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Proteomics of Muscle-Specific Beef Color Stability

Poulson Joseph,[†] Surendranath P. Suman,*[,†] Gregg Rentfrow,[†] Shuting Li,[†] and Carol M. Beach[‡]

[†]Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky 40546, United States

[‡]Proteomics Core Facility, University of Kentucky, Lexington, Kentucky 40506, United States

ABSTRACT: The objective of the present study was to differentiate the sarcoplasmic proteome of color-stable (Longissimus lumborum; LL) and color-labile (Psoas major; PM) beef muscles. LL and PM muscles from seven beef carcasses (24 h post-mortem) were fabricated into 2.54 cm steaks, aerobically packaged, and assigned to refrigerated retail display for 9 days. LL steaks demonstrated greater ($P < 0.05$) color stability and lower ($P < 0.05$) lipid oxidation than PM steaks. Proteome analyses identified 16 differentially abundant proteins in LL and PM, including antioxidant proteins and chaperones. Proteins demonstrating positive correlation with redness (aldose reductase, creatine kinase, and β-enolase) and color stability (peroxiredoxin-2, peptide methionine sulfoxide reductase, and heat shock protein-27 kDa) were overabundant in LL, whereas the protein overabundant in PM (mitochondrial aconitase) exhibited negative correlation with redness. The color stability of LL could be attributed to the overabundance of antioxidant proteins and chaperones, and this finding suggests the necessity of developing muscle-specific processing strategies to improve beef color.

KEYWORDS: *beef color, color stability, Longissimus lumborum, Psoas major, sarcoplasmic proteome*

INTRODUCTION

Meat color is a major quality attribute that influences the purchasing decisions of consumers at the point of sale.[1] Consumers often consider the cherry-red color of fresh meat as a reliable indicator of wholesomeness. During retail display, fresh red meats undergo discoloration, resulting in lost value due to price reduction. It is estimated that the U.S. meat industry incurs an annual revenue loss of $1 billion due to discoloration-induced price discounts.[2]

Although various preharvest and postharvest factors contribute to meat color,[1,3] the inherent muscle biochemical profile is critical to beef color stability.[4−6] Beef muscles are categorized as color-stable or color-labile on the basis of color stability during retail display. Researchers have differentiated beef Longissimus lumborum (LL) and Psoas major (PM) muscles with respect to meat color attributes and biochemical characteristics.[4−7] LL exhibited greater color stability during retail display and demonstrated greater surface redness (a^* value) and metmyoglobin-reducing activity, and lower oxygen consumption rate and lipid oxidation, compared to PM.[4,5,7] Thus, LL (color-stable) and PM (color-labile) muscles offer themselves as valuable models to investigate meat color stability with reference to muscle biochemistry.

Beef muscles respond differently to modified atmosphere packaging (MAP) and cooking with regard to meat color attributes. Whereas surface redness was greater for LL than PM in high-oxygen MAP, the reverse was true in carbon monoxide MAP and vacuum packaging, indicating packaging-specific responses of beef muscles in color stability.[8] On the other hand, cooked meat color demonstrated a different trend in LL and PM;[9] PM steaks cooked to 71 °C had greater internal redness, indicating a lower susceptibility to premature browning compared to LL.

In the postgenomic era, proteomic tools such as two-dimensional gel electrophoresis coupled with mass spectrometry are utilized extensively to elucidate the biochemical mechanisms influencing muscle-to-meat conversion[10,11] and meat tenderness.[12−14] However, relatively few investigators have exploited proteomics to examine color biochemistry in post-mortem skeletal muscles.[10,15] Hwang et al.[15] observed that 12 proteins in porcine LL muscle, including α-actin, myosin light chain 1, cofilin 2, and troponin T, were negatively correlated to L^* value, whereas three proteins (adenylate kinase, troponin T, and ATP-dependent proteinase SP-22) were negatively correlated to drip loss. Proteome analyses for the molecular basis of color difference in porcine semi-membranosus muscle revealed that oxidative metabolic enzymes were overexpressed in animals with dark muscle, whereas glycolytic enzymes were abundant in animals with light muscle.[10] The aforementioned investigations were undertaken in pork, and limited information is available on proteome basis of fresh beef color.

Sarcoplasmic proteome, comprising soluble proteins including myoglobin and enzymes, constitutes 30% of total proteins in skeletal muscle and governs different biochemical processes influencing meat color stability. The interinfluential interactions between myoglobin and sarcoplasmic proteins are critical to meat color stability.[16,17] The sarcoplasmic proteome might be differentially abundant in color-labile and color-stable beef muscles. However, the differential abundance of sarcoplasmic proteomes in color-stable and color-labile beef muscles and its influence on beef color stability have not been characterized. Therefore, the objective of the present study was to differentiate the sarcoplasmic proteomes of color-stable (LL) and color-labile (PM) beef muscles and to correlate the differences in protein abundance with variations in color attributes and biochemical traits influencing meat color.

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■ MATERIALS AND METHODS

Beef Fabrication and Sample Collection. LL and PM muscles were harvested from seven ($n = 7$) beef (*Bos taurus*) carcasses (USDA Select, 24 h post-mortem). Each muscle was fabricated into 10 2.54-cm steaks, kept on styrofoam trays, overwrapped with polyvinyl chloride (PVC) film (15500−16275 $cm^3/m^2/24$ h oxygen transmission rate at 23 °C), and assigned randomly for retail display under constant, cool white fluorescent lighting (1300 lx) at 2 °C for 0, 5, and 9 days. One steak from each muscle, collected on day 0, was frozen at −80 °C for proteome analyses. For each storage time point, three steaks were utilized for the evaluation of color traits and various biochemical attributes.

