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Textural and rheological properties of Pacific whiting surimi as affected by nano-scaled fish bone and heating rates

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ABSTRACT

Textural and rheological properties of Pacific whiting (PW) surimi were investigated at various heating rates with the use of nano-scaled fish bone (NFB) and calcium chloride. Addition of NFB and slow heating improved gel strength significantly. Activity of endogenous transglutaminase (ETGase) from PW surimi was markedly induced by both NFB calcium and calcium chloride, showing an optimal temperature at 30 °C. Initial storage modulus increased as NFB calcium concentration increased and the same trend was maintained throughout the temperature sweep. Rheograms with temperature sweep at slow heating rate (1 °C/min) exhibited two peaks at ~35 °C and ~70 °C. However, no peak was observed during temperature sweep from 20 to 90 °C at fast heating rate (20 °C/min). Protein patterns of surimi gels were affected by both heating rate and NFB calcium concentration. Under slow heating, myosin heavy chain intensity decreased with NFB calcium concentration, indicating formation of ε-(γ-glutamyl) lysine cross-links by ETGase and NFB calcium ion.

1. Introduction

The United States Pacific whiting (PW) fisheries averaged harvests of 199,000 metric tons (MT) annually from 2007 to 2011 (NMFS, 2013). The majority of the harvest has been used for surimi production due to its bland taste, white color, low cost, and abundance. An intrinsic protease problem in Pacific whiting, which contributes to gel textural softening, has been resolved by addition of food grade protease inhibitors (such as egg white, whey protein concentrate and potato extract) or adopting fast cooking (for example, ohmic cooking) (Yongsawatdigul, Hemung, & Choi, 2014). A lower concentration of calcium ions in the flesh was thought to be a primary factor for relatively lower gel values of PW surimi after setting compared to those of Alaska pollock (AP) surimi (Park, 2005). Gordon and Roberts (1977) reported the calcium content of Pacific whiting, at 8.7 mg/100 g meat, while pollock contained 63 mg calcium/100 g meat (Sidwell, 1981). Consequently, the effectiveness of calcium compound addition was more pronounced with PW surimi than with AP surimi (Lee & Park, 1998).

It is generally accepted that calcium ion improves gel texture, mainly by inducing endogenous transglutaminse (ETGase), which is capable of catalyzing acyl transfer reactions by introducing non-disulfide covalent cross-links among myosin molecules (Lanier, Carvajal, & Yongsawatdigul, 2005). Yongsawatdigul and Sinsuwan (2007) reported that cross-links of myosin heavy chain were not disrupted by SDS-urea-2-mercaptoethanol solution and increased with calcium ion concentration. In addition to activating TGase, calcium ion has also been found to have a direct effect on conformational changes of myosin by inducing myosin unfolding. As a result, more exposure of the reactive residues favoured TGase-mediated reactions and led to enhancement of hydrophobic interactions (Yongsawatdigul & Sinsuwan, 2007). Furthermore, calcium ions have a divalent positive charge (Ca^{2+}), which may form ionic linkages between negatively charged sites in two adjacent myofibrillar proteins, resulting in gel texture improvement (Lanier et al., 2005).

Fish bone is the main solid leftover of the surimi processing industry, accounting for 10–15% of total fish biomass. Fish bone is rich in calcium compounds, which have been reported to possess high bioavailability (Malde et al., 2010). Despite increasing efforts to obtain new products from fish bone, the majority is still used for fishmeal, which is of low economic value. To make fisheries more sustainable and profitable, efforts should be focussed on utilizing this calcium-rich material as a high value-added product.

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Calcium compounds from fish bone have been reported to activate ETGase from different fish species (Hemung, 2013; Yin & Park, 2014). In addition, fish bone particles, downsized to a nanoscale, are capable of being imbedded in the surimi gel matrices without disrupting the myofibrillar gel network (Yin, Reed, & Park, 2014). Accordingly, nano-scaled fish bone (NFB) may be useful for calcium enrichment and gel texture enhancement of Pacific whiting surimi seafood. However, the effects of NFB addition on the properties of Pacific whiting surimi gel have not been investigated.

