Comparison of Assays for Metmyoglobin Reducing Ability in Beef Inside and Outside Semimembranosus Muscle

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ABSTRACT: The relationships of 6 assays for metmyoglobin (Metmb) reducing ability to color stability and the chemical differences between the inside (ISM) and outside (OSM) beef semimembranosus (SM) muscle after 5 or 14 d storage were investigated. The ISM had less ($p < 0.05$) color stability than the OSM regardless of time post mortem, and both muscle portions were more color-stable when stored for 5 d rather than 14 d. Among the assays, aerobic reducing ability correlated best with visual color scores ($r = -0.58$) and Metmb accumulation ($r = -0.61$) in the SM. The ISM had less reducing ability than the OSM, which can be attributed partially to lower oxygen consumption rate and NAD concentrations ($p < 0.05$).

Keywords: color stability, semimembranosus, metmyoglobin, reducing ability

Introduction

UNDERSTANDING MUSCLE CHEMISTRY AND THE CHANGES THAT OCcur following slaughter is essential to maximizing meat color and color stability. In the living animal, myoglobin (Mb) must be in the reduced form to remain active in the transport and storage of oxygen. Many oxidants have the capability of oxidizing Mb, thus making it physiologically inactive. However, in living systems, practically no metmyoglobin (Metmb) is present because of an inherent Metmb-reducing system (Giddings 1977). Although chemical properties change when muscle is converted to meat, Mb can still be reduced. However, the relationship between metmyoglobin reducing ability (MRA) and meat color stability is unclear.

Logically, the more MRA a muscle retains, the better its color stability will be. Several different MRA assays are available, but they generate conflicting results. Early MRA research measured reducing ability by oxidizing meat with potassium ferricyanide then following the subsequent pigment reduction spectrophotometrically. O'Keefe and Hood (1982) and Renerre and Labas (1987) found differing MRAs among muscles but were unable to find a significant correlation between MRA and color stability. Potassium ferricyanide facilitates electron transfer and forms a complex with Mb when used to oxidize pigments; therefore, true Metmb reduction capacity may not be measured (Faustman and Cassens 1990). Ledward (1972) developed an aerobic reducing ability (ARA) method that involved the oxidation of meat in 1% oxygen then monitoring the subsequent pigment reduction. This method was correlated highly to color stability; the most color-stable muscles had the highest MRA. However, both methods have been criticized because of the induction of high amounts of Metmb on the surface of meat, which may not correspond with the natural formation of Metmb.

Other MRA assays were developed to measure the actual enzyme activity. Echevarne and others (1990) and Lanari and Cassens (1991) reported the highest MRA in least color-stable muscles. However, they used methylene blue as an indicator, which can be reduced by a number of nonspecific diaphorases capable of transferring hydrogen from NADH to the redox dye. Therefore these assays may not correctly represent muscle Metmb reducing capacity. Measurement of Metmb reductase without the use of artificial indicators shows a direct relationship between MRA and color stability, with the more color-stable muscles having the greatest reducing activity (Madhavi and Carpenter 1993; Reddy and Carpenter 1991). Numerous conflicting conclusions from different MRA assays have limited the understanding of reducing activity in meat.

The beef semimembranosus (SM) is a large, thick muscle that extends from the surface of the carcass to the femur. Because of its location, the outer portion (OSM) chills more rapidly than the inner portion (ISM), resulting in different temperature/pH conditions following slaughter. Tarrant (1977) reported pH values for the ISM below 6 while the carcass temperature was still above 30 °C. As a result, the ISM is less color-stable than the OSM, yet it is unknown how slow chilling of the ISM affects MRA. Therefore, the objective of this study was to compare MRA assays and determine their relationship to chemical characteristics and color stability of the ISM and OSM.

Materials & Methods

TWENTY VACUUM PACKAGED INSIDE ROUNDS (NAMP #168) WERE obtained from a commercial slaughter plant and were stored at 2 ± 2 °C until 5 (n = 10) or 14 (n = 10) d post mortem. Following storage, the adductor and gracilis were removed and the SM was cut into 6 steaks, each 2.54 cm thick. Each steak was packaged on a 4S Styrofoam tray containing a Dri-loc® meat pad (Sealed Air Corp., Saddle Brook, N.J., U.S.A.) and overwrapped with polyvinylchloride (23,250 cc O_2 /m^2/24 h °C/%RH). Steaks were placed in display at 0 ± 2 °C with continuous fluorescent lighting of 1612 lux (150 foot candles; Philips, 34 Watt, Ultralume 30) in open-top display cases (Unit Model DMF8; Tyler Refrigeration Corp., Niles, Mich., U.S.A.). Cases were programmed to defrost at 12-h intervals. The steaks from each SM were assigned randomly for analysis over 6 d (d 0 through d 5). Samples were removed from both the inside (the medial inner 1/3 closest to the femur) and outside (the lateral outer 1/3 closest to the surface of the carcass) portions of the steak. The middle 1/3 portion was not used in the ex-

periments. Only the lateral (top) 1.27 cm of each steak was analyzed for MRA. Metmyoglobin reducing ability was determined using 6 assays at each evaluation time. Concentrations of NAD, NADH and Mb, and pH, were determined on d 0, and oxygen consumption rates were determined on d 1. Instrumental and visual color was measured on steaks displayed for the full 6 d.

