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Gelling properties of surimi as affected by the particle size of fish bone

Running Head: Surimi Gels by Nano/Micro Fish Bone

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ABSTRACT

The effects of fish bone with two different particle sizes (micro and nano) on Alaska pollock surimi gels prepared by two heating procedures were investigated. Heating procedures (with or without setting) resulted in significantly different gel texture values. Nano-scaled fish bone (NFB) effectively increased gel breaking force and penetration distance (up to 1 g/100 g) while micro-scaled fish bone (MFB) did not. Endogenous transglutaminase (TGase) activity of surimi paste increased obviously as the concentration of NFB increased, indicating calcium ions readily released from NFB and assisting gel formation through TGase-induced covalent bonds. With MFB, TGase activity increased slightly, but not significantly. Scanning electron microscopy (SEM) results revealed NFB was capable of being imbedded in the gel matrices without disrupting the myofibrillar gel network. Surimi with MFB formed a discontinuous and porous network with pores near the size of MFB. Lightness (L*) and whiteness (L*-3b*) of NFB gels were higher than those of MFB.

Keywords: surimi, nano-scaled fish bone, particle size, transglutaminase, microstructure

1. Introduction

Commercial fisheries and aquaculture supplied the world with approximately 148 million tons of fish in 2010, of which over 20 million tons was discarded as processing leftovers, including trimming, fins, bones, head, skin, and viscera (FAOSTAT, 2012). Despite increasing efforts to obtain new products from this waste, the majority is still used for fishmeal (Péron, Mittaine, & Gallic, 2010). In an attempt to make fisheries more environmentally sustainable, efforts should be focused on converting and utilizing a higher proportion of the waste material for value added products. Researchers have been investigating by-products from fish waste such as collagen, oil, carbohydrates, and nucleic acids, to improve their functional properties (Hughes et al., 2012; Jongjareonrak et al., 2010; Kim & Mendis, 2006). Fish bones are the main solid by-product from fillet and surimi processing. Bones account for 10-15 g/100 g fish weight and are a potential source of low priced calcium. Calcium compounds from fish bone have been reported to possess high bioavailability (Malde et al., 2010). However, there are few studies on the utilization of fish bone as a natural calcium source in functional foods or as food supplements.

Setting (suwari in Japanese) is the phenomenon of gel formation in surimi paste subjected to incubation at either 25 or 40 °C depending on fish species (Niwa, 1992). The setting process is reported to be meditated by endogenous transglutaminase (TGase), which is capable of catalyzing acyl transfer reactions by introducing ε-(γ-glutamyl) lysine cross-links between proteins (Lanier,

Carvajal, & Yongsawatdigul, 2005). These cross-links play an important role in determining surimi gel texture. Endogenous TGase is a Ca^{2+}-dependent enzyme, and addition of calcium ion to fish protein paste has been confirmed to increase TGase activity, resulting in gel texture improvement (Yongsawatdigul, Worratao, & Park, 2002). Furthermore, calcium from fish bone has been reported to activate endogenous TGase (Hemung, 2013; Yin & Park, 2014). Thus, addition of fish bone powder into surimi seafood is likely to provide additional dietary calcium and improve gel texture. However, investigations on improving of surimi gel texture by addition of fish bone are rarely reported.

Particle size of fish bone is a vital factor to determine its properties and applications. Researches have shown that as the particle size of fish bone powder decreased it resulted in an increase in calcium ion release, fluidity, solubility, electric conductivity, and water holding capacity (Fan, Chen, Xiong, & Yang, 2008; Wu, Zhang, Wanga, Mothibe, & Chen, 2012). Grittiness of fish bone powder can be minimized by decreasing its particle size. According to our preliminary study, the inclusion of fish bone powder with a particle size below 150 μm into surimi gel did not give any negative sensory, i.e., grittiness.

Physicochemical properties of fish bone particle depend on its particle size. Our objective was to determine the effects of fish bone particle size (micro and nano) on the gelling properties of surimi.

