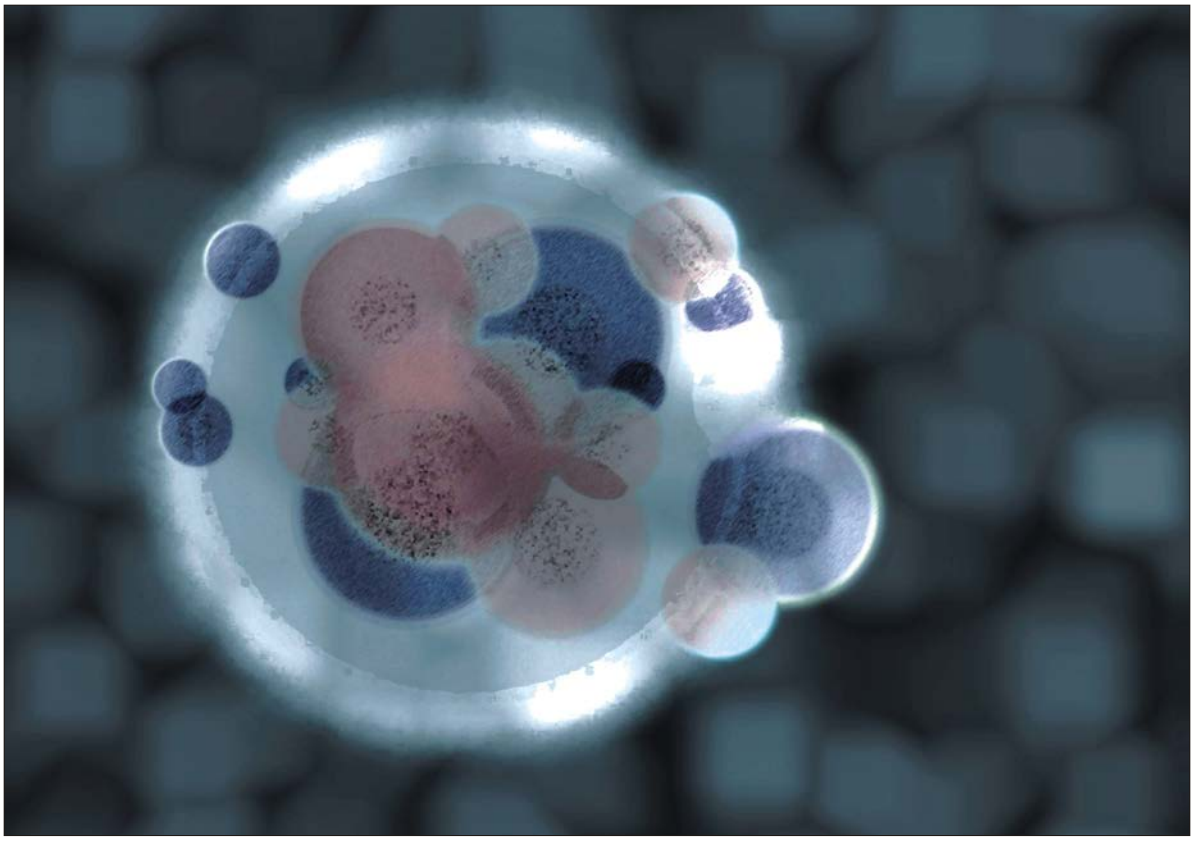

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Microstructure of White Croaker Surimi Protein Gels Set at Low Temperature under the Inhibition of the Polymerization and Degradation of Protein

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Abstract: The role of non-covalent aggregation of protein in the gelation of white croaker surimi during heating process, as well as the difference in the gel formation of white croaker and walleye pollack surimi were investigated by the examination of microstructure of white croaker surimi gels under the inhibition of the polymerization and degradation of protein myosin heavy chain (MHC). In the inhibitory condition, the gel strength of 1-step heating and 2-step heating gels set at 40°C increased with setting time and reached maximum value for 180 min-setting gel. The gel strength of 2-step heating gels set at 50°C reached the maximum value for 20 min-setting. Furthermore, the internal structure of gels set at 40°C was regular and compact in both 1-step and 2-step heating gels. A regular, dense and compact gel network with thick strands was formed in gels set at 50°C. Thus, it was concluded that the non-covalent thermal aggregation of white croaker surimi protein during setting could form a fine and compact gel network with thick strands and this gel network was maintained or reinforced by non-covalent bonding during subsequent heating at 80°C for 20 min under the inhibition of the polymerization and degradation of MHC.