Our objectives were to evaluate the role of NFB on textural and rheological properties of Pacific whiting surimi heated at various heating rates.

2. Material and methods

2.1. Materials

Pacific whiting (*Merluccius productus*) surimi, made with 4% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate without food-grade protease inhibitor, was obtained from Trident Seafoods (Seattle, WA, U.S.A.). Frozen surimi was cut into about 800 g blocks, vacuum-packaged, and stored in a freezer (−18 °C) throughout the experiments. Dried Pacific whiting fish bone was obtained from Trident Seafoods (Newport, OR, U.S.A.).

Dried egg white (EW) was obtained from Henningsen Foods (K-200, Omaha, NE, U.S.A.). Calcium chloride, N, N′-dimethylated casein (DMC), and monodansylcadaverine (MDC), were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Dithiothreitol (DTT) was purchased from Fluka (Buchs, Switzerland). Reagents used for gel electrophoresis were obtained from Bio-Rad (Hercules, CA, U.S.A.). All other chemicals were of analytical grade.

2.2. NFB preparation

Preparation of NFB was carried out according to the method described by Yin and Park (2014). Briefly, fish bone was soaked in sodium hydroxide solution (pH 12) for 2 h and rinsed with tap water three times to remove myofibrillar proteins and dried in an oven (105 °C) overnight. Dried fish bone was mixed with deionized water and further processed to nano-scaled fish bone emulsion (NFB) by Custom Processing Services (Reading, PA, U.S.A.), using a wet mill (Labstar, Netzsch Premier Technologies, Co., Exton, PA, U.S.A.). D_{50} of the fish bone particle in the emulsion was 280 nm, which was analyzed by Custom Processing Services (Reading, PA, U.S.A.), using a Laser particle size analyzer (LA-950 V2, Horiba Co., Kyoto, Japan). Total calcium concentration was 32.4 mg/g emulsion, as analyzed by Universal Testing Company (Quincy, IL, U.S.A.), using inductively coupled plasma (ICP) spectrometry.

2.3. Gel preparation

Frozen surimi was tempered at room temperature for 1 h before being cut into about 3 cm cubes. Surimi cubes, with approximately −5 °C core temperature, were chopped at 1, 800 rpm for 1 min, using a silent cutter (UM 5 universal, Stephan Machinery Co., Columbus, OH, U.S.A.) equipped with an ethylene glycol chilling system. Chopping was continued at 1, 800 rpm for 1 min with 2% salt. NFB emulsion (at 0, 1, 3, 5, 10 mg calcium/g surimi paste) or calcium chloride (at 1 mg calcium/g surimi paste) was added to the salted surimi paste. 1% DEW was then added as protease inhibitor with the assumption of protease inhibition by 80–90% (Hunt, Park, & Handa, 2009). Moisture content was adjusted to 77%, using ice water (0 °C) and sucrose before chopping at 1800 rpm for a further 1 min. Sucrose was added to the treatments without NFB or with reduced NFB as an inert ingredient to substitute for NFB while maintaining equal moisture content. For the final 3 min, chopping continued at 3, 600 rpm while a vacuum was maintained at 0.5–0.6 bar. The final temperature was approximately 15 °C, which is the optimum final chopping temperature for PW surimi (Poowakanjana & Park, 2014). The paste prepared above was packed into a polyethylene bag and subjected to a vacuum packaging machine (Reiser VM-4142, Roescher Werke, Osnabrueck, Germany) to remove air pockets. The paste was stuffed into a nylon tube (Nylatron MC 907, Quadrant Engineering Plastic Products, Reading, PA, U.S.A.) with a 3 cm inner diameter and approximately 15 cm long. The paste was heated, using an ohmic heating apparatus, as described by Yongsawatdigul, Park, Kolbe, Dagga, and Morrissey (1995). Samples were heated from ~15 to 90 °C with five different heating rates: 0.5, 1, 5, 20, or 80 °C/min. The approximate applied voltages for each heating rate were 25 V (0.5 °C/min), 35 V (1 °C/min), 50 V (5 °C/min), 80 V (20 °C/min), and 140 V (80 °C/min), giving voltage gradients (V/cm) of 1.67, 2.33, 3.33, 5.33, and 9.33, respectively. Gels were placed in plastic bags, submerged in cold iced water for 15 min before storing overnight in a refrigerator (4 °C).