2. Materials and Methods

2.1 Materials

Alaska pollock surimi (FA grade), stored at -18 °C for approximately 3 months, was obtained from American Seafoods (Seattle, WA, USA). Surimi was cut into about 1,000 g blocks, vacuum-packaged, and stored in a freezer (-18 °C) throughout the experiments. Dried fish bone made from Pacific whiting was obtained from Trident Seafoods (Newport, OR, USA). It was soaked in alkaline solution (pH 12) for 2 h and rinsed with tap water three times to remove myofibrillar protein and dried in an oven (105 °C) over night. Dried fish bone was ground using a silent cutter (UM 5 universal, Stephan Machinery Corp, Columbus, OH, USA) at 3,600 rpm. Micro-scaled fish bone (MFB) was obtained using a sieve with pores of diameter <150 μm and further processed to nano-scaled fish bone (NFB) emulsion by Custom Processing Services (Reading, PA, USA) using a wet mill (Labstar, Netzsch Premier Technologies, Co., Exton, PA, USA). D50 of the NFB particle was 280 nm, which was analyzed by Custom Processing Services (Reading, PA, USA) using a Laser particle size analyzer (LA-950 V2, Horiba Co., Kyoto, Japan). Calcium concentration of the emulsion was 32.4 mg/ g, which was analyzed by Universal Testing Company (Quincy, IL, USA) using inductively coupled plasma (ICP) spectrometry. The moisture content of the emulsion was 87.47 g/100 g (AOAC, 2000). N, N'-Dimethylated casein (DMC) and monodansylcadaverine (MDC) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Dithiothreitol (DTT) was purchased from Fluka (Buchs, Switzerland). All other chemicals used were of analytical grade.

Endogenous calcium ion concentration in Alaska pollock surimi was not counted in this experiment. However, Gordon and Roberts (1977) reported the calcium ion concentration in Alaska pollock flesh is 63 mg/100 g meat.

2.2 Gel preparation

Frozen surimi was partially thawed at room temperature for 1 h before being cut into approximately 3 cm cubes. Surimi cubes were chopped at 1,800 rpm for 1 min using a silent cutter (UM 5 universal, Stephan Machinery Corp, Columbus, OH, USA). Chopping continued at 1,800 rpm for 1 min with addition of sodium chloride (2 g/100 surimi paste) to extract myofibrillar proteins. NFB or MFB, at 0, 0.1, 0.25, 0.5, 1.0, and 2.0 g /100 g paste calculated based on dried fish bone, was added into the salted surimi. Moisture content was adjusted to 77 g/100 g paste using ice water (0 °C) and sugar before chopping at 1,800 rpm for another 1 min. Sugar was added to the treatments without or with reduced fish bone as an inert ingredient to substitute for fish bone and to maintain moisture content equally. For the last 3 min, chopping continued at 3,600 rpm while a vacuum was maintained at 0.5 - 0.6 bar. During chopping, cold temperature (< 5 °C) was maintained continuously using a NesLab chiller (NesLab, Portsmouth, NH, USA). The paste prepared above was packed into a polyethylene bag and subjected to a vacuum machine (Reiser VM-4142; Roescher Werke, Osnabrueck, Germany) to remove air pockets. The paste was stuffed into a nylon casing (Nylatron MC 907; Quadrant Engineering Plastic Products, Reading, PA, USA) with a 3 cm inner

diameter and approximately 15 cm length using a sausage stuffer (The Sausage Maker, Buffalo, NY, USA). The samples were then heated with two different thermal treatments, respectively: (1) 90 °C for 30 min; (2) 25 °C for 3 h setting (pre-incubation) followed by 90 °C heating for 30 min. Gels were submerged in ice water for 15 min after cooking, and stored overnight in a refrigerator (4 °C).

2.3 Fracture gel evaluation

Fracture gel evaluation was performed using a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a spherical plunger (diameter 5 mm, crosshead speed of 60 mm/min). Cold gels (4 °C) were placed at room temperature for 2 h prior to gel testing. Cylinder-shaped samples with a length of 2.5 cm were obtained and subjected to fracture evaluation. Breaking force (g) indicating gel strength and penetration distance (mm) denoting deformability, at gel fracture, were recorded. For each sample, at least 10 measurements were obtained.

