Research Article

Effects of Ozone Treatments on the Physicochemical Changes of Myofibrillar Proteins from Silver Carp (*Hypophthalmichthys molitrix*) during Frozen Storage

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Physicochemical changes of myofibrillar proteins from silver carp surimi during frozen storage as affected by two manners of ozone treatments were investigated. For preparation of surimi treated with ozone, ozone water (8 mg/L) was used in either the first (To1) or the second (To2) cycle of rinsing. As compared with control samples (Tc) (rinsing two cycles with water), myofibrillar proteins from To1 surimi showed slightly lower free sulfhydryl contents and higher surface hydrophobicity throughout frozen storage and lower Ca²⁺-ATPase activities after 30 d. To2 did not significantly ($P > 0.05$) affect these physicochemical properties, indicating that myofibrillar proteins structure was well maintained. Consequently, To1 significantly ($P > 0.05$) decreased breaking force of surimi gels while To2 did not significantly ($P > 0.05$) affect gel breaking force. In addition, the whiteness of surimi gels was increased more obviously by To2 than by To1. The results indicate that To2 could be used as a mild oxidation treatment for improving white color of silver carp surimi without negatively affecting gel texture.

1. Introduction

Silver carp (*Hypophthalmichthys molitrix*) is one of the main freshwater fish species farmed in China. In 2014, total production output was 4.23 million t [1]. Utilization of silver carp has increased in recent years due to massive overexploitation of sea-water fish and the resulting shortage of raw material for frozen surimi. The output of silver carp surimi was estimated at about 30,000 t in 2013 [2] and has rapidly grown; reaching over 40,000 t in 2015 [3]. However, silver carp surimi possesses an earthy-musty off-odour [4], which is generally thought to be associated with geosmin (GEO) and 2-methylisoborneol (MIB) [5]. Furthermore, whiteness of silver carp surimi is inferior to that of fish species that are traditionally used for high-quality surimi products [6]. Consequently, these defects negatively affect consumer perception of surimi products made from silver carp.

Ozone, which has regulatory approval and is generally recognized as environmentally friendly, has been broadly used in water treatment, sanitization, cleaning, and disinfection of equipment, in off-odour removal, and for processing various food products [7]. Ozone also shows great potential for improving the quality of aquatic products with regard to shelf life, sensory, and so forth [8]. Zhang et al. [9] reported that about 42–69.19% GEO in bighead carp (*Hypophthalmichthys nobilis*) meat was removed by ozone water (3.3–7.6 mg/L) rinsing for 5–20 min. According to the study by Wang et al. [4], the muddy flavours of silver carp surimi were effectively eliminated after washing for 20 min using ozone water with an initial concentration of 0.96 mg/L. In addition, ozone is an oxidant that possesses a bleaching effect, which helps to increase the whiteness of surimi and other aquatic products [10, 11]. Accordingly, the defects of

silver carp surimi in sensory quality and color may be alleviated by applying appropriate ozone treatment.

Myofibrillar proteins are the major components of surimi and are responsible for the formation of gel texture upon heating. During the rinsing process of surimi production, oxidation of myofibrillar proteins by ozone treatment may cause the formation of intra- and/or intermolecular disulfide bonds, peptide bond cleavage, amino acid residue modification, unfolding of protein molecules, and alternation in protein functionality [7]. These changes to protein functionality ultimately affect the textural properties of surimi gels.

Recently, researchers have begun to investigate the effects of ozone treatment on the properties of surimi and surimi products [4, 9, 12, 13]. Zhang et al. [9] reported that ozone water treatment was a mild oxidation protocol to enhance the functionality of myofibrillar proteins from bighead carp. Ozone treatment was found to significantly ($P < 0.05$) increase salt solubility, Ca^{2+} -ATPase activity, carbonyl content, sulfhydryl content, and gel textural values of proteins without increasing peroxide values too much. However, deformation of mackerel surimi gels with ozone treatment was found to be significantly ($P < 0.05$) lower than that of control samples (without ozone treatment) and gradually decreased with increased ozonation time [14]. Xie et al. [12] also found that textural values of silver carp surimi gels significantly ($P < 0.05$) decreased after rinsing with ozone water. Textural properties of surimi gels as affected by ozone water treatment, therefore, may be influenced by differences between fish species and/or ozonation conditions.

