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Research Article

Effects of Micron Fish Bone with Different Particle Size on the Properties of Silver Carp *(Hypophthalmichthys molitrix)* Surimi Gels

Tao Yin,[1,2,3] Jae W. Park,[3,4] and Shanbai Xiong[1,2]

[1]*College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei Province 430070, China*
[2]*National R&D Branch Center for Conventional Freshwater Fish Processing, Wuhan 430070, China*
[3]*Oregon State University Seafood Research and Education Center, 2001 Marine Drive, Room 253, Astoria, OR 97103, USA*
[4]*Department of Food Bioscience and Technology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, Republic of Korea*

Correspondence should be addressed to Shanbai Xiong; xiongsb@mail.hzau.edu.cn

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Qualities of silver carp surimi (SCS) gels incorporated with micron fish bone of different particle size (22 to 0.12 μm) were evaluated. Textural values, whiteness, and water holding capacity of the SCS gels with setting significantly increased ($P < 0.05$) as the micron fish bone particle size decreased. As the particle size decreased, more calcium ion was apparently released from the fish bone ($P < 0.05$). Consequently the released calcium ion increased the activity of endogenous transglutaminase (TGase) and resulted in the formation of more myosin heavy chain (MHC) cross-links in the SCS gel with setting. Fish bone with particle size below 0.48 μm was steadily trapped in the three-dimensional SCS gel network without disrupting the matrices. Results indicated that size reduction of the incorporated micron fish bone improved qualities of the SCS gel with setting by the means of releasing more calcium ion and maintaining better gel matrices.

1. Introduction

Fish bone in some cuisines or processed products is traditionally eaten and regarded as an important calcium sources in Southeast Asia [1]. Fish bone is rich in calcium (234 g/kg dry bone), which is mainly in the form of hydroxyapatite (HA) and calcium carbonate [2]. However, so far fish bone generated from production process of numerous aquatic products (fillet and surimi, etc.) is conventionally used for fishmeal and fertilizer production or directly discarded into the sea, river, and estuaries, resulting in environmental pollution. The fish bone can be converted into nutritive foods or additives for humans by reducing its particle size. According to the reports, micron fish bone powders with a minimum average particle size of 7.65 and 1.75 μm were prepared using superfine grinding and dry media milling, respectively [3, 4]. Particle size of fish bone was further reduced to submicron range (1 to 0.1 μm) using high-energy wet media milling [5]. Consequently, there was about an eightfold increase in calcium release as the particle size decreased from micron to submicron range [5, 6]. Size reduction improved properties of fish bone particle in solubility, water holding capacity [3], calcium bioavailability [7], and sensory quality (i.e., grittiness) as well. Recently, researchers have paid attention to developing calcium-fortified food products using the downsized fish bone [8, 9].

Surimi is an intermediate product of the concentrated myofibrillar proteins produced through several times of washing and dewatering which inevitably remove the majority of minerals contained in the original fish flesh. Gelling of the myofibrillar proteins is a vital process of forming desired texture for surimi products. During gelling process, functional groups imbedded inside of the protein molecular are exposed, which subsequently form intra and/or intermolecular bonds, resulting in a three-dimensional gel network [10]. Addition of calcium compounds has been reported to improve gel functionality of surimi [11–13]. Generally, calcium ion released from those compounds induces

endogenous transglutaminase (TGase), which catalyzes the formation of ε-(γ-glutamyl) lysine cross-links (isopeptide covalent bonds) between myofibrillar proteins during setting, and thus improves the texture of surimi gel [10]. Furthermore, calcium ion enhances the unfolding of myosin and forms "calcium bridge" among the negatively charged myofibrillar proteins [10, 14], which may contribute to the improvement of gel texture as well.

Silver carp is one of the main aquacultured freshwater fish in China with a total production of 4.23 million t in 2014 [15]. In recent years, surimi production from marine fish in China has been limited possibly due to overfishing. The Chinese surimi producers have compensated for the shortage of marine fish by using silver carp. The production of surimi from silver carp was estimated at around 30,000 t in 2013 [16] and has grown fast; reaching over 40,000 t in 2015 [17]. Calcium compounds from fish bone with a particle size of 0.28 μm have been reported to improve gel texture of Alaska pollock surimi [18]. According to the reports, impacts of adding calcium compounds on gel properties vary to fish species used for preparing surimi [13, 19]. Micron fish bone with an appropriate particle size may be used for texture enhancement of silver carp surimi products while providing additional dietary minerals. However, there have been no studies investigating the effect of micron fish bone on the gelation properties of silver carp surimi. The production cost, properties, and applications of fish bone products highly depend on its particle size. Therefore, the objective of this study is to investigate the effects of micron fish bone with different particle size on the qualities of silver carp surimi gels.