2.4 TGase activity

TGase activity was analyzed by the method of Takagi, Saito, Kikuchi, and Inada (1986) with slight modifications. 3 g of surimi paste (with or without 3 h setting) prepared as described above was homogenized in 4 volumes of extraction buffer (10 mmol/ L NaCl and 10 mmol/ L Tris-HCl, pH 7.5). The homogenate was centrifuged at 16,000 × *g* (Sorvall, DuPont Co., Newton, CT) at

4 °C for 30 min. Supernatant was used as a crude extract. The assay mixture contained 1.0 mg/ ml N, N'-Dimethylated casein (DMC), 15 μmol/ L monodansylcadaverine (MDC), 3 mmol/ L dithiothreitol (DTT), 50 mmol/ L Tris-HCl (pH 7.5). The mixture was incubated at 25 °C for 5 min. Crude enzyme (100 μl) was added and further incubated at 25 °C for 10 min. After incubation, EDTA solution was added to a final concentration of 20 mmol/ L to stop the reaction. The fluorescence intensity was measured with excitation and emission wavelengths of 350 and 480 nm, respectively, using a Shimadzu spectrofluorometer (RF-1501; Shimadzu Co., Kyoto, Japan). The enhancing factor, indicating the degree of fluorescence enhancement of the dansyl group after incorporation into DMC, was determined from our study to be 1.66. One unit of TGase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of MDC into DMC per min. TGase activity was measured in triplicate and expressed as unit/ ml extract.

2.5 Scanning electron microscopy (SEM)

Sample for scanning was prepared according to the method of Liu, Zhao, Xiong, Xie, and Qin (2008). The cooked surimi gel was cut into 1–2 mm cubes, fixed in DI water with 2.5 g/100 g glutaraldehyde and 0.1 mol/ L Na phosphate (pH 7.3) at 4 °C overnight, and then washed three times using 0.1 mol/ L Na phosphate (pH 7.3). The post-fixation stage involved exposure to DI water with 1 g/100 g osmium tetroxide for 2 h followed by three further washes using 0.1

mol/ L Na phosphate (pH 7.3). The washed sample was dehydrated in DI water with 10 g/100 g dimethyl sulphoxide (DMSO) for 6 h and then freeze-dried. Dried sample was mounted on a bronze stub, and sputter-coated with gold. The specimen was observed with a scanning electron microscope (Quanta 3D Dual Beam, FEI Co., Tokyo, Japan) at an acceleration voltage of 10 kV.

2.6 Gel whiteness

Surimi gel color was determined according to a method described by Park (1994) using a CR-300 Minolta colorimeter (Osaka, Japan). For each sample, 6 measurements were obtained. L* (lightness), a* (redness to greenness) and b* (yellowness to blueness) were measured and whiteness was calculated as follows:

Whiteness = L*- 3b*

2.7 Statistical analysis

Analysis of variance (ANOVA) was analyzed using the SAS program (SAS Institute Inc, Carry, NC, USA). Differences among mean values were established using the Duncan multiple range test (DMRT) at $p < 0.05$.