Instrumental and visual color evaluations

A MiniScan XE spectrophotometer (3.18-cm-diameter aperture and 10 degree standard observer; Hunter Associates Laboratory, Inc., Reston, Va., U.S.A.) was used to determine CIE L*a*b* values (CIE 1976) for Illuminant A and reflectance from 400 to 700. The spectrophotometer was calibrated using the white and black standard plates. Three readings on the OSM and 2 readings on the ISM (due to its smaller surface area) were taken through the packaging film, and averages were calculated. Hue angle [$\tan^{-1}(b^*/a^*)$, detects shifts from red to brown as values decrease]; saturation index [$(a^{*2} + b^{*2})^{1/2}$, greater color saturation as values increase]; a*/b* value (detects shifts from red to brown as values decrease); percent reflectance ratio, 630 nm/580 nm and reflectance difference, 630 nm–580 nm difference (both measurements indicate greater redness as values increase); and percentages of oxymyoglobin (Oxymb) and Metmb were calculated (AMSA 1991). Visual color was evaluated by a trained panel (n = 9) using a 5-point scale (1 = very bright cherry red or very bright pinkish red; 2 = bright cherry red or bright pinkish red; 3 = dull red to brown or dull pink to tan; 4 = moderately dark red to brown or moderately tan; 5 = dark red to brown or tan). Different descriptors were used for the ISM and OSM because 1 set of descriptors was not sufficient to describe the different portions. However, a specific number on each scale represented the same point of discoloration or acceptability of the ISM and OSM. A score of 3.5 or greater represented the point where panelists would discriminate against the color at retail. All panelists passed the Farnsworth Munsell 100-hue test (Macbeth, Newburgh, N.Y., U.S.A.) as recommended by AMSA (1991). This test requires no training, but does mandate normal color vision and the ability to place in correct order a series of colored samples varying in hue and intensity. All panelists were experienced color evaluators and they were oriented to this study during sessions in which SM steaks varying in color deterioration were evaluated to increase uniformity and accuracy of scoring.

Assays of metmyoglobin reducing ability

Total reducing activity: total reducing activity (TRA) was determined as described by Lee and others (1981). Briefly, a 2-g sample of muscle was homogenized in buffer and mixed with potassium ferricyanide for 1 h. Then, ammonium sulfamate and lead acetate were added. Distilled water and trichloroacetic acid were added and the solution was filtered using a 0.45-micron syringe filter. Absorbance of the sample and control (excluded muscle homogenate) was read at 420 nm. The calculation of activity was modified such that it was expressed as a unitless value of absorbance of control - absorbance of sample filtrate.

Aerobic reducing ability: aerobic reducing ability was determined as described by Ledward (1972) on a $3 \times 2 \times 1.27$ cm^3 sample. Metmyoglobin formation was induced in a 1% oxygen-99% nitrogen environment for 24 h and reflectance from 400-700 nm was measured at 24 h using a Hunter LabScan 2000 (1.27-cm-diameter aperture and 10 degree standard observer; Hunter Associates Laboratory, Inc., Reston, Va., U.S.A.). Subsequent reduction was determined after another 24 h in atmospheric oxygen. The MRA was calculated as: (observed decrease in Metmb concentration ÷ initial Metmb concentration) × 100.

Reduction of nitric oxide metmyoglobin: a modified procedure of Watts and others (1966) involving the oxidation of muscle with nitric oxide was used to determine MRA. Sample cubes ($3 \times 2 \times 1.27$ cm^3, rather than 50 g of ground product) were oxidized in 50 mL of 0.3% sodium nitrite at room temperature for 30 min. The samples then were blotted to remove excess solution, vacuum-packaged, and incubated at 30 °C. Reflectance from 400-700 nm was measured every 30 min for 2 h using a Hunter LabScan 2000 (1.27-cm-diameter aperture. The MRA was calculated as: (observed decrease in nitric oxide Metmb concentration ÷ initial nitric oxide Metmb concentration) × 100.

Reduction of dichlorophenolindophenol: a 5-g sample was homogenized in 20 mL of 0.2 mM sodium phosphate buffer (pH 5.6) and centrifuged at 25000 × *g* for 30 min at 4 °C. The supernatant was decanted and filtered through a 0.45-micron syringe filter. Reduction of dichlorophenolindophenol (DCPIP) was measured by a change in absorbance at 600 nm (Hultquist 1978). The DCPIP reagent consisted of 20 mL of 50 mM Tris-HCl buffer (pH 8.1), 1.4 mg of 2,6-DCPIP (Sigma D-2908) and 3.7 mg of disodium EDTA of which 1.2 mL was added to a 1.5-mL microcuvette. The reaction was initiated by simultaneously adding 100 μL of 1 mM NADH (Sigma N-8129) and 200 μL of muscle extract to the cuvette. As DCPIP was reduced by the muscle extract, absorbance decreased. Reducing activity was calculated from the linear phase of the assay using Beer's law with an extinction coefficient of 21,000 $M^{-1}cm^{-1}$ for DCPIP. The MRA was reported as nmoles reduced/min/g of muscle.

Reduction of horse and bovine metmyoglobins: Reddy and Carpenter (1991) reported species specificity of the reductase system. However, bovine Metmb is not commercially available as is horse Metmb. Therefore, assays were conducted with both horse and bovine Metmb to compare results and determine whether horse Metmb can be substituted for bovine Metmb while maintaining accuracy of reducing activity measurements. Horse Metmb was obtained from Sigma Chemical Co (M-0630; St. Louis, Mo., U.S.A.). Bovine Metmb was extracted from heart muscle by homogenizing 15 g of heart with 45 mL of buffer (10 mM Tris-HCl and 1 mM EDTA) and centrifuging at 10,000 × *g* for 10 min at 4 °C. The supernatant was filtered through Whatman No.1 filter paper and concentrated to about 0.1 mM in a Millipore Centricone Plus-80 (UFC5LGC02; Fisher Scientific, Atlanta, Ga., U.S.A.) by centrifugating at 500 × *g*. Two crystals of potassium ferricyanide were added to oxidize the Mb solutions. The MRA was measured using procedures described by Madhavi and Carpenter (1993). A 5-g muscle sample was homogenized in 20 mL of 0.2 mM sodium phosphate buffer (pH 5.6) and centrifuged at 25,000 × *g* for 30 min at 4 °C. The supernatant was decanted and filtered through a 0.45-micron syringe filter. The reaction mixture consisted of 100 μL of 5 mM EDTA, 100 μL of 50 mM citrate buffer, 100 μL of 3.0 mM potassium ferrocyanide and 200 μL of 0.75 mM horse Metmb and 200 μL of deionized water or 400 μL of 0.1 mM bovine Metmb. The reaction was initiated by simultaneously adding 100 μL of 1 mM NADH (Sigma N-8129) and 200 μL of muscle extract to a 1.5-mL microcuvette. As Metmb was reduced by the muscle extract, the absorbance at 580 nm increased. Reducing activity was calculated from the linear phase of the assay using Beer's law with an extinction coefficient of 12,000 $M^{-1}cm^{-1}$ for Oxymb. The MRA was reported as nmoles reduced/min/g of muscle.

