PROTEOME BASIS OF ANIMAL EFFECT ON COLOR STABILITY OF BEEF *LONGISSIMUS LUMBORUM* STEAKS

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Abstract – Our objective was to characterize the correlation between the sarcoplasmic proteome and the color stability in beef *Longissimus lumborum* to explain animal-to-animal variation. *Longissimus lumborum* (36 h post-mortem) were obtained from 73 beef carcasses demonstrating similar marbling scores, aged for 13 days, and fabricated to 2.5 cm steaks. From each carcass, one steak was aerobically packaged and stored under refrigerated retail display. Instrumental color was evaluated on days 0 and 11. Another steak was immediately vacuum packaged and frozen at –80°C for proteome analysis. Steaks were ranked based on redness and color stability on day 11, and ten (n = 10) color-stable and color-labile steaks were selected. Sarcoplasmic proteome from the corresponding frozen steaks was analyzed using two-dimensional electrophoresis and tandem mass spectrometry. Twelve protein spots were differentially abundant in color-stable and color-labile steaks. Three glycolytic enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2) were over-abundant ($P < 0.05$) in color-stable steaks and positively correlated ($P < 0.05$) to a^* value (redness) and color stability. These results indicated that the variation in sarcoplasmic proteome profile between animals is associated with differences in the beef color stability.

Key Words – Color stability; Sarcoplasmic proteome; *Longissimus lumborum*

I. INTRODUCTION

One of the most important attributes for consumer acceptance of fresh beef is the color (1). Discoloration leads to more than $1 billion revenue loss per year to the meat industry in the United States (2). Myoglobin is the pigment responsible for meat color, and its redox stability is governed by endogenous and exogenous factors (3). Among the endogenous factors, muscle biochemistry has been extensively studied. *Longissimus lumborum* (LL) is a color-stable beef muscle exhibiting low oxygen consumption (4) and high metmyoglobin reducing activity (5, 6), which favor the formation of ferrous redox forms of myoglobin. Color stability of beef LL during retail display is affected by animal-to-animal variation (7, 8). However, the fundamental basis of this variation is not completely understood, and further studies are required to explain the underlying molecular mechanisms.

Proteomic tools were successfully used to elucidate the role of sarcoplasmic proteome in color stability of different beef muscles (9, 10). However, such an approach is yet to be undertaken to investigate the animal effect on beef color stability. Therefore, the objectives of the present study were to characterize the sarcoplasmic proteome of beef LL steaks demonstrating differential color stability during retail display and to correlate the differentially abundant sarcoplasmic proteins with color parameters.

II. MATERIALS AND METHODS

LL muscles were obtained from seventy-three beef carcasses (36 h post-mortem) exhibiting similar marbling scores. The muscles were wet-aged at 1°C for 13 days in vacuum packaging and fabricated into 2.5 cm thick steaks. One steak was placed in white polystyrene display tray with soaker pad, over-wrapped with oxygen-permeable polyvinylchloride film, and allotted to simulated retail display for 11 days. Another steak was immediately vacuum packaged and frozen at –80°C for proteome analysis.

The *a** value (redness) and the ratio of reflectance at 630 nm to 580 nm (R630/580) were measured at two random surface locations on each steak on days 0 and 11 using a HunterLab Miniscan XE Plus colorimeter (Hunter Associates Laboratory, Reston, VA, USA) with 2.54 cm diameter aperture, illuminant A, and 10° standard observer (11). On day 0, the steaks were bloomed for at least 2 h (after packaging) before color evaluation. The instrumental color data on day 11 were used to rank the steaks based on *a** value and R630/580. Two different groups were identified based on the ranking of these parameters; ten (n = 10) LL steaks were characterized as color-stable, and another ten (n = 10) considered as color-labile. The frozen counterparts of these color-stable and color-labile LL steaks (total twenty steaks) were used to investigate the molecular basis of animal-to-animal variation in color-stability.

Sarcoplasmic proteome was extracted (9) from the frozen samples corresponding to the color-stable and color-labile steaks. In the first dimension isoelectric focusing (IEF), the proteins were separated using immobilized pH gradient strips (pH 5–8, 17 cm) passively rehydrated with 1200 µg of the sarcoplasmic protein extracts for 16 h, and focused in a Protean IEF cell system (Bio-Rad, Hercules, CA, USA). For the second dimension, the proteins were separated utilizing 13.5% SDS–PAGE. Extracts were analyzed in duplicate, resulting in forty gels. The gels were stained with Colloidal Coomassie Blue. The gel images were digitalized using VersaDoc (Bio-Rad) and analyzed using PDQuest software (Bio-Rad). Detected spots were matched with the aid of landmarks. Matched spots were considered differentially abundant between the two groups when they exhibited 1.5-fold or more intensity difference associated with 5% statistical significance ($P < 0.05$) in the Student's t-test (9).

Differentially abundant spots were subjected to in-gel tryptic digestion, and the 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 2.0 (Applied Biosystems) in the UniProt and National Center for Biotechnology Information (NCBI) database to identify the proteins.

The data of instrumental color parameters (*a** values, and R630/580) on 0 and 11 days of retail display were analyzed using the PROC MIXED procedure with repeated measures (12). Differences between means were detected using least square difference (LSD) at 5% of significance level. To determine the Pearson's correlation between differentially abundant protein spots and instrumental color parameters, PROC CORR procedure was used (12).