Key words: Gel network, white croaker, surimi, non-covalent bonding, thermal aggregation

INTRODUCTION

Heat-induced gel of muscle is formed from the myofibrillar proteins, primarily myosin and actin, the most abundant in muscle protein (Katoh *et al*., 1979; Yasui *et al*., 1980). A gel network is formed by interactions or bonds that occur among myofibrillar proteins after solubilization with salt, to give a firm, elastic network by heating. Gelling involves unfolding of the proteins and the establishment of bonds among them. It is believed that hydrogen bonds (Lanier *et al*.,1982), hydrophobic interactions (Sano *et al*., 1988; Funatsu *et al*., 1993), disulfide bridges (Itoh *et al*., 1979; Kishi *et al*., 1995; Hossain *et al*., 2001; Sano *et al*.,1988) and covalent bonds other than disulfide (Wan *et al*., 1994; Seki, 1990) intervene differently in the formation of the network depending on the various parameters involved.

In processing surimi-based product, salted surimi paste is subjected to setting (20-40°C) before cooking at a high temperature (80-90°C) to form a strong and elastic gel. The low temperature gelation during setting is termed setting or suwari. The gel strength of cooked gel with setting becomes higher than that without setting. The setting process closely correlated with polymerization of myosin heavy chain (MHC) by transglutaminase (TGase), a Ca-dependent and sulfhydryl (SH) enzyme (Wan *et al*., 1994). Non-covalent bonding also contributes to low-temperature setting as well (Nowsad *et al*., 1996).

The internal structure of fish meat gel is one of the most important factors used to judge gel quality (Okada and Migita, 1956; Kubota *et al*., 2006). Thus, many researches involving the relation between microstructure of gel network and the characteristic of gel have been carried out (Alvarez *et al*., 1999; Sato and Tsuchiya, 1992; Jafarpour and Gorczyca, 2008). The microstructure properties are controlled by the internal structure of the gel. The internal network and porous structures of surimi gel can easily be observed by natural scanning electron microscope (N-SEM) (Kubota *et al*., 2003, 2006).

Our previous study (Phu *et al*., 2010) on gel-forming characteristic of white croaker surimi under the inhibition of the polymerization and degradation of MHC showed that suwari occurred during setting at 40 and 50°C even under the inhibitory condition and contributed to the gel strength of final cooked gel (2-step heating gel). Thus, it was obvious that the gel formation under the inhibition of the polymerization and degradation of MHC of white croaker surimi differed from that of walleye pollack surimi,

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in which suwari occurred during setting at 40°C under the inhibitory condition, but did not contribute to the gel strength of final cooked gel (Hossain *et al*., 2001, 2010).

In this research, the microstructure of white croaker surimi gels set at low temperature was examined in order to clarify the gel formation of white croaker surimi by non-covalent aggregation, as well as the difference in the gel formation between white croaker and walleye pollack surimi under the inhibition of the polymerization and degradation of MHC.

MATERIALS AND METHODS

Materials: White Croaker Surimi (SA grade) was imported from Pacific Fish Processing Co., Ltd., (Thailand) by Maruha-Nichiro Co.Ltd. (Japan) and purchased in 2008. Samples were packed in a box and transported to the Aquatic Product Utilization Laboratory, Kochi University (Japan) within 30 min. Frozen surimi was vacuum-packed and kept at -55°C until use.

Chemical reagents: EDTA was obtained from Dojindo (Kumamoto, Japan). Leupeptin was purchased from Peptide Institute (Osaka, Japan). All chemicals were of analytical grade.

Gel preparation: White croaker surimi was thawed at 5°C overnight in cold room, then adjusted to a moisture content of 80% and mixed with 3% NaCl for 4 min by mixer (Model MK-K48, National, Japan) in the presence or absence of inhibitors (10 mmol kg^{-1} EDTA, 100 mg kg^{-1} leupeptin). The resulting pastes were stuffed into stainless steel cases (3.1 cm in diameter, 3.0 cm in height) and then wrapped in polyvinylidene chloride film. Two sets of heat-induced gels (1-step heating gels and 2-step heating gels) were prepared by the following method: the 1-step heating gels were prepared by incubating the salted surimi paste in a water-bath at 40 and 50°C for 20 min, 1, 2 and 3 h. Two-step heating gels were also prepared from one-step heating gels, followed by further heating at 80°C for 20 min. The heated gels were cooled immediately in ice water before being stored at 5°C.