Oxygen consumption rate

Muscle samples ($3 \times 3 \times 1.3$ cm^3) not previously exposed to air were removed, placed immediately in a 250-mL filtering flask with a side arm, and flushed with 100% nitrogen for 15 s. Then a mixture of 1% oxygen and 99% nitrogen was used to flush the flask for 10 s to achieve an atmosphere of < l% oxygen. A rubber septum was placed over the side arm and sealed with vacuum grease, and a rubber stopper was inserted in the top of the flask. A Mocon PacCheck™ (Model 650; Mocon Inc., Minneapolis, Minn., U.S.A.) oxygen analyzer (zirconium sensor accurate to ± 0.05% at concentrations used) was used to determine the concentration of oxygen in the flask. A sample of air from the flask was removed and analyzed for oxygen by puncturing the side arm septum with a needle attached to the automatic pump of the analyzer. The flask was placed in an incubator (35 °C) and measurements were taken over a 2-h period. Oxygen consumption was reported as nmoles consumed/cube of sample/2 h.

NAD and NADH

The NAD and NADH were extracted in acid and alkaline, respectively, as described by Klingenberg (1974), and their concentrations were assayed following procedures outlined by McCormick and Lemuel (1971). All reagent volumes were halved, and the reactions were carried out in 1.5-mL microcuvettes.

pH

The pH was determined using a Sentron Red-Line LanceFet probe (Sentron Europe BV, Roden, The Netherlands) connected to an Accumet portable AP61 pH meter (Fisher Scientific, Atlanta, Ga., U.S.A.) by a Sentron 701 ISFET pH adapter. The probe was inserted twice in both the ISM and OSM before MRA samples were removed, and the 2 readings were averaged for each portion.

Myoglobin concentration

A 5-g sample of pulverized muscle was homogenized in 15 mL of deionized water at 4 °C. The homogenate was centrifuged at 30000 × *g* for 30 min. The supernatant was filtered with a 0.45-micron syringe filter and the absorbance was measured at 525 nm. Calculations were made using a molar extinction coefficient of 7.6 (Bowen 1949) and a molecular weight of 16,110 Da for myoglobin (Drabkin 1978).

Statistical analysis

Data were analyzed as a completely randomized split plot design where the SM muscle was the whole plot and storage was the whole plot treatment. The 6 steaks from each SM were the split-plots and muscle location (ISM versus OSM) was the split-split plot. Proc Mixed procedures of SAS (2000) were used to determine treatment differences, and means were separated ($P < 0.05$) using least significant differences.

Results & Discussion

Characteristics of the inside and outside semimembranosus

The ISM had less ($p < 0.05$) NAD, a lower oxygen consumption rate (OCR) and a slightly higher pH than the OSM (Table 1). No differences in Mb concentration or NADH occurred between the ISM and OSM; however, a significant decrease in NADH occurred in the ISM and OSM from 5 to 14 d post mortem. Most research on the SM has not differentiated between the ISM and OSM; therefore, comparing these results with results of other studies is difficult. Hunt and Hedrick (1977) and Sammel and others (2002) measured some chemical characteristics on both portions of the SM and found the ISM was softer, and more exudative, had more denatured protein, and had a lower water holding capacity than the OSM. Furthermore, succinic dehydrogenase activity, lipid oxidation, Mb, heme iron, and nonheme iron concentrations, did not explain differences in color stability between the ISM and OSM (Sammel and others 2002).

Table 1—Mean chemical characteristics of the outside (OSM) and inside (ISM) semimembranosus

Trait	OSM	ISM	Standard Error
NAD, nmol/g	0.16[a]	0.10[b]	0.02
NADH, nmol/g	0.102	0.124	0.009
OCR[c], nmol/cube/2 h	1388[a]	1013[b]	134
pH	5.54[b]	5.59[a]	0.02
Myoglobin, mg/g	3.64	3.51	0.13
	5 d storage	14 d storage	
NADH, nmol/g	0.195[a]	0.031[b]	0.009

[a–b] Means within a row with a different superscript letter differ ($P < 0.05$).
[c] OCR = oxygen consumption rate of a cube ($3 \times 3 \times 1.3$cm^3) over 2h.

Color stability

Visual color of the ISM and OSM are presented in Figure 1. On d 0 of display, the ISM was a brighter ($p < 0.05$) cherry-red than the OSM (Figure 1a). No differences were found on d 1, and the OSM was redder (lower visual scores) than the ISM on d 2

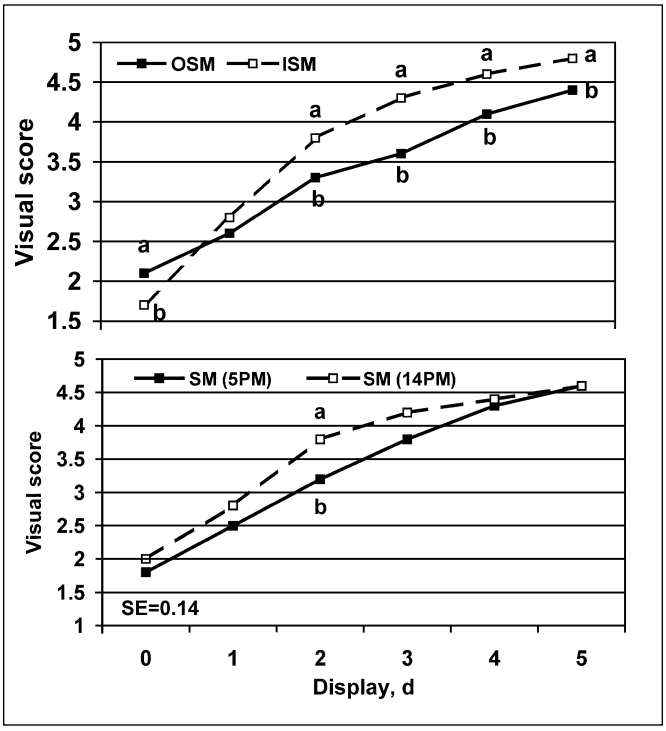

Figure 1—Visual color scores[c] for the semimembranosus (SM). a) Outside (OSM) and inside (ISM) SM. b) SM stored for 5 d (5PM) and 14 d (14PM) post mortem
[a–b] Values on the same display d with a different superscript letter differ ($p < 0.05$).
[c] Score of 1 = very bright cherry red or bright pinkish red; 2 = bright red or bright pinkish red; 3 = dull red to brown or dull pink to tan 4 = moderately dark red to brown or moderately tan; 5 = dark red to brown or tan.