III. RESULTS AND DISCUSSION

The color-labile LL steaks demonstrated greater ($P < 0.05$) *a** values (redness) than the color-stable ones (Table 1) on day 0, although they were numerically close. In addition, R630/580 values were similar ($P > 0.05$) for the two groups on day 0 (Table 1). After 11 days refrigerated display, both groups exhibited lower ($P < 0.05$) *a** value and R630/580 than the day 0 readings. Nevertheless, color-labile steaks demonstrated a greater ($P < 0.05$) decline in *a** and R630/580 than their color-stable counterparts. Furthermore, color-stable steaks demonstrated greater ($P < 0.05$) *a** value and R630/580 than the color-labile samples on day 11 (Table 1). This trend essentially separated the groups based on the color stability traits.

Table 1. Surface redness (*a** value) and color stability (R630/580) of color-stable and color-labile *Longissimus lumborum* steaks during refrigerated retail display.

		Retail display days	
Parameter	Category	0	11
*a**	Color-stable	34.65 ^{ay}	31.49 ^{bx}
	Color-labile	36.43 ^{ax}	12.22 ^{by}
R630/580	Color-stable	8.71 ^{ax}	6.97 ^{bx}
	Color-labile	9.45 ^{ax}	1.17 ^{by}

Means without common superscripts (a–b) in a row are different ($P < 0.05$).
Means without common superscripts (x–y) in a column within a parameter are different ($P < 0.05$).

Twelve differentially abundant protein spots were identified in color-stable and color-labile LL steaks. Nine spots over-abundant ($P < 0.05$) in color-stable samples were identified as proteins involved in glycolysis

(phosphoglucomutase-1 in 2 different spots; glyceraldehyde-3-phosphate dehydrogenase in 3 different spots; and pyruvate kinase M2), ATP metabolism (creatine kinase M-type), and skeletal muscle contraction (myosin regulatory light chain 2 and myosin light chain 1/3). In addition, three different spots over-abundant ($P < 0.05$) in color-labile samples were the proteins involved in ATP metabolism (adenylate kinase isoenzyme 1), signaling pathways (phosphatidylethanolamine-binding protein 1), and oxygen transport (myoglobin).

Two proteins (phosphoglucomutase-1 and glyceraldehyde-3-phosphate dehydrogenase) were identified in multiple spots with different isoelectric pH, possibly due to post-translational modifications such as phosphorylation, which was previously reported (13–15). The over-abundance ($P < 0.05$) of three different glycolytic enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2) in the color-stable group suggested possible variations in metabolism between LL steaks with differential color-stability. These enzymes can increase glycolytic metabolism through NAD+ and pyruvate generation, promoting NADH regeneration (16). NADH regeneration can in turn increase metmyoglobin reduction and improve color-stability. Analyses of correlation (Table 2) indicated that the aforementioned enzymes were positively correlated ($P < 0.05$) to a^* value (0.55–0.61) and R630/580 (0.54–0.65). In partial agreement, a previous study documented that two glycolytic enzymes (β-enolase and triose phosphate isomerase) were over-abundant in color-stable beef LL muscle than in color-labile *Psoas major*, and β-enolase was positively correlated to a^* value (9). In the present study, two protein spots related to muscle contraction (myosin regulatory light chain 2 and myosin light chain 1/3) were over-abundant ($P < 0.05$) in color-stable group and were positively correlated ($P < 0.05$) to a^* value (0.52–0.69). Furthermore, myosin regulatory light chain 2 was positively correlated ($P < 0.05$) to R630/580 (0.62). These myofibrillar proteins are fast-type suggesting a predominance of fast-twitch type IIb muscle fibers in the color-stable LL steaks, which can result in an increased glycolytic metabolism (17) and improved color-stability (4).

Phosphatidylethanolamine-binding protein 1 was over-abundant ($P < 0.05$) in color-labile LL steaks (Table 2) and demonstrated a negative correlation ($P < 0.05$) with a^* value (–0.58) and R630/580 (–0.59).

Table 2. Correlation between color parameters on day 11 of refrigerated retail display and differentially abundant sarcoplasmic proteins in color-stable and color-labile *Longissimus lumborum* steaks.

Protein	Over-abundant group	Color parameter	Correlation coefficient
PGM-1	Color-stable	a^* value	+ 0.57
GAPDH	Color-stable	a^* value	+ 0.61
PK M2	Color-stable	a^* value	+ 0.55
MRLC 2	Color-stable	a^* value	+ 0.69
MLC 1/3	Color-stable	a^* value	+ 0.52
PEB-1	Color-labile	a^* value	– 0.58
PGM-1	Color-stable	R630/580	+ 0.62
GAPDH	Color-stable	R630/580	+ 0.65
PK M2	Color-stable	R630/580	+ 0.54
MRLC 2	Color-stable	R630/580	+ 0.62
PEB-1	Color-labile	R630/580	– 0.59

PGM-1 = Phosphoglucomutase-1
GAPDH = Glyceraldehyde-3-phosphate dehydrogenase
PK M2 = Pyruvate kinase M2
MRLC 2 = Myosin regulatory light chain 2
MLC 1/3 = Myosin light chain 1/3
PEB-1 = Phosphatidylethanolamine-binding protein 1

IV. CONCLUSION

The results of the present study demonstrated that animal-to-animal variation influences sarcoplasmic proteome profile and potentially affects beef LL color stability during retail display. The over-abundance of glycolytic enzymes in color-stable LL steaks can possibly contribute to improved color stability through NADH regeneration in post-mortem muscles.

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