2. Material and Methods

2.1. Materials. Silver carp *(Hypophthalmichthys molitrix)* surimi (AAA grade) with cryoprotectants (6% sucrose and 0.3% sodium tripolyphosphate) was obtained from a local surimi plant (Jingli Aquatic Product Co., Ltd., Honghu, China). Frozen surimi was cut into about 800 g blocks, vacuum-packaged, and stored in a freezer (−18°C) throughout the experiments. Silver carp backbone was collected after the deboning process of surimi production. It was cleaned and stored in a freezer (−18°C) before use.

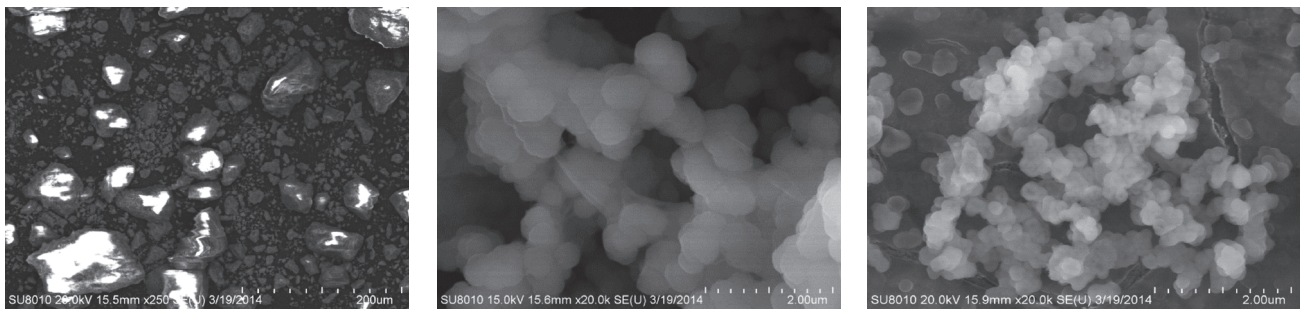
N,N′-Dimethylated casein (DMC), monodansylcadaverine (MDC), and glutaraldehyde used for TGase activity test were purchased from Sigma Chemical Company (St. Louis, MO, USA). Reagents used for gel electrophoresis were obtained from Bio-Rad (Hercules, CA, USA). All other chemicals were of analytical grade.

2.2. Preparation of Micron Fish Bone (MFB). Frozen fish bone was thawed with running tap water and then heated at 121°C for 60 min (ZM-100, GBPI Packaging Test Instruments Co., Ltd., Guangzhou, China). The heated fish bone was rinsed with tap water 5 times and drained off before grinding (MKCA6-2, Masuko Co., Tokyo, Japan). The bone paste was dried at 105°C for 6 h and then coarsely milled (RT-08HK, Kaichuangtonghe Technology Development Co., Ltd., Beijing China). Average particle size of the obtained fish bone power was determined to be about 22 μm using a Mastersizer 2000 analyzer (Malvern Instruments Ltd., Worcestershire, UK). The fish bone powder was mixed with deionized water (DI) and further diminished using a high-energy wet bead mill (MiniZeta 03, Netzsch, Selb, Germany) according to the method as described by Yin et al. [5]. Average size of the fish bone particles in the emulsion after milling for 1, 2, 4, and 6 h was about 0.48, 0.30, 0.18, and 0.12 μm, respectively, which were analyzed using a Nano ZS90 analyzer (Malvern Instruments Ltd., Worcestershire, UK). Morphologies of the fish bone particles observed using field emission scanning electron microscope (ULTRA PLUS-43-13, Zeiss, Germany) were shown in Figure 1.

2.3. Preparation of Surimi Gel. The vacuum-packaged frozen surimi was tempered at room temperature for 40 min before being cut into approximately 2 cm × 2 cm × 4 cm cubes. Surimi 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 protein. Fish bone of different size (22, 0.48, 0.30, 0.18, and 0.12 μm), at 1 g dried fish bone/100 g surimi paste, was added. 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 stuffed into a polyethylene sausage casing (2.5 cm diameter) with one end presealed using a sausage stuffer (Tre-mss7kh, Trs Spade, Italy). After stuffing the other end was sealed with U-shape aluminum wire clips using a clipper (Hk12, Hakanson, Sweden). The samples were heated with two different thermal treatments: (1) 90°C for 30 min (direct cooking); (2) 40°C for 1 h setting followed by 90°C cooking for 30 min. Cooked gels were submerged in ice water for 15 min and stored overnight in a refrigerator (4°C).