Table 2—Mean[g] color traits for the outside (OSM) and inside (ISM)semimembranos

Trait	Muscle Portion	Display, d 0	1	2	3	4	5
L*	OSM	42.3^{az}	41.3^{az}	40.1^{bz}	40.2^{bz}	41.7^{az}	39.6^{bz}
	ISM	51.4^{ay}	49.0^{cy}	48.3^{cy}	48.1^{cy}	50.2^{by}	48.7^{cy}
a*	OSM	32.8^{az}	28.8^{by}	25.2^{cy}	22.5^{dy}	18.4^{ey}	17.5^{ey}
	ISM	35.9^{ay}	27.4^{bz}	21.7^{cz}	18.6^{dz}	15.7^{ez}	15.3^{ez}
b*	OSM	24.8^{az}	22.1^{bz}	19.9^{cz}	18.8^{dz}	15.7^{ez}	16.9^{fz}
	ISM	28.9^{ay}	23.9^{by}	21.2^{cy}	20.1^{cy}	18.1^{dy}	19.2^{cdy}
Oxymyoglobin[j]	OSM	94.4^{az}	82.9^{b}	69.3^{cy}	56.7^{dy}	29.9^{ey}	27.6^{ey}
%	ISM	106.7^{ay}	78.0^{b}	51.2^{cz}	34.9^{dz}	14.2^{ez}	13.6^{ez}
Metmyoglobin[j]	OSM	13.9^{fy}	27.4^{ez}	37.0^{dz}	46.5^{cz}	56.1^{bz}	64.0^{az}
%	ISM	9.6^{fz}	36.3^{ey}	56.4^{dy}	68.7^{cy}	74.6^{by}	79.6^{ay}
Saturation[h] index	OSM	41.4^{az}	36.2^{b}	32.2^{cy}	29.1^{d}	24.1^{e}	24.2^{e}
	ISM	46.3^{ay}	36.5^{b}	30.4^{cz}	27.7^{d}	24.2^{e}	24.7^{e}
Hue angle[i]	OSM	37.4^{cz}	37.2^{cz}	38.5^{bcz}	39.7^{bz}	40.7^{bz}	44.3^{az}
	ISM	39.0^{fy}	41.2^{ey}	44.5^{dy}	47.8^{cy}	49.6^{by}	51.8^{ay}
630nm/ 580nm	OSM	7.5^{ay}	5.3^{by}	4.2^{cy}	3.4^{dy}	2.5^{ey}	2.4^{ey}
	ISM	6.9^{az}	4.0^{bz}	2.6^{cz}	2.1^{dz}	1.7^{ez}	1.6^{ez}
630nm-580nm	OSM	27.8^{az}	21.4^{bz}	16.7^{c}	14.1^{dy}	11.3^{ey}	9.1^{f}
	ISM	40.5^{ay}	24.1^{by}	16.1^{c}	11.7^{dz}	9.1^{ez}	7.5^{e}

[a–f]Means within a row with a different superscript letter differ (P < 0.05).
[g]Standard errors: L* = 1.24; a* = 0.72; b* = 0.58; oxymyoglobin = 3.85; metmyoglobin = 2.26; saturation index = 0.79; hue angle = 0.69; 630 nm/580 nm = 0.18; 630 nm-580 nm = 1.03.
[h]Saturation index = $(a^2 + b^2)^{-1/2}$.
[i]Hue angle = $(b/a)^{tan-1}$.
[j]Estimates for percentage oxymyoglobin and metmyoglobin are accurate to only 6 to 7%, therefore their sum may be over 100% on a given day.
[y–z]Means within a trait Pearson correlation coefficients (r values)[a] for 6 assays for metmyoglobin (Metmb) reducing ability[b]

Table 3—Pearson correlation coefficients (r values)[a] for 6 assays for metmyoglobin (Metmb) reducing ability[b]

	TRA	ARA	NOMetmb	DCPIP	Horse
ARA	0.49				
NOMetmb	0.28	0.51			
DCPIP	0.59	0.49	0.45		
Horse	0.27	NS[c]	NS	0.23	
Bovine	–0.18	–0.27	–0.13	NS	NS

[a]r values presented (p < 0.05)
[b]TRA = total reducing activity; ARA = aerobic reducing ability; NOMetmb = nitric oxide. Metmb reduction; DCPIP = dichlorophenolindophenol reduction; Horse = horse Metmb reduction; Bovine = bovine Metmb reduction.
[c]Not significant

through 5, indicating a slower rate of discoloration. A score of 3.5 or greater represented panelist rejection based upon visual color. By d 2 of display, the ISM was determined visually unacceptable by panelists (color score = 3.8), whereas the OSM still had a score of 3.6 (borderline acceptable) on d 3 and was not severely discolored until d 4. Panelists also detected differences in discoloration due to storage post mortem. As expected, muscles stored for only 5 d had less discoloration (lower scores) on d 0 through 3 of display than muscles stored for 14 d (Figure 1b). On d 2 of display, SM stored for 5 d scored below 3.5, whereas SM stored for 14 d scored above 3.5 ($p < 0.05$).

On d 0 of display, the ISM had more Oxymb and less Metmb than the OSM (Table 2). Apparently, the lower OCR of the ISM allowed more oxygen penetration into the muscle, resulting in a greater bloom. Furthermore, MacDougall (1977) showed that slow chilling of the ISM denatured proteins, causing them to scatter more light and thus a paler appearance. However, after d 0 of display, the color of the ISM deteriorated rapidly. The ISM had more Metmb and less Oxymb than the OSM on d 2 through d 5 of display. By d 2, the ISM had 56% Metmb and appeared brown, whereas the OSM had only 37% Metmb and still retained red color. In addition, hue angles and percent reflectance 630 nm/ 580 nm ratios for the ISM and OSM were different ($p < 0.05$) over display, denoting the faster discoloration of the ISM.