2.4. Fracture gel evaluation

Fracture gel evaluation of the gels was performed using a TA-XT texture analyzer (Stable Micro Systems, Surrey, U.K.). Cold gels (4 °C) were placed at room temperature for 2 h prior to gel testing. Cylinder-shaped samples with lengths of 2.5 cm were prepared and subjected to fracture analysis. Breaking force and penetration distance were measured, using the texture analyzer equipped with a spherical plunger (diameter 5 mm) at a crosshead speed of 60 mm/min. For each treatment, mean values were obtained from at least four measurements.

2.5. Oscillatory dynamic measurement

The surimi paste was subjected to a temperature sweep to monitor heat-induced gelation, using a CVO rheometer (Malvern Instruments Ltd., Worcestershire, U.K.). Sample paste was applied between a cone (4° and 40 mm diameter) and plate with a gap of 150 μm. Sample was covered by a trapper with moistened sponge to avoid the sample drying out during heating. Samples were subjected to temperature sweep (from 20 to 90 °C) at two different heating rates of 20 °C/min and 1 °C/min, respectively, shear stress of 100 Pa, which was in the linear viscoelastic region through shear stress sweep, and a fixed frequency of 0.1 Hz.

2.6. TGase activity

TGase activity was assayed by the method of Yongsawatdigul, Worratao, and Park (2002) with slight modifications; 5 g of surimi were homogenized in 4 volumes of extraction buffer (10 mM NaCl and 10 mM Tris-HCl, pH 7.5). The homogenate was centrifuged at 16, 000×*g* (Sorvall, DuPont Co., Newton, CT, U.S.A.) at 4 °C for 30 min. The supernatant was used as crude extract. The assay mixtures contained 1.0 mg/ml DMC, 15 μM MDC, 3 mM DTT, and 50 mM Tris-HCl (pH 7.5). NFB or $CaCl_2$ solution was added to the mixtures and vortexed immediately. The mixtures were incubated at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C and 70 °C for 5 min. 100 μl of crude enzyme were added and further incubated for 10 min. After incubation, EDTA solution was added to a final concentration of 20 mM to stop the reaction of TGase immediately (Yongsawatdigul et al., 2002). 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). 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 units/ml of extract.

2.7. SDS–PAGE

Protein pattern, by molecular weight, was measured, using SDS–PAGE, as described by Laemmli (1970). Gel samples were solubilized in 5% sodium dodecyl sulfate solution (90 °C) according to Reed and Park (2008). Stacking and separating gels were prepared, using 4% (w/v) and 10% (w/v) acrylamide, respectively. Acrylamide gel was fixed and stained with 0.125% Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA, U.S.A.), and destained in a solution containing 50% methanol and 10% acetic acid. The molecular weight of the protein band was determined, based on the relative mobility of standard proteins (Kaleidoscope precision plus, Bio-Rad Laboratories, Hercules, CA, U.S.A.).

2.8. Statistical analysis

The entire study was repeated twice and every measurement was conducted using at least duplicate samples. Analysis of variance (ANOVA) was analyzed, using the SAS program (SAS Institute Inc, Carry, NC, U.S.A.). Differences among mean values were established, using the Duncan multiple range test (DMRT) at $P < 0.05$.

