Proteome basis of muscle-specific beef color stability

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Abstract— **The objective of this study was to differentiate the sarcoplasmic proteomes of color-stable (Longissimus lumborum; LL) and color-labile (Psoas major; PM) beef muscles and to correlate the differences in protein abundance with color stability attributes. LL and PM muscles were harvested from seven beef carcasses (USDA Select grade, 24 h post-mortem), and samples for proteome analyses were collected and frozen at -80°C. Muscles were fabricated into 2.54-cm steaks; steaks were aerobically packaged and assigned to refrigerated retail display for 0, 5, and 9 days. On respective storage days, *a** value (redness) and ratio of reflectance at 630 nm to 580 nm (R630/580; indicator for surface color stability) were measured. LL exhibited greater (P < 0.05) *a** value and R630/580 than PM during retail display indicating greater color stability. Two-dimensional gel electrophoresis and tandem mass spectrometry identified sixteen differentially abundant proteins, which included antioxidant and chaperone proteins and enzymes involved in energy metabolism. Proteins exhibiting positive correlation with *a** value (aldose reductase, creatine kinase, and beta-enolase) and R630/580 (peroxiredoxin-2, dihydropteridine reductase, and heat shock protein-27 kDa) were over-expressed in LL than in PM. On the other hand, mitochondrial aconitase was more abundant in PM than in LL and correlated negatively to *a** value. The greater color stability of beef LL compared to PM could be attributed to the over-expression of antioxidant (peroxiredoxin-2, dihydropteridine reductase, aldose reductase) and chaperone (heat shock protein-27 kDa) proteins. Our result suggests the necessity to develop muscle-specific antioxidant and packaging strategies to improve beef color stability.**

Keywords— **Beef, Color stability, Sarcoplasmic proteome**

I. INTRODUCTION

Previous research documented that inherent muscle biochemical profile influences beef color stability, and beef muscles have been categorized based on color stability [1, 2, 3]. Longissimus lumborum (LL) is a color-stable beef muscle, while Psoas major (PM) is color-labile [2].

Sarcoplasmic proteome comprises of soluble proteins, including myoglobin and enzymes, and constitutes 30% of total proteins in skeletal muscle [4]. The biomolecular interactions between myoglobin and sarcoplasmic proteins influence meat color stability [5, 6, 7, 8, 9]. However, the differential abundance of sarcoplasmic proteomes in color-stable and color-labile beef muscles and its role in 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 meat color traits.

II. MATERIALS AND METHODS

A. Beef fabrication and instrumental color evaluation

LL and PM muscles from seven (n = 7) carcasses (USDA Select grade, 24 h post-mortem) were fabricated into 2.54-cm steaks. Steaks were placed individually on trays, over soaker pads, and were over-wrapped with oxygen-permeable polyvinyl chloride fresh meat film. Individually packaged steaks were assigned for retail display at 4°C for 0, 5, and 9 days. Samples for proteome analyses were collected on 0 d and frozen at -80°C. On respective storage days, *a** value (redness) and the ratio of reflectance at 630 nm to 580 nm (R630/580) were measured on the steak surfaces at four random locations 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 [10].

B. Sarcoplasmic proteome analyses

Sarcoplasmic proteomes were extracted [11], and protein samples (900 µg) were loaded on immobilized pH gradient (IPG) strips (pH 5-8, 17 cm) and passively rehydrated for 16 h. Proteins were separated in first dimension isoelectric focusing (IEF) utilizing a Protean IEF cell system (Bio-Rad, Hercules, CA, USA) and in second dimension using 12% SDS-PAGE. Three gels per muscle sample were produced, and the gels were stained with Colloidal Coomassie Blue. The gel images were analyzed using PDQUEST software (Bio-Rad, Hercules, CA, USA). Detected and matched spots were normalized by expressing the relative quantity of each spot as the ratio of individual spot quantity to the total quantity of valid spots. For each spot in a muscle, spot quantity values in triplicate gels were averaged and considered significant at $P < 0.05$ in pairwise Student t test [11]

C. Tandem mass spectrometry

The differentially abundant spots were excised and subjected to in-gel tryptic digestion. Peptide extracts were analyzed by tandem mass spectrometry (4800 MALDI TOF-TOF Proteomics Analyzer, Applied Biosystems, Foster City, CA, USA). Tandem mass spectra were analyzed using Protein Pilot (Applied Biosystems Foster City, CA) in the National Center for Biotechnology Information (NCBI) database to identify the proteins.

D. Statistical analysis

The experiment was a completely randomized design with seven replicates (n = 7). The data from retail display experiment were analyzed using PROC MIXED option [12], and the differences among means were detected using the least significance difference (LSD) at 5% level. The correlation of differential abundance of proteins with meat color attributes was evaluated with PROC CORR option [12].

III. RESULTS AND DISCUSSION

LL demonstrated greater ($P < 0.05$) surface redness (*a** value) than PM on days 5 and 9 of retail display (Fig. 1), whereas R630/580 was greater ($P < 0.05$) for LL than for PM throughout the retail display (Fig. 2).

