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The impact of stunning methods on stress conditions and quality of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4 °C during 72 h postmortem

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ABSTRACT

This study aimed to evaluate different stunning methods [percussion (T1), immersion in ice/water slurry (T2), and gill cut (T3)] on quality and stress conditions of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4 °C in 72 h postmortem. Rigor index (RI%), behavioral analysis, levels of lactic acid and muscle glycogen were measured for stress level evaluation. Meanwhile, sensory assessment, texture properties, cooking loss, adenosine triphosphate (ATP) related compounds, adenosine monophosphate deaminase (ADA) activity, and acid phosphatase (ACP) activity were analyzed. The least stress condition, significantly ($P < 0.05$) higher initial glycogen content was observed in T1. Ice/water stunning reduced the rate of ATP degradation, reflected in the lowest K value during 72 h. Aversive behaviors, significantly ($P < 0.05$) higher cooking loss, hypoxanthine riboside (HxR) content, and lower sensory score were observed in T3. The results indicated that gill cut in aquatic processing industry should be avoided for inferior quality and aversive reactions during stunning.

1. Introduction

Stunning methods are usually recognized as a crucial step that influences the quality of fish for consumption (Rasco, Down, & Ovissipour, 2015). According to the opinion of the European Food Safety Association panel, slaughter/stunning methods for farmed fish should be performed in an appropriate way, which requires a rapid loss in sensibility and consciousness in terms of fish welfare (European Food Safety Association, 2009). Stress caused by anxiety, suffering, and distress during stunning procedures leads to hormonal, biochemical, osmoregulatory, immune, and energetic alterations, in which plasma cortisol-secreting and anaerobic glycolysis occurred, followed by the formation of lactic acid, and changes in muscular activity can subsequently affect the quality of fish fillets (Barton, 2002; Poli, Parisi, Scappini, & Zampacavallo, 2005).

Fish species are considered to experience suffering and pain, but current stunning methods are accepted primarily for their economic benefits. Percussion, immersion in ice/water slurry and gill cut are commonly used stunning methods in the aquatic processing industry. When fish were faced with external stimuli, stress induced by the stunning process significantly affected attributes of muscle quality (Digre et al., 2011), protein degradation (Tulli et al., 2015), lipid oxidation, and metabolism (Secci, Parisi, Dasilva, & Medina, 2016) in several fish species under different storage conditions. However, effects of different stunning methods on the changes of *rigor mortis* were rarely investigated. Postmortem biochemical changes are strongly correlated to stress circumstances during the stunning procedure. Lyu, Huang, Liu, Zhou, and Ding (2015) found that Chilean jack mackerel (*Trachurus*

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purphyi) slaughtered by asphyxia in air, asphyxia in ice water, and stunning on fish head resulted in different onset time in *rigor mortis* and resolution. Different developments of *rigor mortis* were also observed in tench (*Tinca tinca*) that were subjected to various slaughter methods (Gasco, Gai, Rotolo, & Parisi, 2014).

Silver carp (*Hypophthalmichthys molitrix*) is one of the most important farmed freshwater fish in China, due to its fast growth rate, low feed demand, high nutritional value, and low price. The gross yield of silver carp increased dramatically from 4,099,663 tons in 2010 to 4,967,739 tons in 2014 (FAO, 2016). However, freshwater fish species are easily perishable after death, because of the high water content, microbial activity, and abundant nutrients (Fan, Luo, Yin, Bao, & Feng, 2014). The postmortem process has a strong impact on texture properties and flavor, which will eventually affect consumer acceptance for fish. The combination of actin and myosin, dissociation in actomyosin, proteolysis caused by cathepsin, and relevant changes in texture properties occurred during postmortem, especially in *rigor mortis*, strongly affect the flesh quality of fish (Wang et al., 2016). Nevertheless, only a few studies have focused on the changes in *rigor mortis* of silver carp, which mainly concentrated on effects of different concentrations of sucrose and salt (Shi, Cui, Luo, & Zhou, 2014). Few studies have investigated how stunning methods affect *rigor mortis* in silver carp.

Therefore, the objective of this study was to investigate the influence of three commercially used stunning methods (percussion, immersion in ice/water slurry, and gill cut) on stress conditions and quality of silver carp fillets stored at 4 °C in 72 h postmortem based on the physicochemical evaluation and sensory assessments, and provide proper stunning methods for aquatic processing (especially for silver carp) in terms of fish welfare and quality indicators.