3. Results and Discussion

3.1 Fracture gel properties

194 The breaking force and penetration distance of the Alaska pollock surimi gels
195 prepared by different thermal treatments with NFB or MFB at different
196 concentrations are illustrated in Fig. 1. Breaking force and penetration distance
197 of gels with setting at 25 °C for 3 h increased with NFB concentration ($P< 0.05$)
198 up to 1 g/100 g paste and then decreased. The addition of 1 g/100 g NFB
199 resulted in increased breaking force and penetration distance by approximately
200 31 % and 16 %, respectively, over the control (without fish bone). Breaking force
201 of gels with setting increased slightly with MFB concentration ($P< 0.05$) up to 0.5
202 g/100 g paste and then decreased, while penetration distance remained similar
203 to MFB concentration ($P>0.05$). Increasing texture values of gels prepared with
204 NFB addition and setting was thought primarily related to endogenous TGase
205 and calcium ion from NFB. NFB calcium ion affected the activity of endogenous
206 TGase (Fig. 2). The formation of myosin heavy chain (MHC) cross-links through
207 ε-(γ-glutamyl) lysine covalent bond after setting with NFB was confirmed by our
208 previous study (Yin & Park, 2014). Furthermore, addition of NFB containing
209 calcium ions in conjunction with setting might induce the unfolded myosin which
210 favored a TGase-mediated reaction and promoted a higher degree of
211 hydrophobic interaction (Hemung & Yongsawatdigul, 2005; Yongsawatdigul &
212 Sinsuwan, 2007). Both ε-(γ-glutamyl) lysine covalent bond and hydrophobic
213 interactions might be enhanced by addition of NFB calcium ion, consequently
214 increased texture values of surimi gels. Enhancement of gel texture was
215 significantly higher by NFB than MFB (Fig. 1). This was attributed to higher free

calcium ion concentration of NFB compared to that of MFB. MFB had a negative effect on surimi gel's microstructure due to its large particle size (Fig. 3), resulting in weaker gels (Fig. 1). MFB, a relatively large particle, likely disconnected myofibrillar protein's gel network while NFB was small enough to be imbedded finely without impeding the gel network.

Breaking force and penetration distance of gels without setting increased gradually with NFB concentration ($p<0.05$). This may be due to the combined effects of ionic bonds and TGase-mediated covalent bonds. Myofibrillar proteins carry an overall net negative charge at the normal pH of surimi. Calcium ions, having a divalent positive charge (Ca^{2+}), can thus form a 'salt bridge' between negatively charged sites on two adjacent proteins (Lanier et al., 2005). However, breaking force and penetration distance of gels without setting were relatively the same regardless of MFB concentration, which were due to a low free calcium ion concentration of MFB. Cooking surimi paste in 3-cm diameter casing in a water bath (90^{o}C) even without pre-incubation (setting) is a slow heating process (Park, Yoon, and Kim, 2014). As Alaska pollock surimi is in favor of slow heating (Yongsawatdiful and Park, 1996), endogenous TGase was able to be activated and contributed to gradual gel texture enhancement as NFB concentration increased.

3.2 TGase activity

Effects of NFB and MFB on endogenous TGase activity in Alaska pollock

surimi paste were monitored by means of the incorporation of MDC into DMC (Fig. 2). TGase activity of surimi paste before setting increased from 7.6 to 17.6 unit/ml extract as the concentration of NFB increased from 0 to 2 g/100 g, respectively. With increasing MFB, TGase activity increased slightly (from 7.6 to 9.5 unit/ml extract). It was postulated that calcium ion activates endogenous TGase by inducing a conformational change to the enzyme, which exposes the TGase active site to a substrate (Nozawa, Cho, & Seki, 2001). The data indicated that calcium ion from Pacific whiting fish bone was released to activate endogenous TGase. It was consistent with the result reported by Hemung (2013) that endogenous TGase extracted from tilapia fillet was activated by calcium from tilapia fish bone powder. Calcium compounds from fish bone are hard to dissolve; nevertheless, Kim, Yeum, and Joo (1998) reported more soluble calcium (105.0 mg/100 g) was contained in mackerel surimi gels made with 0.9 g/100 g Alaska pollock fish bone powder than the control surimi gel (2.9 mg/100 g). Increased TGase activity for surimi mixed with NFB confirmed that NFB had a higher concentration of free calcium ions than MFB.

TGase activities of the pastes with NFB and MFB decreased significantly after setting (Fig. 2) as the result of cross-linking by TGase. Endogenous TGase contains 38 glutaminyl and 42 lysyl residues, which allows autocatalytical cross-linking (homoaggregates) when calcium ions are present (Wilhelm, Meinhardt, & Seitz, 1996).