Other researchers determined that different chilling rates significantly impacted the color traits of the ISM and OSM (Nichols and Cross 1980; Taylor and others 1980-81). Sammel and others (2002) found that the ISM was exposed 2 to 4 times longer to a pH 6.0 and a meat temperature of 25 °C than the OSM. Although the OSM retained acceptable color longer than the ISM, consumers discriminate against the whole SM once the ISM is discolored. Therefore, the SM has a display life of only 1 to 2 d unless the postmortem treatment of the muscle is modified.

Metmyoglobin reducing assays

Identifying an MRA assay that correlates highly to color stability was a prime objective of this study. Results from 6 assays are presented in Figure 2 to 7. The TRA assay is not specific for MRA or any other muscle-reducing chemistry, but it does indicate the muscle's overall reductive state. Few differences existed between the ISM and OSM at the same time post mortem for TRA (Figure 2). However, this assay did identify significant decreases in reducing capacity between SM displayed after 5 and 14 d of storage. Muscles stored for 5 d exhibited a higher TRA on d 0 of display, but it decreased significantly until d 4, whereas muscles stored for 14 d had a low initial TRA which decreased only slightly with display. These results, as expected, indicated that reducing ability of the SM was greater with shorter postmortem storage. This corresponds with visual scores, indicating lower color stability of muscles stored for 14 rather than 5 d, but not with visual differences between the OSM and ISM.

The ARA spectrophotometrically measures the muscle's ability to reduce Metmb; therefore, it is not surprising that this assay differentiated the ISM and OSM, which visually discolor at different rates. The OSM stored for 5 d had significantly higher reducing ability for 3 display d than the OSM stored for 14 d or the ISM

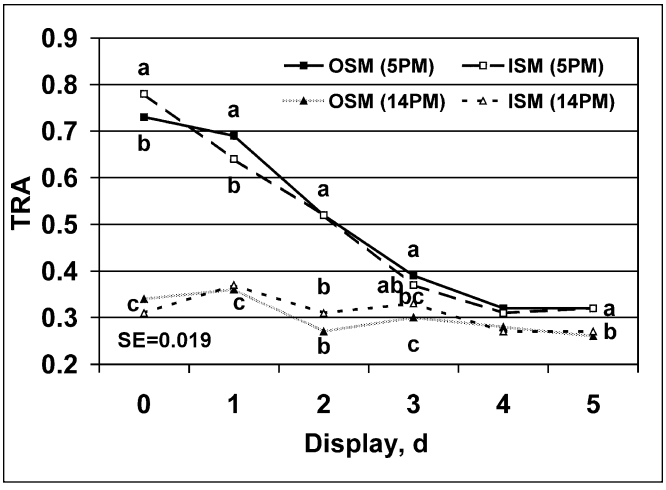

Figure 2—Total reducing activity of the outside (OSM) and inside (ISM) semimembranosus after postmortem storage of 5 d (5PM) and 14 d (14PM).
[a–d]Values on the same display d with different superscript letters differ (p < 0.05).

Table 4—Overall Pearson correlation coefficients (r values)[a] of assays for metmyoglobin (Metmb) reducing ability[b] and d 3 display correlations for aerobic reducing ability (ARA) and reduction of nitric oxide metmyoglobin (NOMetmb) and color traits

	TRA	DCPIP	Horse	Bovine
Visual	–0.58	–0.53	–0.13	0.48
L*	NS[c]	–0.18	0.20	NS
a*	0.57	0.52	0.14	–0.45
b*	0.55	0.39	0.31	–0.29
Oxymyoglobin, %	0.55	0.52	0.11	–0.45
Metmyoglobin, %	–0.54	–0.51	–0.09	0.48
a*/b* value	0.36	0.47	–0.13	–0.46
Hue angle	–0.36	–0.46	0.13	0.46
Saturation index	0.58	0.49	0.21	–0.41
630 nm/580 nm	0.58	0.53	0.18	–0.40
630 nm–580 nm	0.51	0.42	0.17	–0.44
	Overall		d 3 display	
	ARA	NOMetmb	ARA	NOMetmb
Visual	–0.58	–0.42	–0.46	–0.68
L*	–0.28	–0.47	0.51	–0.49
a*	0.56	0.40	0.43	0.63
b*	0.35	NS	0.41	NS
Oxymyoglobin, %	0.56	0.42	0.45	0.66
Metmyoglobin, %	–0.61	–0.49	–0.61	–0.70
a*/b* value	0.57	0.59	0.71	0.70
Hue angle	–0.55	–0.57	–0.68	0.70
Saturation index	0.50	0.31	NS	0.48
630 nm/580 nm	0.64	0.48	0.54	0.70
630 nm–580 nm	0.42	0.24	NS	0.41

[a]r values presented ($p < 0.05$).
[b]TRA = total reducing activity; DCPIP = dichlorophenolindophenol reduction; Horse = horse Metmb reduction; Bovine = bovine Metmb reduction.
[c]Not significant.

with either storage time (Figure 3). The ARA of the OSM stored for 5 d remained high for 1 d of display before decreasing to a level similar to that of the ISM at d 4 of display. Before display, the OSM stored for 5 d had more reducing ability than the OSM stored 14 d. Therefore, the OSM stored 5 d had a larger decline in ARA once displayed whereas the OSM stored 14 d had a smaller decline in ARA during display because it had already exhausted half of its reducing ability during storage. The OSM stored for 14 d had greater ARA than the ISM at either storage time, although the differences were significant only at d 1 and 3 of display. No differences ($p>0.05$) in ARA due to postmortem storage were detected in the ISM. The results for ARA correlated with color traits showing that the OSM was more color-stable than the ISM regardless of storage time.

Reduction of nitric oxide Metmb is another method for measuring MRA that Watts and others (1966) used as an alternative to oxidizing meat with potassium ferricyanide. They observed a slower and more linear reduction when nitric oxide was used as the oxidizing agent. However, they used this method only on ground beef and did not make comparisons among muscles. Our results for the reduction of nitric oxide Metmb are comparable to those for ARA. The MRA's of OSM stored for 5 and 14 d were equivalent until d 2 of display, and then the OSM stored for 14 d had significantly less activity (Figure 4). No MRA differences ($p>0.05$) were found between ISM stored for 5 and 14 d, and activities were lower ($p < 0.05$) than those of the OSM during display, except on d 5.