3. Results and discussion

3.1. Textural properties influenced by NFB

Effects of NFB and calcium chloride on the breaking force and penetration distance of Pacific whiting surimi gels cooked ohmically at different heating rates are illustrated in Fig. 1. The control (neither DEW nor calcium added), heated at 0.5 °C/min and 1 °C/min, did not form a gel. At 5 °C/min, the gel was soft and mushy. No gel or weak gels were primarily due to the activity of cathepsin L, which caused proteolytic degradation of the myofibrillar components (An, Weerasinghe, Seymour, & Morrissey, 1994) upon slow or extremely slow heating. Texture of the cooked gel under slow heating (0.5–5 °C/min) was significantly improved by addition of 1% DEW ($P < 0.05$), demonstrating that DEW could effectively inhibit protease. Breaking force and penetration distance of the control gels increased markedly when heating rate was raised to 5–20 °C/min ($P < 0.05$). This was attributed to the rapid inactivation of protease by fast heating. Consequently, as in the previous study (Yongsawatdigul et al., 1995), a greatly reduced degradation of myosin heavy chain was observed (Fig. 4). Results confirmed that both a protease inhibitor, e.g. DEW, and fast heating could be effectively used to control the protease activity in Pacific whiting surimi. Yin et al. (2014) confirmed that the microstructures of gels made with NFB were consistent, demonstrating no disrupts in myofibrillar gel.

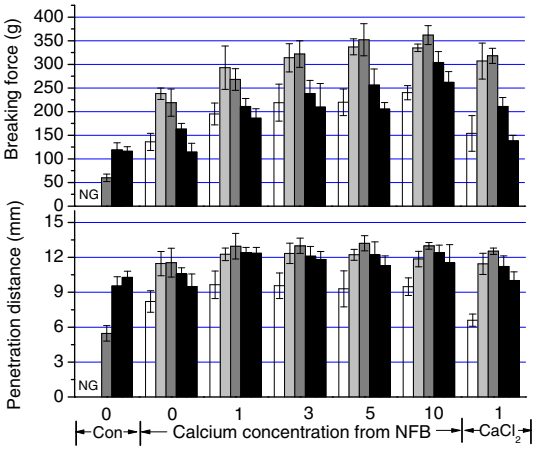

Fig. 1. Breaking force and penetration distance of Pacific whiting surimi gels with NFB calcium and calcium chloride heated ohmically at different heating rates. NFB = nano-scaled fish bone, Con = control surimi gel made without dried egg white (DEW), NFB, and calcium chloride. Numbers on the *x*-axis designate calcium concentration (mg/g surimi paste). NG indicates no gel formation at 0.5 °C and 1 °C/min heating rates. □ = 0.5 °C/min, ▫ = 1 °C/min, ▩ = 5 °C/min, ■ = 20 °C/min, ■ = 80 °C/min.

Breaking force of the gels with 1% DEW increased significantly with addition of calcium chloride (1 mg calcium/g surimi) ($P < 0.05$), and it increased as the calcium concentration of NFB increased up to 10 mg calcium/g surimi at all studied heating rates ($P < 0.05$). Maximum breaking forces of gels with NFB and calcium chloride, obtained at a heating rate of 5 °C/min, were 1.52 and 1.34 times, respectively, greater than the maximum breaking force of the control gel made at a heating rate of 1 °C/min. The texture improvement of gels with NFB under slow heating was primarily related to endogenous TGase (ETGase). Since ETGase is Ca^{2+}-dependent, calcium ions from NFB likely activated ETGase (Fig. 3), enhancing the formation of non-disulfide covalent bonds among myosin molecules (Yongsawatdigul et al., 2002). In addition, increased breaking force under fast heating (5–80 °C/min) ($P < 0.05$) might be attributed by hydrophobic interactions and ionic linkages among protein molecules by the role of calcium from NFB (Lanier et al., 2005; Yongsawatdigul & Sinsuwan, 2007).

