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Proteome basis for intramuscular variation in color stability of beef semimembranosus

Mahesh N. Nair [a], Surendranath P. Suman [a,*], Manish K. Chatli [a,b], Shuting Li [a], Poulson Joseph [a,c], Carol M. Beach [d], Gregg Rentfrow [a]

[a] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA
[b] Department of Livestock Products Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 241004, Punjab, India
[c] Kalsec, Inc., Kalamazoo, MI 49005, USA
[d] Proteomics Core Facility, University of Kentucky, Lexington, KY 40506, USA

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ABSTRACT

The objective of the present study was to characterize the proteome basis for intramuscular color stability variations in beef semimembranosus. Semimembranosus muscles from eight carcasses ($n = 8$) were fabricated into 2.54-cm thick color-labile inside (ISM) and color-stable outside (OSM) steaks. One steak for sarcoplasmic proteome analysis was immediately frozen, whereas other steaks were allotted to retail display under aerobic packaging. Color attributes were evaluated instrumentally and biochemically on 0, 2, and 4 days. Sarcoplasmic proteome was analyzed using two-dimensional electrophoresis and tandem mass spectrometry. ISM steaks demonstrated greater ($P < 0.01$) abundance of glycolytic enzymes (fructose-bisphosphate aldolase A, phosphoglycerate mutase 2, and beta-enolase) and phosphatidylethanolamine-binding protein 1 than their OSM counterparts. Possible rapid post-mortem glycolysis in ISM, insinuated by over-abundance of glycolytic enzymes, could lead to rapid pH decline during early post-mortem, which in turn could potentially compromise its color stability. These results indicated that differential abundance of sarcoplasmic proteome contributes to intramuscular variations in beef color stability.

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1. Introduction

Proteomic investigations play a major role in meat research because the ultimate objective of livestock rearing is the production of high quality protein for human consumption (D'Alessandro & Zolla, 2013; Gobert, Sayd, Gatellier, & Santé-Lhoutellier, 2014; Joseph, Nair, & Suman, 2015). Innovative techniques in proteomics have been extensively applied to explain the underlying molecular mechanisms of meat quality attributes such as color (Joseph, Suman, Rentfrow, Li, & Beach, 2012; Sayd et al., 2006; Suman, Faustman, Stamer, & Liebler, 2007) and tenderness (Anderson, Lonergan, & Huff-Lonergan, 2014; Jia et al., 2009; Picard et al., 2014). Color stability is critical to fresh beef retailing (Mancini & Hunt, 2005; Suman & Joseph, 2013; Suman, Hunt, Nair, & Rentfrow, 2014). Surface discoloration during retail display causes consumer rejection, leading to an annual revenue loss of more than $1 billion to the United States beef industry (Smith, Belk, Sofos, Tatum, & Williams, 2000).

Beef muscle profiling studies indicated that physico-chemical and biochemical characteristics of individual muscles in a carcass vary significantly (Von Seggern, Calkins, Johnson, Brickler, & Gwartney, 2005) and thus have a profound effect on color attributes (McKenna et al., 2005; Seyfert et al., 2006; King, Shackelford, & Wheeler, 2011). Furthermore, single-muscle cuts provide meat industry the opportunity to market individual muscles based on their physico-chemical characteristics as well as quality traits (McKenna et al., 2005; Von Seggern et al., 2005). Beef semimembranosus is a sizeable round muscle, which exhibits two-toned color and can be separated to color-labile inside (ISM) and color-stable outside (OSM) semimembranosus based on the location within the carcass (Sammel, Hunt, Kropf, Hachmeister et al., 2002). Variations in the rate of chilling have been attributed as one probable cause for the differential color stability of beef semimembranosus. Because of its internal location ISM chills at a slower rate than its OSM counterpart, and the greater post-mortem temperature in ISM can lead to accelerated glycolysis and a rapid pH decline (Sammel, Hunt, Kropf, Hachmeister, Johnson, 2002). Furthermore, Tarrant (1977) observed pH values below 6.0 in ISM when carcass temperature was above 30 °C. The differential rates of temperature fall and pH decline can affect metmyoglobin reducing systems resulting in differential color stability in beef semimembranosus; high temperature and low pH can compromise myoglobin stability and color stability in ISM (Sammel, Hunt, Kropf, Hachmeister, Johnson, 2002). Further research by Sammel, Hunt, Kropf, Hachmeister, et al. (2002) supported this explanation; these authors applied partial hot boning and observed