Studies on the properties of fish myofibrillar proteins or mince as affected by ozone water rinsing are limited. In the majority of published literature, fish myofibrillar proteins were immediately heated to form a gel after being washed with ozone water and then subjected to penetration test for evaluation of the ozone treatment on gel texture [4, 9, 12]. Ozone treatment enhances unfolding of fish myofibrillar proteins, which may contribute to stronger gel formation during the heating step [11]. However, surimi is an intermediate product that is typically mixed with cryoprotectants and then subjected to a period of frozen storage prior to being manufactured into different products. Therefore, partially unfolding proteins with ozone treatment before freezing may result in promoting protein aggregation during storage, which ultimately results in a weaker surimi gel being formed in the finished product [26]. The impacts of ozone-induced oxidation on the physicochemical changes of fish myofibrillar proteins during frozen storage, however, have not been reported.

Currently, in the production of silver carp surimi, it is common to use water: mince ratios of 5:1 to 3:1 with two rinsing cycles. The majority of the water-soluble proteins, primarily sarcoplasmic protein, and lipids are removed after the first rinsing cycle [15]. Oxidization of fish myofibrillar proteins may be influenced by the presence or absence of sarcoplasmic proteins and lipids [16, 17]. In order to determine appropriate application of ozone treatment for improving silver carp surimi quality, this study investigated the application of ozone water in the first or second cycle of

rinsing on the physicochemical changes of fish myofibrillar proteins during frozen storage and subsequent gelation properties.

2. Materials and Methods

2.1. Materials. Silver carp (*Hypophthalmichthys molitrix*), approximate 1.5 kg, was obtained from a local fish farm (Wuhan, China). Reagents used for SDS-PAGE were purchased from Bio-Rad (Hercules, CA, USA). Adenosine triphosphate (ATP), 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), and 1-anilino-8-naphthalenesulfonate (ANS) were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Sugar and sodium tripolyphosphate were purchased from Guangshengyuan Food Co., Ltd. (Wuhan, China) and Xingfa Group Co., Ltd. (Wuhan, China), respectively. All other chemicals were of analytical grade.

2.2. Preparation of Surimi Treated with Ozone. Silver carp was headed, gutted, and thoroughly cleaned prior to deboning the carcass by a roll-type fish meat separator (YBYM-6004-B, Yingbo Food Machinery Co., Ltd., Xiamen, China). The obtained fish mince was subjected to two rinsing cycles with a water: mince ratio and rinsing time at 4:1 and 10 min, respectively. A total of 3 rinsing treatments were conducted: (1) two washing cycles using ice water only (Tc); (2) 1st and 2nd cycle using ice water containing 8 mg/L ozone and ice water, respectively (To1); (3) 1st and 2nd cycle using ice water and ice water containing 8 mg/L ozone, respectively (To2). Ozone water, containing an initial concentration of 8 mg/L, was prepared according to the method by Zhang et al. [11] using a corona discharge ozone generator (SY-SB40, Sheng Ya Co., Ltd., Xuzhou, China). After rinsing, fish mince was wrapped in cheesecloth and centrifuged (SS-300, Runxin Machinery Works, Zhangjiagang, China) at 15,000 rpm to remove excess water. The concentrated myofibrillar proteins were mixed with cryoprotectants (6% sucrose and 0.3% tripolyphosphate), vacuum packaged (~600 g each bag), and stored in a freezer (-18°C) until used (0, 7, 15, 30, 60, and 90 days). Room temperature during all of the aforementioned operations was maintained below 10°C .