Instrumental Color. On respective storage days, steaks were removed from the packages, and CIE L^*, a^*, b^*, hue, and chroma values were measured on light-exposed steak surfaces with a HunterLab LabScan XE colorimeter (Hunter Associates Laboratory, Reston, VA, USA) using a 2.54 cm diameter aperture, illuminant A, and 10° standard observer.[18] In addition, the ratio of reflectance at 630 nm and at 580 nm (R630/580) was determined to evaluate surface color stability; a greater ratio indicates a lesser amount of metmyoglobin and thus greater color stability. The instrument was calibrated with standard black and white plates every 8 h. The color attributes were measured at four random locations on each steak.

Oxygen Penetration Depth (OPD). OPD in the steaks was determined.[7] Briefly, thin meat slices was removed by cutting perpendicular to the steak surface, and OPD (mm) was measured using digital calipers. Six measurements were taken on each steak and averaged. Greater OPD indicates low oxygen consumption.

Metmyoglobin Reducing Activity (MRA). MRA was evaluated according to the method of Sammel et al.[19] Triplicate 2.54 cm cubes were removed from the light-exposed steak surfaces and submerged in 100 mL of 0.3% sodium nitrite solution for 20 min, to induce metmyoglobin formation. Samples were blotted dry, vacuum packaged, and immediately scanned from 700 to 400 nm, on the light-exposed surface with HunterLab LabScan XE colorimeter to obtain the reflectance data. The samples were incubated at 30 °C for 2 h to induce reduction of metmyoglobin and then rescanned. Surface metmyoglobin was calculated using K/S ratios and established formulas.[18] MRA was calculated as

$$\begin{aligned} \text{MRA} = 100 \times [&(\text{pre-incubation \% surface metmyoglobin} \\ &- \text{post-incubation \% surface metmyoglobin}) \\ &\div \text{pre-incubation \% surface metmyoglobin}] \end{aligned}$$

Total Reducing Activity (TRA). TRA was measured according to the method of Lee et al.[20] Briefly, 2 g of meat sample was homogenized (Polytron PT 10/35 GT, Kinematica, Luzernerstrasse, Switzerland) in 10 mL of 25 mM PIPES buffer, and 5 mL of the homogenate was transferred to a 10 mL volumetric flask. Two milliliters of 5 mM potassium ferricyanide was mixed with the homogenate and chilled at 2 °C for 1 h with occasional stirring. Later, 0.1 mL of ammonium sulfamate (0.5%) and 0.2 mL of lead acetate (0.5 M) were added, and the mixture was incubated at room temperature for 5 min. Further, 2.5 mL of 20% trichloroacetic acid (TCA) was added, and the solution was brought to volume (10 mL) with distilled water. The solution was filtered after 5 min, and the absorbance was measured at 420 nm (A_{420}) with a Shimadzu UV-2401 spectrophotometer (Shimadzu Inc., Columbia, MD, USA). A standard (1 mM potassium ferricyanide) was prepared, and TRA was expressed as the A_{420} of the standard minus A_{420} of sample filtrate.

Meat pH. The pH values of raw steak samples were determined according to the method of Strange et al.[21] Duplicate 5 g samples were homogenized with 30 mL of distilled deionized water, and the pH was measured using an Accumet AR25 pH-meter (Fisher Scientific, Pittsburgh, PA, USA).

Lipid Oxidation. Secondary products of lipid oxidation were measured using the thiobarbituric acid assay.[22] Briefly, 5 g samples were mixed with trichloroacetic acid (11%), homogenized, and filtered. One milliliters of aqueous filtrate was mixed with 1 mL of aqueous thiobarbituric acid (20 mM) and incubated at 25 °C for 20 h. The absorbance of the samples measured at 532 nm (A_{532}) using a Shimadzu UV-2401 spectrophotometer was reported as thiobarbituric acid reactive substances (TBARS).

Isolation of Sarcoplasmic Proteome. The sarcoplasmic proteomes from LL and PM muscles were extracted according to the method of Sayd et al.[10] with minor modifications. Frozen muscle samples were thawed at 4 °C and homogenized (Polytron PT 10/35 GT, Kinematica) in an ice-cold extraction buffer (40 mM Tris, 5 mM EDTA, pH 8.0) at the ratio of 1:4 (w/v). The homogenate was centrifuged at 10000*g* for 10 min at 4 °C. The supernatant (sarcoplasmic proteome) was filtered through a 0.45 μm membrane filter (Fisher Scientific) and utilized for subsequent analysis.

Two-Dimensional Electrophoresis (2-DE). The protein concentration of the sarcoplasmic proteome was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). The sarcoplasmic proteome (900 μg) was mixed with rehydration buffer (Bio-Rad) optimized to 9 M urea, 4% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte, and 0.001% Bromophenol blue, loaded onto immobilized pH gradient (IPG) strips (pH 5−8, 17 cm), and subjected to passive rehydration for 16 h. Further, the IPG strips was subjected to first-dimension isoelectric focusing (IEF) in a Protean IEF cell system (Bio-Rad) by applying a linear increase in voltage initially and a final rapid voltage ramping to attain a total of 80 kVh. After IEF, the IPG strips were equilibrated in SDS-containing buffers, first with equilibration buffer I (6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2% (w/v) DTT) followed by equilibration buffer II (6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2.5% (w/v) iodoacetamide), each for 15 min. The proteins were separated in the second dimension on 12% SDS-PAGE (38.5:1 ratio of acrylamide to bis(acrylamide)) using a Protean XL system (Bio-Rad). The gels were stained with Colloidal Coomassie Blue for 48 h and destained until sufficient background clearance was obtained. The LL and PM samples from one carcass were run under the same conditions, and three gels per muscle sample were produced.