Measurement of gel strength: Gel strength (g cm^{-2}) was assessed by multiplying breaking strength (g cm^{-2}) and elongation that were measured by a tensile test using a rheometer (Model CR-200D, Sun Scientific Co. Ltd., Tokyo, Japan) according to the method of Shimizu *et al*. (1981). The gel samples were sliced and cut into rings of 5 mm in thickness and were applied to gel strength measurement. Five tests were conducted for each sample. The mean value and standard deviation of results obtained were calculated.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): The heated gel (0.1 g) was homogenized with 4.5 mL of 0.05 M sodium phosphate buffer (pH 7.2) containing 8 M urea, 2% SDS and 0.5 mL of 0.09% N-ethylmaleimide (NEM) and then heated in boiling water for 2 min to make unreduced sample. To make reduced sample, 2-mercaptoethanol was added to final concentration of 10%. An aliquot of 12 μL from each sample was subjected to SDS-PAGE using 5% polyacrylamide gel according to the method of Weber and Osborn (1969).

Autolysis: Three grams of the 2-step heating gel were homogenized with 15 mL of 5% trichloroacetic acid (TCA) solution at 10,000 rpm for 5 min using an Ace homogenizer (Nihon Seiki Kaisha Ltd., Tokyo, Japan). The filtrate of the resulting homogenate was used as an extract. TCA-soluble free amino acids and peptides were determined according to the Lowry method (Lowry *et al*., 1951). Tyrosine (Tyr) was used as a standard. The amount of TCA-soluble free amino acids and peptides was expressed as μg Tyr-equivalent/g sample.

Natural scanning electron microscopy: Microstructure of the gel was observed by using a natural scanning electron microscope (S-2380N, Hitachi Ltd., Tokyo, Japan) according to the method of Kubota *et al*. (2006). The gels were cut into small pieces (5 mm×5 mm×1 mm) by a razor blade. The samples were mounted on the copper specimen holder with adhesive of vinylacetate. Samples were observed by S-2380N with an N-SEM mode (Sample stage, -10°C; pressure, 5-10 Pa; accelerating voltage, 15 kV).

RESULTS

Changes in the gel strength: The changes in the gel strength of white croaker surimi in the presence or absence of inhibitors at 40°C were shown in Fig. 1a, b. In the samples without inhibitors (control), the gel strength of both 1-step and 2-step heating gels increased with the heating time up to 2 h and then reached a plateau. Subsequent heating at 80°C resulted in the increase in gel strength. In the presence of EDTA and leupeptin, the gel strength of the 1-step heating gels set at 40°C increased with the increase in the setting time and reach highest value after set for 3 h. In the 2-step heating gel, the gel strength steadily increased when set up to 1 h and then slightly increased. However, the extent of gel-strength

