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# Effects of tea polyphenols on the post-mortem integrity of large yellow croaker (*Pseudosciaena crocea*) fillet proteins

Jin Zhao [a,c], Weijin Lv [c], Jinlin Wang [c], Jianrong Li [b,c,*], Xiaoxiang Liu [c], Junli Zhu [c]

[a] Zhejiang Marine Food Processing Quality Control Techniques and Instrumentation Engineering Laboratory, College of Life Science, China Jiliang University, Hangzhou 310018, China
[b] College of Chemistry, Chemical Engineering and Food Safety, Bohai University, Jinzhou 121013, China
[c] College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, China

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ABSTRACT

Tea polyphenols (TP) are known to be important for the post-mortem deterioration of fish muscle and can enhance food quality. To shed light on the influence of TP on the status of large yellow croaker muscle proteins, control and treated fillets (0.1% TP, 0.2% TP and 0.3% TP, w/v) were analysed periodically for myofibrillar protein functional properties ($Ca^{2+}$-ATPase activity, surface hydrophobicity, total sulfhydryl content, emulsion stability index and rheological behaviour). Degradation of the myofibrillar protein myosin could be clearly observed; several proteins were also observed to vary in abundance following post-mortem storage for 25 days. The present study offers new evidence that TP have an effective impact on muscle protein integrity post-mortem.



## 1. Introduction

The large yellow croaker (*Pseudosciaena crocea*) is a commercially important marine fish species in south-eastern China, due to its good taste and high nutritional value. The aquaculture production of the large yellow croaker in China was 82,853 tonnes in 2012 (Anonymous, 2012). Fish are generally more perishable than other muscle foods, and a considerable number of fish are spoiled due to a lack of good preservation. One of the most unfavourable changes related to fish muscle quality is the post-mortem freshness of the muscle, which promotes a progressive loss in quality. The fish flesh quality is determined by various factors, such as the species, stress prior to death, post-mortem handling and temperature of post-mortem storage. Therefore, the biochemical processes have been extensively studied, either to identify potential quality indicators or to control post-mortem degradation. With the consumption of processed and seasoned fish fillets rapidly increasing, the development of reliable methods for quality assessment is of great interest in the field of aquaculture.

Nowadays, fish flesh quality is an indispensable factor for marketing. Considerable research has focused on improving fillet quality, such as high-pressure treatment (Ko, Jao, Hwang, & Hsu, 2006), salting (Hong, Luo, Zhou, & Shen, 2011), partial freezing (Song, Luo, You, Shen, & Hu, 2012), and modified atmosphere (Genç, Esteves, Aníbal, & Diler, 2013). Several recent studies have focused on using natural ingredients to enhance fillet quality during cold storage (Fan, Chi, & Zhang, 2008; Li et al., 2012). Tea polyphenols (TP) are popular plant extracts that have been widely used in various foods (Perumalla & Hettiarachchy, 2011). TP have attracted attention in recent years due to their enzyme inhibition, antibacterial and antioxidant activity (Cho, Schiller, & Oh, 2008; Erol, Sari, Polat, & Velioglu, 2009; Gondoin, Grussu, Stewart, & McDougall, 2010; Miura et al., 2000; Sivarooban, Hettiarachchy, & Johnson, 2007).

Proteomics has been increasingly used to address different questions concerning aquacultured products, with respect to nutrition, health, quality, and safety. In recent years, the increasing number of proteomic studies have provided insight into the biochemical processes occurring post-mortem in fish species (Addis, Cappuccinelli, Tedde, & Pagnozzi, 2010; Addis et al., 2012; Alves, Cordeiro, Silva, & Richard, 2010; Delbarre-Ladrat, Cheret, Taylor, & Verrez-Bagnis, 2006; Forne, Abia, & Cerda, 2010), thus facilitating the identification of the markers of fish muscle quality. The 2-D PAGE/MS identification approach is very well suited for studies on degradation patterns and also enables information to be obtained on the molecular weight and isoelectric point of the protein spots of interest. Therefore, 2-D PAGE/MS was used to trace the changes in the protein profiles of large yellow croaker muscle proteins over 25 days of post-mortem storage with TP treatment, with the aim of identifying potential biochemical markers, evaluating

* Corresponding author at: College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, China. Tel./fax: +86 416 3400008.
*E-mail addresses:* zhaojin@cjlu.edu.cn (J. Zhao), lijianrong@mail.zjgsu.edu.cn (J. Li).

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the effects of TP on protein functional properties of fish muscle and monitoring the fillet quality during superchilling storage at −2 °C.

## 2. Materials and methods

### 2.1. Fish and tissue collection

The experiment was performed at the College of Food Science and Biotechnology of Zhejiang Gongshang University (Hangzhou, China). TP were purchased from the Zhejiang University Tea Scientific Co., Ltd. (Tea polyphenol purity >98%, including catechin content >70%, EGCG content of 45%; Hangzhou, Zhejiang province, China).

Live, commercial-sized large yellow croaker were obtained from an aquatic farm in Zhoushan City, Zhejiang province, China. At the beginning of the experiment, 40 fish (average body weight 500 ± 30 g) were removed from the water and sacrificed by hypothermia (30 min of immersion in ice-cold water). Fillets were prepared at the time of death by taking alternately from both sides (right and left) of the epiaxial muscular quadrant of each fish and avoiding contamination.