Gel Image Analysis. The stained gels were analyzed using PDQUEST software (Bio-Rad) for image analysis. The spots in LL and PM were detected, and matched spots were normalized by expressing the relative quantity of each spot (in ppm) as the ratio of individual spot quantity to the total quantity of valid spots. For each spot in a given muscle sample, spot quantity values in triplicate gels were averaged for statistical analysis. A spot was considered to be significant in differential abundance when it was associated with $P < 0.05$ in a pairwise Student *t* test.[10]

Protein Identification by Tandem Mass Spectrometry. The spots identified were excised from the gel using pipet tips, destained by two 30-min washes with 50 mM NH_4HCO_3/50% CH_3CN followed by 10 min of vortexing, and dried in a vacuum centrifuge. Proteins in the gel were reduced by the addition of 50 mM NH_4HCO_3 containing 10 mM DTT and incubation at 57 °C for 30 min. After discarding the reducing liquid, proteins were alkylated by the addition of 50 mM NH_4HCO_3 containing 50 mM iodoacetamide and incubated for 30 min in the dark at room temperature. The gel was washed twice with 50 mM NH_4HCO_3 and once with CH_3CN, and partially dried in a vacuum centrifuge. Dried gel was rehydrated with a solution of 40 mM NH_4HCO_3/9% CH_3CN, containing proteomic grade trypsin (Sigma, St. Louis, MO, USA) at a concentration of 20 ng/μL, on ice for 1 h. Additional 40 mM NH_4HCO_3/9% CH_3CN was added to cover the sample, and the gel was incubated for 18 h at 37 °C. Peptides were extracted from the gel in 0.1% trifluoroacetic acid by sonication for 10 min followed by vortexing for 10 min. Extraction was repeated with 50% acetonitrile/0.1% trifluoroacetic acid. The extracts were combined, and the volume was reduced to eliminate most of the acetonitrile. Peptide extracts were desalted and concentrated by solid-phase extraction using a 0.1−10 μL pipet tip (Sarstedt, Newton, NC, USA) packed with 1 mm of Empore C-18 (3M, St. Paul, MN, USA), and peptides were eluted in 5 μL of 50% CH_3CN/0.1% trifluoroacetic acid.

Desalted peptide extracts (0.3 μL) were spotted onto an Opti-TOF 384 well insert (Applied Biosystems, Foster City, CA, USA) with 0.3

μL of 5 mg/mL α-cyano-4-hydroxycinnamic acid (Aldrich, St. Louis, MO, USA) in 50% CH_3CN/50% 0.1% trifluoroacetic acid. Crystallized samples were washed with cold 0.1% trifluoroacetic acid and were analyzed using a 4800 MALDI TOF-TOF Proteomics Analyzer (Applied Biosystems). An initial MALDI MS spectrum was acquired for each spot (400 laser shots per spectrum), and a maximum of 15 peaks with a signal-to-noise ratio of >20 were automatically selected for MS-MS analysis (1000 shots per spectrum) by postsource decay. Peak lists from the MS-MS spectra were submitted for database similarity search using Protein Pilot version 2.0 (Applied Biosystems), and the search was performed in the National Center for Biotechnology Information (NCBI) database to identify the proteins.

Statistical Analysis. The experimental design was a split-plot with randomized block design in the whole plot with seven replicates (n = 7). The muscles from each carcass served as blocks. The effects of muscle source (LL and PM) and storage period (0, 5, and 9 days) were analyzed using the PROC MIXED option in SAS,[23] and the differences among means were detected using the least significance difference (LSD) at a 5% level. The correlation of differential abundance of proteins with meat color attributes was evaluated with the PROC CORR option.[23]

RESULTS AND DISCUSSION

Instrumental Color and Biochemical Attributes. LL demonstrated greater (P < 0.05) surface redness (a* value) than PM on days 5 and 9 during retail display (Figure 1), reiterating that LL is a color-stable muscle, whereas PM is color-labile.[4−6] The L* value (lightness) and b* value (yellowness) were not different (P > 0.05) between LL and PM. However, the b* value decreased (P < 0.05) during retail display in both LL and PM (Table 1). R630/580 was greater (P < 0.05) in LL than in PM during retail display (Figure 2). A greater ratio (R630/580) indicates lower metmyoglobin (brown color) formation, and thus the results indicated greater color stability of LL than PM. LL exhibited greater (P < 0.05) chroma and lower (P < 0.05) hue than PM during retail display (Table 1). Our results for raw surface color attributes are in agreement with previous research.[4−7]