260 **3.3 Microstructure**

261 Microstructures of surimi gels with and without fish bone under different
262 thermal treatments are illustrated in Figure 3. Control gels (without fish bone)
263 and gels with 1 g NFB/100 g paste formed continuous networks, while surimi
264 with MFB particles formed a discontinuous and porous network with large pores
265 (Fig. 3a MFB)

266 Two step heating (setting and cooking) made surimi gel networks looking
267 smoother and denser (Fig. 3b) compared to gels prepared without setting (Fig.
268 3a). Heat-induced gels in three-dimensional network are formed in an ordered
269 fashion and exhibit smooth texture when unfolded proteins are associated in
270 slow heating fashion (i.e., setting). However, when aggregation is done rapidly,
271 gel matrixes tend to be rough and coarse (Hermansson, 1979). Consequently, a
272 more compact and denser gel network was developed after setting. With
273 addition of 1 g NFB/100 g paste and setting, gel's microstructure appeared
274 definitely smoother and denser (Fig. 3b NFB) due to the formation of ε-(γ-
275 glutamyl) lysine covalent bonds enhanced by TGase and NFB calcium.

276

277 **3.4 Color**

278 The effects of fish bone on color of surimi gels with and without setting are
279 shown in the Table 1. As the concentration of fish bone, regardless of NFB or
280 MFB, increased, the L* values and whiteness of surimi gels decreased ($P<0.05$).
281 This was due to water-insoluble fish bone particles which inhibit the light's pass

through. In addition the lightness values of MFB gels were lower than those of NFB at equal concentrations ($P<0.05$). As fish bone concentration and particle size increased, more light was inhibited from going through the gel, resulting in decreased lightness. Yellowness (+b*) increased gradually as the fish bone concentration increased ($P<0.05$). Gels with MFB possessed higher yellowness ($P<0.05$). Whiteness values of NFB gels were higher than those of MFB gels ($P<0.05$), indicating that NFB addition more positively contributes to the lightness of surimi gel than MFB does.

As setting induced gels to be more translucent, the lightness (L*) and the yellowness (b*) of the gels with NFB and MFB decreased after setting (<0.05). This may be due to trapped water molecules as the result of an ordered gel structure formed after setting.

4. Conclusion

Physicochemical properties of gels fortified with fish bone were significantly influenced by the particle size of fish bone. NFB was more readily incorporated into the fish myofibrillar protein gel than MFB. In addition, NFB incorporation did not disrupt the integrity of gel networks. Enhancement of endogenous TGase activity was much more pronounced by NFB than MFB, confirming calcium ions from NFB were more readily available. The addition of NFB up to 1 g /100 g of surimi seafood paste, if processed in slow heating, can effectively enhance gel texture and possible calcium enrichment.

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Figures Captions

Figure 1 - Breaking force (g) and penetration distance (mm) of Alaska pollock gel with NFB (a) and MFB (b). ■ – gel cooked at 90 °C for 30 min. ■ – gel incubated at 25 °C for 3 h followed by 90 °C heating for 30 min.

Figure 2 - TGase activities (unit/ ml extract) of Alaska pollock surimi pastes with NFB and MFB. NB = paste with NFB before setting, MB = paste with MFB before setting, NA = paste with NFB after setting, MA = paste with MFB after setting.

Figure 3 - Microstructures of gels without (a) and with setting (b). Con = control gel without fish bone, NFB = gel with 1 g/100 g NFB, MFB = gel with 1 g/100 g MFB.