2.4. Determination of Gel Strength. Gel strength of the silver carp surimi gels incorporated with MFB of different particle sizes was determined by the method as described by Cao et al. [20]. The chilled surimi gels were equilibrated at room temperature (~25°C) for 2 h. Samples were cut to 2.5 cm cylinder and subjected to fracture by penetration 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). Breaking force (g) indicating gel strength and penetration distance (mm) denoting deformability were recorded.

2.5. Determination of Calcium Ion Concentration. Calcium ion concentration in the surimi paste incorporated with MFB of different particle sizes was measured according to the method as described by Yin et al. [5] with some modification. Surimi paste prepared as described above was added with 4 times the volumes of DI water and homogenized at 5,000 rpm for 1 min (IKA T18, Cole-Parmer, Shanghai, China). The homogenate was then centrifuged at 10,000 ×g for 30 min (J-26XP, Beckman Coulter Inc., Fullerton, CA, USA). After centrifugation, the supernatant was filtrated (Number 1



(a) (b) (c)

Figure 1: Morphologies of fish bone particles. (a) Micro fish bone powder, (b) micron fish bone particles in the emulsion with 1 h of high-energy wet media milling, and (c) micron fish bone particles in emulsion with 6 h of milling.

Waterman, Xinhua, Filter Paper Co., Ltd., Hangzhou, China) and diluted with DI water. The concentration of calcium ion in the dilution was analyzed using an atomic absorption spectrophotometer (AA-6300c, Shimadzu, Kyoto, Japan).

2.6. Determination of TGase Activity. TGase activity was measured by the method of Yin and Park [18] with slight modifications. Silver carp surimi was added with 4 volumes of extraction buffer (10 mM NaCl and 10 mM Tris-HCl, pH 7.5) and homogenized (Ika T18, Cole-parmer, Co., Ltd., Shanghai, China) at 5,000 rpm for 1 min. The homogenate was centrifuged (J-26XP, Beckman Coulter Inc., Fullerton, CA, USA) at 16,000 ×g for 30 min under 4°C. The supernatant was filtrated and used as crude extract. Fish bone emulsion prepared as described above was centrifuged at 10,000 ×g for 30 min (Beckman Coulter Inc., Fullerton, CA, USA) and filtrated. Filtrate was added to an assay mixture (15 μM MDC, 1.0 mg/mL DMC, 3 mM DDT, and 50 mM Tris-HCl, pH 7.5) at a volumetric ration of 1 to 4. For the control sample, calcium chloride was added to the assay mixture and reached a concentration of 0.17 mM. The crude enzyme was added and vortexed. After incubating the mixture at 40°C for 10 min, EDTA solution was added to terminate the catalytic reaction. Fluorescence intensity of the mixture was immediately measured (RF-1501, Shimadzu Co., Kyoto, Japan).

2.7. SDS-PAGE. Protein patterns of all surimi gel samples were revealed using SDS-PAGE according to Laemmli [21] with some modification. Surimi gel samples were 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). Stacking and separating gels were made using 5% (w/v) and 12% (w/v) acrylamide, respectively. Each lane was loaded with 10 μg 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. Scanning Electron Microscopy (SEM). Surimi gel was cut into pieces (5 mm × 5 mm × 1 mm) and fixed with 2.5% glutaraldehyde in 0.2 M phosphate (pH 7.2) for 2 h at room temperature. The sample was rinsed three times using 0.2 M phosphate (pH 7.2). The fixed sample was dehydrated in graded ethanol solution with serial concentrations of 30%, 50%, 70%, 80% 95%, and 100%. Samples were submerged in acetic acid isopropyl ester (substituting ethanol) and then critical-point-dried (HCP-2, Hitachi Koki Co., Ltd., Tokyo, Japan) using CO_2 as the transition fluid. Dried sample was mounted on a bronze stub and sputter-coated with gold. The specimen was observed using a scanning electron microscope (Quanta 3D Dual Beam, FEI Co., Tokyo, Japan) at an acceleration voltage of 15 kV.

2.9. Determination of Water Holding Capacity (WHC). WHC of gel sample was measured according to the method of Shi et al. [22]. Cylindrical gel samples were cut into a thickness of about 5 mm, weighed accurately, and placed between two layers of filter paper (Number 1 Waterman paper, Xinhua, Filter Paper Co., Ltd., Hangzhou, China). Sample was then placed at the bottom of a centrifuge tube (50 mL) and centrifuged at 3,000 ×g for 15 min (TDL-5A, Fulgor Instruments Ltd., Shanghai, China). WHC was calculated as percentage of water retained after centrifugation.