Breaking force and penetration distance of gels with DEW increased as heating rate decreased from 80 °C/min to 1 °C/min. As the heating period became extended, more covalent bonds catalyzed by ETGase were formed, resulting in increased gel strength. On the other hand, rapid unfolding of proteins, followed by slow aggregation, tended to form a stronger gel compared to fast-cooked gel. This is because heat-denatured proteins are aligned in an ordered fashion to form a fine gel network when aggregation is slow (Yongsawatdigul, Park, Virulhakul, & Viratchakul, 2000). Significantly decreased breaking force and penetration distance were observed at 0.5 °C/min ($P < 0.05$), which was due to proteolytic degradation of proteins involved in the formation of the gel network by endogenous protease during slow heating.

3.2. Rheological properties influenced by NFB

Changes in the storage modulus (G′) of Pacific whiting surimi with different calcium concentrations during temperature sweeps at 1 °C/min and 20 °C/min are shown in Fig. 3. An increase in storage modulus (G′), which represents energy recovered per cycle of sinusoidal shear deformation, indicated an increase in rigidity of the sample associated with the formation of elastic gel structure (Park, Yoon, & Kim, 2014). Two quite distinct patterns in gelation, under slow and fast heating, were observed (Fig. 2a and b). Heat-induced gelation of Pacific whiting surimi under slow heating could be characterized into 4 different stages: (1) the first increase in G′ at 20 to 36 °C, (2) the slight decrease in G′ or "gel weakening" that reached a minimum at 45 °C, (3) the second increase in G′ or "gel strengthening" beginning at 45 °C and extending to a maximum at 66 to 70 °C, and (4) the second decrease in G′ at the final stage of heating (Fig. 2a).

In the case of gelation under fast heating, G′ constantly decreased from 20 to 55 °C then steadily increased to 90 °C. No G′ peak was observed throughout the heating process from 20 to 90 °C at 20 °C/min (Fig. 2b). Unlike fracture analysis, where gels prepared by fast, ohmic heating demonstrated high gel values (Fig. 1), with dynamic rheology the heating rates, were too fast

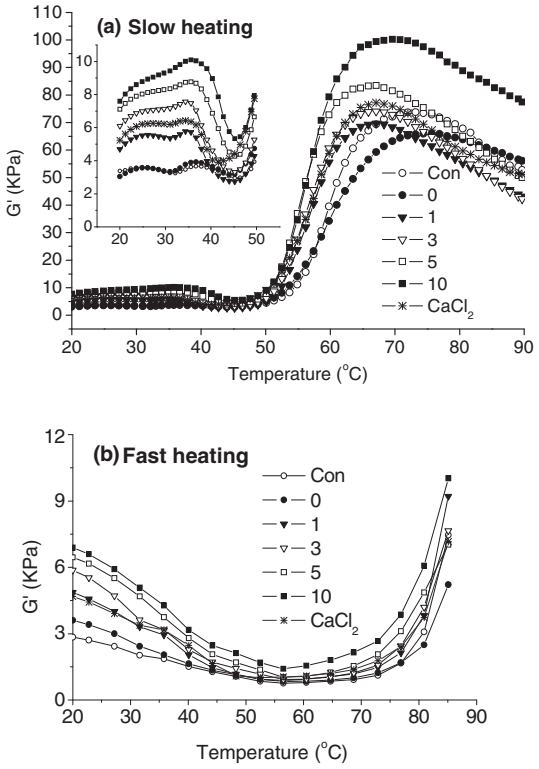

Fig. 2. Elastic modulus of Pacific whiting surimi with NFB and calcium chloride at 1 °C/min (a) and 20 °C/min (b). Con = control surimi gel made without dried egg white (DEW), NFB (nano-scaled fish bone), and calcium chloride. Numbers in the legend designate calcium concentration (mg/g surimi paste).

to detect the aggregation of unfolded proteins in this sinusoidal sweeping process. The final G′ of surimi cooked at 20 °C/min was much lower than that of surimi cooked at 1 °C/min. This was in agreement with the breaking force of gels cooked at these two heating rates (Fig. 1).