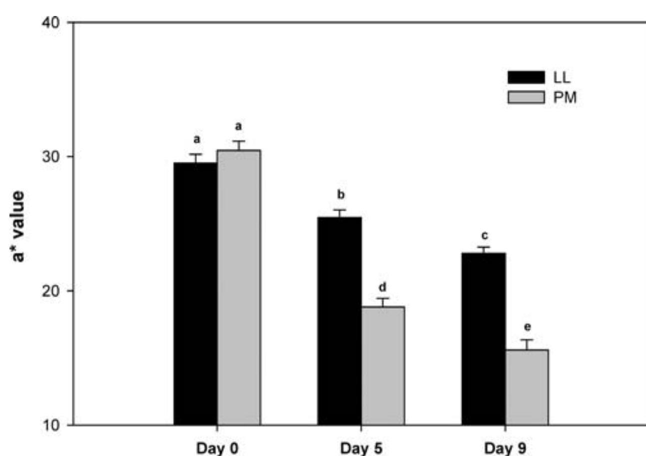

Figure 1. Surface redness (a* value) of beef Longissimus lumborum (LL) and Psoas major (PM) steaks during refrigerated retail display for 9 days under aerobic packaging. Standard error bars are indicated. Means without common letters are different (P < 0.05).

PM exhibited greater (P < 0.05) pH than LL during retail display. Nevertheless, pH increased (P < 0.05) in both muscles during retail display (Table 1). The greater pH of PM compared to LL has been previously documented.[5] LL exhibited greater (P < 0.05) MRA than PM throughout the retail display (Figure 3), and this observation correlated with the color stability of LL. MRA decreased during retail display in both LL and PM. However, the decrease in MRA was more pronounced in PM than in LL. This observation was in agreement with previous studies.[4,19] PM demonstrated greater (P < 0.05) lipid oxidation (TBARS) than LL on day 9 (Figure 4). Nevertheless, TBARS increased during retail display in both muscles. Similar results were reported in LL and PM by McKenna et al.[4] LL demonstrated lower (P < 0.05) OPD than PM during retail display, and OPD increased significantly (P < 0.05) during retail display in both muscles (Table 1). In contrast, previous research documented greater OPD in color-stable muscles than in color-labile muscles during 5 days of retail display.[4] The lack of agreement between our result on OPD and the previous paper[4] may be attributed to the longer retail display period (9 days) in the present study. Although PM exhibited greater (P < 0.05) TRA than LL during retail display, the trend was inconsistent with respect to meat color stability. TRA decreased (P < 0.05) in both LL and PM after day 5 (Table 1). Seyfert et al.[5] suggested that TRA is an inadequate estimate of the reducing power of meat and, therefore, cannot be interpreted accurately in relation with meat color stability.

The color attributes (a* value, chroma, hue, and R630/580) confirmed that LL is a color-stable muscle, whereas PM is color-labile, in support with previous investigations.[4−6] MRA correlated well with the color stability in beef muscles. Furthermore, LL exhibited greater MRA and lower surface discoloration than PM, indicating that MRA could be employed as a reliable indicator of color stability.[4,19] Lower lipid oxidation, measured as TBARS, in LL corresponded to its greater color stability, compared to PM. TBARS estimates the formations of secondary reactive lipid oxidation products, such as aldehydes, which form covalent adducts with myoglobin, accelerating heme oxidation and subsequent meat discoloration.[24,25] In the present study, TRA and pH did not exhibit correlation with color stability attributes of LL and PM steaks. In agreement, previous research also reported that these parameters may not always correlate with beef color stability.[4,5]

Sarcoplasmic Proteome Analysis. Colloidal Coomassie blue staining of 2-DE gels revealed the presence of 180 protein spots, similar to the previous reports on porcine[10] and bovine[26] skeletal muscle proteome. Statistical analyses indicated that 17 protein spots are differentially abundant (P < 0.05) in LL and PM, with spot intensity difference of 1.5-fold or more.[10] The protein spots with differential abundance are indicated in the gel image (Figure 5). Fourteen spots were overabundant in LL, whereas three were overabundant in PM. The differentially abundant protein spots were excised from the gel, subjected to in-gel tryptic digestion, and analyzed with tandem MS. The tandem mass spectra were utilized by Protein Pilot software for searching protein identity in NCBI database. The protein matching is expressed as ProtScore in Protein Pilot; a score of 2 indicates 99% confidence in peptide identification. The protein identity revealed that all proteins matched to the bovine family (*Bos taurus*/*Bos grunniens*) in the NCBI database. The proteins identified by tandem MS are presented in Table 2 with their NCBI accession number, species, molecular weight (MW), isoelectric pH (pI), and sequence coverage. The differentially abundant proteins exhibited a molecular mass range of 11−79 kDa. Similar results were reported in porcine sarcoplasmic proteome[10] and bovine muscle proteome[11,12] analyses. In general, the observed MW and pI for the protein spots in the

Table 1. Instrumental Color and Biochemical Attributes of Beef Longissimus lumborum (LL) and Psoas major (PM) Steaks during Refrigerated Retail Display for 9 Days under Aerobic Packaging[a]