Reduction of DCPIP by Metmb reductase is a quick and easy assay to measure enzyme activity without having to isolate Mb. On day 0 and 1 of display, the OSM stored for 5 d had higher ($p < 0.05$) reductase activity than the ISM stored for 5 d; activities were equivalent on d 2 and 3, and then the OSM activity was significantly higher on d 4 and 5 of display (Figure 5). The OSM and ISM stored for 14 d exhibited no differences in reductase activity, which was lower than activity of muscle stored for 5 d.

Reductase activity measured with horse and bovine Metmb showed inconsistent results, which may have been due to methodology (Figure 6 and 7). The reductase reaction is influenced by the amount of Metmb and enzyme added to the reaction cuvette (Hagler and others 1979). As display time increased, Metmb concentrations in the muscles and muscle homogenates increased, thus, possibly changing the rate of reduction in the reaction cuvette. Metmyoglobin concentrations in ISM and OSM homogenates also varied, making it difficult to determine whether differences in MRA were due to differences in reducing capacity of the muscles or just differences in Metmb concentrations. Other researchers (Reddy and Carpenter 1991; Madhavi and Carpenter 1993) oxidized the muscle homogenate with potassium ferricya-

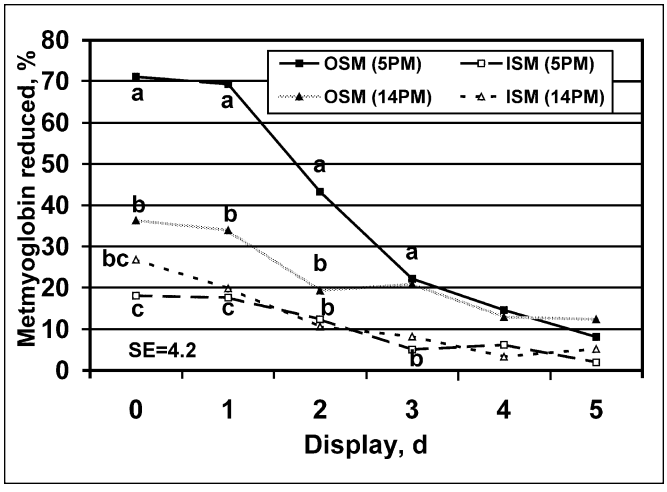

Figure 3—Aerobic reducing ability of the outside (OSM) and inside (ISM) semimembranosus after postmortem storage of 5 d (5PM) and 14 d (14PM).
[a–d]Values on the same display d with different superscript letters differ ($p < 0.05$).

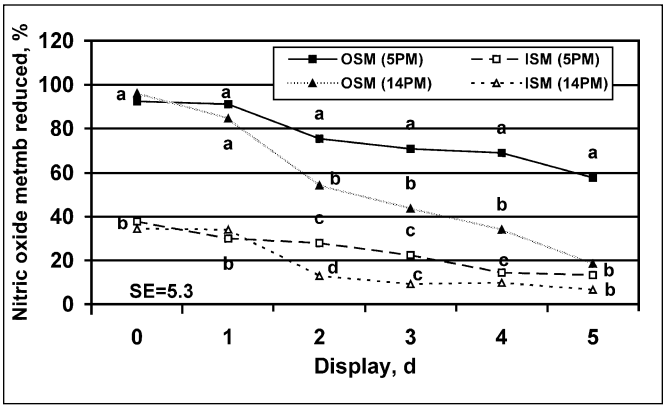

Figure 4—Reduction of nitric oxide metmyoglobin on the outside (OSM) and inside (ISM) semimembranosus after postmortem storage of 5 d (5PM) and 14 d (14PM).
[a–d]Values on the same display d with different superscript letters differ ($p < 0.05$).

nide before running the reaction, but that oxidation was omitted in this study. However, Madhavi and Carpenter (1993) were unable to show a decrease in MRA in the LD and PM over 7 d of display. Addition of NADH to the reaction cuvette could be responsible for the inconclusive results. It may be the limiting factor in the reduction reaction *in vivo*, because it is lost quickly post mortem (DeVore and Solberg 1975). However, the reaction will not run *in vitro* without the addition of NADH (Reddy and Carpenter 1991). Therefore, the reduction of horse or bovine Metmb *in vitro* may differentiate reducing capacity among muscles; but changes in MRA over display were not evident because the limiting factor was present in excess. From d 0 of display, before Metmb concentrations in the ISM and OSM differed greatly, the activities for the reduction of horse Metmb were: OSM stored for 5 d > OSM stored for 14 d > ISM stored for 14 d > ISM stored for 5 d. When bovine Metmb was reduced, the OSM with both storage times reduced more ($p < 0.05$) Metmb than the ISM with both storage times. Therefore, our results indicated the OSM had more MRA than the ISM, although we were unable to detect how MRA changed over display time.

Relationships among MRA, muscle chemistry, and color stability

Pearson correlation coefficients for MRA assays are given in Table 3. Total reducing activity and the reduction of DCPIP were the most highly correlated (r = .59), and ARA and reduction of nitric oxide Metmb had a slightly lower correlation (r = .51). Reduction of horse and bovine Metmb did not correlate well to the other 4 assays.

Overall Pearson correlation coefficients for MRA assays and color traits are given in Table 4 as well as correlation coefficients for ARA and reduction of nitric oxide Metmb with color traits on d 3. Among the MRA assays, TRA and ARA correlated best with visual color scores. The ARA also correlated best with %Metmb accumulation and the 630 nm/580 nm ratio. Furthermore, ARA had a high correlation with a*/b* value and hue angle on d 3 of display when the OSM was slightly discolored and the ISM was severely discolored. Although ARA has been criticized because of the induction of high amounts of Metmb on the surface of meat, it is measured on intact muscle and does not require the addition of any chemicals to measure activity. Thus, it is not likely that we are altering the inherent reducing system before or during measurement. Aerobic reducing activity is a relatively simple assay that can differentiate between muscles of different color stability and changes with time post mortem, while correlating well with visual color scores. However, the assay does not specifically measure the reducing enzyme, and it takes 48 h to get results.