* Corresponding author.
E-mail address: spsuma2@uky.edu (S.P. Suman).

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that hot-boned ISM and OSM chilled at the same rate, demonstrated similar pH decline, and exhibited similar color stability attributes.

Sarcoplasmic proteome plays a critical role in fresh meat color stability (Renerre, Dumont, & Gatellier, 1996). Joseph et al. (2012) investigated the role of sarcoplasmic proteome on intermuscular variations in beef color stability and observed a greater abundance of antioxidant proteins and chaperones in color-stable (longissimus lumborum) than in color-labile (psoas major) beef muscles. Furthermore, antioxidant proteins and chaperones were positively correlated to surface redness, color stability, and metmyoglobin reducing activity (MRA). On the other hand, the proteome basis for the intramuscular variations in the color stability of beef semimembranosus is yet to be investigated. We hypothesize that the sarcoplasmic proteome profiles of beef ISM and OSM are different and contribute to the intramuscular variation in color stability. Therefore, our objective was to identify the differentially abundant proteins in the sarcoplasmic proteome of ISM and OSM, and to examine their relationship with intramuscular variation in color attributes.

2. Materials and methods

2.1. Beef fabrication

Beef inside rounds (USDA Select grade; 48 h post-mortem; IMPS # 168) from eight carcasses (n = 8) were obtained from a commercial packing plant. In order to effectively simulate the commercial beef retail marketing conditions in the United States, the carcasses utilized in this study were from cattle of unknown backgrounds. The vacuum-packaged cuts were transported on ice to the USDA-inspected meat laboratory at University of Kentucky. External fat and adjacent muscles were excised to separate the semimembranosus muscles. Each semimembranosus muscle (n = 8) was then fabricated into ten 2.54-cm ISM and OSM steaks. One steak assigned for proteome profiling and determining myoglobin concentration was vacuum packaged and frozen immediately at −80 °C until further analysis. The remaining nine steaks were individually placed on Styrofoam trays and aerobically overwrapped with oxygen-permeable polyvinyl chloride film (15,500–16,275 $cm^3/m^2/24$ h oxygen transmission rate at 23 °C). Individually packaged steaks were assigned randomly for 0, 2, and 4 days of refrigerated retail display (2 °C) under constant, cool white fluorescent lighting (1300 lx). For each time point, three steaks were utilized for evaluation of instrumental color and biochemical traits.

2.2. Instrumental color

CIE lightness (L^*), redness (a^*), yellowness (b^*), hue, and chroma values were measured at three random locations on the light-exposed steak surfaces with a HunterLab LabScan XE colorimeter (Hunter Associates Laboratory, Reston, VA, USA) using 2.54 cm diameter aperture, illuminant A, and 10° standard observer (AMSA, 2012). The instrument was calibrated with standard black and white plates. In addition, the ratio of reflectance at 630 nm and 580 nm (R630/580) was calculated as an indirect estimate of surface color stability.

2.3. Meat pH

The pH of steaks was determined according to the method of Strange, Benedict, Smith, and Swift (1977) with modifications. Duplicate 5-g samples were homogenized in 30 mL distilled deionized water, and the pH was measured using an Accumet AR25 pH-meter (Fisher Scientific, Pittsburgh, PA, USA).