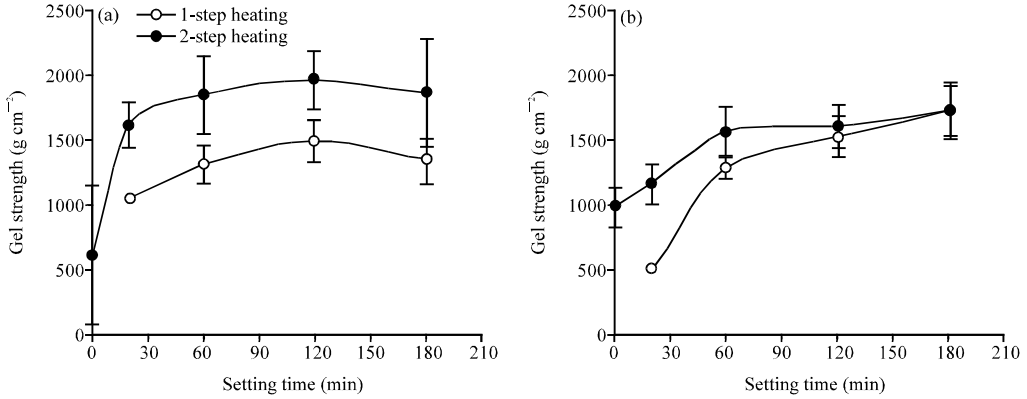

Fig. 1: Change in gel strength of white croaker surimi in the presence or absence of inhibitors of gels set at 40°C. 1-step heating gels setting at 40°C for 20 min, 1, 2 and 3 h and 2-step heating gels at 80°C for 20 min after setting at 40°C for 20 min, 1, 2 and 3 h. (a) Control and (b) EDTA + Leupeptin

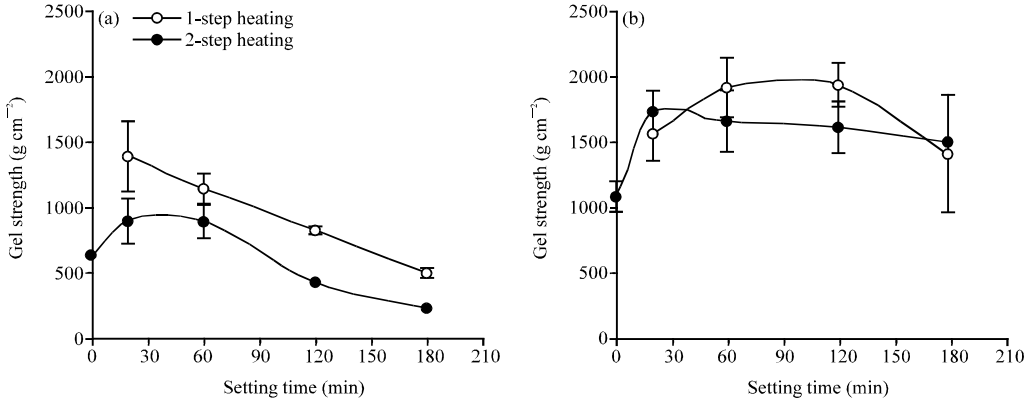

Fig. 2: Change in gel strength of white croaker surimi in the presence or absence of inhibitors of gels set at 50°C. 1-step heating gels setting at 50°C for 20 min, 1, 2 and 3 h and 2-step heating gels at 80°C for 20 min after setting at 50°C for 20 min, 1, 2 and 3 h. (a) Control and (b) EDTA + Leupeptin

increment lessened with prolonged setting time. The gel strength of gel set for 3 h in the 1-step heating and 2-step heating was similar (1,800 g cm^{-2}).

Figure 2a and b showed the changes in the gel strength of white croaker surimi in the presence and absence of inhibitors at 50°C. In the control samples, the gel strength of the 1-step and 2-step heating gels decreased with prolonged setting time. In the presence of EDTA and leupeptin, the gel strength of the 1-step heating gels increased up to 2 h and then decreased. The gel strength of the 2-step heating gel showed the highest value at the gel set for 20 min (1,800 g cm^{-2}) and it decreased slightly with heating time. However, it was markedly higher than that of gel heated directly at 80°C without setting.

SDS-PAGE: To clarify the behavior of the polymerization and degradation of protein in the heated gels, the experiment with SDS-PAGE was conducted (Fig. 3a, b). At 40°C, in unreduced and reduced samples of the control gels, the MHC bands markedly decreased with prolonged setting time. The substances between MHC and actin bands (MHC-A) increased with setting time. In the presence of EDTA and leupeptin, the intensity of MHC bands of all samples was observed to be almost similar regardless of setting time in the unreduced and reduced samples. The MHC-A bands were only slightly produced after setting for 3 h. This indicated that the degradation of MHC was largely inhibited. To elucidate the polymerization of MHC more clearly, SDS-PAGE using 3% polyacrylamide gel was carried out. In the control gels, a large amount of polymer was observed at the top of gel patterns. On the contrary, the polymer band of samples containing inhibitors was not observed (data not shown). Therefore, it was confirmed that the polymerization of MHC was inhibited.