Reduction of nitric oxide Metmb is another relatively easy assay that can be conducted in about 2 h. Although the assay's overall correlations with color traits were lower than those for ARA, it had a high correlation with many of the color traits on d 3 of display. Therefore, the reduction of nitric oxide Metmb may be the best assay for distinguishing MRA differences between muscles at different stages of discoloration. Reduction of DCPIP had a slightly lower correlation to visual color panels than ARA, yet the assay measures Metmb reductase activity without isolating Metmb and takes about 45 min. Correlations between MRA assays and color traits were not as high as expected, possibly because many other factors including Mb autoxidation and OCR influence discoloration. However, MRA assays that do not involve potassium ferricyanide or methylene blue show a direct relationship with color stability, indicating the importance of MRA in fresh meat (Madhavi and Carpenter 1993; Reddy and Carpenter 1991).

Differences in NAD and OCR between the ISM and OSM may be responsible for the differences in MRA and color stability. Oxygen consumption rate and ARA were correlated significantly on d 0 (r = 0.39) and d 3 (r = 0.50, data not shown) of display. Although the correlations were not high, they do indicate a definite relationship which has been observed by other researchers (Watts and others1966; Atkinson and Follett 1973; Ledward 1985; Arihara and others 1996). The significant differences in NAD concentration within the SM may be partially responsible for the differences in MRA, because Metmb reductase requires NADH as reducing equivalents (Arihara and other 1990). Although muscles with greater OCR have been characterized as having lower color stability (Lanari and Cassens 1991; O'Keefe and Hood 1982; Renerre and Labas 1987), the citric acid cycle utilizes NAD and generates NADH, which can be used in oxidative phosphorylation or the reduction of Metmb. Arihara and others (1996) inhibited Metmb reduction with the addition of glycolytic pathway in-

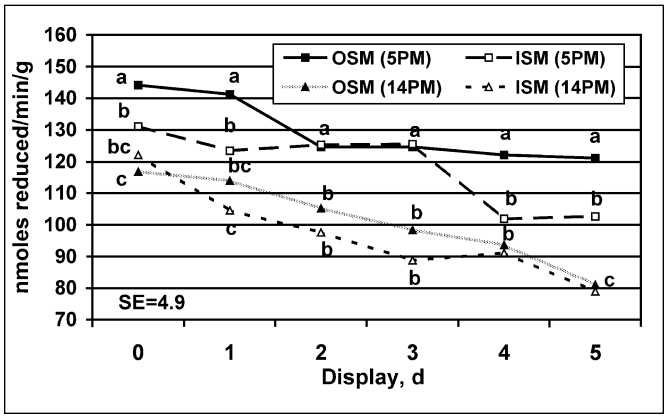

Figure 5—Reduction of dichlorophenolindophenol on the outside (OSM) and inside (ISM) semimembranosus after postmortem storage of 5 d (5PM) and 14 d (14PM).
[a–d] Values on the same display d with different superscript letters differ ($p < 0.05$).

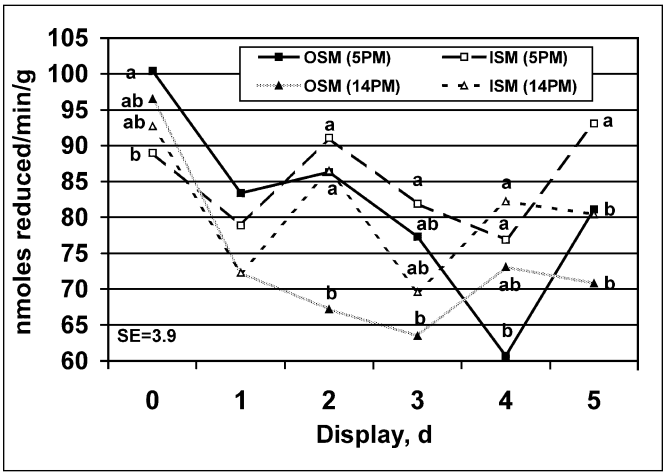

Figure 6—Reduction of horse metmyoglobin on the outside (OSM) and inside (ISM) semimembranosus after postmortem storage of 5 d (5PM) and 14 d (14PM).
[a–d] Values on the same display d with different superscript letters differ ($p < 0.05$).

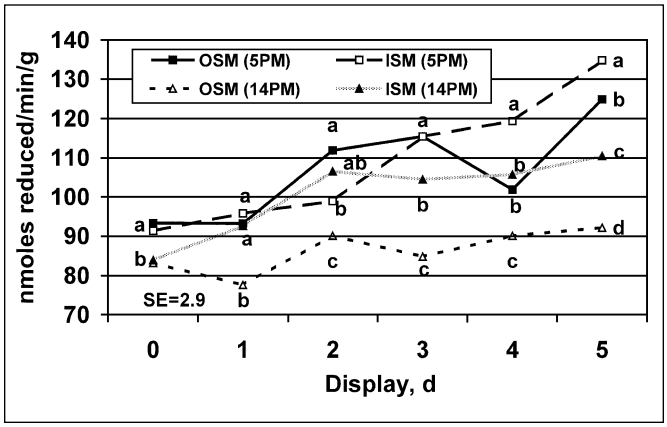

Figure 7—Reduction of bovine metmyoglobin on the outside (OSM) and inside (ISM) semimembranosus after postmortem storage of 5 d (5PM) and 14 d (14PM).
[a–d] Values on the same display d with different superscript letters differ (p < 0.05).

hibitors to cell suspensions. Thus, without some mitochondrial activity, NADH will not be generated. Furthermore, NADH was correlated to DCPIP reduction (d 0: r = 0.43; d 3: r = 0.67) and TRA (d 0: r = 0.85; d 3: r = 0.47, data not shown). However, high consumption of oxygen reduces the oxygen partial pressure at the surface of meat, resulting in a thin layer of Oxymb (O'Keefe and Hood 1982). Perhaps the significantly lower OCR of the ISM indicates less generation of NADH as display time increases, leading to less MRA, while the OSM still has the capability of generating NADH. Therefore, a very high or very low OCR may have a negative impact on color stability, whereas an intermediate OCR may positively affect color stability.

