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Changes in microbiological, physicochemical and muscle proteins of post mortem large yellow croaker (*Pseudosciaena crocea*)

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ABSTRACT

This study was devoted to the identification of changes in microbiological, physicochemical and proteins which could be used as freshness indicators in large yellow croaker. The parameters that proved to be most sensitive to variations over storage time were total viable count, *Shewanella putrefacents*, *Photobacterium phosphoreum*, TVB-N, PV and *K* value. They might therefore be considered as good indicators for evaluating spoilage of large yellow croaker during refrigerated storage for 20 days. The proteins including MHC, actin, tropomyosin, protein bands of 14 ~ 15 kDa, 32 ~ 33 kDa, 35 ~ 36 kDa and 41 ~ 42 kDa could be used as the potential freshness markers of large yellow croaker. The results of these analyses indicated optimal quality for large yellow croaker stored under these conditions and established a shelf-life of 12 days.

1. Introduction

Large yellow croaker (*Pseudosciaena crocea*) is widely cultured in China because of its delicious taste, nutritional quality, as well as its high economic value (Xie, Ai, Mai, Xu, & Ma, 2011). At present, the annual yield of large yellow croaker ranks first in China, that exceeds any other single netcage-farmed marine species (Yu, Mu, Ao, & Chen, 2010; Zhou, Zhang, Wang, Zou, & Xie, 2010). Studies on large yellow croaker have related to artificial breeding (Mai et al., 2006), genome (Cui, Liu, Li, You, & Chu, 2009), immunology (Ai et al., 2007), as well as preservation technology (Li, Hu, et al., 2012). However, little information is available on the postmortem quality changes of large yellow croaker during storage.

The changes that take place in fish muscle post mortem have a significant influence on the aquaculture industry due to their crucial impacts on quality and consumer preference. Fresh fish spoilage can be very fast after it is caught. Immediately after death the fish muscle is absolutely relaxed and the limp elastic texture usually lasts for some hours, then the muscle will contract. Rigor mortis is the phase through which fish loses its flexibility on account of stiffening of fish muscles after several hours of its death. This condition usually lasts one day or more and then rigor resolves. Deterioration of quality, followed by spoilage, is the result of a combination of microbiological, physiological and chemical processes (Verrez-Bagnis, Ladrat, Morzel, Noël, & Fleurence, 2001). The initial reactions in deterioration consist of proteolysis, catalysed by endogenous enzymes, which produce nutrients that cause bacterial proliferation. During fish spoilage, there is a degradation or breakdown of various components and formation of new compounds. These new compounds are responsible for the changes in colour, odor, flavor and texture of the fish muscle (Ghaly, Dave, Budge, & Brooks, 2010).

Nowadays, ways for evaluating freshness and quality of different marine species are based on postmortem changes associated with sensory and non-sensory changes. Sensory methods are still the most satisfactory for assessing the fish freshness (Connel, 1995). Non-sensory methods, using physical and biochemical methods, microbiological growth, as well as proteolysis, are also used to assess the freshness quality of fish (Delbarre-Ladrat, Chéret, Taylor, & Verrez-Bagnis, 2006; Gill, 1992). Physical and biochemical methods evaluate the concentrations of breakdown products by bacterial or enzymatic activity. A lot of spoilage indicators have been used, including trimethylamine (TMA), total volatile basic nitrogen (TVB-N) and formation of biogenic amines (Hebard, Flick, & Martin, 1982). Indices of quality based on nucleotide degradation have achieved special attention for monitoring fishery products freshness during handling and processing. The concentration of major adenine nucleotides and their related compounds in

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postmortem fish muscle correlates well with the loss of freshness in a wide category of fish. Total molar concentration of ATP and related compounds in fish muscle, as well as the change ratios and pattern in their levels during storage are species-dependent and even muscle-dependent. *K* value is calculated from the ATP concentration and its products of degradation. This index is universally used to evaluate the fish freshness and shows a very good correlation with the storage time (Ocaño-Higuera et al., 2011). Recent studies on proteins from different frozen fish species such as cod and hake have indicated changes in the solubility of α-actin and myosin heavy chain, which can be responsible for some protein solubility alterations happening during frozen storage (Kjærsgård, Nørrelykke, & Jessen, 2006).

Large yellow croaker is a very important and economic species in China. However, studies about the postmortem changes during refrigerated storage are still limited. This study reports issue on postmortem quality changes of large yellow croaker to improve the freshness and spoilage indexes. Basic knowledge of chemical composition and storage characteristics of large yellow croaker would greatly benefit its further utilization as human food. Thus, the present study was to evaluate changes in microbiological, physicochemical and muscle proteins of large yellow croaker and set up better processing and marketing strategies for domestic consumption and for export.