2.10. Color Evaluation. Color parameters, L^* (lightness), a^* (redness to greenness), and b^* (yellowness to blueness), were measured using a CR-400 colorimeter (Konica Minolta, Osaka, Japan). Whiteness was calculated according to an equation developed by Park [23] for surimi gel.

2.11. Statistical Analysis. All the data were obtained from at least triplicate measurements. Analysis of variance (ANOVA) was carried out 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. Results and Discussion

3.1. Gel Texture. Effects of added MFB with different particle size on breaking force and penetration distance of silver carp surimi gels prepared with two thermal treatments are illustrated in Figure 2. Breaking force and penetration

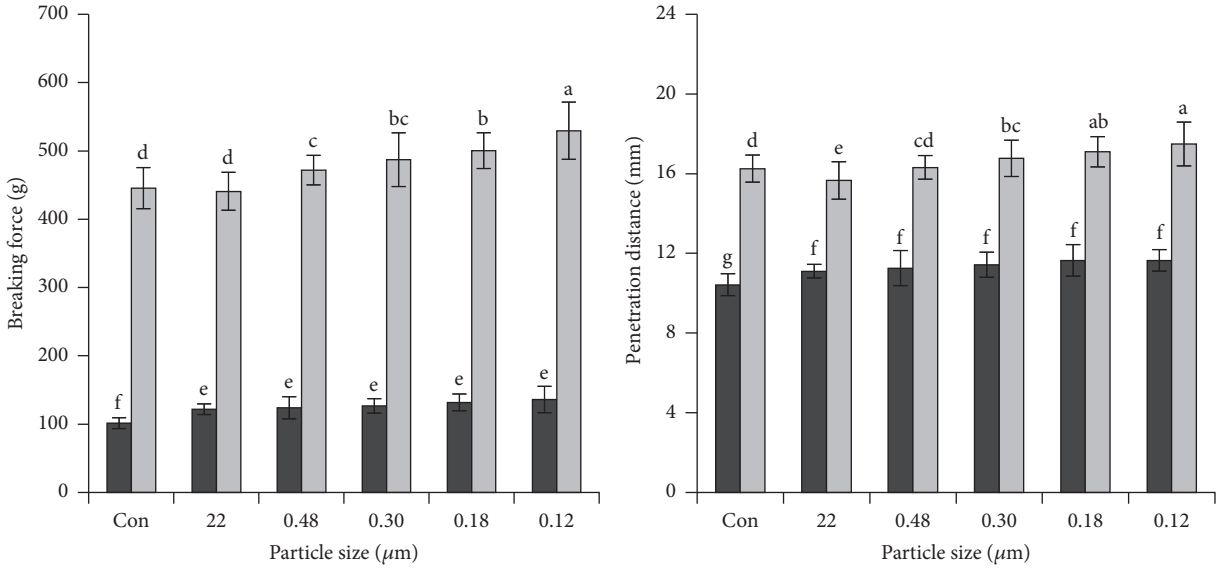


Figure 2: Breaking force and penetration distance of surimi gels with different size of fish bone particles and thermal treatments. Con: control sample, without added fish bone. Black bars: gel cooked at 90°C for 30 min. Grey bars: gel incubated at 40°C for 1 h followed by 90°C cooking for 30 min. Different lowercases above the error bar indicate significant differences among samples with fish bone of different particle size ($P < 0.05$).

distance of directly cooked gel (90°C/30 min) containing MFB were ($P < 0.05$) higher than the control. When gels were prepared with setting before cooking (40°C/1 h + 90°C/30 min), MFB with an average particle size between 0.48 and 0.12 μm significantly ($P < 0.05$) increased breaking force and penetration distance. However, MFB with an average particle size of 22 μm had no effect ($P > 0.05$) on breaking force while significantly ($P < 0.05$) decreasing penetration distance.

