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Comparison of gel properties and biochemical characteristics of myofibrillar protein from bighead carp (*Aristichthys nobilis*) affected by frozen storage and a hydroxyl radical-generation oxidizing system

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ABSTRACT

We wanted to clarify whether gel properties can be affected by *in vivo* or *in vitro* myofibrillar protein oxidation and, thus, to provide relevant information and a scientific foundation for the processing of gel products. To accomplish this, we measured the changes in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), total disulfide (SS) content, surface hydrophobicity (So-ANS), carbonyl content, and gel texture and water-holding capacity (WHC) of isolated myofibrillar protein from bighead carp fillets during frozen storage and under different H_2O_2 concentrations, which were used to represent *in vivo* and *in vitro* conditions, respectively. The results indicated that a certain range in content of disulfide crosslinks (0.91 mol/10^5 g protein) would promote gel hardness. Mild protein oxidation caused by a certain degree of frozen storage and hydroxyl radicals can promote gel texture and WHC. Based on those results, freezing bighead carp for a certain period can be used to produce gel products.

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1. Instruction

Frozen storage is one of the most important techniques for long-term preservation of fish products. Although microbial spoilage can be terminated effectively by frozen storage, some structural and physicochemical changes can occur, such as toughness and loss of protein functional properties, which might be caused by oxidation of myofibrillar protein (Rodríguez Herrera, Pastoriza, & Sampedro, 2000). Oxidative processes that act on proteins can decrease the number of sulfhydryl groups by forming disulfide bridges. Active oxygen species also attack the side chains of basic amino acids (histidine, arginine, and lysine) and they can convert them into carbonyl derivatives. These carbonyl groups can react with free amino groups to form amide bonds (Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008). These oxidative changes are implicated in quality deterioration of oxidized meat products, including off-flavor, discoloration, destruction of nutrients, the formation of toxic compounds, and reduced consumer acceptability (Kanner, 1994). It is now recognized that protein oxidation is an important parameter for quality loss during processing and storage of muscle foods (Ooizumi & Xiong, 2004; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004). The impact of protein oxidation on the functionality of myofibrillar protein has received more attention recently, especially in terms of gel properties (Ooizumi & Xiong, 2004; Rowe et al., 2004). Extended frozen storage of threadfin bream (*Nemipterus bleekeri*), bigeye snapper (*Priacanthus tayenus*), lizardfish (*Sauruda micropectoralis*) and croaker (*Pennahai macrophthalmus*) at −18 °C caused the loss in gel-forming ability (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005).

Hydrogen peroxide (H_2O_2) is generated in all aerobic organisms as a result of normal cellular metabolism. Thus, electrons that leak from the electron transport chain of mitochondria cause the univalent reduction of molecular oxygen to the superoxide anion, which then dismutates spontaneously or enzymatically to H_2O_2 (Fridovich, 1997). Hydrogen peroxide is thought to contribute to various cellular functions (Kamata & Hirata, 1999). It is converted readily to hydroxyl radicals (OH·) by the Fenton reaction in the presence of iron (or copper) and cellular reductants, and these radicals induce irreversible oxidative damage to various cellular components (Stadtman, 1992). In particular, hydroxyl radicals are known to be a major oxidizing agent that impact biochemical and functional properties of myofibrillar proteins in many *in vitro*

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experiments. According to Chen, Diao, Li, Chen, and Kong (2016), increased oxidation time with hydroxyl radicals was accompanied by increased carbonyl content, reduced Ca-ATPase activity, and increased protein susceptibility to thermal aggregation, which induced deterioration of the structural and rheological properties of porcine myofibrillar protein. Li and her colleagues (2013) reported that the hydroxyl radical-generating system destroyed the springiness and hardness of gels in the myofibrillar protein of common carp and that it decreased their water-binding capacity and whiteness. However, a previous report indicated that moderate oxidative modification could improve the texture as well as WHC of the protein gel (Zhou, Zhao, Zhao, Sun, & Cui, 2014). Xiong, Blanchard, Ooizumi, and Ma (2010) also advised that we can take advantage of mild oxidation to alter the mode of myosin aggregation to form an elastic gel network.

Researchers tend to use the hydroxyl radical system to imitate the actual environment where muscles are oxidized to understand better the mechanisms of protein oxidation that occur in complex muscle food systems. But we do not know the real difference of myofibillar protein changes that was induced by oxidation under *in vitro* and *in vivo* conditions.

Therefore, in our study, frozen storage at −20 °C and the hydroxyl radical-generation oxidizing system were used to represent *in vivo* and *in vitro* conditions, respectively. We used bighead carp (*Aristichthys nobilis*) fillets which is one of the main freshwater fish species in the world, as our experimental samples. In addition, we measured the changes in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), total disulfide (SS) content, surface hydrophobicity (So-ANS), carbonyl content, and gel properties (texture and water-holding capacity (WHC)) of isolated myofibrillar protein from bighead carp fillets during frozen storage and under different H_2O_2 concentrations.