attribute	muscle	day 0	day 5	day 9
L^*	LL	36.77 ± 0.62	38.00 ± 0.82	37.77 ± 0.85
	PM	40.63 ± 0.46	38.49 ± 0.55	39.57 ± 0.72
b^*	LL	21.84 ± 0.50 a	20.55 ± 0.42 a	18.61 ± 0.35 b
	PM	23.48 ± 0.46 a	17.69 ± 0.34 b	16.82 ± 0.41 b
chroma	LL	36.72 ± 0.82 ax	32.89 ± 0.69 bx	29.29 ± 0.57 cx
	PM	38.48 ± 0.81 ax	25.86 ± 0.67 by	22.84 ± 0.73 cy
hue	LL	36.52 ± 0.28 ax	38.70 ± 0.26 bx	39.48 ± 0.23 bx
	PM	37.69 ± 0.33 ax	43.58 ± 0.67 by	48.25 ± 1.24 cy
OPD[b] (mm)	LL	1.0 ± 0.02 ax	3.4 ± 0.27 bx	5.0 ± 0.21 cx
	PM	1.0 ± 0.02 ax	4.4 ± 0.30 by	6.2 ± 0.39 cy
TRA[c]	LL	0.39 ± 0.02 ax	0.42 ± 0.03 ax	0.33 ± 0.01 bx
	PM	0.44 ± 0.02 ay	0.45 ± 0.02 ay	0.38 ± 0.02b y
pH	LL	5.50 ± 0.04 ax	5.49 ± 0.03 ax	5.61 ± 0.04 bx
	PM	5.59 ± 0.02 ay	5.67 ± 0.03 by	5.71 ± 0.03 by

[a]Results are expressed as the mean ± standard error. Means without common letters (a−c) in a row are different ($P < 0.05$). Means without common letters (x, y) in a column within an attribute are different ($P < 0.05$). [b]OPD, oxygen penetration depth. [c]TRA, total reducing activity.

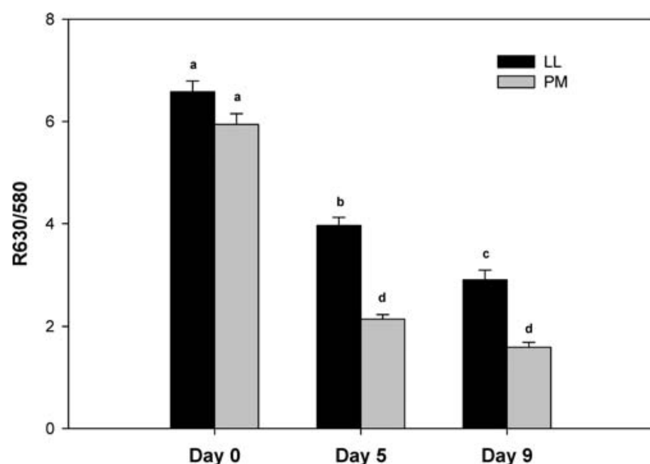

Figure 2. Surface color stability (R630/580) of beef Longissimus lumborum (LL) and Psoas major (PM) steaks during refrigerated retail display for 9 days under aerobic packaging. Standard error bars are indicated. Means without common letters are different ($P < 0.05$).

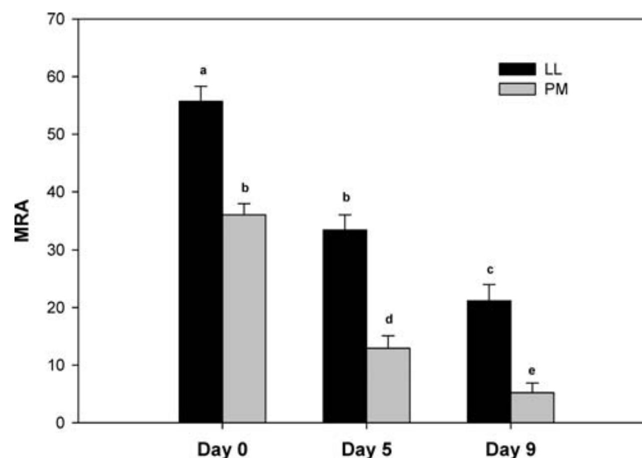

Figure 3. Metmyoglobin reducing activity (MRA) of beef Longissimus lumborum (LL) and Psoas major (PM) steaks during refrigerated retail display for 9 days under aerobic packaging. Standard error bars are indicated. Means without common letters are different ($P < 0.05$).

gel matched their theoretical values, suggesting that the identified protein spots were full-length proteins (Table 2). Few proteins demonstrated a shift from theoretical p*I*, which may be due to possible post-translational modifications.[12] In addition, two spots (10 and 11) were observed for the same protein (creatine kinase), which is not an uncommon phenomenon in 2-DE gels that could be attributed to protein fragmentation or post-translational modifications.[12,27]

The functional roles of the differentially abundant proteins and the muscle in which these proteins are overabundant are provided in Table 3. The identified proteins included antioxidant proteins, chaperones, binding proteins, and enzymes involved in energy metabolism. Overall, LL exhibited overabundance of 13 proteins, whereas 3 proteins were overabundant in PM. Noticeably, LL demonstrated over-abundance of antioxidant proteins, such as thioredoxin, peroxiredoxin-2, dihydropteridine reductase, and peptide methionine sulfoxide reductase.

