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Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in M. *semitendinosus* of Chinese *Luxi* yellow cattle

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ABSTRACT

Exploration of the potential predictors of meat discoloration is a challenging task, especially in *Luxi* yellow cattle that are highly appreciated breed in China. In the present study, the variations in meat color attributes and sarcoplasmic proteome of M. *semitendinosus* during post-mortem storage (0, 5, 10 and 15 days) were evaluated and analyzed via integrating proteomics and bioinformatics. The proteomics results revealed that meat discoloration gradually occurred during storage periods, while sarcoplasmic proteome experienced complex changes. Among the differential proteins identified by comparison between day 0 and days 5, 10 and 15, triosephosphate isomerase, L-lactate dehydrogenase A chain isoform, fructose-bisphosphate aldolase A isoform, peroxiredoxin-6, and pyruvate kinase isozymes M1/M2 isoform are highly related to meat color parameters, which may serve as the candidate predictors for monitoring of meat discoloration during post-mortem. Furthermore, Gene Ontology and protein–protein interaction analyses indicated that they are representative proteins of the glycolytic metabolism and the redox process during post-mortem storage, suggesting a potential relationship between post-mortem discoloration and these proteins. The present results provide the theoretic basis of searching novel strategies to maintain the stability of meat color.

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1. Introduction

As a superior protein source of human diet, beef has played an indispensable role in our daily life. According to the latest statistics, the worldwide consumption of beef has up to 9.6 kg/capita/year, followed by pork and poultry (FAOSTAT, 2014). *Luxi* yellow cattle, as one of the most important cattle breeds in China, enjoy an excellent global reputation due to high meat qualities and production (Liu et al., 2008).

To date, numerous researches on beef have focused on meat qualities, such as tenderness, color and water holding capacity (D'Alessandro, Marrocco, Zolla, D'Andrea, & Zolla, 2011; Wu, Fu, Therkildsen, Li, & Dai, 2015), among which meat color is one of the most critical attributes that can influence the purchase desire of consumers, who prefer the bright cherry-red color to the brown color of meat (Font & Guerrero, 2014). Myoglobin is an essential pigment in post-mortem muscles, which imposes a crucial influence on the meat color. There are three redox forms of myoglobin, namely deoxymyoglobin (DeoMb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb) that are responsible for the purplish-red, cherry-red and brown color, respectively. In addition, the synergy of intrinsic (breed, age, sex, the ultimate pH and metabolism, etc.) and extrinsic factors (temperature, O_2 availability and light, etc.) lead to the final color state of post-mortem skeletal muscles (Bekhit & Faustman, 2005). Thus, the reasons accounting for the meat discoloration are extremely intricate and remain to be explored.

Historically, studies concerning meat color have been emphasized on biochemistry and redox dynamics of myoglobin (Ramanathan, Mancini, Joseph, & Suman, 2013), on modified atmosphere packaging (Mancini, Suman, Konda, & Ramanathan, 2009) and on measurement method of color (Tapp, Yancey, & Apple, 2011). In recent decades, owing to the rapid development of high-throughput analysis tools in the post-genome era, proteomics has been applied for exploration of molecular mechanisms of meat quality, especially in meat tenderness (D'Alessandro, Marrocco, Rinalducci, et al., 2012; D'Alessandro & Zolla, 2013) and muscle-to-meat conversion (Jia et al., 2009; Lametsch, Roepstorff, & Bendixen, 2002). Nonetheless, few researchers have clarified the fundamental mechanism of meat discoloration at the proteome level. Furthermore, the available literature with regard to the reliable protein biomarkers related to meat color is still limited. Sayd et al.

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(2006) compared the difference in proteome between two groups of porcine M. *semimembranosus* that were categorized by high or low L^* values and found that the composition of sarcoplasmic proteome has a tight relationship with the color stability. Sarcoplasmic proteome is constituted by some soluble proteins including, myoglobin and enzymes, which can participate in different biochemistry processes that may exert impacts on the stability of meat color. Recently, Joseph, Suman, Rentfrow, Li, and Beach (2012) reported the difference in sarcoplasmic proteome between color-stable and color-labile bovine muscles and successfully identified sixteen differentially abundant proteins. However, the underlying reasons responsible for changes at level of sarcoplasmic proteome in the same muscle during post-mortem storage have not been fully elucidated. Therefore, the objective of this study is to explore the variations in sarcoplasmic proteome leading to the discoloration of M. *semitendinosus* of Chinese *Luxi* yellow during a period of storage (0, 5, 10 and 15 days). The correlation between the differentially expressed proteins and parameters of meat color was investigated. In addition, Gene Ontology (GO) and protein–protein interaction analyses were performed to support the significance of differentially abundant proteins as potential predictors of meat discoloration of Chinese *Luxi* yellow cattle.

2. Materials and methods

2.1. Preparation of samples

The muscle samples were collected from Chinese *Luxi* yellow cattle, which were slaughtered at the age of around 36 months and with the live weight of 400 ± 5 kg. In this experiment, M. *semitendinosus* muscles were collected from four (n = 4) (*Bos taurus*) carcasses at 24 h post-mortem. Each muscle was fabricated into 2.54 cm steak and put on the styrofoam trays, and wrapped with polyethylene film (18,500 ± 40% $cm^3/m^2/24$ h oxygen transmission rate). Then the sample trays were stored in a refrigerator at 0 °C and taken out to analyze after 0, 5, 10 and 15 days' storage, respectively. The steaks were utilized for the subsequent determination of biochemical indices of meat color attributes. The samples for proteome analysis were frozen in liquid nitrogen for further analysis.