We have two aims in this study: 1) To determine whether gel properties can be promoted by mild protein oxidation that is caused by hydroxyl radicals, specifically *in vitro* protein oxidation. 2) To determine whether protein oxidation *in vivo* affected gel properties as *in vitro*; if the effects are similar, then we can take advantage of the positive effects on gel properties. If the effects are different, then we need to build a more comprehensive *in vitro* system to imitate the real environment. Whatever results we obtained in this study could provide an overview of gel properties under *in vitro* and *in vivo* environments to improve gel quality during processing.

2. Materials and methods

2.1. Fish

Live samples of bighead carp (weight 1883 ± 201 g, length 49 ± 1 cm) were purchased from a local aquatic market in Beijing, China, and transported to the laboratory by fish van in water with dissolved oxygen. They were killed by a physical blow to the head, eviscerated, washed, and cut into fillets, in accordance with the Guidance on Treating Experimental Animals developed by China's Ministry of Science & Technology in 2006 and Regulations issued by China State Council in 1988. The fish fillets were packed in polyvinyl chloride bags and subjected to two treatments. There were 15 fillets stored under −20 °C (three fillets were stored for 3, 7, 13, and 30 weeks each, the extra three fillets were kept in case there was a failure during measurement), and another 25 fillets were stored under 4 °C and used within 24 h. Three of the 25 fillets were defined as samples at week 0 under −20 °C, and 18 of the remaining fillets were used for measuring changes in myofibrillar protein under the hydroxyl radical-generation oxidizing system (three fillets each for control and for 0.1, 0.5, 1, 5, 10 mM H_2O_2, the extra four fillets were kept in case there was a failure during measurement).

2.2. Preparation of myofibrillar protein from bighead carp fillets

Frozen fillets were thawed at 4 °C overnight prior to the preparation of myofibrillar protein, as described by Xiong et al. (2010), with some modifications. Approximately a 50 g sample of muscle was minced and homogenized in 4 volumes of 10 mM potassium phosphate buffer solution at pH 7.0 that contained 100 mM NaCl, 2 mM MgCl2, and 1 mM EGTA using a homogenizer (FM200, Fluko company, Shanghai, China) at 10000 rpm for 30 s in an ice bath. The mixture was centrifuged at 5000*g* for 15 min and the pellets were washed twice with the buffer solution and once with 100 mM NaCl at pH 7. The pellets were myofibrillar protein and kept in tightly capped tube, stored in ice, and used within 24 h. The protein concentration was measured according to the Biuret method using BSA as a standard.

2.3. Incubation of myofibrillar protein under hydroxyl radical-generation oxidizing system

The method of incubation was according to Xiong et al. (2010), with some modifications. Myofibrillar protein (30 mg/mL) was suspended in 15 mM piperazine-*N*,*N* bis (2-ethane sulfonic acid) (PIPES) buffer at pH 7.0 that contained 0.6 M NaCl, and the sample was incubated at 4 °C for 36 h with an iron-catalyzed oxidizing system that generates hydroxyl radicals (10 μM $FeCl_3$, 0.1 mM ascorbic acid, 0.1, 0.5, 1, 5, 10 mM H_2O_2 respectively). Oxidation was terminated by washing the mixture with 3 volumes of 15 Mm PIPES buffer twice and with cold, deionized water once to remove hydroxyl radicals. The pellets contained oxidized myofibrillar protein, which was obtained by centrifugation at 10,000*g* for 15 min. The control was the myofibrillar protein that was treated only with the washing process.

2.4. Determination of SS content

The myofibrillar protein pellets were suspended in 15 mM sodium phosphate that contained 0.6 M NaCl at pH 7 and the protein concentration was adjusted to 4 mg/ml. The SS content in the myofibrillar protein was determined using the 2-nitro-5-thiosulfobenzoate (NTSB) assay, according to the method of Thannhauser, Konishi, and Scheraga (1987) with a few modifications. To a 0.5 mL sample of the myofibrillar protein solution we added 4.5 mL of 0.2 M Tris-HCl buffer (pH 8.0), which contained 8 M urea, 1% SDS, 3 mM EDTA, and 0.1 M Na_2SO_3. Four milliliters of the mixture was added to 0.5 mL of 0.2 M Tris-HCl buffer (pH 9.5) that contained 8 M urea, 1% SDS, 3 mM EDTA, 0.1 M Na_2SO_3, and 10 mM NTSB. The mixture was incubated in the dark at 40 °C for 25 min. The absorbance at 412 nm was measured. A blank was developed by replacing the sample with 0.6 M NaCl (pH 7.0).

SS content was expressed as mol/10^5 g protein and calculated as follows: SS content (mol/10^5g) = A × D/B × C, where A is the absorbance, B is the concentration of the myofibrillar protein, C is the molar extinction of 13, 600 mol/cm/L, and D is dilution 11.25.

2.5. Determination of So-ANS

The surface hydrophobicity of myofibrillar protein was determined according to You, Pan, Shen, and Luo (2012) using ANS (8-anilino-1-naphthalene sulfonate) as a probe by a fluorescence spectrophotometer (RF-5301PC, Shimadzu Corporation, Japan) at an excitation wavelength of 390 nm and an emission wavelength of 470 nm, and a 5 nm width for both the excitation and emission slits. The myofibrillar protein pellets were suspended in 15 mM

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