2.3. Extraction of Myofibrillar Proteins. Myofibrillar proteins were extracted from surimi according to the method of Poowakanjana and Park [18] with slight modification. Briefly, 1 g surimi was added to 29 mL buffer (0.6 M KCl, 20 mM Tris-HCl, and pH 7.0) and homogenized (FJ-200, Shanghai Specimen and Models Factory, China) at 8,000 rpm for 1 min. The homogenate was centrifuged at $15,000 \times g$ (J-26XP, Beckman Coulter Inc., Fullerton, CA, USA) at 4°C for 30 min. After centrifugation, the supernatant was filtered and used for analyzing free sulfhydryl content, Ca^{2+} -ATPase activity, and surface hydrophobicity as detailed below. Protein concentration of the supernatant was measured using the Lowry method [19].

2.4. Determination of Free Sulfhydryl Content. Free sulfhydryl content was determined according to the method of Jiang et al. [13] using Ellman's reagent (DTNB) with some

modifications. Protein concentration of the myofibrillar protein sample, as described above, was diluted to 0.5 mg protein/mL using 0.6 M KCl in 20 mM Tris-HCl buffer (pH 7.0). The diluted sample (0.5 mL) was mixed with 2 mL of 8 M urea in 0.2 mM Tris-HCl buffer (pH 7.0) and 50 μ L of 0.1 M sodium phosphate buffer (pH 7.0) containing 10 mM DTNB and 0.2 mM EDTA. The mixture was incubated at 40°C for 15 min before measuring absorbance at 412 nm (722 s, Shanghai Precision and Scientific Instrument Co., Ltd., China). Free sulfhydryl content was calculated using the extinction coefficient of 13600 M⁻¹ cm⁻¹ and expressed as mol per 10⁵ g protein.

2.5. Determination of Ca²⁺-ATPase Activity. Determination of Ca²⁺-ATPase activity was performed according to the method of Benjakul et al. [20] with some modifications. The myofibrillar protein sample (1 mL) was mixed with 0.5 mL of 0.5 M Tris-maleate buffer (pH 7.0) and 0.5 mL of 0.1 M CaCl₂. Deionized water was added to a total volume of 9.5 mL. Subsequently, 0.5 mL of 20 mM ATP was added to initiate the reaction. The mixture was incubated at 25°C for 8 min and then terminated by adding 5 mL of chilled trichloroacetic acid (15 g/100 mL). The reaction mixture was centrifuged at 3,500 $\times$ g for 5 min and filtered. Inorganic phosphate liberated in the filtrate was measured by the method of Fiske and Subbarow [21]. Specific activity was expressed as μ M inorganic phosphate (Pi) released/mg protein/min.

2.6. Determination of Surface Hydrophobicity. Surface hydrophobicity was measured using ANS probe according to the method of Poowakanjana and Park [18] with slight modification. Protein concentration of the myofibrillar proteins was diluted to 0.1, 0.2, 0.3, and 0.5 mg protein/mL using 0.6 M KCl in 20 mM Tris-HCl buffer (pH 7.0). Then 4 mL of sample with different protein concentrations was mixed with 20 μ L of 0.1 M phosphate buffer (pH 7.4) containing 8 mM ANS and left at room temperature for 10 min. Fluorescence intensity was immediately measured using a spectrofluorometer (RF-1501, Shimadzu, Kyoto, Japan) with excitation and emission wavelengths of 390 nm and 470 nm, respectively. The surface hydrophobicity was calculated from the initial slope of the net relative fluorescence intensity versus the myofibrillar proteins concentration.