2.2. Instrumental color determination

A CR-400 Minolta colorimeter (Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA) with illuminant D_{65} was applied to determine the CIE lightness (L^*), redness (a^*) and yellowness (b^*). Before the measurement, this instrument was calibrated with white plate. Sample color was evaluated randomly at five locations on the surface of beef samples (Dai et al., 2014).

2.3. pH value measurement

Meat samples (5 g) were homogenized with 50 mL deionized water for 30 s. Then the pH values of the homogenate were measured utilizing a FE-20 pH meter (Mettler Toledo, Zurich, Switzerland) (Strange, Benedict, Smith, & Swift, 1977). Each sample was determined in triplicates and for further statistical analysis.

2.4. Determination of myoglobin redox forms

The proportion of myoglobin redox forms was evaluated according to Tang, Faustman, and Hoagland (2004) with minor modification. Muscle samples (5 g) were homogenized in 25 mL phosphate buffer (40 mM, pH 6.8) for 25 s. The homogenate was centrifuged at 4 °C, 4500 *g* for 30 min. Prior to measuring the absorbance at 503, 525, 557 and 582 nm using a spectrometer (Evolution 60s, Thermo Scientific, USA), the supernatant was filtered and replenished with the same phosphate buffer to 25 mL. The percentage of DeoMb, OxyMb and MetMb was calculated as the following formulas:

$$[\mathrm{DeoMb}] = \frac{C_{DeoMb}}{C_{Mb}} = -0.543R_1 + 1.594R_2 + 0.552R_3 - 1.329$$

$$[\mathrm{OxyMb}] = \frac{C_{OxyMb}}{C_{Mb}} = 0.722R_1 - 1.432R_2 - 1.659R_3 + 2.599$$

$$[\mathrm{MetMb}] = \frac{C_{MetMb}}{C_{Mb}} = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520$$

Where $R_1 = \frac{A_{582}}{A_{525}}$, $R_2 = \frac{A_{557}}{A_{525}}$, $R_3 = \frac{A_{503}}{A_{525}}$.

2.5. Metmyoglobin reductase activity (MRA)

MRA of samples was spectrophotometrically determined as described by Mikkelsen, Juncher, and Skibsted (1999). 10 g of skeletal muscle was homogenized with 20 mL cold phosphate buffer (2 mM, pH 7.0) for 60 s at 4 °C. After centrifugation at 10,000 *g* for 20 min at 4 °C, a qualitative filter paper was applied to remove the fat from the supernatant. Excessive $K_3Fe(CN)_6$ was used to oxidize the remaining solution and the solution was further dialyzed (14,000 MW cut-off) against cold phosphate buffer for 24 h. Afterwards, the resulting solution was centrifuged (35,000 *g*) for 20 min at 4 °C and the supernatant was considered as metmyoglobin reductase. The absorbance changes of the reaction mixture were measured at 580 nm. MRA was evaluated as the reduction of nM MetMb per minute per g of meat.

2.6. Thiobarbituric acid reactive substance (TBARS)

Thiobarbituric acid assay was applied to determine the secondary products of lipid oxidation (Soyer, Özalp, Dalmış, & Bilgin, 2010). 1 g of sample was homogenized with 10 mL deionized water. Then 1 mL of the homogenate was mixed with 2 mL of stock solution of trichloroacetic acid/thiobarbituric acid (TCA/TBA) that contained 15% TCA and 0.375% TBA in 0.25 M HCl; 3 mL of 2% butylated hydroxytoluene (BHT) was prepared in absolute ethanol (w/v). After incubation in boiling water for 15 min, running water was immediately used to cool the mixture. Subsequently, the absorbances of upper layer of samples were measured at 532 nm using a spectrophotometer (Evolution 60s, Thermo Scientific, USA) after centrifugation at 3500 *g* for 10 min. The final value of TBARS was shown as mg of malondialdehyde (MDA) per kg of meat.

2.7. Analysis of proteomics

2.7.1. Sarcoplasmic proteome isolation

The sarcoplasmic proteome was extracted from M. *semitendinosus* according to the procedure of Sayd et al. (2006) with a minor revision. 2 g of frozen samples were homogenized in the cold extraction buffer (40 mM Tris, 2 mM EDTA, pH 8.0) for 60 s. The homogenate was centrifuged at 4 °C, 15,000 *g* for 20 min. Then the supernatant (sarcoplasmic proteome) was distributed and stored at −80 °C for the subsequent analysis.

2.7.2. Two-dimensional gel electrophoresis (2-DE)

After determining the concentration of sarcoplasmic proteome by Bradford assay (Bradford, 1976), 1 mg of samples was mixed with 4.5 μL ampholytes, 4.5 μg dithiothreitol (DTT) and the rehydration buffer was added until the volume up to 450 μL. Then the buffer was loaded on the immobilized pH gradient (IPG) straps (pH 3–10, 24 cm) for 60 min. Furthermore, the first dimension-Isoelectric Focusing (IEF) of IPG strips was performed on Protean IEF Cell (Bio-Rad). Low voltage (30 V) was conducted in the initial hydration, followed by a linear increase to 10,000 V and attained a total of 12,000 V. After finishing the

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