The first G′ peak of Pacific whiting surimi paste heated at 1 °C/min had an onset temperature of about 30 °C and it reached its maximum value (first peak temperature) between 34.4 and 36.6 °C. The onset and peak temperature were consistent with the results reported by Yoon, Gunasekaran, and Park (2004). It has been suggested that the formation of this peak relates to the unfolding and cross-linking of light meromyosin chain (LMM) (Reed & Park, 2011). NFB and calcium chloride had no effect on onset and the first peak temperature. However, the magnitude of peak G′ increased significantly with the addition of NFB and calcium chloride ($P < 0.05$), indicating that ETGase is affected by calcium ions in this temperature range.

The slight decrease in G′ before 45 °C was postulated to be due to the helix-to-coil transformation of myosin, which leads to a large increase in fluidity as a result of possible disruption of the semi gel-like protein network (Sano, Noguchi, Tsuchiya, & Matsumoto, 1988). G′ started to rise constantly after 45 °C and reached its maximum value at 66 to 70 °C. This was attributed to both an increase in the number of cross-links between protein aggregates and a deposition of additional denatured proteins in the existing protein networks to strengthen the gel matrix (Xiong, 1997).

Normally a higher G′ value, at the point where gelation is completed, corresponds to higher gel strength. Surimi gel with NFB (10 mg calcium/g surimi paste) showed the highest G′, which was consistent with the highest breaking force of the surimi gel (Fig. 1). G′ declined after heating beyond 66 to 70 °C. The decreased G′ beyond this point was likely due to slipperiness of the gel between the cone and plate (Poowakanjana, Mayer, & Park, 2012).

Surprisingly, the sample treated with 1% DEW had no increase in G′ after 55 °C as observed in the control sample. In addition, a lower G′ value was obtained at the second peak when compared with the control sample. Rawdkuen, Benjakul, Visessanguan, and Lanier (2007) reported that the G′ of Pacific whiting surimi paste decreased with addition of chicken plasma (CP) after 55 °C. They suggested that the result obtained might reflect the dilution effect of muscle proteins by CP addition. In their study, CP was added to surimi paste, as a substitute for the myofibrillar proteins, to obtain a constant protein concentration. Therefore, the concentration of myofibrillar proteins was reduced with increased CP addition. Visessanguan, Benjakul, and An (2000) also found that the addition of porcine plasma resulted in decreased G′ of Pacific whiting actomyosin after 55 °C. Actomyosin concentration used was the same in their study.

The decrease of G′ with the addition of protease inhibitor (Fig. 2a) might be related to changes in the moisture content. Surimi paste, with protease inhibitor, forms a gel with a compact and continuous 3-dimensional structure under slow heating, while surimi paste without protease inhibitor would form a loose and discontinuous 3-dimensional structure (due to hydrolysis of myofibrillar proteins by endogenous protease). Thus, less water would be retained in the control gel as compared with the gel with protease inhibitor. G′ increased as moisture content decreased (Yoon et al., 2004).

3.3. TGase activity induced by NFB

Effects of NFB and calcium chloride on endogenous TGase (ETGase) from Pacific whiting were monitored by means of the incorporation of MDC into DMC. Crude TGase from Pacific whiting showed that calcium ion is an absolute requirement to catalyze a cross-linking reaction (Fig. 3). TGase activity in the absence of added calcium was 4.2 unit/ml of extract at 30 °C. It increased with NFB calcium concentration (up to 2.5 mM) and reached a maximum of 30.6 unit/ml of extract. It was postulated that calcium ion activates ETGase by inducing conformational changes of the enzyme, which consequently exposes the active site to a substrate (Nozawa, Cho, & Seki, 2001). The results clearly demonstrated that the calcium ion from NFB was released to activate ETGase from Pacific whiting surimi. This was consistent with the reports by Hemung (2013) and Yin and Park (2014) that ETGase, from different fish species, was activated by calcium from fish bone. Activity of ETGase with 0.25 mM NFB calcium at 30 °C was 7.7 unit/ml of extract, which was much lower than that with 0.25 mM calcium