2.7. Protein Patterns. The protein pattern of myofibrillar proteins at different storage times (0, 7, 15, 30, 60, and 90 days) was revealed using SDS-PAGE according to Laemmli [22] with some modifications. The sample was homogenized (Ika T18, Cole-Parmer, Co., Ltd., Shanghai, China) at 10,000 rpm for 1 min and solubilized using 5% sodium dodecyl sulfate solution (90°C). Solubilized proteins were centrifuged at 17,000 $\times$ g for 20 min at room temperature. Protein content of the supernatant was measured using the Lowry method [19]. Protein sample (2.5 mg/mL) was dissolved in Laemmli 5x sample buffer with or without β -mercaptoethanol and followed by heating at 100°C for 3 min. β -ME, as a reducing agent, was used to cleave the RS-SR bonds of proteins in the SDS-PAGE analysis. Stacking and separating gels were made

using 5% (w/v) and 12% (w/v) acrylamide, respectively. Each lane was loaded with 10 μ g protein. After running, gels were fixed and stained with 0.125% Coomassie brilliant blue R-250 and destained in DI water containing 50% methanol and 10% acetic acid.

2.8. Preparation of Surimi Gel. Vacuum-packaged frozen surimi was removed at the respective storage time (0, 7, 15, 30, 60, or 90 days) and partially thawed at room temperature for 40 min before being cut into approximately 2 cm $\times$ 2 cm $\times$ 4 cm cubes. The cubes were comminuted using a silent cutter (Multiquick 3, Braun, Germany) at speed 3 for 30 sec. Sodium chloride (2%) was added to extract myofibrillar proteins. Moisture content was adjusted to 78% using ice water (0°C). The mixture was blended and ground in a stainless steel mortar using twin pestles (CA 1, Kinn Shang Hoo Iron Works, Taiwan) at an agitation speed of 45 rpm for 30 min. Final temperature of the paste was below 10°C. The paste was then stuffed into a polyethylene sausage casing (2.5 cm diameter) using a sausage stuffer (Tre-mss7kh, Trs Spade, Italy). Both ends were sealed with U-shaped aluminum wire clips using a clipper (Hkl2, Hakanson, Sweden). The sample was then heated at 90°C for 30 min. Cooked gels were immediately submerged in ice water and then stored overnight in a refrigerator (4°C).

2.9. Texture Analysis. Gel strength of surimi gels was determined by the method described by Yin and Park [23]. Chilled surimi gels were equilibrated at room temperature ($\sim$ 25°C) for 2 h. Samples were then cut into 2.5 cm cylinders and subjected to the penetration test using a TA-XT texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a spherical probe (diameter 5.0 mm and crosshead speed of 1 mm/s).

2.10. Color Measurement. Color parameters, L* (lightness), a* (redness to greenness), and b* (yellowness to blueness), of the surimi gels were measured using a CR-400 colorimeter (Konica Minolta, Osaka, Japan). Whiteness was calculated according to the equation (L-3b*) developed by Park [24] for surimi gel.

2.11. Statistical Analysis. Analysis of variance (ANOVA) was conducted using the SAS program (V8, SAS Institute Inc, Carry, NC, USA). Differences among mean values were evaluated by the Duncan multiple range test (DMRT) using a 95% confidence interval.

3. Result and Discussion

3.1. Free Sulfhydryl Content. Conversion of sulfhydryl groups (R-SH) into disulfide covalent bonds (RS-SR) and other oxidized species through oxidation of sulfhydryl groups or disulfide interchanges is generally considered a good indicator for analyzing the radical-mediated oxidation of proteins [7]. As shown in Figure 1, the free sulfhydryl contents of silver carp myofibrillar proteins with and without ozone treatments decreased significantly ($P < 0.05$) after storing

FIGURE 1: Changes in free sulfhydryl content of myofibrillar proteins extracted from silver carp surimi during frozen storage. Light grey: two washing cycles using ice water only (Tc), dark grey: first and second washing cycle using ice water containing 8 mg/L ozone and ice water, respectively (To1); black: first and second washing cycle using ice water and ice water containing 8 mg/L ozone, respectively (To2). Different letters indicate significant difference among samples (Tc, To1, and To2).

