discoloration[e]	0	1.0 sz	1.0 pz	1.0 rz	1.0 qz	1.0 pz	0.33
	1	1.4 rz	1.0 pz	1.0 rz	1.0 qz	1.0 pz	
	2	2.7 qy	1.0 pz	1.1 rz	2.7 py	1.1 pz	
	3	4.2 py	1.2 opz	1.7 qz	3.6 oy	1.7 oz	
	4	4.8 ox	1.6 noz	2.6 py	4.8 nx	2.0 oyz	
	5	5.7 nw	1.8 nz	3.3 ox	5.1 nw	2.5 ny	
	6	6.1 mw	2.4 mz	3.9 nx	5.8 mw	3.2 my	
	7	6.1 mw	2.7 mz	4.5 mx	5.9 mw	3.5 my	

[a] m,n,o,p,q,r,s = means in a column within a trait with a different letter differ ($p < 0.05$). w,x,y,z = means in a row with a different letter differ ($p < 0.05$). [b] PM = *psoas major*, LL = *longissimus lumborum*, SSM = superficial *semimembranosus*, DSM = deep *semimembranosus*, ST = *semitendinosus*. [c] SE = standard error. [d] 1 = very bright cherry red to 5 = tan to brown. [e] Percentage of surface metmyoglobin: 1 = no discoloration (0%) to 7 = total discoloration (100%).

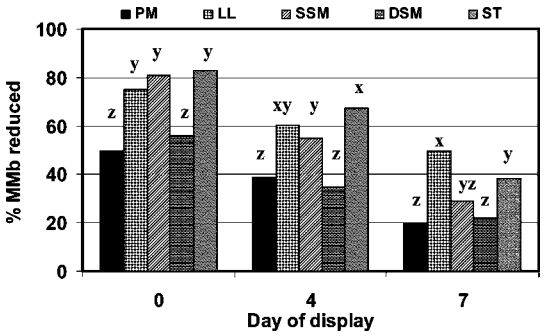

Figure 1. Metmyoglobin-reducing activity (MRA) of five bovine muscles during a 7 d display. PM = *psoas major*, LL = *longissimus lumborum*, SSM = superficial *semimembranosus*, DSM = deep *semimembranosus*, ST = *semitendinosus*. MRA, or % MMb reduced, was calculated as (Δ % surface MMb/preincubation % surface MMb) × 100. Means for a given muscle differ each day ($p < 0.05$). x,y,z = means within a display day with a different letter differ ($p < 0.05$). Standard error = 5.52.

Table 4. Total Reducing Activity (TRA) of Five Bovine Muscles during 7 Days of Display[a]

		muscles[b]					
trait	display day	PM	LL	SSM	DSM	ST	SE[c]
TRA	0	0.59 mxy	0.62 mx	0.54 my	0.57 mxy	0.45 nz	
	4	0.56 mx	0.49 nyz	0.47 nyz	0.52 mxy	0.45 nz	0.032
	7	0.56 mz	0.50 nz	0.53 mz	0.53 mz	0.51 mz	

[a] m,n = means in a column within a trait with a different letter differ ($p < 0.05$). x,y,z = means in a row with a different letter differ ($p < 0.05$). [b] PM = *psoas major*, LL = *longissimus lumborum*, SSM = superficial *semimembranosus*, DSM = deep *semimembranosus*, ST = *semitendinosus*. [c] SE = standard error.

units; SSM = 1.50 units; DSM = 0.87 units; ST = 1.08 units; standard error for the mean = 0.25).

DISCUSSION

Color Stability. Both pH and initial beef color indicate that the muscles in this study were absent of defects such as dark cutters or very pale meat. The ranking of muscle color stability was LL (most stable) > ST > SSM > DSM > PM (least stable), which agreed with previous research regarding muscle color-stability comparisons. The LL was more color stable than the PM (*6*, *27*), with color stability of the SM being intermediate (*28*). The SM was less color stable than the LL and ST, which had similar stability (*29*). O'Keeffe and Hood (*6*) found LL of acceptable color for 6.6 days and PM for 1.5 days, similar to the current research of 7 d for LL and 2 d for PM. Increased color stability of SSM, compared with DSM, has been noted (*19*, *20*, *22*).

Muscle differences can be attributed to differing muscle-fiber type and metabolic functions. Muscles with greater oxidative metabolic activity have greater OCR and are more color labile than those with glycolytic metabolic activity (*6*−*8*). Metabolic enzymes consume oxygen postmortem and reduce the oxygen partial pressure at the meat surface, thereby favoring formation of DMb (*10*), which is more susceptible to oxidation than is oxymyoglobin (*11*). Competition for oxygen is influenced by pH, temperature, and oxygen concentration (*30*), as well as by postmortem age (*31*). Less OCR causes deeper oxygen penetration (*6*, *32*), creating a MMb layer where low oxygen partial pressures exist deeper within the meat. The deeper the MMb layer forms, the longer until it appears on the meat surface (*6*, *33*). Therefore, depth of oxygen penetration is the limiting factor for MMb formation, rather than surface OCR in glycolytic muscles.

Of the muscles in this study, the ST has the greatest amount of α-white fibers, the SSM and DSM have predominantly α-white fibers, the LL has slightly more α-white than red fibers, and the PM is mostly *β*-red fibers (*21*). Consequently, the LL, ST, SSM, and DSM, with predominantly glycolytic muscle fibers, should have the greatest color stability, and the PM, with primarily oxidative fibers, should discolor the fastest. This study supports this conclusion except for the DSM, which discolored rapidly and was similar to the PM.