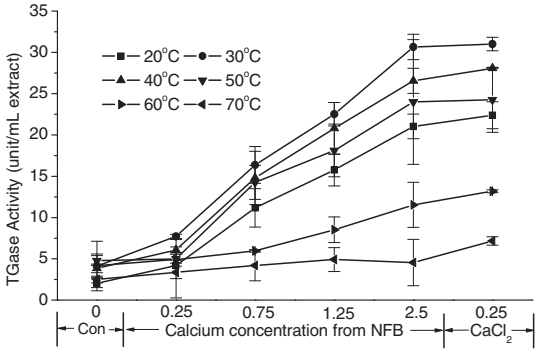

Fig. 3. Activities of TGase extracted from Pacific whiting with addition of NFB (nano-scaled fish bone) and calcium chloride under different incubation temperatures. Numbers designate calcium concentration (mM) in the assay mixtures.

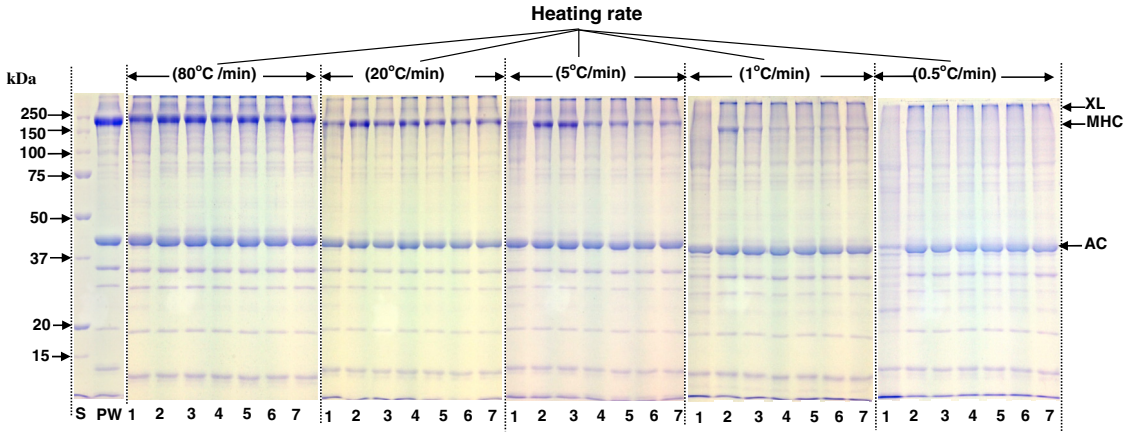

Fig. 4. Protein patterns of surimi gels as affected by NFB and calcium chloride. S = kaleidoscope protein standard (kDa); PW = Pacific whiting surimi; DEW = dried egg white; NFB = nano-scaled fish bone. MHC = myosin heavy chain, AC = actin, XL = cross links. 1 = gel without DEW, NFB, and calcium chloride, 2 = gel with DEW and without NFB and calcium chloride, 3–6 = gels with DEW and NFB with calcium concentrations at 1, 3, 5, 10 mg/g surimi paste, respectively, 7 = gel with DEW and calcium chloride (1 mg calcium ion/g surimi paste).

ion (31.0 unit/ml of extract). This was probably due to a lower solubility of calcium from NFB compared to calcium chloride.