2.4. Lipid oxidation

Lipid oxidation was analyzed employing thiobarbituric acid assay (Yin, Faustman, Riesen, & Williams, 1993). Briefly, 5 g representative samples, taken from multiple locations, were mixed with trichloroacetic acid, homogenized in a blender, and filtered using Whatman No. 1 filter paper. One mL of filtrate was mixed with 1 mL of aqueous thiobarbituric acid (20 mM) and incubated at 25 °C for 20 h. The absorbance of samples at 532 nm measured spectrophotometrically (UV-2401 spectrophotometer, Shimadzu Inc., Columbia, MD, USA) was reported as thiobarbituric acid reactive substances (TBARS).

2.5. Metmyoglobin reducing activity (MRA)

MRA was evaluated according to the method described by Sammel, Hunt, Kropf, Hachmeister, Johnson (2002). Cubes ($2.5 \times 2.5 \times 2.5$ cm^3) of meat were removed from the light-exposed surfaces and submerged in a solution of 0.3% sodium nitrite for 20 min at room temperature to induce metmyoglobin formation. Samples were blotted dry, vacuum packaged, and the reflectance spectra from 700 to 400 nm were recorded immediately on the light-exposed surface using a HunterLab LabScan XE colorimeter. The vacuum-packaged samples were then incubated at 30 °C for 2 h to induce reduction of metmyoglobin, and the reflectance data were taken again. Percentage of surface metmyoglobin (pre-incubation as well as post-incubation) was calculated based on K/S ratios and according to established formulas (AMSA, 2012). MRA was calculated using the following equation.

MRA = 100 × [(%pre-incubation surface metmyoglobin − %post-incubation surface metmyoglobin)/%pre-incubation surface metmyoglobin].

2.6. Myoglobin concentration

Myoglobin concentration was determined according to the method of Faustman and Phillips (2001). Duplicate 5 g frozen samples were homogenized in 45 mL ice cold 40 mM sodium phosphate buffer at pH 6.8. The homogenate was filtered using Whatman No. 1 filter paper, and the absorbance of the filtrate at 525 nm (A_{525}) was recorded using a UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA) with sodium phosphate buffer as blank. Myoglobin concentration was calculated using the following equation.

Myoglobin (mg/g) = $[A_{525}/(7.6\ mM^{-1}\ cm^{-1} \times 1\ cm)]$ x $[17{,}000/1000] \times 10$.

where, 7.6 mM^{-1} cm^{-1} = millimolar extinction coefficient of myoglobin at 525 nm; 1 cm = path length of cuvette; 17,000 Da = average molecular mass of myoglobin; 10 = dilution factor.

2.7. Isolation of sarcoplasmic proteome

The sarcoplasmic proteomes from ISM and OSM steaks (n = 8) collected on day 0 were extracted according to the method of Joseph et al. (2012). Frozen samples were thawed at 4 °C, and 5 g of muscle tissue was homogenized in a 25 mL ice-cold extraction buffer (40 mM Tris, 5 mM EDTA, pH 8.0) using a Waring blender (Waring Commercial, Torrington, CT, USA). The homogenate was centrifuged at 10,000 ×g for 10 min at 4 °C. The supernatant (sarcoplasmic proteome extract) was filtered and utilized for subsequent analysis.

2.8. Two-dimensional electrophoresis (2-DE)

The protein concentration of the sarcoplasmic proteome extract from each sample was determined in duplicate employing Bradford assay (Bradford, 1976) using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, USA). The sarcoplasmic proteome (900 μg) was mixed with a rehydration buffer (Bio-Rad) optimized to 7 M urea, 2 M thiourea, 20 mM DTT, 4% CHAPS, 0.5% Bio-Lyte 5/8 ampholyte, and 0.001% Bromophenol blue. The mixture of sarcoplasmic proteome and rehydration buffer was loaded onto immobilized pH gradient (IPG) strips (pH 5–8, 17 cm; Bio-Rad) and was subjected to passive rehydration for 16 h (Joseph et al., 2012). First dimension isoelectric focusing (IEF)

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