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Proteome basis of red color defect in channel catfish (*Ictalurus punctatus*) fillets

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ABSTRACT

The quality attributes, descriptive sensory characteristics, and muscle proteomes (sarcoplasmic and myofibrillar) of normal and reddish channel catfish (*Ictalurus punctatus*) fillets were evaluated. Reddish fillets had greater ($p < 0.05$) a^*(redness) and b^* (yellowness) values and lower L^* (lightness) ($p < 0.05$) than normal ones, but no differences existed ($p > 0.05$) in cooking loss and instrumental texture. The reddish fillets were more ($p < 0.05$) bitter, earthy, grassy, metallic, and oxidized, and had more off-flavor than normal ones, but was less ($p < 0.05$) sweet and juicy than normal fillets. Profiling of muscle proteomes employing two-dimensional electrophoresis and mass spectrometry identified several myofibrillar and sarcoplasmic proteins. The sarcoplasmic proteome revealed differential abundance of the beta subunit of hemoglobin, which was over-abundant ($p < 0.05$) in reddish fillets. On the other hand, no differences ($p > 0.05$) were observed in the abundance of myofibrillar proteome components. The results indicated that the occurrence of red color defect in catfish fillets is primarily due to the over-abundance of hemoglobin.

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1. Introduction

Mississippi produces and processes more channel catfish (*Ictalurus punctatus*) than any other state in the United State (Hargreaves & Tucker, 2004, pp. 1–9). The main steps involved in catfish processing include de-heading, filleting, skinning, trimming, sizing, and chilling (Silva, Ammerman, & Dean, 2001, p. 183). Environmental factors, handling practices, and processing affect the final quality of catfish fillet (Lu, 2008). Catfish muscle is characterized by a predominantly pale/white color with grayish to a slightly red tint. However, researchers have reported that capture, transport, and killing may induce stress that results in undesirable reddish color in catfish fillets (Bosworth & Small, 2004; Lu, 2008; Pottinger, 2000; Silva, Nunez, & Chamul, 2001). These factors induce stress in the fish and affect muscle color by depleting the glycogen reserve and ATP levels, as well as metabolizing proteins and fat, which influences muscle pH and rigor development (Pottinger, 2000). Several studies have focused on determining the factors responsible for change in color in catfish fillets. Silva, Nunez, et al. (2001) reported that stressing channel catfish through pre-slaughter chilling resulted in red, soft and exudative (RSE) catfish fillets. In addition, Bosworth and Small (2004) documented that increases in transport stress increased moisture loss and lightness in catfish fillets due to muscle protein denaturation.

Proteomics has been applied extensively in aquaculture, to determine nutritional issues, authenticity, quality, toxicity and allergen characterization (Pineiro, Barros-Velázquez, Vázquez, Figueras, & Gallardo, 2003; Zhou, Ding, & Wang, 2012). Furthermore, proteomic approaches have also been utilized for investigating muscle food quality, i.e. to understand the relationship between protein expression and meat tenderness (Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006; Lametsch et al., 2003; Laville et al., 2007), muscle-to-meat conversion (Jia et al., 2007; Jia, Hildrum, et al., 2006; Morzel et al., 2004; Sayd et al., 2006), water-holding capacity (Van de Wiel & Zhang, 2007), and meat color (Joseph, Suman, Rentfrow, Li, & Beach, 2012; Sayd et al., 2006). The sarcoplasmic and myofibrillar proteome has been characterized in various meat species in relation with muscle food quality.

Abbreviations: 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; pI, isoelectric point; MS, mass spectrometry.

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Sayd et al., (2006) evaluated color variability in porcine *semimembranosus* muscle (light pale vs. normal) and related color differences to sarcoplasmic protein expression. However, catfish muscle proteome has not been characterized and the biochemistry of red color defect in catfish fillets has not been examined previously.

Therefore, the major focus of this study was to identify catfish fillet quality biomarkers using proteomics that could help the aquaculture industry to minimize the incidence of red fillets. The specific objectives of the present study were: (a) to determine quality parameters such as color, pH, texture, and cooking loss in normal and reddish catfish fillets, (b) to characterize the sensory attributes of normal and reddish catfish fillets using descriptive sensory analysis, and (c) to characterize the myofibrillar and sarcoplasmic proteomes in normal and reddish catfish fillets employing two-dimensional electrophoresis and mass spectrometry.

2. Materials and methods

2.1. Catfish fillet sampling

Catfish fillets were procured from a commercial processing plant (Macon, MS, USA) on three different occasions ($n = 3$). Normal and reddish (with varying degrees of redness) fresh catfish fillets were sampled on the same day of harvest, stored separately in labeled plastic bags, and shipped to Mississippi State University in ice-filled coolers. Instrumental color and pH of the catfish fillets were analyzed 1 day after sampling. The normal and red catfish fillets were vacuum packaged separately (Prime Source Vacuum Nylon/PE 3 mil standard barrier pouches, 8 × 12 inch, Kansas, MO, USA) and stored at −20 °C until tenderness, cooking loss, descriptive sensory analysis, and proteome analysis were conducted.