Conclusion

AEROBIC REDUCING ABILITY CORRELATED BEST WITH COLOR stability over display and appeared to be the best current method for measuring reducing ability. Reduction of nitric oxide Metmb correlated well with d 3 color traits when discoloration differences between the ISM and OSM were greatest. Direct measurement of the reductase enzyme did not correlate to color stability possibly because addition of NADH *in vitro* does not represent the actual Metmb reducing environment in muscle. The ISM had lower OCR and less NAD, MRA and color stability than the OSM, which may have been due to slow chilling rates. Both the ISM and OSM were more color-stable after storage of 5 d rather than 14 d. Early postmortem treatment of the ISM should be modified to enhance its chilling and slow its decline in pH (Sammel and others 2002) to improve its color stability and shelf life.

References

AMSA. 1991. Guidelines for meat color evaluation. Recip Meat Conf Proc 44:1-17.
Arihara K, Indo M, Itoh M, Kondo Y. 1990. Presence of cytochrome b_5-like hemoprotein (OM cytochrome b) in rat muscles as a metmyoglobin reducing system component. Jpn J Zootech Sci 61(9):837-842
Arihara K, Itoh M, Kondo Y. 1996. Contribution of the glycolytic pathway to enzymatic metmyoglobin reduction in myocytes. Int Biochem Mol Biol 38(2):325-331.
Atkinson JL, Follett MJ. 1973. Biochemical studies on the discoloration of fresh meat. J Food Technol 8(1):51-58.
Bowen WJ. 1949. The absorption spectra and extinction coefficients of myoglobin. J Biol Chem 179(2):235-245.
CIE. 1976. Recommendations on uniform color spaces-color difference equations, psychometric color terms. Supplement No. 2 to CIE publications No. 15 (E-1.3.1) 1978, 1971/(TC-1-3). Commission Internationale de Eclairage, Paris.
Devore DP, Solberg M. 1975. A study of the rate-limiting factors in the respiratory oxygen consumption of intact post-rigor bovine muscle. J Food Sci 40(3):651-652.
Drabkin DL. 1978. Selected landmarks in the history of porphyrins and their biologically functional derivatives. In Dolphin D, editor. The porphyrins, Vol 1. New York: Academic Press. p 29-83.
Echevarne C, Renerre M, Labas R. 1990. Metmyoglobin reductase activity in bovine muscles. Meat Sci 27(2):161-172.
Faustman C, Cassens RG. 1990. The biochemical basis for discoloration in fresh meat: A review. J Muscle Foods 1(3):217-243.
Giddings GG. 1977. The basis of color in muscle foods. J Food Sci 42(2):288-294.
Hagler L, Coppes RI Jr, Herman RH. 1979. Metmyoglobin reductase. J Biol Chem 254(14):6505-6514.
Hultquist, DE. 1978. Methemoglobin reduction system of erythrocytes. In: Fleischer S, Packer L, editors. Methods in Enzymology. Biomembranes LII. New York: Academic Press. p 465-466.
Hunt MC, Hedrick HB. 1977. Profile of fiber types and related properties of five bovine muscles. J Food Sci 42(2):513-517.
Klingenberg M. 1974. Nicotinamide-adenine dinucleotides (NAD, NADP, NADH, NADPH). Spectrophotometric and fluorimetric methods. In: Bergmeyer HU, editor. Methods of enzymatic analysis, vol 4. New York and London: Academic Press. p. 2045-2048.
Lanari MC, Cassens RG. 1991. Mitochondrial activity and beef muscle color stability. J Food Sci 56(6):1476-1479.
Ledward DA. 1972. Metmyoglobin reduction and formation in beef during aerobic storage at 1 °C. J Food Sci 37(4):634-635.
Ledward DA. 1985. Post-slaughter influences on the formation of metmyoglobin in beef muscles. Meat Sci 15(2):149-171.
Lee M, Cassens RG, Fennema OR. 1981. Effect of metal ions on residual nitrite. J Food Proc Preserv 5(4):191-205.
MacDougall DB. 1977. Colour of meat. In: Birch GC, Brennan JG, Parker KJ, editors. Sensory properties of foods. London: Applied Science Publishers, Ltd. p 59-69.
Madhavi DL, Carpenter CE. 1993. Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. J Food Sci 58(5):939-942.
McCormick DB, Lemuel DW. 1971. Nicotinic acid: Analogs and coenzymes. In: Colowick SP, Kaplan NO, editors. Methods in Enzymology. Vitamins and Coenzymes XVIII. New York: Academic Press. p 26-27.
Nichols JE. Cross HR. 1980. Effects of electrical stimulation and early postmortem muscle excision on pH decline, sarcomere length and color in beef muscles. J Food Prot 43(7):514-519.
O'Keefe M, Hood DE. 1982. Biochemical factors influencing metmyglobin formation on beef from muscles of differing colour stability. Meat Sci 7(3):209-228.
Reddy IM, Carpenter CE. 1991. Determination of metmyoglobin reductase activity in bovine skeletal muscles. J Food Sci 56(5):1161-1164.
Renerre M, Labas R. 1987. Biochemical factors influencing metmyoglobin formation in beef muscles. Meat Sci 19(2):151-165.
Sammel LM, Hunt MC, Kropf DH, Hachmeister KA, Kastner CL. 2002. Influence of chemical characteristics of beef inside and outside semimembranosus on color traits. J Food Sci (Forthcoming).
SAS. 2000. SAS user's guide: Basic statistical analysis. SAS Institute, Inc., Cary, NC.
Tarrant VP. 1977. The effect of hot-boning on glycolysis in beef muscle. J Sci Food Agric 28(10):927-930.
Taylor AA, Shaw BG, MacDougall DB. 1980-81. Hot deboning beef with and without electrical stimulation. Meat Sci 5(2):109-123.
Watts BM, Kendrick J, Zipser MW, Hutchins B, Saleh B. 1966. Enzymatic reducing pathways in meat. J Food Sci 31(1):855-861.
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