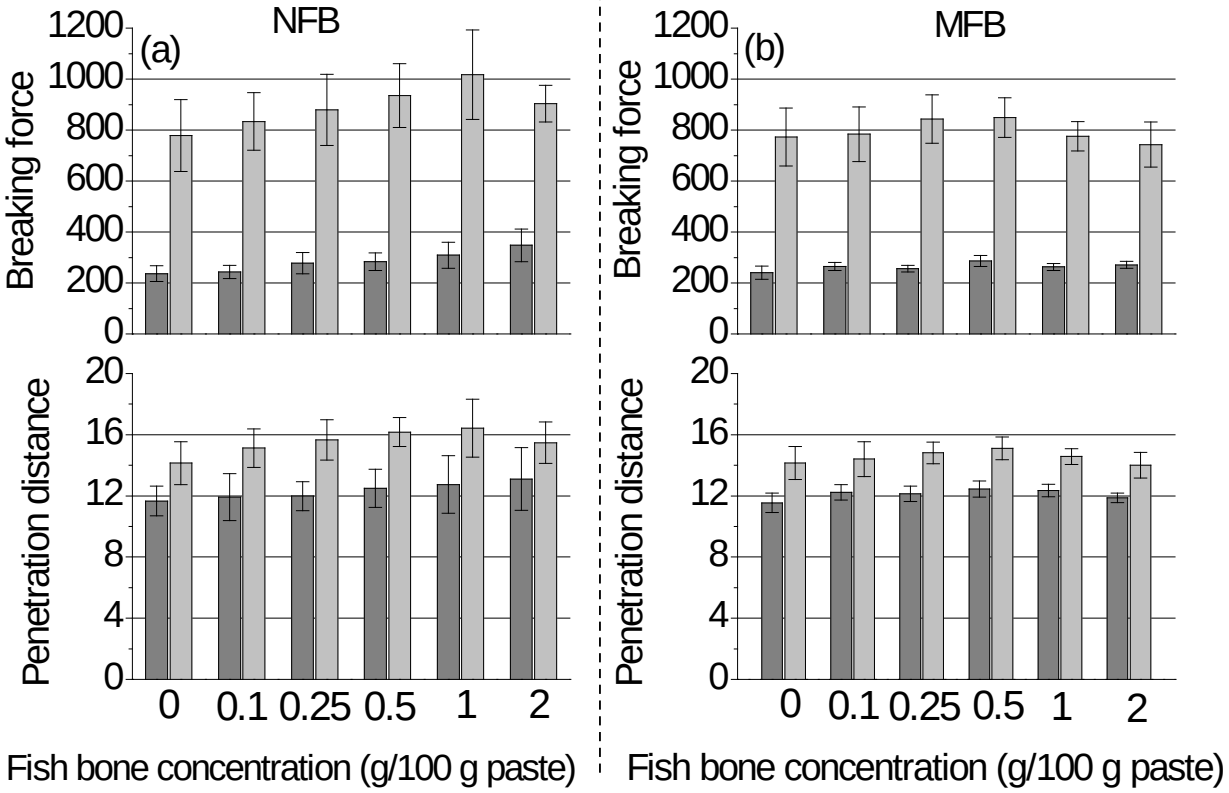
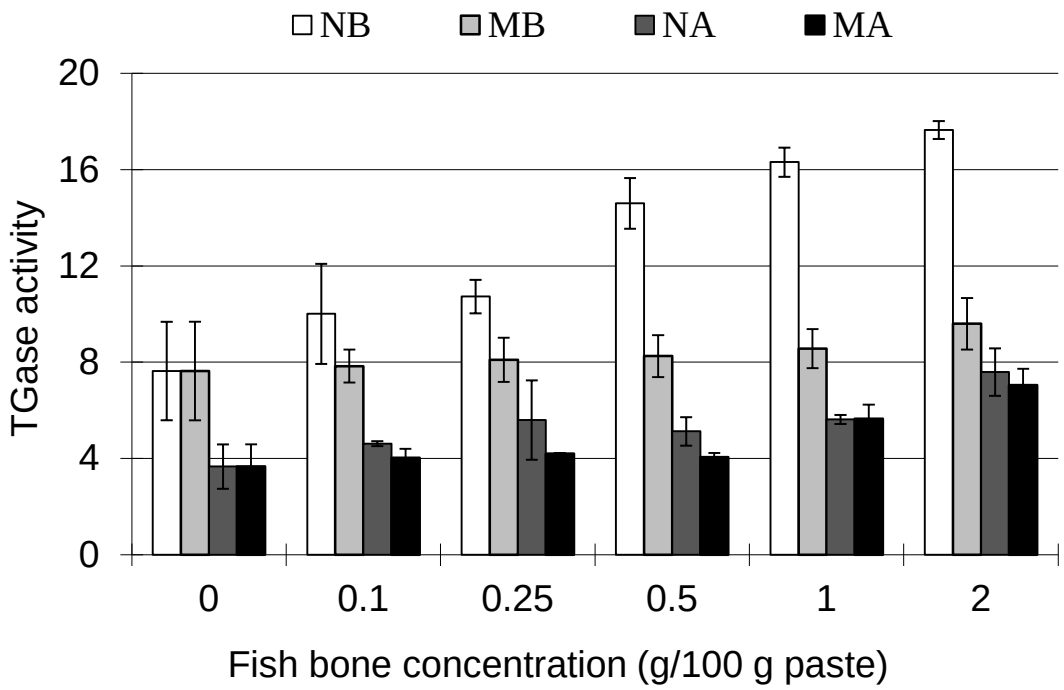