(-18°C) for 7 d. The sulfhydryl contents remained constant ($P > 0.05$) during frozen storage from day 7 to day 30, and then continued to decrease ($P < 0.05$) at day 60. Reduction of free sulfhydryl content resulted from the formation of disulfide covalent bonds (RS-SR), as evidenced by changes of the protein patterns (Figure 4(a)). Myofibrillar proteins exhibited a reduction of about 23%, 24%, 24%, and 62%, respectively, in the sulfhydryl contents after 7 d, 15 d, 30 d, and 60 d of storage.

Changes in the sulfhydryl content of silver carp proteins during frozen storage were similar to that of croaker, threadfin bream, and bigeyes snapper as reported by Benjakul et al. [20]. Myofibrillar proteins are mainly composed of myosin (~55%) and actin (~20%), which contain about 42 and 12 sulfhydryl groups, respectively [25]. The sulfhydryl groups include active sulfhydryl groups on the surface and the hidden sulfhydryl groups in the protein interior. Oxidization of the active sulfhydryl groups on the surface reduced sulfhydryl content during early storage (<7 d). Subsequently, myofibrillar proteins unfolded during extended storage; thus, some of the original hidden sulfhydryl groups were exposed to the surface. These exposed sulfhydryl groups were then activated, which caused sulfhydryl content to decrease further (>30 d). The decrease in the sulfhydryl content coincided with an increase in surface hydrophobicity (Figure 3), which represents change in the tertiary structure of the protein. Surprisingly, the surface hydrophobicity significantly ($P < 0.05$) increased (Figure 3) at day 90 while the sulfhydryl content was not significantly ($P > 0.05$) changed. This observation may be due to the masking of sulfhydryl groups by aggregation of partially unfolded myofibrillar proteins [20].

Although cryoprotectants are mixed into surimi to maintain protein structure during frozen storage, fish myofibrillar proteins continue to gradually unfold and subsequently aggregate during frozen storage [26].

Before frozen storage (0 d), the free sulfhydryl content of the samples in the descending order was Tc, To2, and To1, respectively. But the differential was not significant ($P > 0.05$). However, Zhang et al. [11] reported that the free sulfhydryl content of myofibrillar proteins recovered from big-head carp decreased about 12% after rinsing with 7.6 mg/L ozone water. The reason might be due to a much longer rinsing time (20 min) used in their study. This order of the three samples (Tc, To2, To1), with regard to free sulfhydryl content, was maintained throughout frozen storage. Free sulfhydryl content of To1 was lower than that of To2; however the difference was not significant ($P > 0.05$). It might be related to the formation of lipid radicals and peroxide during the ozone water rinsing which enhanced the oxidation of the myofibrillar proteins [16, 17]. It has been reported that peroxy radicals from lipids abstracted hydrogen atoms from molecules of protein leading to a radical-mediated chain reaction similar to that of lipid oxidation [27]. And Lund et al. [28] reported that oxidation of lipids in meat systems took place faster than that of myofibrillar proteins and, hence, it was more likely that lipid derivatives (radicals and peroxides) promoted proteins oxidation than the other way round. For the ozone treatment, the To1 sample was rinsed in ozone water with the presence of a high concentration of lipids. As for the To2 sample lipids were majorly removed prior to the second cycle of rinsing using ozone water.

3.2. Ca^{2+} -ATPase Activity. Ca^{2+} -ATPase activity is widely used as an index of the denaturation of fish myofibrillar proteins during storage and processing [29]. As shown in Figure 2, Ca^{2+} -ATPase activity of silver carp myofibrillar proteins with and without ozone treatment gradually declined ($P < 0.05$) during frozen storage. These results were consistent with the report by Cao et al. [30] that Ca^{2+} -ATPase activity of silver carp surimi with various types of cryoprotectants decreased with frozen storage (-80°C) up to 90 d. The oxidation of sulfhydryl groups, especially in the head region (SH1 and SH2), caused Ca^{2+} -ATPase activity to decline. Moreover, inter- and/or intramolecular interactions of myofibrillar proteins during frozen storage could also contribute to decreased Ca^{2+} -ATPase activity [26].