Protein Function and Correlation with Meat Color Attributes. Statistical analyses revealed that nine proteins among the differentially abundant proteins demonstrated correlation ($P < 0.05$) with different color attributes in LL and PM muscles. The correlation between differentially abundant sarcoplasmic proteins and fresh meat color attributes in LL and PM is presented in Table 4. The proteins overabundant in LL (aldose reductase, creatine kinase, β-enolase, pyruvate dehydrogenase) demonstrated positive correlation (range of 0.64−0.72) with a^* value (redness). On the other hand, mitochondrial aconitase, overabundant in PM, exhibited a negative correlation (−0.59) with a^* value. Five

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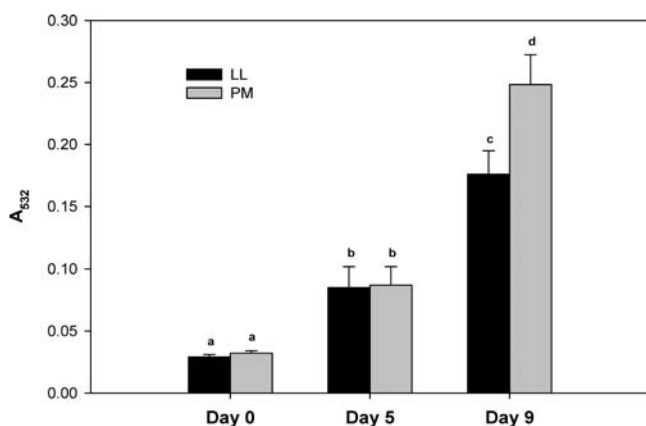

Figure 4. Lipid oxidation (TBARS) in beef Longissimus lumborum (LL) and Psoas major (PM) steaks during refrigerated retail display for 9 days under aerobic packaging. Standard error bars are indicated. Means without common letters are different ($P < 0.05$).

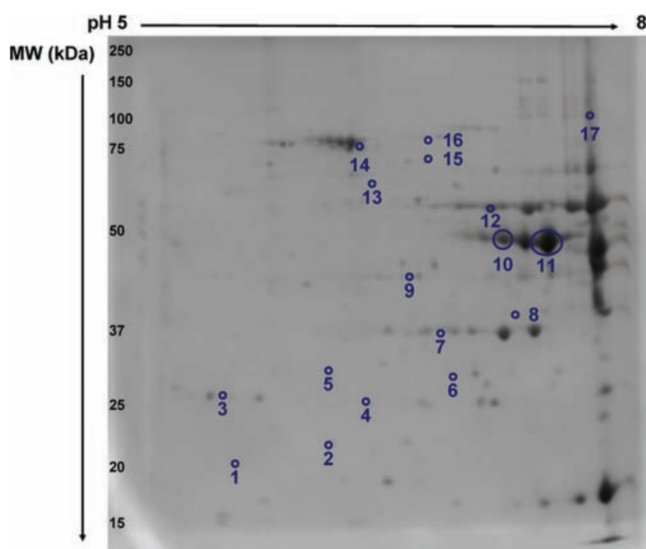

Figure 5. Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from beef Longissimus lumborum (LL) muscle. Seventeen protein spots, differentially abundant in LL and Psoas major (PM) muscles, are numbered.

proteins overabundant in LL (pyruvate dehydrogenase, peroxiredoxin-2, heat shock protein-27 kDa, peptide methionine sulfoxide reductase, stress-induced phosphoprotein-1) demonstrated positive correlations with color stability (R630/580). Furthermore, peptide methionine sulfoxide reductase (overabundant in LL) correlated positively with MRA, suggesting its possible role in meat color stability.

The 16 identified proteins can be grouped into four classes, namely, antioxidant proteins, chaperone proteins, binding proteins, and metabolic enzymes. Among the metabolic enzymes, peptide methionine sulfoxide reductase, dihydropteridine reductase, and aldose reductase could also be considered as proteins possessing antioxidant function. Previous reports suggested several proteins identified in the present study, including heat shock protein-27 kDa,[13] heat shock protein-70 kDa, protein DJ-1, and peroxiredoxin,[12] as potential biomarkers for beef tenderness. Nine proteins demonstrated correlation with meat color stability parameters (a^* value, R630/580, and MRA), with the majority of them (eight proteins) being overabundant in LL. In general, LL exhibited overabundance of antioxidant and chaperone proteins and metabolic enzymes. The significance of these proteins in meat color stability could be attributed to their functionality in cellular systems.

Antioxidant Proteins. Peroxiredoxin-2 functions in the redox regulation and protects the cells from oxidative stress. Other common names for this protein are thiol-specific antioxidant protein and thioredoxin-dependent peroxide reductase 1 (www.unitprot.org). Peroxiredoxin-2 belongs to a group of antioxidant enzymes known as peroxiredoxins,[28] which catalyze the inactivation of peroxides. During this process, the active site in peroxiredoxins (cysteine at position 51) undergoes oxidation and is regenerated by cellular thiols such as thioredoxin.[29] Therefore, the overabundance of thioredoxin in LL correlates with the abundance of peroxiredoxin-2 (Table 3). The role of peroxiredoxin and thioredoxin in eliminating peroxides could inhibit lipid oxidation, which will, in turn, minimize lipid oxidation-induced myoglobin oxidation,[25] leading to improved color stability in LL. Furthermore, peroxiredoxin-2 and peroxiredoxin-6 have been identified in post-mortem pork[15] and beef[12] longissimus muscles as candidate proteins for early tenderness prediction.