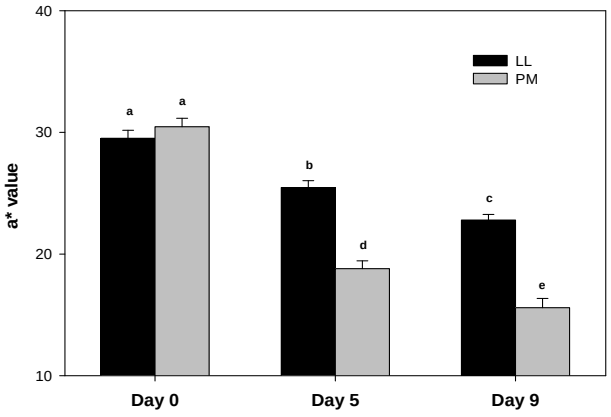

Fig. 1 Surface redness (*a** value) of aerobically packaged LL and PM steaks during retail display at 4°C.
a-e Means with different letters differ ($P < 0.05$).

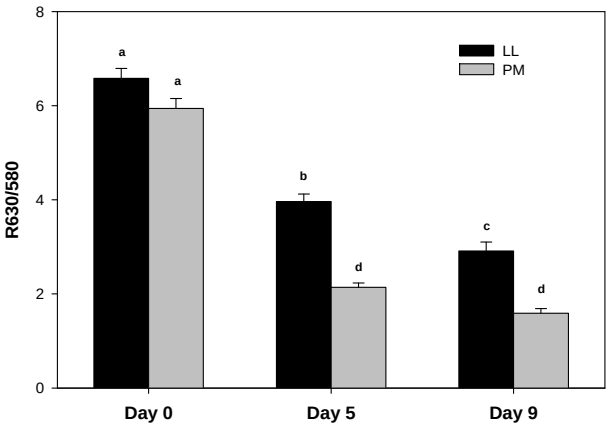

Fig. 2 Ratio of reflectance at 630 nm to 580 nm (R630/580) of aerobically packaged LL and PM steaks during retail display at 4°C.
a-d Means with different letters differ ($P < 0.05$).

Greater R630/580 in LL indicated lower surface metmyoglobin formation and greater color stability. These results were in agreement with previous reports [2, 3] and confirmed that LL is a color-stable muscle, while PM is color-labile. Nonetheless, *a** value and

R630/580 decreased ($P < 0.05$) progressively in LL and PM steaks during retail display (Fig. 1 and 2).

Proteome analysis revealed that thirteen proteins were over-abundant in LL, whereas three were over-abundant in PM (data not presented). The differentially abundant proteins were in a molecular mass range of 11 kDa to 79 kDa. Similar results were reported for porcine sarcoplasmic proteome [11] and bovine muscle proteome [13]. These differentially abundant proteins were subjected to in-gel tryptic digestion and tandem mass spectrometry to reveal the identity, and all proteins were matched to bovine family in NCBI database.

Table 1 Differentially abundant sarcoplasmic proteins in beef LL and PM muscles correlated to meat color traits

Protein	Muscle	Color trait	Correlation coefficient
Aldose reductase	LL	*a** value	+0.64
Creatine kinase	LL	*a** value	+0.72
Beta-enolase	LL	*a** value	+0.64
Mitochondrial aconitase	PM	*a** value	−0.59
Peroxiredoxin-2	LL	R630/580	+0.92
Heat shock protein-27 kDa	LL	R630/580	+0.87
Dihydropteridine reductase	LL	R630/580	+0.50

Proteins correlated ($P < 0.05$) to color attributes are presented in Table 1. The identified proteins included antioxidant proteins (peroxiredoxin-2, dihydropteridine reductase, aldose reductase), chaperone protein (heat shock protein-27 kDa), and enzymes involved in energy metabolism (creatine kinase, beta-enolase). Noticeably, LL demonstrated over-abundance of antioxidant proteins compared to PM (Table 1). The proteins over-abundant in LL (aldose reductase, creatine kinase, beta-enolase) demonstrated positive correlation (0.64 to 0.72) with *a** value (redness). In addition, peroxiredoxin-2, dihydropteridine reductase, and heat shock protein-27 kDa, which are over-abundant in LL, demonstrated positive correlation (0.50 to 0.92) with R630/580. Peroxiredoxin-2 inhibits the formation of peroxides and thereby retards lipid oxidation, which accelerates myoglobin oxidation and meat discoloration [14]. Chaperone proteins prevent protein aggregation and protein denaturation [11], two processes that can compromise myoglobin stability. On the other hand, mitochondrial aconitase was over-abundant in PM and exhibited a negative correlation (−0.59) with *a** value. Mitochondrial aconitase is a redox indicator of reactive oxygen species [15] and releases iron from its active site when reacted with superoxide. Greater abundance of aconitase in PM than in LL could be therefore correlated with increased free radical- and iron-catalyzed lipid oxidation, and subsequent pigment oxidation, resulting inferior meat color stability.

IV. CONCLUSIONS

Proteome approach was utilized to examine the muscle-specificity in beef color stability. The over-abundance of the proteins that inhibit lipid and pigment oxidation and prevent protein aggregation and denaturation can be attributed to the greater color stability in LL than in PM. The results of the present study suggest the need to engineer muscle-specific antioxidant, injection-enhancement, and packaging strategies to improve beef color stability.

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