Figure 1 - Breaking force (g) and penetration distance (mm) of Alaska pollock gel with NFB (a) and MFB (b). ■ – gel cooked at 90 °C for 30 min. ■ – gel incubated at 25 °C for 3 h followed by 90 °C heating for 30 min.



418

419 Figure 2 - TGase activities (unit/ml extract) of Alaska pollock surimi pastes with

420 NFB and MFB. NB = paste with NFB before setting, MB = paste with MFB before

421 setting, NA = paste with NFB after setting, MA = paste with MFB after setting.

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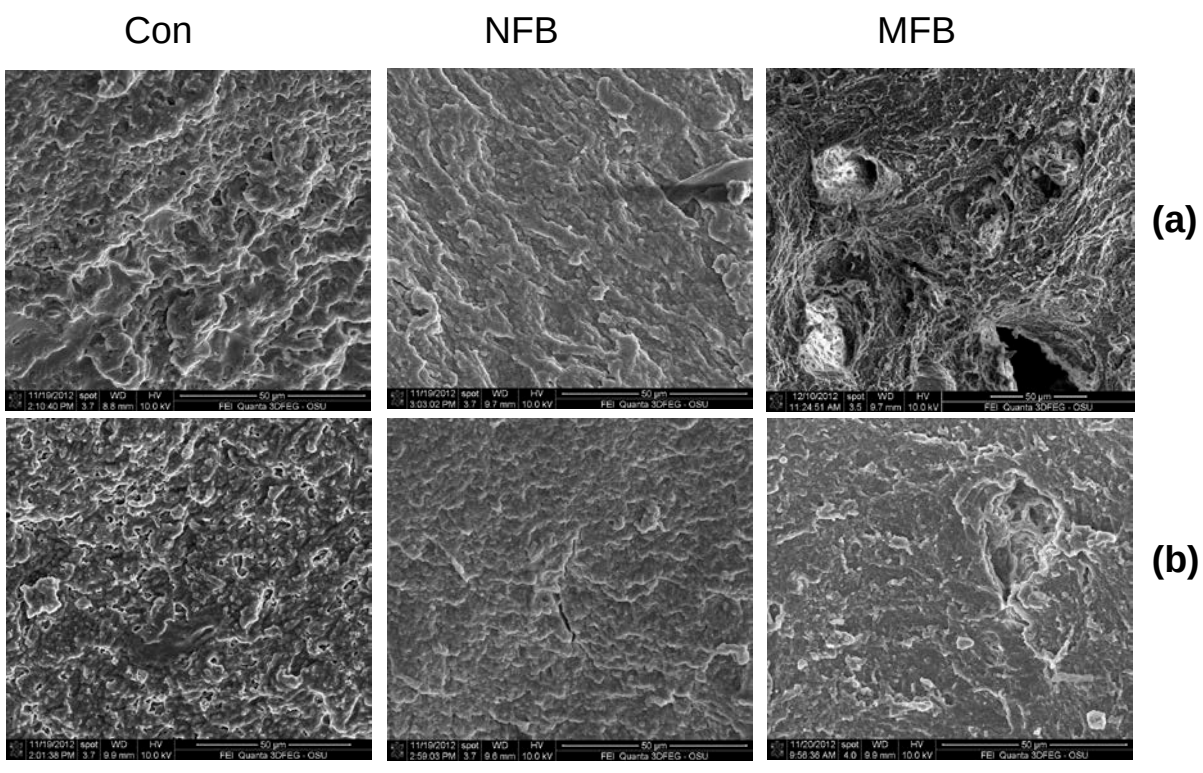