Peptide methionine sulfoxide reductase (PMSR) counteracts oxidative damage to methionine residues in proteins and free methionine, thus functioning against protein oxidation. Oxidation of methionine to methionine sulfoxide results in the loss of biological activity of a variety of proteins, which could be reverted by PMSR with a cysteine residue (at position 72) at its active site.[30] Protein oxidation is implicated in loss of meat quality attributes such as color,[25] tenderness,[31] and water-holding capacity.[32] Therefore, overabundance of PMSR could offer protection against oxidative damage in sarcoplasmic proteins related to color stability in LL and is evident by the positive correlation of PMSR with surface color stability (R630/580) and MRA in LL (Table 4). Furthermore, PMSR is considered to be an important antioxidant mechanism for scavenging reactive oxygen species through thioredoxin-dependent reduction of methionine sulfoxide,[33] a process that can inhibit lipid oxidation and subsequent myoglobin oxidation. In biological systems, PMSR received significant attention relative to aging in human beings,[34] and an increase in PMSR activity has been associated with an increase in human lifespan and low incidence of Alzheimer's disease.[33]

Metabolic Enzymes. The overabundance of glycolytic enzymes (β-enolase, triose phosphate isomerase) is indicative of a pronounced glycolytic mechanism in LL. This is in agreement with previous research,[27] which reported that LL muscle composition, with predominantly fast twitch type IIa fibers, is suggestive of predominantly glycolytic metabolism. Improved color stability was documented in muscles with glycolytic metabolism.[7,35] On the other hand, PM, a color-labile muscle, is predominantly oxidative in metabolism.[36] Metabolic intermediates such as lactate are utilized in injection enhancement solution to improve beef color stability.[8,17] The proposed mechanisms of action of lactate include regeneration of reducing equivalents (NADH), which favor metmyoglobin reduction and thus minimize meat discoloration.[17] The differential abundance of metabolic enzymes indicates that lactate could be metabolized in post-mortem beef muscles at differential rates and suggests the need to develop muscle-specific injection enhancement strategies utilizing metabolic intermediates. Furthermore, our results support the possibility

Table 2. Differentially Abundant Sarcoplasmic Proteins in Beef Longissimus lumborum (LL) and Psoas major (PM) Muscles Identified by Tandem Mass Spectrometry[a]

spot	protein	accession no.	species	ProtScore/matched peptides	sequence coverage (%)	MW_T/pI_T	MW_E/pI_E
1	thioredoxin	gil27806783	*Bos taurus*	9.84/6	74.3	11.81/4.97	11.80/5.00
2	phophohistidine phosphatase-14 kDa	gil115497372	*Bos taurus*	2.00/1	16.0	13.93/5.49	12.60/5.37
3	peroxiredoxin-2	gil27807469	*Bos taurus*	4.00/2	18.1	21.95/5.37	20.22/4.99
4	protein DJ-1	gil62751849	*Bos taurus*	6.81/4	41.3	20.03/6.84	19.60/5.99
5	heat shock protein-27 kDa	gil85542053	*Bos taurus*	10.53/7	54.2	22.39/5.98	22.40/5.98
6	peptide methionine sulfoxide reductase	gil27806537	*Bos taurus*	10.18/7	37.8	25.85/7.15	22.34/6.16
7	triose phosphate isomerase	gil61888856	*Bos taurus*	6.06/4	26.5	26.69/6.45	27.78/6.12
8	dihydropteridine reductase	gil115496448	*Bos taurus*	13.70/7	53.7	25.51/6.90	29.81/6.49
9	aldose reductase	gil60302887	*Bos taurus*	8.04/5	39.9	36.05/5.88	36.09/6.00
10	creatine kinase (M chain)	gil60097925	*Bos taurus*	18.49/10	42.8	42.99/6.63	44.10/6.42
11	creatine kinase (M chain)	gil60097925	*Bos taurus*	14.49/8	43.3	42.99/6.63	42.90/6.63
12	*β*-enolase	gil77736349	*Bos taurus*	13.44/7	36.4	47.09/7.60	51.47/6.41
13	pyruvate dehydrogenase	gil115496095	*Bos taurus*	6.26/4	17.6	53.89/8.64	56.87/5.99
14	heat shock protein 1 B-70 kDa	gil75077423	*Bos grunniens*	19.46/11	20.9	70.25/5.54	67.67/5.99
15	stress induced phosphoprotein-1	gil78369310	*Bos taurus*	15.07/9	24.7	62.48/6.08	63.47/6.04
16	serotransferin	gil2501351	*Bos taurus*	14.18/8	18.0	77.75/6.75	71.40/6.03
17	mitochondrial aconitase 2	gil90970312	*Bos taurus*	15.00/8	24.2	78.55/6.67	78.60/6.68

[a]Spot number refers to the numbered spots in gel image (Figure 5). For each spot, different parameters related to protein identification are provided (NCBI accession number; species; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry; theoretical protein mass (MW_T; kDa) and isoelectric pH (pI_T); experimental protein mass (MW_E; kDa) and isoelectric point (pI_E).

Table 3. Functional Roles of Differentially Abundant Sarcoplasmic Proteins in Beef Longissimus lumborum (LL) and Psoas major (PM) Muscles Identified by Tandem Mass Spectrometry[a]

spot	protein	functional category	overabundant in muscle	spot ratio	significance
1	thioredoxin	antioxidant	LL	3.31	$P < 0.05$
2	phosphohistidine phosphatase-14 kDa	enzyme	LL	1.60	$P < 0.01$
3	peroxiredoxin-2	antioxidant	LL	3.67	$P < 0.01$
4	protein DJ-1	chaperone	PM	1.52[b]	$P < 0.05$
5	heat shock protein-27 kDa	chaperone	LL	2.23	$P < 0.01$
6	peptide methionine sulfoxide reductase	enzyme	LL	4.21	$P < 0.01$
7	triose phosphate isomerase	enzyme	LL	1.95	$P < 0.01$
8	dihydropteridine reductase	antioxidant	LL	2.30	$P < 0.01$
9	aldose reductase	enzyme	LL	2.58	$P < 0.05$
10	creatine kinase (M chain)	enzyme	LL	2.25	$P < 0.01$
11	creatine kinase (M chain)	enzyme	LL	2.25	$P < 0.01$
12	*β*-enolase	enzyme	LL	2.01	$P < 0.05$
13	pyruvate dehydrogenase	enzyme	LL	1.56	$P < 0.01$
14	heat shock protein-1 B-70 kDa	chaperone	LL	2.80	$P < 0.01$
15	stress-induced phosphoprotein-1	chaperone	LL	2.24	$P < 0.05$
16	serotransferin	binding	PM	1.63[b]	$P < 0.01$
17	mitochondrial aconitase 2	enzyme	PM	2.05[b]	$P < 0.05$