Within 15 d of frozen storage, Ca^{2+} -ATPase activity of To1 was higher ($P < 0.05$) than the control (Tc) (Figure 2). The results generally coincided with the findings of Zhang et al. [9, 11], in which the Ca^{2+} -ATPase activity of bighead carp proteins increased after rinsing with 5.1 mg/L ozone water for 20 min followed by rinsing with distilled water. A change in the tertiary structure of the myosin head region or an increase in its flexibility, owing to a light extent of denaturation, may result in increased Ca^{2+} -ATPase activity [31]. The results of Zhang et al. [9] also indicated that the tertiary structure of the myosin head region was slightly influenced by ozone water rinsing. Rinsing with ozone exposed the globular myosin

FIGURE 2: Changes in Ca^{2+} -ATPase activity of myofibrillar proteins extracted from silver carp surimi during frozen storage. Light grey: two washing cycles using ice water only (Tc), dark grey: first and second washing cycle using ice water containing 8 mg/L ozone and ice water, respectively (To1); black: first and second washing cycle using ice water and ice water containing 8 mg/L ozone, respectively (To2). Different letters indicate significant difference among samples (Tc, To1, and To2).

head, which is typically buried within the protein structure, outside the tertiary structure [9].

After 30 d of frozen storage, our results showed the Ca^{2+} -ATPase activity of the To1 sample was lower ($P < 0.05$) than that of Tc (Figure 2). As storage duration extended, negative effects (sulfhydryl oxidation and/or protein interactions) dominated and Ca^{2+} -ATPase activity continued to decline. Before frozen storage, Ca^{2+} -ATPase activity of To2 was lower ($P < 0.05$) than To1 (Figure 2). However, after 30 d of frozen storage the Ca^{2+} -ATPase activity of To2 was higher ($P < 0.05$) than To1 (Figure 2). This result might be due to the lesser extent of oxidation in To2, which minimally influenced the physiological activity of myosin.

3.3. Surface Hydrophobicity. The changes in surface hydrophobicity of silver carp myofibrillar proteins with different ozone treatments during frozen storage are illustrated in Figure 3. The surface hydrophobicity of the three samples (Tc, To1, and To2) increased significantly ($P < 0.05$) after storing for 7 d, remained unchanged ($P > 0.05$) for up to 30 d, and then subsequently increased ($P < 0.05$) for up to 90 d. After storing for 90 d, the surface hydrophobicity of the control sample increased by approximately 87%. Similar tendencies were also found in the surface hydrophobicity of croaker, threadfin bream, and bigeye snapper myofibrillar proteins during frozen storage [16]. The increase of surface hydrophobicity during extended frozen storage is connected to the exposure of the hydrophobic bonds of myofibrillar proteins, which are located in the interior of the protein structure [20]. Frozen storage directly altered the tertiary structure of protein molecules, which results in functionality loss as observed by a decline in gelling ability (Figure 5).

FIGURE 3: Changes in surface hydrophobicity of myofibrillar proteins extracted from silver carp surimi during frozen storage. Light grey: two washing cycles using ice water only (Tc), dark grey: first and second washing cycle using ice water containing 8 mg/L ozone and ice water, respectively (To1); black: first and second washing cycle using ice water and ice water containing 8 mg/L ozone, respectively (To2). Different letters indicate significant difference among samples (Tc, To1, and To2).

Surface hydrophobicity is an effective indicator for reflecting the conformational change of protein from its native structure [26]. Surface hydrophobicity of To1 was higher than that of Tc, but not significantly ($P > 0.05$), during 90 d of frozen storage (Figure 3). In addition, the surface hydrophobicity of To2 was comparable to Tc. Results demonstrated that the oxidation in this study is mild. Changes of the surface hydrophobicity could be used to explain changes of sulfhydryl content (Figure 1) and Ca^{2+} -ATPase activity (Figure 2) as affected by ozone water rinsing and frozen storage.