Figure 3 - Microstructures of gels without (a) and with setting (b). Con = control gel without fish bone, NFB = gel with 1 g/100 g NFB, MFB = gel with 1 g/100 g MFB.

438 Table 1 - Color parameters (lightness (L), yellowness (b*) and whiteness (W)) of
439 Alaska pollock surimi gels with NFB and MFB at different concentration (g/100 g
440 paste).

441

Color parameter [1]		Without setting		With setting	
		Micro	Nano	Micro	Nano
L	0.00	81.51±0.29^{aA}	81.51±0.29^{aA}	80.16±0.28^{aB}	80.16±0.28^{aB}
	0.10	80.45±0.65^{bA}	80.50±0.29^{bA}	79.00±0.55^{bB}	78.35±0.50^{bcC}
	0.25	79.87±0.64^{bcA}	80.64±0.35^{bA}	78.45±0.66^{bcB}	78.11±0.33^{cC}
	0.50	79.66±0.23^{cB}	80.85±0.44^{abA}	78.26±0.67^{cD}	78.87±0.73^{bC}
	1.00	78.46±0.57^{dC}	80.98±0.17^{abA}	77.00±0.52^{dD}	78.90±0.69^{bB}
	2.00	76.58±0.84^{eC}	80.43±0.49^{bA}	75.60±0.31^{eD}	78.62±0.42b^{cB}
b*	0.00	2.45±0.05^{fA}	2.45±0.05^{eA}	1.82±0.11^{eB}	1.82±0.11^{eB}
	0.10	2.71±0.07^{eA}	2.38±0.08^{eB}	1.96±0.05^{dC}	1.54±0.10^{fD}
	0.25	3.54±0.04^{dA}	2.88±0.07^{dB}	1.95±0.10^{dC}	1.95±0.13^{dC}
	0.50	4.30±0.07^{cA}	3.38±0.07^{cB}	3.59±0.07^{cB}	2.60±0.05^{cC}
	1.00	5.82±0.12^{bA}	4.83±0.10^{bC}	5.08±0.14^{bB}	3.75±0.10^{bD}
	2.00	7.98±0.10^{aA}	6.84±0.13^{aC}	7.58±0.15^{aB}	6.23±0.13^{aD}
W	0.00	74.17±0.27^{aB}	74.17±0.27^{aB}	74.70±0.37^{aA}	74.70±0.37^{aA}
	0.10	72.31±0.61^{bC}	73.34±0.13^{bB}	73.12±0.49^{bBC}	73.72±0.50^{bA}
	0.25	69.24±0.67^{cD}	71.99±0.30^{cB}	70.74±0.56^{cC}	72.23±0.32^{cA}
	0.50	66.77±0.33^{dD}	70.69±0.36^{dB}	67.49±0.66^{dC}	71.07±0.68^{dA}
	1.00	58.20±0.51^{eD}	66.48±0.38^{eB}	61.76±0.72^{eC}	67.66±0.68^{eA}
	2.00	52.64±0.69^{fB}	59.89±0.10^{fA}	52.85±0.23^{fB}	59.93±0.14^{fA}

442 [1] Within the same column, different lowercases indicate significant differences
443 between the gels with different fish bone concentration ($P < 0.05$). Within the
444 same row, different capitals indicate significant differences between the gels
445 with different thermal treatments and fish bones particle size ($P < 0.05$).

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