Breaking force and penetration distance of gels with setting increased as MFB particle size decreased ($P < 0.05$). More calcium ions released from smaller fish bone particles increased the activity of TGase in silver carp surimi (Figure 3). Increased calcium ion release contributed to the formation of MHC cross-links in the surimi gel (Figure 4). In addition to being an endogenous TGase activator, calcium ions, in conjunction with setting (40°C for 1 h), possibly enhanced the unfolding of silver carp myofibrillar proteins. Consequently, more exposure of the reactive residues imbedded inside the myofibrillar proteins might contribute to the formation of more ε-(γ-glutamyl) lysine cross-links and a higher degree of hydrophobic interactions [14]. On the other side, reduction of fish bone particle size contributed positively to maintaining better surimi gel matrices (Figure 5). The addition of 1% MFB with an average particle size at 0.12 μm resulted in increased breaking force and penetration distance of gel with setting by approximately 19% and 8%, respectively, over the control (without added fish bone). The effectiveness of MFB (0.28 μm) addition on improvement of breaking force and penetration distance was more pronounced with Alaska pollock surimi gel (25% and 14%) [18]. It might be related to different endogenous TGase activity, myosin reactivity, and endogenous calcium content from different

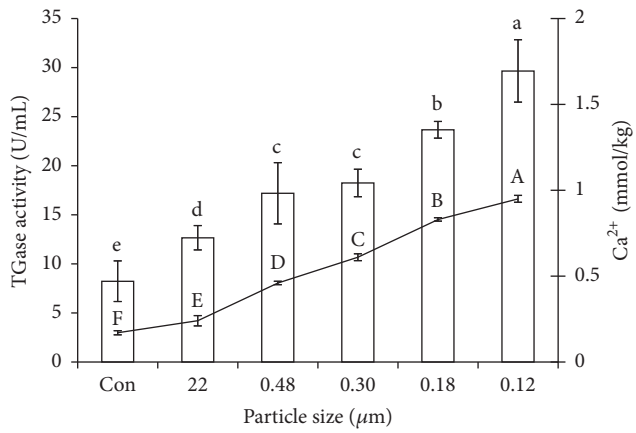


Figure 3: Endogenous TGase activity and calcium ion concentration from surimi paste as affected by added fish bone particle size. Column: TGase activity; line: calcium ion concentration. Con: control sample, without added fish bone. Different letters above the error bar indicate significant differences among samples with fish bone of different particle size ($P < 0.05$).

fish species. Compared to silver carp, myosin from Alaska pollock is reported to be more reactive and tends to form larger polymers during cross-linking reaction [10].

Breaking force and penetration distance of surimi gel without setting increased gradually but not significantly ($P > 0.05$) as MFB particle size decreased (Figure 2). This slight increase might have been attributed to the formation of a "calcium bridge" between negatively charged residues on two adjacent myofibrillar proteins. Generally, the strength of ionic bonds in surimi gel is much weaker than that of hydrophobic interactions and covalent bonds [10].

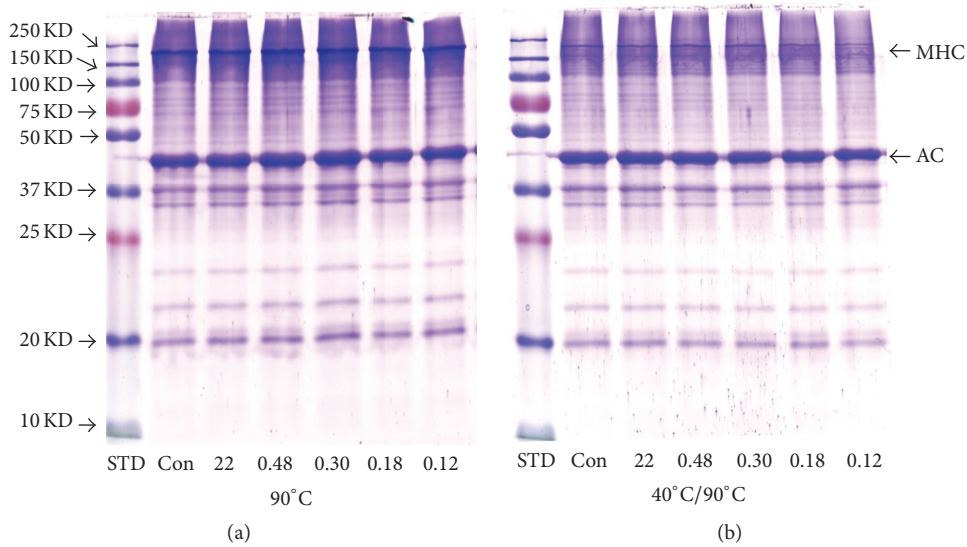


Figure 4: SDS-PAGE patterns of silver carp surimi gels with different size of fish bone particles and thermal treatments. (a) Gel cooked at 90°C for 30 min; (b) gel incubated at 40°C for 1 h followed by 90°C cooking for 30 min. Numbers designate average fish bone particle size (μm). STD: protein standard. Con: control sample, without added fish bone. MHC: myosin heavy chain. AC: actin.