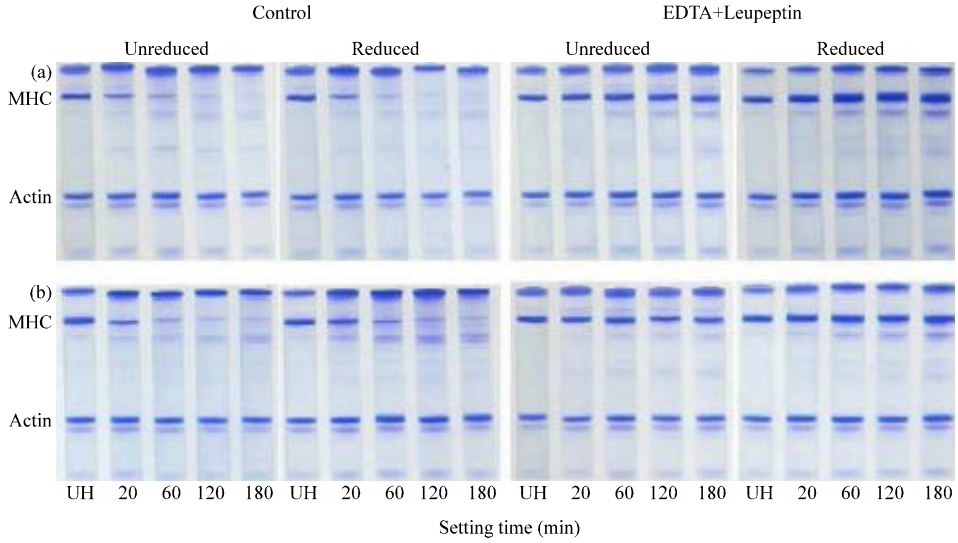

Fig. 3: SDS-PAGE patterns using 5% polyacrylamide gel of reduced and unreduced samples of white croaker surimi gels in the presence or absence of inhibitors of gels set at 40°C. (a) 1-step heating gels at 40°C for 20 min, 1, 2 and 3 h and (b) 2-step heating gels at 80°C for 20 min after setting at 40°C for 20 min, 1, 2 and 3 h. MHC: Myosin heavy chain; UH: Unheated surimi

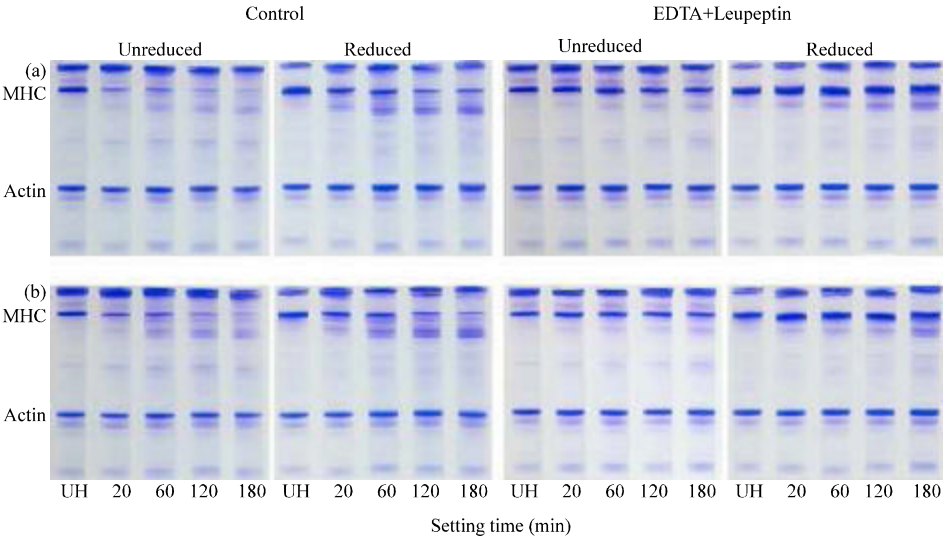

Fig. 4: SDS-PAGE patterns using 5% polyacrylamide gel of reduced and unreduced samples of white croaker surimi gels in the presence or absence of inhibitors of gels set at 50°C. (a) 1-step heating gels at 50°C for 20 min, 1, 2 and 3 h and (b) 2-step heating gels at 80°C for 20 min after setting at 50°C for 20 min, 1, 2 and 3 h. MHC: Myosin heavy chain; UH: Unheated surimi

The SDS-PAGE patterns of gels set at 50°C were shown in Fig. 4a and b. During setting process, the quantity of MHC decreased along with the formation of MHC-A substances in the control sample. In addition, the production of MHC-A substances during setting was higher than that at 40°C. In the presence of the inhibitors, the intensity of MHC bands was almost constant during setting. The MHC-A substances were observed a little in samples set over 2 h.