The DSM and SSM differ widely in color stability; the DSM chills more slowly and has a more rapid pH decline postmortem than the SSM does, resulting in denatured protein and reduced enzymatic activity and OCR (*19*, *20*). With less OCR, the muscle blooms brightly at the initiation of display as oxygen binds to myoglobin without mitochondrial competition. This was observed on day 0 in the current study. A lack of mitochondrial function, however, limits NADH production, a key component of MRA systems (*18*), and MRA decreases rapidly during display because no reducing equivalents are generated. Thus, it seems that although greater OCR is less conducive to color stability than lesser OCR, an intermediate OCR is needed to ensure that MRA occurs (*19*) and color stability is maintained.

Reducing-Activity Assays. Data for MRA corresponded well with visual and instrumental color. Muscles with greater color stability (LL, ST, and SSM) had more MRA than muscles that were less color stable (PM and DSM) throughout display. These results were expected because of the different color stabilities among muscles of differing oxidative potential, but research documenting muscle differences for the specific MRA assay used in this study is lacking. Most researchers have used different methodology to quantify MRA. Nevertheless, their findings for relative differences among muscles are similar to those in the present study.

Ledward (*27*) placed PM, LL, and SM after display into anaerobic environments and measured decreases in MMb by reflectance. The LL exhibited more MRA than the PM did, and MRA of the SM was intermediate. Sammel et al. (*19*, *20*) used the same MRA procedure as the current study and found that SSM had more MRA than DSM did. O'Keeffe and Hood (*6*) used ferricyanide to oxidize finely comminuted samples and used changes in reflectance to measure MMb changes to demonstrate that MRA decreased from LL to SM to PM and as time progressed. Reddy and Carpenter (*34*) used muscle extracts, ferrocyanide, and metmyoglobin in solution with NADH to show that the LL had more MRA than the SM did, which had marginally more MRA than the PM had. Madhavi and Carpenter (*33*) had similar results for intact and ground LL and PM, but grinding the muscles did not result in color stability differences because OCR increased. They concluded that OCR was the primary determinant of color stability during the first 7 days postmortem, whereas MRA was most important after 7 days.

Results for TRA were not consistent among muscles or display days. Because there were no clear trends for TRA, and because of its low correlation with visual and instrumental color, this method does not seem sufficient for relating reducing activity to muscle-color stability. This could be attributed to the TRA procedure's quantification of total reducing capacity rather than reducing capacity specifically associated with pigment stability. The method seemed to differentiate between reducing activity at different display times, but it did not differentiate between DSM and SSM (*19*, *20*). Similarly, Suman et al. (*35*) found no significant difference in TRA between ground beef made from *longissimus* and *psoas major* sources, whereas raw *psoas major* patties were more discolored during storage than *longissimus* patties. Reports of other comparisons of TRA to color stability for different muscles are lacking.

Overall, measures of MRA are reliable in determining muscle color-stability differences. This study's method to measure MRA shows good correlation with visual and instrumental color measurements, but changes in TRA during display do not account for as much variability in muscle color stability as changes in MRA do.

Cytochrome *c* Oxidase Activity. Differences in cytochome *c* oxidase activity among the muscles did not correspond to color differences exhibited during display. It is likely that the assay used in this procedure needs further refinement for this type of application. The amount of cytochome *c* oxidase activity should relate to the color stability because mitochondria are the principle source of postmortem oxygen consumption, which is related to myoglobin redox stability (*10*).

Of particular interest to meat-color researchers is the difference between the SSM and DSM muscles. Our results suggested

that the SSM had almost twice as much cytochrome *c* oxidase activity as the DSM had. Although a large difference in intramuscle cytochrome *c* oxidase activity might partly explain color differences between the SSM and DSM, we speculate that differing postmortem conditions between the two SM regions led to denaturation and deactivation of cytochrome *c* oxidase because the DSM would have had a slower postmortem temperature decline and an accelerated pH decline, resulting in greater protein and enzymatic denaturation (*19*, *20*, *22*). A procedure that can accurately quantify mitochondria within postmortem muscle, regardless of their activity, would be useful in studies designed to assess the relationships among muscle mitochondria content, enzyme activity, oxygen consumption, and color stability.

Compared with the SSM, the DSM had a brighter bloomed color at the beginning of display, but rapidly discolored with extended display. The DSM may have had a bright cherry-red color because enzymes within the mitochondria, particularly cytochrome *c* oxidase, were denatured by postmortem conditions, resulting in a decrease in oxygen consumption rate (*20*). But the decreased oxygen consumption rate did not lead to increased color stability (*8*, *9*) because the enzymes that mediate MRA likely also would have been denatured (*19*).

Beef muscles differ greatly in their color, color stability, and endogenous metmyoglobin-reducing activity. Measures of metmyoglobin-reducing activity are more useful than measures of total reducing activity in relating meat reducing activity to color differences. Muscle variations lead to differences in how muscles should be handled and merchandised at retail. Methods that optimize each muscle's time in retail display should be explored.

ABBREVIATIONS USED

PM, *psoas major*; LL, *longissimus lumborum*; SM, *semimembranosus*; SSM, superficial *semimembranosus*; DSM, deep *semimembranosus*; ST, *semitendinosus*; MRA, metmyoglobin-reducing activity; TRA, total reducing activity; OCR, oxygen consumption rate; MMb, metymyoglobin; USDA, United States Department of Agriculture; DMb, deoxymyoglobin; CCO, cytochrome *c* oxidase.

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