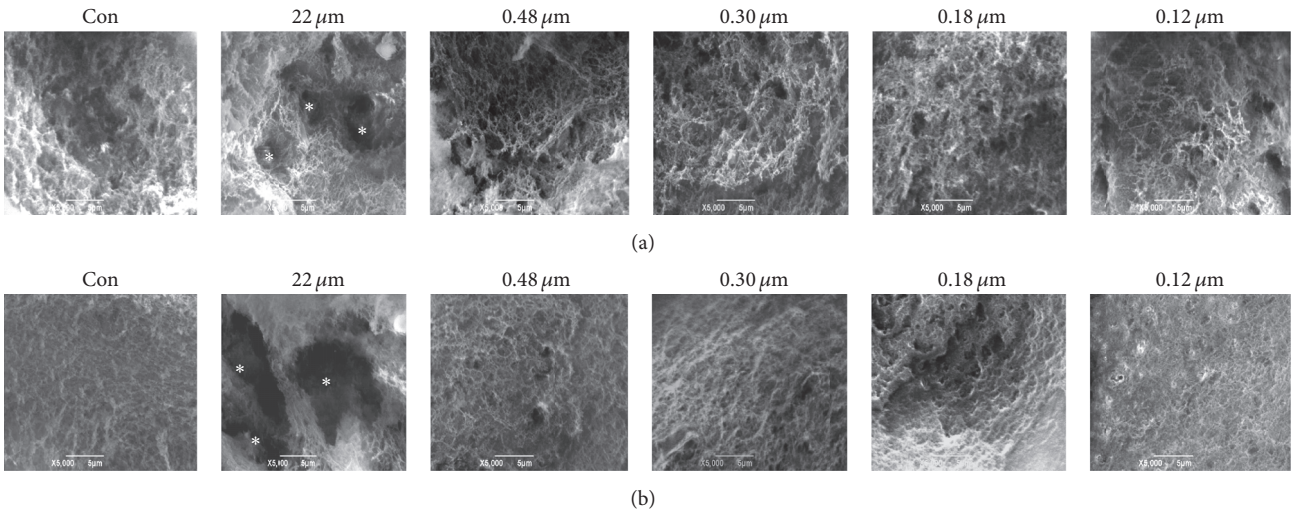


Figure 5: SEM images of silver carp surimi gels with different size of fish bone particles and thermal treatments. (a) Gel cooked at 90°C for 30 min; (b) gel incubated at 40°C for 1 h followed by 90°C cooking for 30 min. Con: control sample, without added fish bone. Numbers designate average size of fish bone particles.

Results suggested that effects of MFB on surimi gel texture might vary by fish bone particle size, thermal treatments, and fish species.

3.2. TGase Activity. As shown in Figure 3, addition of micron fish bone obviously activated TGase from silver carp surimi. It was consistent with the reports by Yin and Park [18] and Hemung [24]. Activity of crude TGase extracted from silver carp surimi without added fish bone was 8.23 U/mL extract at 40°C. It significantly ($P < 0.05$) increased as the added fish bone particle size decreased. Activities of crude TGase extract incubated in assay with fish bone particle size at 22, 0.48, 0.30, 0.18, and 0.12 μm were 12.66, 17.19, 18.23, 23.66, and 29.66 U/mL, respectively. Increased activity of endogenous TGase with decreased fish bone particle size was due to the release of more calcium ions in the surimi paste (Figure 3). Calcium compounds in the fish bone possess low solubility. Furthermore, they are imbedded in the collagen matrix, which makes them even harder to dissolve in water. During the wet milling process, specific surface area of fish bone particle markedly increases, and the collagen matrix is destroyed, facilitating the release of calcium ion [6]. The

calcium ion concentration in the surimi paste without fish bone was 0.17 mmol/kg. Calcium ion concentration significantly increased with decreasing of fish bone particle size and reached the maximal at 0.95 mmol/kg. Optimal calcium ion concentration for full activation of endogenous TGase extracted from carp, however, depends on the purification procedure. It has been reported that optimal calcium ion concentrations for crude TGase extract and purified TGase from carp were at 5 mM and 50 mM, respectively [25, 26].