Autolysis: To clarify the effect of inhibitors on the degradation of MHC, the TCA-soluble free amino acid

and peptides content of white croaker surimi at different temperatures and times was represented in terms of increment of tyrosine (Fig. 5). The increment of Tyr (μg g^{-1} sample) was defined as the difference between the amount of TCA-soluble of heated gel and that of unheated gel. TCA-soluble of the control samples increased with setting time. In the presence of inhibitors, the production of TCA-soluble was almost suppressed. Therefore, it was confirmed that the degradation of the surimi proteins was suppressed.

TCA-soluble content of the control gels set at 50°C markedly increased with setting time (Fig. 6). In the presence of inhibitors, production of TCA-soluble was largely suppressed, although it slightly increased with prolonged heating time.

From the above results, it was indicated that the maximum and the similar gel strength is obtained by setting at 40°C for 3 h and at 50°C for 20 min under the inhibition of polymerization and degradation of surimi proteins including MHC.

Microstructure: Microstructure of the 1-step heating and 2-step heating gels set at 40°C in the presence or absence of inhibitors was shown in Fig. 7a and b. In the control samples, the microstructure of the 1-step heating gel became more regular and denser with setting time. The strand width of gel set for 2 h was 5.7±2.1 μm. The 2-step heating gel set for 20 min exhibited a fine and regular structure with thin strands (width 7.1±3.2 μm). When setting was prolonged up to 2 h, the strands of the 2-step heating gel became markedly thicker (width 19±8.1 μm),

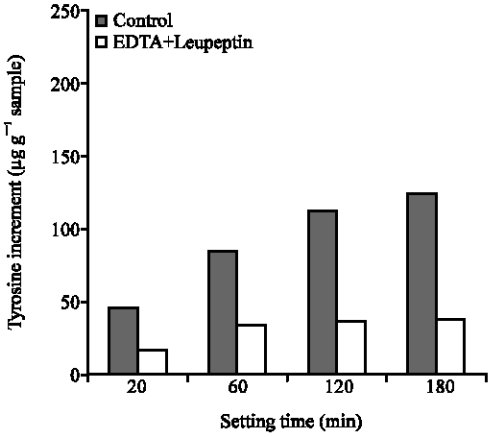

Fig. 5: TCA-soluble free amino acids and peptides in white croaker surimi in the presence or absence of inhibitors of gels set at 40°C for 20 min, 1, 2 and 3 h; followed by heating at 80°C for 20 min

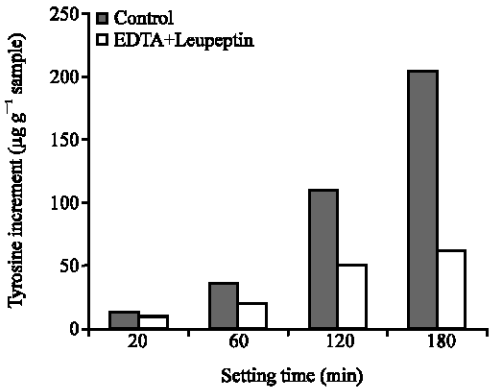

Fig. 6: TCA-soluble free amino acids and peptides in white croaker surimi in the presence or absence of inhibitors of gels set at 50°C for 20 min, 1, 2 and 3 h; followed by heating at 80°C for 20 min