The fillets were divided into four groups. The first group was used as the control, the second group was immersed in a solution of 3 g of TP in 1 L of distilled water for 60 min (0.3% TP), the third was immersed in a solution of 2 g of TP in 1 L of distilled water for 60 min (0.2% TP), and the fourth was immersed in a solution of 1 g of TP in 1 L of distilled water for 60 min (0.1% TP). The four groups of fillets were kept on a plastic net for 30 min to drain at 4 °C. Next, the samples were vacuum-packed in a polyethylene bag and were immediately transferred to a low temperature freezer (SNOWSONG SYX-313, Guangzhou, China) at −2 °C for temperature equalisation and storage for as long as 25 days. Parameter analyses were performed at 5-day intervals, with each analysis repeated three times using three fillets, and the averages were used to evaluate the overall quality of the fillets.

### 2.2. Myofibrillar protein extraction

A previously published extraction procedure (Chen, Wang, Lai, & Lin, 2003) was followed with certain modifications. Two grams of each fillet (control, 0.1% TP, 0.2% TP and 0.3% TP) were added to 20 mL extraction buffer A (0.1 M KCl, 20 mM Tris–HCl, pH 7.5) and then homogenised. The homogenate was centrifuged at 5000*g* using an Avanti J-25 Beckman centrifuge (Beckman Coulter, Inc., Fullerton, CA) at 4 °C for 10 min. The supernatant was removed, and the precipitate was washed twice. The precipitate was added with 15 mL extraction buffer B (0.6 M KCl, 20 mM Tris–HCl, pH 7.0) and then homogenised. The homogenate was extracted at 4 °C for 30 min and centrifuged at 5000*g* using an Avanti J-25 Beckman centrifuge at 4 °C for 20 min. The obtained supernatant was the myofibrillar protein solution used in this study. Relevant parameters, such as $Ca^{2+}$-ATPase activity, surface hydrophobicity, total sulfhydryl content, emulsion stability index, and rheological behaviour of myofibrillar protein, were determined every 5 days. Tests were performed in triplicate per sample.

### 2.3. Total protein extraction

Proteomic experiments were performed at the Proteomics Laboratory of the College of Life Science (China Jiliang University, Hangzhou, China). The proteins were extracted from the fillet using a Tissue Lyser mechanical homogeniser (Chuan Xiang Co., Ltd., Shanghai, China) as reported previously (Zhao, Li, Wang, & Lv, 2012). Briefly, a small portion (100 mg) of minced frozen fish muscle was placed in a mortar containing 5.0 mL of lysis buffer [8 M urea, 2 M thiourea, 4% CHAPS (w/v), 65 mM DTT, 0.8% v/v carrier ampholyte (pH 3–10) and a protease inhibitor cocktail]. Each sample was then processed with a Tissue Lyser mechanical homogeniser. All of the extracts were clarified for 15 min at 12,000*g* at 4 °C, quantified using the Bradford method using a BSA standard curve, tested for quality and quantity by SDS–PAGE and then stored at −80 °C until further analysis.

### 2.4. Determination of myofibrillar protein functional properties

Myofibrillar proteins are important structural proteins implicated in the freshness of fish flesh (Lonergan, Zhang, & Lonergan, 2010; Lund, Heinonen, Baron, & Estevez, 2011; Pazos, Maestre, Gallardo, & Medina, 2013). Therefore, information related to myofibrillar protein degradation patterns is of great importance in meat technology. In view of their capacity to monitor subtle changes in the chemical and physical state of the protein, the $Ca^{2+}$-ATPase activity, hydrophobicity, total sulfhydryl content, emulsion stability index and rheological behaviour are suitable parameters with which to estimate protein degradation and denaturation.

#### 2.4.1. Determination of the $Ca^{2+}$-ATPase activity of myofibrillar protein

The $Ca^{2+}$-ATPase activity of myofibrillar protein was determined according to the instructions for the $Ca^{2+}$-ATPase kit (Beyotime Institute of Biotechnology, Shanghai, China). The absorbance value was recorded at 636 nm. The $Ca^{2+}$-ATPase activity of myofibrillar protein was expressed as μmol (pi) $mg^{-1}$ (pro) $min^{-1}$ (pi = inorganic phosphorus, pro = protein).

#### 2.4.2. Determination of the surface hydrophobicity of myofibrillar protein

The surface hydrophobicity was determined as described by Chelh, Gatellier and Santé-Lhoutellier (2006). The protein content of each sample was determined by the BCA method and adjusted to identical concentrations (2 mg/mL), and the absorbance was measured at 595 nm. The surface hydrophobicity of the myofibrillar protein was expressed as bound bromophenol blue (BPB) (μg).

#### 2.4.3. Determination of the total sulfhydryl content of myofibrillar protein

The total sulfhydryl content of myofibrillar protein was determined according to the instructions of the corresponding kit (Beyotime Institute of Biotechnology, Shanghai, China). The absorbance was measured at 412 nm. The total sulfhydryl content of the myofibrillar protein was expressed as mol $g^{-1}$ (pro) (pro = protein).

#### 2.4.4. Determination of the emulsion stability index (ESI) of myofibrillar protein

The emulsifying activity and stability index of the protein were determined as by others (Uzun, Ibanoglu, Catal, & Ibanoglu, 2012), with certain modifications. The protein content of each sample was determined by the BCA method and adjusted to identical concentrations (0.4 mg/mL), and a 0.1-mL aliquot of the emulsion was mixed with 5 mL of 0.1% (w/v) SDS solution. The absorbance of the diluted emulsion was then determined at 500 nm by spectrophotometer (Biomate 3; Thermo, Waltham, MA). The emulsifying activity (EAI) was determined by measuring the absorbance immediately after emulsion formation, and the emulsion stability index (ESI) was estimated by following the time-dependent change in the absorbance readings. The result was expressed as surface area per unit weight of protein ($m^2$/mg) used in the emulsions. The emulsion experiments were replicated two times:

$$\text{EAI } (m^2/mg) = 2T\ [A_0 \times \text{dilution factor}/C \times \Phi \times 10000]$$