3.3. MHC Cross-Linking. Influence of fish bone particle size on the cross-linking of MHC during gel formation was analyzed using SDS-PAGE. Compared to gel without setting (Figure 4(a)), MHC of silver carp surimi gel markedly disappeared after setting (Figure 4(b)). Reduction of MHC after setting could be attributed to the formation of ε-(γ-glutamyl) lysine cross-links and/or proteolytic degradation. Ogata et al. [27] reported that the degradation of MHC was observed in the carp surimi sample incubated at 37°C for 10 min with addition of endogenous protease (cathepsin L) and accompanied by the appearance of the resultant product, which had a molecular weight of ~27 KD. No obviously visible band with a molecular weight of ~27 KD was detected on the SDS-PAGE after setting (Figure 4), implying that the reduction of MHC in this study was mainly related to the cross-linking reaction.

Gradual reduction of MHC from the surimi gel with setting was observed as MFB particle size decreased due to the activation of endogenous TGase (Figure 3), resulting in the formation of more ε-(γ-glutamyl) lysine cross-links of MHC. MHC intensity of gel without setting remained constant as the particle size of fish bone changed. These results were in agreement with the report by Wang et al. [28] that the intensities of MHC cross-links from silver carp surimi incubated at 35°C for various time significantly increased with calcium ion concentration up to 1.80 mmol/kg surimi paste. However, MHC cross-links of gel without setting were not significantly affected by calcium ion concentration ranging from 0 to 5.40 mmol/kg surimi paste. Changes of MHC on the SDS-PAGE gel corresponded well with the changes of gel texture values (Figure 2).

The significant reduction of MHC band's intensity was noted when setting was employed; neither MHC cross-links nor protease-induced small molecular bands were present (Figure 4). As discussed above, no visible band for protease-induced ~27 kDa [27] was observed. MHC cross-links, which are not shown above MHC on the SDS-PAGE, possibly could not enter the polyacrylamide gel system because the size of cross-links was too large.

3.4. Microstructure. Figure 5 shows scanning electron microscopy (SEM) images of the internal structures of surimi gels added with different particle size of MFB and prepared with two thermal treatments. Fibrous matrix, which is a characteristic of heat-induced protein gel, was clearly observed in the surimi gels. Control gels (without added fish bone) and gels with MFB of particle size between 0.48 and 0.12 μm formed continuous structures (Figure 5), while surimi with MFB particles size of 22 μm formed discontinuous structures with large pores (asterisks in Figure 5). In contrast to the porous and coarse networks of directly heated gel (Figure 5(a)), networks of surimi gel prepared with two-step heating (setting and cooking) were denser and more compact along with a concomitant appearance of homogeneous surface (Figure 5(b)). Heat-denatured surimi proteins align in an ordered fashion to develop a fine gel network when rapidly unfolded proteins are associated in slow heating fashion (i.e., setting) [29]. In addition, the formation of larger amount of ε-(γ-glutamyl) lysine cross-links after setting contributed to the stabilization of the gel network.

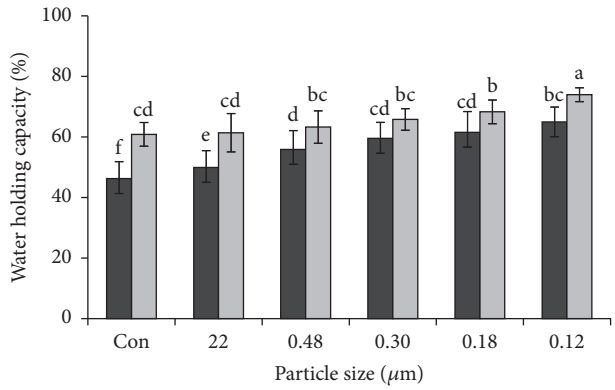


Figure 6: Water holding capacity of the surimi gels with different size of fish bone particles and thermal treatments. Black bars: gel cooked at 90°C for 30 min. Grey bars: gel incubated at 40°C for 1 h followed by 90°C cooking for 30 min. Different lowercases indicate significant differences among different treatments ($P < 0.05$).

3.5. Water Holding Capacity (WHC). Higher WHC values indicate less expressible water in the surimi gel. During the heat-induced gelling process, the gel network formed while binding water and entrapping other ingredients. WHC of the gels prepared with setting was significantly ($P < 0.05$) higher than that of gel without setting for all particle sizes (Figure 6) as setting induced more compact and denser gel networks by the function of endogenous TGase (Figure 3). WHC increased from 46.20% to 64.96% and 60.84% to 73.93% for the samples without and with setting, respectively, as MFB particle size decreased from 22 μm to 0.12 μm. It might be attributed to the increased WHC contributed by fish bone addition [3]. As mud obviously holds more water than sand, the size of particle is a significant factor affecting WHC.