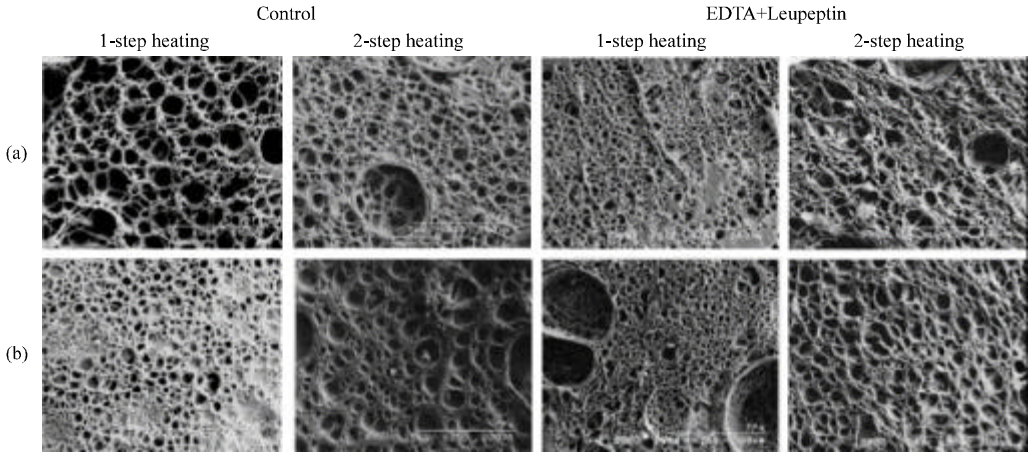

Fig. 7: Microstructure of white croaker surimi in the presence or absence of inhibitors of gel set at 40°C for 20 min and 2 h; followed by heating at 80°C for 20 min. (a) setting for 20 min and (b) setting for 2 h. Magnification = x250, Bar = 200 μm

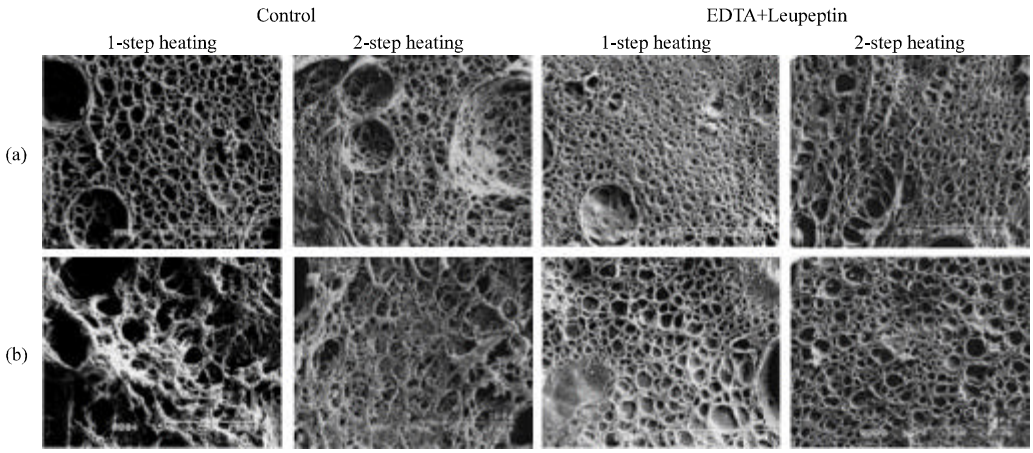

Fig. 8: Microstructure of white croaker surimi in the presence or absence of inhibitors of gel set at 50°C for 20 min and 2 h; followed by heating at 80°C for 20 min (a) setting for 20 min and (b) setting for 2 h. Magnification = x250, Bar = 200 μm

producing a very compact structure. In the presence of inhibitors, the microstructure of 1-step heating gel for 20 min and 2 h was similar. The strands width of these structures was 3.4±1.2 and 4.6±1.7 μm, respectively. The gel network of the 2-step heating gel set for 20 min was less regular and had thinner strand (width 6.4±2.6 μm) compared with that of the control. The gel network became more regular and finer in the gel set for 2 h (strand width 9.5±3.5 μm).

Microstructure of the 1-step heating and 2-step heating gels set at 50°C in the presence or absence of the inhibitors was shown in Fig. 8a and b. The fine gel network of the control samples was obtained in the gel set for 20 min in the 1-step heating gel (strand width 6.4±2.5 μm). After 2-step heating, the gel network became less fine and less regular (strand width 5.1±2.1 μm). When setting at 50°C was prolonged, the gel network was ragged and the three-dimentional gel network was destroyed. In the presence of inhibitors, a dense and regular gel network with thick strand (width 12.7±2.4 μm) was observed in the 1-step heating gel set for 20 min. This structure was still maintained after heated at 80°C for 20 min. The gel network became less dense and less regular in the 1-step heating gel after setting for 2 h. After being heated at 80°C, the gel network exhibited a fine filamentous and uniform porous (strand width 9.8±4.2 μm).