2. Materials and methods

2.1. Preparation of fish samples and storage conditions

Live commercial-sized large yellow croaker with an average weight of 400–450 g were obtained from Jinjiang Aquatic Market (Hangzhou, Zhejiang province, China) and transferred to Food Processing Laboratory of Zhejiang Gongshang University within 1 h. Fish were killed in slurry ice and kept at 0 °C before being used. Each sample was drained and packed in polyethylene bag and kept at 4 °C for 20 days. These methods of fish handling and storage conditions largely imitate normal commercial practice employed by the fish farming industry in China. Samples were taken on day 0, 4, 8, 12, 16 and 20 of storage. The fish were divided into four lots, and three fish from each lot were taken on each sampling day to make the different analyses. Two lots of six fish were used for microbiological and physicochemical analyses, and the other two for the protein and sensory analysis.

2.2. Microbiological analysis

The measurement of the total bacteria amounts indicated as aerobic plate counts (APCs) followed Chinese National Standard (2008) (GBT 4789.2-2008). A sample (10 g) taken from the muscle of the anterior-dorsal region of each whole fish was transferred aseptically into a stomacher bag containing 90 ml of 8.5 g/l sterile NaCl water and homogenized for 60 s. Further decimal dilutions were made, and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar (Base Bio-Tech, Hangzhou, China) plates. They were then incubated for 72 h at 30 °C. *Pseudomonads* were enumerated on cetrimide fusidin cephaloridine agar (CFC, Base Bio-Tech, Hangzhou, China) and incubated at 30 °C for 48 h. For lactic acid bacteria (LAB), Enterobacteriaceae and H_2S-producing bacteria (typical of *Shewanella putrefaciens*), samples (1.0 ml) of decimal serial dilutions were inoculated into 10 ml of molten de Man Rogosa Sharpe agar (MRS, pH 6.2, Base Bio-Tech, Hangzhou, China), violet red bile glucose agar (VRBGA, Base Bio-Tech, Hangzhou, China) and iron agar (Base Bio-Tech, Hangzhou, China), respectively. After setting, a 10 ml overlay of molten media was added and plates were incubated at: 37 °C for 3 days for MRS plates; 30 °C for 24 h for VRBGA plates; 20 °C for 48 h for iron agar plates. Three replicates of at least three appropriate dilutions on the sampling day were enumerated. *Photobacterium phosphoreum* growth medium contained (in g L^{-1}): Lab-lamco powder, 10.0; bacto peptone, 10.0; NaCl, 10.0; yeast extract, 3.0; KCl, 0.75; $MgSO_4 \cdot 7H_2O$,0.75; $FeSO_4 \cdot 7H_2O$, 0.014; Tris–HCl, 6.00; TMAO dihydrate,4.0; 0.04 ml of formaldehyde (37% sol.); agar (15 g L^{-1}). The pH was adjusted to 6.6. Plates were incubated for 5 days at 15 °C. Bacteria analysis was achieved by spreading a total of 1.0 ml inoculum of the 1:10 dilution into one Petri plate separately and made three parallel samples. All plates were examined visually for typical colony shape and morphology characteristics associated with each growth medium.

2.3. Physicochemical analyses

2.3.1. Proximate composition

A proximate analysis was performed on 6 fish on day 0 of storage. Proximate analyses (the moisture content, total crude protein, ash content and lipid content) of the whole fish muscle were based on the procedures set by AOAC (1997).

2.3.2. pH, total volatile basic nitrogen, peroxide value and thiobarbituric acid

The pH was measured using a digital 320 pH meter (Mettler Toledo, Zurich, Switzerland). Total volatile basic nitrogen (TVB-N, mg N/100 g muscle) were estimated by the FOSS method (2002). The lipids were extracted from fish samples (50 g) with a mixture of water, methanol, and chloroform (25:100:100). The peroxide values of the extracts were measured using the modified method of Lea (1952). The thiobarbituric acid value was determined colorimetrically by the method of Porkony and Dieffenbacher as described by Kirk and Sawyer (1991, p. 640, 643).

2.3.3. ATP related compounds and K value

Determinations of nucleotides and related compounds were determined by a reverse phase high-performance liquid chromatography method described by Ryder (1985). The identification of nucleotides, nucleosides, and bases was obtained by comparing their retention times with those of commercially obtained standards and by adding or spiking of standards. The *K* value was defined as follows:

$$K\ \text{value}(\%) = [(\text{HxR} + \text{Hx})/(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})] \times 100.$$

2.3.4. Texture measurements

Texture profile analyses (TPA) were performed using a TA-XT2i Texture Analyzer (Stable Micro System, Surrey, UK). The samples, measuring 2.0 × 2.0 × 1.0 cm, were compressed perpendicularly using a 5 mm cylindrical probe (P/5). The testing conditions were two consecutive cycles at 25% compression, cross-head movement at a consistent speed of 0.5 mm/s. Texture variables (hardness, adhesiveness, springiness, chewiness, gumminess, cohesiveness and resilience) were calculated as reported by Bourne (1978).

2.4. Extraction of muscle proteins and SDS-PAGE

The total soluble proteins of large yellow croaker was extracted according to the method of Sriket, Benjakul, and Visessanguan (2010) with slight modifications. The dorsal muscles (3 g) were transferred into polyethylene tube, and 27 ml of 50 g L^{-1} SDS solution heated to 85 °C were added. The mixture was then