3.6. Color Attributes. Color parameters of gels from silver carp under different thermal treatments with various fish bone particle size are shown in Table 1. L^* value of control gel and gel with MFB of particle size below 0.48 μm slightly but significantly ($P < 0.05$) increased after setting while that of gel with MFB of particle size at 22 μm slightly decreased ($P < 0.05$). Yellowness value ($+b^*$) decreased ($P < 0.05$) and whiteness value ($L^* - 3b$) increased ($P < 0.05$) after setting for all fish bone particle sizes. L^* values of gels with MFB of particle size below 0.48 μm, regardless of setting,

Table 1: Color parameters (lightness (L), greenness (a^*), yellowness (b^*), and whiteness (w)) of silver carp surimi gels with different size of fish bone particles (μm) and thermal treatments.

Color parameter		L	a^*	b^*	w
Without setting	Con	74.27 ± 0.49^{g}	-2.05 ± 0.07^{a}	2.44 ± 0.21^{g}	66.95 ± 0.60^{b}
	22	74.79 ± 0.65^{f}	-2.71 ± 0.09^{cd}	9.06 ± 0.43^{a}	47.60 ± 1.28^{i}
	0.48	76.52 ± 0.48^{a}	-2.67 ± 0.06^{c}	8.26 ± 0.21^{b}	51.74 ± 0.54^{g}
	0.30	76.35 ± 0.23^{ab}	-2.74 ± 0.45^{de}	7.81 ± 0.20^{c}	52.93 ± 0.61^{f}
	0.18	76.10 ± 0.35^{bc}	-2.81 ± 0.03^{f}	7.78 ± 0.37^{c}	52.74 ± 0.93^{f}
	0.12	75.85 ± 0.43^{cd}	-2.80 ± 0.03^{ef}	7.36 ± 0.33^{d}	53.77 ± 0.90^{e}
With setting	Con	75.49 ± 0.47^{de}	-2.36 ± 0.04^{b}	1.91 ± 0.26^{h}	69.76 ± 0.58^{a}
	22	75.11 ± 0.51^{ef}	-2.94 ± 0.07^{h}	8.27 ± 0.18^{b}	50.30 ± 0.32^{h}
	0.48	76.27 ± 0.48^{ab}	-2.88 ± 0.05^{g}	7.53 ± 0.26^{d}	53.68 ± 0.59^{e}
	0.30	75.81 ± 0.46^{cd}	-3.03 ± 0.05^{i}	7.31 ± 0.26^{d}	53.88 ± 0.67^{e}
	0.18	75.70 ± 0.29^{cd}	-3.10 ± 0.05^{j}	6.85 ± 0.32^{e}	55.13 ± 0.93^{d}
	0.12	75.59 ± 0.27^{d}	-3.09 ± 0.05^{j}	6.36 ± 0.22^{f}	56.50 ± 0.56^{c}

The different lowercases in the same column indicate the significant differences ($P < 0.05$). Data are expressed as means ± standard deviations.

were significantly ($P < 0.05$) higher than that of gel with MFB of particle size at 22 μm. As particle size decreased, L^* values of gels with MFB of particle size below 0.48 μm, regardless of setting, gradually decreased ($P < 0.05$). This might be due to the decreased light scattering effect of water molecular as a result of increased WHC of the gel (Figure 6). Yellowness value of gels with and without setting decreased gradually ($P < 0.05$) and whiteness value significantly ($P < 0.05$) increased with decreasing MFB particle size. Thus, both thermal treatment and fish bone particle size affected color attributes of silver carp surimi gels.

4. Conclusions

Gel properties of silver carp surimi were significantly affected by thermal treatments and MFB particle size. Textural values, WHC, and whiteness of gels prepared with setting increased ($P < 0.05$) as the MFB particle size decreased. Improvement of gel texture and WHC resulted from reduced bone particle size which was primarily due to the function of endogenous TGase and calcium ion from the MFB. In addition, reduction of MFB particle size contributed positively to maintaining integrity of the three-dimensional myofibrillar gel networks. MFB with an average particle size below 0.48 μm possesses the potential to be used to improve gel quality while providing additional dietary minerals for silver carp surimi products.

Additional Points

Practical Applications. Fish bone is a natural calcium source. Fish bone incorporated into silver carp surimi gels exhibited different properties, which were affected by its particle size and heating methods subjected to surimi paste. Improvement of the surimi gel texture was achieved by addition of the fish bone with particle size below 0.48 μm when combined with setting. The practical application of this work is providing a theoretical foundation and basic data support for using calcium-enriched fish bone to improve gel texture while providing additional dietary calcium for silver carp surimi products.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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