3.4. Protein Patterns. SDS-PAGE was performed to monitor polymerization or degradation of the myofibrillar proteins as affected by ozone oxidation and frozen storage. Bands of myosin heavy chain (MHC 200 kDa) and actin (AC 45 kDa) with high densities were clearly visible on all SDS-PAGE gels (Figure 4). In addition, bands assigned to troponin-T (TN T 35 kDa), myosin light chain 1 (MLC 1 21 kDa), tropomyosin (TM 40 kDa), and several other proteins were also observed, but with relatively lower densities. In the absence of β -mercaptoethanol, bands with molecular weight above 200 kDa were noticed on the SDS-PAGE gel (Figure 4(a)). However, those bands (>200 kDa) seemed to disappear from SDS-PAGE gel when β -mercaptoethanol was present (Figure 4(b)).

β -mercaptoethanol is a reducing agent that possesses the ability to cleave disulfide covalent bonds (RS-SR) of proteins. Therefore, the obvious difference in the protein patterns caused by β -mercaptoethanol could be mainly attributed to myosin heavy chain (MHC) polymer formation through

FIGURE 4: Change in protein patterns of silver carp surimi during frozen storage. Tc: two washing cycles using ice water only; To1: first and second washing cycle using ice water containing 8 mg/L ozone and ice water, respectively; To2: first and second washing cycle using ice water and ice water containing 8 mg/L ozone, respectively. (a) Protein sample was dissolved in loading buffer without β -mercaptoethanol; (b) protein sample was dissolved in loading buffer with β -mercaptoethanol; STD: kaleidoscope protein standard; MHCXL: cross-links of myosin heavy chain; MHC: myosin heavy chain; AC: actin; TM: tropomyosin; TN T: troponin-T; MLC 1: myosin light chain 1.

disulfide covalent bonds (RS-SR). Myosin contains three kinds of active sulfhydryls, including SH1, SH2, and SHa. SH1 and SH2 are located in the globular myosin head and are closely related to Ca^{2+} -ATPase activity. SHa is distributed in the light meromyosin chain (LMM) and is related to the oxidation of the myosin heavy chain (MHC) and polymer formation [11]. The number of bands (>200 kDa) increased with frozen storage time up to 90 d (Figure 4(b)). Results confirmed the formation of disulfide covalent bonds during frozen storage, which also coincided with the reduction of free sulfhydryl content (Figure 1).

Regardless of β -mercaptoethanol, there was no considerable difference among samples rinsed with and without ozone water when compared at the same frozen storage period. This indicates that the ozone treatments used in this study did not induce detectable polypeptide chain breakage or RS-SR cross-linking. Zhang et al. [9] compared the effects of two manners of ozone treatments (washing with ozonized water and

ozone-flotation) and various treatment times on myofibrillar proteins from bighead carp. They found that protein patterns among all samples did not behave differently under ozone water rinsing of different time. However, densities of bands with molecular weights between 80 and 200 kDa clearly increased with ozone-flotation time. Results in this study confirmed, once again, that rinsing silver carp myofibrillar proteins with 8 mg/L ozone water for 10 min was a mild oxidation process.

3.5. Gel Texture. Gel-forming ability is an important index for surimi quality. The integrity of myofibrillar proteins is essential to form a strong gel. Breaking force and penetration distance of silver carp surimi gels with and without ozone treatments decreased ($P < 0.05$) gradually during frozen storage (Figure 5). After 90 d of frozen storage, breaking force and penetration distance significantly ($P < 0.05$) declined by 7–18% and 13–21%, respectively. The decrease

FIGURE 5: Changes in breaking force (a) and penetration distance (b) of silver carp surimi gels during frozen storage. Light grey: two washing cycles using ice water only (Tc); dark grey: first and second washing cycle using ice water containing 8 mg/L ozone and ice water, respectively (To1); black: first and second washing cycle using ice water and ice water containing 8 mg/L ozone, respectively (To2). Different letters indicate significant difference among samples (Tc, To1, and To2).

in textural values was in accordance with decreased Ca^{2+} -ATPase activity (Figure 2), which can be used as an indicator for the integrity of the myosin molecules.