[a]Spot number refers to the numbered spots in gel image (Figure 5). For each protein, functional category of the protein, muscle with greater abundance of the protein, and significance level are indicated. [b]Spot ratio of PM/LL; otherwise spot ratio of LL/PM.

of the interactions between mitochondria and sarcoplasmic proteome[37] governing meat color stability.

Chaperone Proteins. The chaperone proteins identified in the study include heat shock protein (HSP)-70 kDa, HSP-27 kDa, and stress-induced phosphoprotein 1. Among these, correlation with meat color attributes was observed for the latter two. In previous research,[10] HSP-27 kDa has been reported to be overabundant in dark porcine semimembranosus muscles. HSP-27 kDa protein plays a critical role in the stabilization of myofibrillar proteins by protecting actin filaments and cytoskeletal proteins from stress-induced fragmentation.[38] Furthermore, chaperone proteins prevent protein aggregation and protein denaturation,[10] two processes happening during the muscle-to-meat conversion that could compromise myoglobin stability and meat color. However, the specific role of chaperone proteins in meat color stability is yet to be completely understood.

Miscellaneous Proteins. Protein DJ-1, overabundant in PM in this study, exhibited a negative correlation with drip loss in pork[39] and a positive correlation with tenderness in beef.[12] Its direct implication in meat color stability is not completely understood. However, protein DJ-1 is associated with water-holding capacity[39] and thereby is indirectly related to the occurrence of pale, soft, and exudative condition in pork.[10] Mitochondrial aconitase, overabundant in PM, contains a [4Fe-4S]$^{+2}$ cluster in its active site that catalyzes reversible

Table 4. Correlation of Differentially Abundant Sarcoplasmic Proteins in Beef Longissimus lumborum (LL) and Psoas major (PM) Muscles with Color Attributes

protein	overabundant in muscle	trait	correlation coefficient
aldose reductase	LL	*a** value	+0.64
creatine kinase	LL	*a** value	+0.72
β-enolase	LL	*a** value	+0.64
pyruvate dehydrogenase	LL	*a** value	+0.65
mitochondrial aconitase 2	PM	*a** value	−0.59
pyruvate dehydrogenase	LL	R630/580	+0.67
peroxiredoxin-2	LL	R630/580	+0.92
heat shock protein-27 kDa	LL	R630/580	+0.87
peptide methionine sulfoxide reductase	LL	R630/580	+0.88
stress-induced phosphoprotein-1	LL	R630/580	+0.75
peptide methionine sulfoxide reductase	LL	MRA	+0.63

conversion of citrate to isocitrate in the TCA cycle. Mitochondrial aconitase activity has been reported to be a sensitive redox indicator of reactive oxygen species in cells. Furthermore, when reacted with superoxide, iron is released from the active site of mitochondrial aconitase, forming the inactive $[3Fe\text{-}4S]^{+1}$ aconitase and hydrogen peroxide, thus facilitating hydroxyl radical formation by the Fenton reaction.[40] The overabundance of mitochondrial aconitase in PM could therefore be correlated with an increased susceptibility to free radical-induced and iron-catalyzed lipid oxidation and subsequent pigment oxidation, resulting in poor meat color stability. The presence of mitochondrial aconitase in sarcoplasmic proteome could be attributed to its disassociation from mitochondria as a result of apoptosis.[14] In agreement, previous research documented the presence of mitochondrial enzymes and proteins in the soluble proteome of beef[11] and pork[10] muscles.

Mass spectrometry and 2-DE were utilized to interpret the muscle-specific biochemistry of meat color in color-stable (LL) and color-labile (PM) beef muscles. Proteins overabundant in LL were correlated positively with color attributes, such as *a** value, R630/580, and MRA. The overabundance of proteins functioning as antioxidants, to inhibit lipid and pigment oxidation or prevent protein aggregation, can be attributed to the greater color stability in LL than in PM. The results of the present study suggest the necessity to engineer muscle-specific antioxidant, injection enhancement, and packaging strategies to improve beef color stability.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1-(859)-257-3248. Fax: +1-(859)-257-7537. E-mail: spsuma2@uky.edu.

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Notes

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■ ABBREVIATIONS USED

LL, Longissimus lumborum; PM, Psoas major; MRA, metmyoglobin reducing activity; TRA, total reducing activity; OPD, oxygen penetration depth; 2-DE, two-dimensional gel electrophoresis; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; MALDI-TOF MS, matrix-assisted laser desorption ionization−time-of-flight mass spectrometry; MW, molecular weight; p*I*, isoelectric pH; PMSR, peptide methionine sulfoxide reductase; HSP, heat shock protein; TBARS, thiobarbituric acid reactive substances.

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