DISCUSSION

The three-dimensional structure of fish meat gel is an important factor affecting the textural properties of the gel (Sato and Tsuchiya, 1992; Yamada and Yoshioka, 1999; Yamaguchi *et al*., 2000; Shikha *et al*., 2006; Jafarpour and Gorczyca, 2009). As for white croaker surimi, gel network structure of the 1-step heating gel set at 40°C for 2 h in the samples without inhibitors were regular and dense, accompanied with the formation of larger amount of polymer by TGase cross-linking which induced gelation and stabilized the gel network (Seki, 1990; Tsukamasa and Shimizu, 1991; Kamath *et al*., 1992). As a result, subsequent heating at 80°C enhanced the gel formation and induced a fine and compact gel network with thick strands, correlated with the highest gel strength. This result agreed with Alvarez *et al*. (1999) that by heating the gels with setting to form cooked gels, the gel structure was rearranged and additional aggregation of proteins was formed in sardine cooked (kamaboko) gel. The structure of 2-step heating gels set at 40 and 50°C for 20 min showed a finer and more compact network structure than that of 1-step heating gels and the strands became thicker. This was consistent with the report of Iwasaki *et al*. (2005) that the rheological characteristics of heat- and pressure-induced myosin filament strands determined the rheological properties of whole gels. The microstructure of the 1-step heating gels set at 50°C was disrupted with prolonged setting time. The strands of the three-dimensional network of gel were destroyed by setting for 2 h, corresponding to the very weak gel. This disruption of gel network was related to the degradation of MHC by proteases (Saeki *et al*., 1995; Makinodan *et al*., 1987; Cao *et al*., 1999).

In the presence of the inhibitors, a regular and dense gel network was obtained in gel set at 40 and 50°C. The finer and more compact gel network structure was formed during setting at 40°C in both 1-step and 2-step heating gels. It indicated that the gel network structure developed

with setting time. This correlated well with the increase of gel strength of gels set at this temperature. The gel network of both 1- and 2-step heating gel set at 50°C for 20 min was dense and compact (with thick strands). The highest gel strength was also obtained at this temperature. These results affirmed the important role of non-covalent bonding on the formation of the structure of gel network in white croaker surimi during heating. Prolonged setting time, the structure of 1-step gels network became less dense and less compact. The inhibitors used could not inhibit the activity of protease completely when set at 50°C. Then, the gel network structure might be destabilized after set for longer time. Therefore, the gel strength was decreased with further heating at 80°C. This result affirmed the importance of the stability of gel network during setting on gel formation. This also agreed with the report of Chang-Lee *et al*., (1989) and Sankar and Ramachandran, (2002) that further cooking process of gel degraded by protease would not enhance the gel strength.

Obviously, the white croaker surimi could form a regular, dense and compact gel network with thick strands during setting even under the inhibition of the polymerization and degradation of MHC. Due to these properties, this gel network was maintained or reinforced by non-covalent bonding during subsequent heating at 80°C for 20 min. On the contrary, Hossain *et al*. (2010) reported that the fine network was observed up to 40°C during setting in walleye pollack surimi under the inhibition of the polymerization and degradation of MHC. However, after a second step heating at 80°C, the microstructure was disrupted and it resembled the cooked gels regardless of the setting temperatures. Moreover, under the inhibition of the polymerization and degradation of MHC, the highest gel strength of the 1-step heating gel of white croaker surimi was 2-fold higher that of walleye pollack surimi (data not shown). The obtained results suggested that the formation of gel network structure of white croaker surimi by non-covalent thermal aggregation during setting was stronger and more stable than that of walleye pollack surimi.

CONCLUSIONS

The texture of the gel was related to continuity of the three-dimensional network and the thickness of strands. The non-covalent thermal aggregation of white croaker surimi protein could form a regular and compact gel network with thick strands during setting at low temperature and this gel network was maintained or reinforced during subsequent heating at 80°C for 20 min under the inhibition of the polymerization and degradation of MHC. The difference in gel formation between white croaker and walleye pollack surimi under the inhibitory condition was due to the difference in the stability of gel network structure, which was formed by non-covalent aggregation of protein during setting.

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