Optimum temperature for ETGase from Pacific whiting was 30 °C (Fig. 3). The optimum temperature of ETGase activity, however, varies with fish species. Yongsawatdigul et al. (2002) reported that the optimum temperature of ETGase purified from threadfin bream was 55 °C, while purified ETGase from bigeye snapper, common carp, oil sardine and tilapia showed optimum activities at 37 °C, 37 °C, 37 °C and 50 °C, respectively (Binsi & Shamasundar, 2012). The difference in optimal temperature for activity of ETGase from different fish species may be related to their habitat temperature. Nearly 77% of activity was retained at 50 °C, but it rapidly decreased to 42% at 60 °C. The decreased activity was due to denaturation of ETGase at high temperatures.

3.4. Non-disulfide covalent cross-linking catalyzed by endogenous TGase and NFB

In order to clarify the contribution of NFB calcium concentration to the cross-linking of myosin heavy chains (MHC) and resulting gel formation, surimi gels were analyzed by SDS–PAGE. MHC band intensities of gels, with and without 1% DEW, heated at 80 °C/min, were similar (Fig. 4). This was due to the rapid inactivation of protease with fast heating. However, as heating rate decreased, MHC intensity of gel without 1% DEW was much lower than that of gel with 1% DEW. An et al. (1994) reported that the intensity of the MHC band from Pacific whiting on SDS–PAGE gel was substantially reduced within 5 min of incubation at 55 °C and it completely disappeared within 20 min. Band intensity of a degradation product (around 30 kDa) was proportional to the degree of hydrolysis and increased with duration of reaction time.

As shown in Fig. 4, MHC and cross-links (XL) of the gels without DEW were completely gone at 0.5 and 1 °C/min. Simultaneously, small-molecular-weight proteins ranging from 20 kDa to 45 kDa were darker in density, indicating a higher level of these proteins. Surimi without DEW cooked at 0.5 °C/min and 1 °C/min showed no MHC band (Fig. 4) and could not form a gel (Fig. 1). This emphasized the importance of myosin as a major component responsible for gel-forming ability of surimi.

MHC band intensity of gels with 1% DEW decreased as heating rate decreased from 80 °C/min to 0.5 °C/min. Since 1% DEW did not completely inhibit proteolysis (Hunt et al., 2009), the decreased MHC intensity was attributed to formation of non-disulfide covalent cross-linking and proteolytic degradation. Depending on which process dominates, stronger or weaker gels will be formed. When heating rate was above 1 °C/min, MHC decreased and fewer or no degraded products were observed, implying that the cross-linking reaction dominated. New bands, ranging from 20 to 45 kDa, clearly formed as heating rate decreased to 0.5 °C/min, indicating that the monomers and polymers of MHC were greatly degraded by protease. Under slow heating, MHC band intensity decreased with addition of calcium chloride or NFB, due to the activation of ETGase, resulting in the formation of more ε-(γ-glutamyl) lysine cross-links of MHC. Changed gel texture (Fig. 1) could be well explained by protein patterns after SDS–PAGE (Fig. 4).

Actin band intensities of gels with 1% DEW, regardless of heating rate and calcium addition, were relatively stable. This indicated that actin affected gel functionality to a lesser extent. However, actin band intensity of the gel without DEW decreased as heating rate decreased. One band overlapping the actin site was observed in the protein patterns of gels made with DEW. This band corresponded to albumin from DEW (Reed & Park, 2008).

4. Conclusions

Textural and rheological properties of Pacific whiting surimi were significantly influenced by DEW, heating rate, and calcium addition. Breaking force and penetration distance of Pacific whiting gels were maximally increased, by 52% and 12%, respectively, with addition of 1% DEW and 10 mg NFB calcium/g surimi paste, at a heating rate of 5 °C/min. NFB improved gel texture, primarily by activating endogenous transglutaminase, which catalyzed the formation of MHC cross-links. Addition of NFB to Pacific whiting surimi seafood could lead to both calcium enrichment and gel texture enhancement.

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