2.2. pH measurement

The pH of the catfish fillet samples was determined using a pH meter (Model Accumet 61, Fisher Scientific, Hampton, NH, USA) with an attached meat penetrating probe (Penetration tip, Cole Palmer, Vernon Hills, IL, USA), which was inserted directly into the muscle at three different locations (Kin et al., 2009, 2010). Five fillets were utilized for each treatment per replication.

2.3. Instrumental color evaluation

Instrumental color was determined using a Chroma meter (Model CR-400, Minolta Camera Co Ltd, Osaka, Japan) with 8 mm port size, 2° observer, and illuminant D65. Calibration of the instrument was carried out using a standard white Minolta calibration plate (Model No 20933026, Japan). Color of normal and red fillets were measured and expressed as CIE L^* (lightness), a^* (redness), b^* (yellowness), Chroma and Hue (Kin et al., 2009, 2010). For each fillet sample, color was determined at three different locations (5 fillets for each treatment per replication) and averaged prior to statistical analysis.

2.4. Cooking loss

Cooking loss for normal and red catfish fillets was measured according to Kin et al. (2009, 2010). The whole fillets were thawed at 4 °C, placed separately in aluminum foil trays on a baking sheet, and were cooked to an internal temperature of 65 °C in an oven (Viking, Greenwood, MS, USA) at 149 °C. After cooking, the fillets were allowed to rest for 10–15 min. Prior to weighing the cooked fillets; excess moisture was wiped with a paper towel. Cooking loss percentage (5 fillets for each treatment per replication) was expressed as: [(raw weight − cooked weight)/(raw weight)] × 100.

2.5. Texture evaluation

Texture of the normal and red catfish fillets were analyzed according to previously reported methods (Kin et al., 2009, 2010; Marroquin, Silva, Koo, Wannapee, & Kim, 2004) using an Instron Universal Testing Center (Model 3300, Instron, Norwood, MA, USA) equipped with a Kramer shear compression cell (CS-2) at a chart and crosshead speed of 100 mm/min. Normal and red catfish fillets were first cooked as described in the Cooking loss section. After cooking, a 25 g square piece from each fillet was placed individually in the Kramer cell and subjected to shear force. The shear force was recorded in N/g, which corresponds to the highest peak in the texturegram, and the area under the peak was reported as total energy (J/g) (Kin et al., 2009, 2010).

2.6. Descriptive sensory analysis

Descriptive sensory analysis was conducted using both raw and cooked catfish fillets at the Garrison Sensory Lab (Mississippi State University). The trained panelists ($n = 6$), with more than 50 h experience in muscle food sensory evaluation and 20 h experience with descriptive analysis of catfish, were trained in three separate sessions with respect to catfish fillets that varied in color from light gray to dark red. For raw samples, the panelists evaluated the odor (off-odor, spoilage), appearance (pinking intensity, sliminess, and color intensity) and physical compression (firmness) of normal and red fillets on a 0–15 cm scale. For the descriptive analysis of cooked fillets, the normal and red catfish fillets were cooked as described previously in the cooking loss section. After cooking, the catfish fillets were cut into 8–10 pieces (2 cm × 2 cm size) and individually placed in plastic cups that were coded with three-digit random numbers. During each descriptive panel, each panelist was randomly assigned 4 pieces (two pieces per normal and red catfish fillets). For descriptive analysis of cooked fillets, 0–15 cm line scales were used where '0' represented 'none' and '15' represented 'very strong' for the particular attribute measured (Garner, 2002, 71 pp.). Panelists evaluated the cooked fillet samples in terms of taste, texture, appearance and odor. Sweet, salty, sour, bitter, hardness and juiciness were the taste and texture attributes that were evaluated. The appearance was measured in terms of cooked appearance, pinking intensity and exudates while particular flavor evaluated were metallic/bloody flavor, oxidized flavor and off-flavor. A total of 3 replications were conducted for descriptive analysis of raw and cooked catfish fillets.

2.7. Muscle proteome isolation

Frozen normal and red catfish fillets were thawed at 4 °C. The sarcoplasmic proteome was isolated from catfish according to a procedure that was adapted from Sayd et al. (2006). A two-gram sample was cut from the center of each fillet. Catfish muscle tissue (2 g) was homogenized (Polytron Brinkmann, Westbury, NY, USA) in 8 ml (0.25 g/ml) extraction buffer (40 mmol/L Tris, 5 mmol/L EDTA buffer (pH 8, 4 °C) at low speed for 30 s). The homogenate was centrifuged (Sorvall RC-5C Plus Super speed Centrifuge, Newton, Connecticut, USA) at 4 °C for 15 min at 10,000 × *g*. After centrifugation, the supernatant was filtered through 0.45 μm membrane filters (TPP, Trasadingen, Switzerland) and referred to as the sarcoplasmic proteome. Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA).

The myofibrillar proteome was isolated from catfish fillets according to Liu and Xiong (2000). Catfish muscle (2 g) was

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