2. Material and methods

2.1. Fish and experimental design

Forty-eight silver carp with an average weight and length of 1.96 ± 0.15 kg and 49.2 ± 0.2 cm, respectively, were bought from a local aquatic wholesale market in Beijing, China in January 2016, and transported to the laboratory alive in water. In the laboratory, fish were divided randomly into three groups (16 individuals in each group), then allocated to six 60-liter plastic containers (8 fish for each container) and treated with different stunning methods. In the percussion group (T1), fish were kept in containers filled with tap water (temperature = 17 ± 2 °C) for 30 min, and then they were stunned immediately by one or two blows on the head with a wooden club. In the ice/water slurry group (T2), fish were exposed and immersed in ice/water slurry (1:1 w/w ratio, temperature = 1 ± 1 °C) for about 40 min until no opercular movements were observed. In the gill cut group (T3), fish were bled sequentially by cutting gill fragments on both sides with scissors and then placed into water for bleeding (it was necessary to change water every 15 min to remove fish blood) until fish were unconscious. After stunning, all fish (except those for the measurement of rigor index) were scaled, gutted, decapitated, filleted into 4 pieces, and washed in flowing tap water. Rinsed silver carp fillets were placed onto a clean stainless steel frame for draining (within 5 min). Then, samples were packaged in polyethylene bags and stored evenly in refrigerators at 4 ± 1 °C. According to the experimental design, three random samples of each group were taken for analysis.

Behavioral analysis was recorded during the stunning procedure. Analysis for muscle glycogen and lactic acid content was performed at 0, 2, 4, 6, 12, and 24 h and at 0, 6, 12, 24, 36, 48, and 72 h postmortem, respectively. Adenosine triphosphate (ATP) related compounds, adenosine monophosphate deaminase (ADA) activity, and acid phosphatase (ACP) activity, rigor index, pH, K value, cooking loss, texture properties, and sensory assessment were measured at 0, 2, 4, 8, 12, 24, 36, 48, and 72 h postmortem.

2.2. Behavioral analysis and measurement of rigor index (RI %)

As a nondestructive and macroscopical method for the assessment of stress conditions under the presence of external stimuli, behavioral analysis was carried out using the method of Bjorlykke, Roth, Sorheim, Kvamme, and Slinde (2011), with some modifications. Self-initiated behaviors, such as persistence of swimming motility, gill movements, equilibrium ability and escape reaction, were recorded by 10 trained panelists from the laboratory staff during the stunning procedure. Afterward, all descriptions were gathered and summarized (behaviors of T1 were analyzed before percussion).

Developments of *rigor mortis* were measured as rigor index. Rigor index was tested by the method developed by Gasco et al. (2014). Briefly, the upper half of each fish was placed on a flat surface in a refrigerator (temperature = 4 ± 1 °C) with the other half suspended. At specified time intervals, the vertical distance (L, cm) from the flat surface to the caudal fin was measured by a meter ruler calibrated at 0.1 cm and calculated by the following equation: $RI\ (\%) = [(L_0 - L)/L_0] \times 100$, where L_0 is the distance obtained immediately after death.

2.3. Determination of muscle glycogen and lactic acid

Concentration of muscle glycogen was determined by the sulfuric acid anthrone colorimetric method (Carroll, Longley, & Roe, 1956), according to the instructions in the glycogen assay kit for liver and muscle tests (No. A043, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Results were expressed as mg (glycogen)/g (muscle).

The method of estimating lactic acid content in muscle followed the instructions of the corresponding test kit (No. A019-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by lactate dehydrogenase method (Macqueen & Plaut, 1979). Briefly, one gram of muscle sample was homogenized with 9 mL of cold 0.85 % sodium chloride solution for 30 s and then centrifuged for 10 min at 550×*g*. The protein concentration of the supernatant was measured according to the Biuret method (Torten & Whitaker, 1964). Afterward, 20 μL of liquid supernatant and the relevant chemical reagent of test kits were mixed and incubated at 37 °C for 10 min. Absorbance was measured at 530 nm using a UV-2600 spectrophotometer (Unico Instrument Co. Ltd, Shanghai, China). Content of lactic acid was expressed as mmol/g protein.

2.4. ATP related compounds and K value

ATP and derived nucleotide degradation products were extracted according to the method described by Song, Luo, You, Shen, and Hu (2012), with some modifications. One gram of dorsal muscle was added to 2 mL of cold 10% perchloric acid, and then homogenized immediately. The homogenate was centrifuged at 1430×*g* for 5 min at 4 °C. The supernatant was collected and precipitate was washed twice by 5% perchloric acid and then centrifuged again under the same condition. After that, all the liquid supernatant was gathered to adjust the pH to 6.4 using 1 mol/L and 10 mol/L potassium hydroxide followed by centrifugation at 800×*g* for 3 min. The supernatant was made up to 10 mL with neutral perchloric acid (pH 6.4, using ammonium hydroxide for neutralization) and then frozen at −23 °C for further use.