Before storage, breaking force and penetration distance of samples with ozone rinsing (To1 and To2) were significantly lower ($P < 0.05$) than Tc, which was consistent with results reported by Xie et al. [12]. Myofibrillar proteins unfold and then aggregate to form three-dimensional gel networks through intermolecular interactions (hydrophobic interactions, disulfide covalent bonds, ionic bonds, etc.) of exposed functional groups. Conversion of sulfhydryl groups into disulfide covalent bonds before the myofibrillar proteins are well unfolded may result in a weak gel [12]. Breaking force of To1 was significantly lower ($P < 0.05$) than that of Tc during frozen storage. However, breaking force of To2 after 15 d was not significantly ($P > 0.05$) different from that of Tc.

3.6. Whiteness. Whiteness is an important factor affecting customer acceptability of the end surimi products. As shown in Figure 6, whiteness of surimi rinsed with ozone water (To1 and To2) was significantly higher than that of Tc, which could be attributed to the bleaching function of ozone. During ozone water rinsing, the porphyrin structure of the heme pigment is destroyed and consequently discolored [26]. To2 showed a better effect on increasing whiteness than To1. In To2, lipids and heme pigments (mainly myoglobin and hemoglobin) were partially removed after the first cycle of rinsing. Thus, ozone more effectively discolored the reduced amount of remaining pigments.

The whiteness of all samples (Tc, To1, and To2) continuously increased as frozen storage time increased. The decrease of gel-forming ability during frozen storage (Figure 5) might contribute to increased free water contained in the surimi gels, which led to increased reflectivity on the surface of

FIGURE 6: Changes in whiteness of silver carp surimi gel during frozen storage. Light grey: two washing cycles using ice water only (Tc), dark grey: first and second washing cycle using ice water containing 8 mg/L ozone and ice water, respectively (To1); black: first and second washing cycle using ice water and ice water containing 8 mg/L ozone, respectively (To2). Different letters indicate significant difference among samples (Tc, To1, and To2).

cooked gels and resulted in “whitening” of the proteins. However, Benjakul et al. [16] reported that whiteness of surimi made from four kinds of fish species harvested in Thailand gradually decreased with increased frozen storage time. This might be due to different surimi processing methods. In their study, whole fish were subjected to different periods of frozen storage prior to being manufactured into surimi. Denaturation of heme proteins during frozen storage

can result in their irreversible binding to myofibrillar proteins and thus decreased whiteness of surimi [26].

4. Conclusion

The results demonstrated that physicochemical properties of myofibrillar proteins from silver carp surimi during frozen storage were affected by the ozone treatment protocol (Tc, To1, or To2). As compared to only water rinsing (Tc), addition of 8 mg/L ozone in the first cycle of rinsing (To1) enhanced oxidation and denaturation of myofibrillar proteins during frozen storage, resulting in a gel with lower breaking force. Addition of ozone of the same concentration in the second cycle of rinsing (To2) minimally affected the physicochemical properties of myofibrillar proteins, including free sulfhydryl content, Ca^{2+} -ATPase activity, surface hydrophobicity, and gel textural values. In addition To2 treatment significantly increased whiteness of the surimi gel. Addition of ozone in the second rinse cycle is therefore a promising technology to upgrade freshwater fish surimi in color without negatively affecting gelation properties or gel texture.

Additional Points

Practical Applications. Ozone has regulatory approval and is recognized as being environmentally friendly; therefore there is great potential to use ozone in aquatic processing industries. The present results indicated that applying ozone treatment (8 mg/L and 10 min) in the second cycle of rinsing minimally affected the physicochemical properties of myofibrillar proteins during frozen storage. This study provides scientific evidence for using ozone treatment as a mild oxidation treatment to improve the white color of silver carp surimi without negatively affecting gel texture.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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