Effect of Endogenous Transglutaminase on Threadfin Bream Surimi Gelation

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ABSTRACT: Transglutaminase(TGase) activity of threadfin bream mince was 99.6 units/g of dry weight. After washing and screw-pressed dewatering, 44% residual activity was retained. Covalent cross-linking of myosin heavy chain (MHC) was observed at both 25 and 40 °C and supported by increased gel strength. When pre-incubation at 40 °C was prolonged to 4 h, breaking force and MHC decreased due to endogenous proteinase(s). TGase activity towards MHC and synthetic substrates was effectively inhibited by iodoacetic acid (IAA). Autolytic activity and degradation of MHC was inhibited by phenylmethanesulfonyl fluoride (PMSF). Addition of 0.2% Ca^{2+} significantly improved breaking force and increased MHC cross-linking of surimi gels pre-incubated at 40 °C for 2 h.
Keywords: transglutaminase, myosin heavy chain, cross-linking, threadfin bream

Introduction

THREADFIN BREAM (*NEMIPTERUS SPP.*) IS THE 2ND LARGEST RESOURCE used for surimi production, after Alaska pollock. Thailand is one of the major threadfin bream surimi producers in the world with an approximate annual production of over 80000 metric tons. Despite its large production quantity and value, scientific information related to its gelation characteristics is limited. Utilization of threadfin bream surimi in the surimi-seafood industry has primarily relied on the technical information of other species, such as Alaska pollock. However, the intrinsic properties of cold water fish, such as Alaska pollock, are significantly different from the properties of warm water fish, such as threadfin bream.

Setting or "suwari" is a phenomenon describing an increased gel strength after pre-incubation of surimi paste at a certain temperature, between 5 and 40 °C for a specific period of time (Lanier 2000). An improvement of textural properties is attributed to an endogenous transglutaminase (TGase) (R-glutaminyl-peptide: amine γ-glutamyltransferase; EC 2.3.2.13) that catalyzes the cross-linking reaction of muscle proteins, especially myosin (Kimura and others 1991; Kishi and others 1991). The γ-carboxyamide groups of peptide-bound glutamine residues act as acyl donors, while the primary amino groups, including the ε-amino of lysine can be acyl acceptors (Folk 1980). The resulting ε-(γ-glutamyl) lysyl isopeptide bonds are stronger than hydrogen and hydrophobic interactions. Such covalent cross-linkings of myosin formed inter- and intramolecularly, resulting in higher gel elasticity (Lee and others 1997).

Optimum temperature of setting varies with fish species. Klesk and others (2000) reported that setting of Alaska pollock surimi was noticed at 5 and 25 °C for 22 and 3 h, respectively. In contrast, optimal setting for subtropical tilapia surimi was at 40 °C. Maximum cross-linking of myosin heavy chain (MHC) of croaker surimi was also found at 40 °C (Kamath and others 1992). Differences in optimum setting temperature were attributed to conformational changes of myosin (Joseph and others 1994). Optimum setting condition of threadfin bream surimi has not been studied extensively.

Setting conditions of surimi are also affected by calcium content since endogenous TGase is a Ca^{2+}-dependent enzyme (Lee and Park 1998; Kimura and others 1991). The optimal $CaCl_2$ concentration for TGase also varied with the source of the enzyme (Yasueda and others 1994; Kumazawa and others 1996). Therefore, enhancement of gel strength through TGase can be achieved by optimizing the Ca^{2+} concentration to be used for the respective species.

Better understanding of the setting phenomenon of threadfin bream surimi could lead to better quality control and possible enhancement of gel quality. Objectives of our study were to investigate changes of TGase activity during threadfin bream surimi processing. In addition, the effects of temperature and $CaCl_2$ concentration on setting of threadfin bream surimi were examined.

Materials and Methods

Materials and reagents

Fresh threadfin bream (*Nemipterus spp*), washed mince, screw-pressed mince, and threadfin bream surimi (Grade AA) of the same lot of raw fish were collected from a surimi plant at Samutsakorn, Thailand. Samples were packed in a polystyrene box, filled with ice, and immediately transported to the Suranaree University laboratory. TGase activity of washed mince was determined upon arrival. Surimi was cut into 1 kg blocks, vacuum-packed, and kept at –18 °C during the study.

N, N'-Dimethylated casein (DMC), monodansylcadaverine (MDC), iodoacetic acid (IAA), phenylmethanesulfonyl fluoride (PMSF), and N-ethylmaleimide (NEM) were purchased from Sigma Chemical Company (St. Louis, Mo., USA). Ethylene glycol-bis (2-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) and dithiothreitol (DTT) were purchased from Fluka (Buchs, Switzerland). Reagents used for gel electrophoresis were obtained from Bio-Rad (Hercules, Calif., U.S.A.). All other chemicals were of analytical grade.

Preparation of crude TGase extracts

TGase activities of mince, 1st washed, 2nd washed, 3rd washed, and screw-pressed minces were determined. Samples were homogenized, upon arrival, in 4 volumes of extraction buffer (10 mM NaCl, 5 mM EDTA, 2 mM DTT, 10 mM Tris-HCl, pH 7.5). The homogenates were centrifuged at 16000 × g for 20 min (RC 28S; Sorvall Co., Newtown, Conn., U.S.A.) and supernatants were subsequently centrifuged at 100000 × g for 60 min (Beckman Class S; Beckman Co., Palo Alto, Calif., U.S.A.). The supernatants, after ultracentrifugation, were used as crude extract.

TGase activity

TGase activity was assayed by the method of Takagi and others (1986) with slight modifications. The assay mixture contained 1.0 mg/mL DMC, 15 μM MDC, 5 mM $CaCl_2$, 3 mM DTT, 50 mM Tris-HCl (pH 7.5), and 100 μL of crude enzyme. After incubation at 37 °C for 10 min, a time period found to be in a linear range from preliminary study, EDTA solution was added to a final concentration of 20 mM to stop the reaction. The fluorescence intensity was measured with excitation and emission wavelengths of 350 and 380 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 1.93. 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.

The temperature profile of crude TGase was ascertained by pre-incubating the assay mixture at 0, 25, 37, 40, 50, 55, 60, and 70 °C for 10 min. Crude enzyme was added and the mixtures were further incubated for 10 min. The reaction was stopped using EDTA and fluorescence intensity was measured as described above. Protein content of crude enzyme was measured using the Lowry method (Lowry and others 1951).

Study of TGase inhibitors

Inhibitor study of crude TGase was carried out for the same reaction mixtures described above with the addition of PMSF, NEM, and IAA at 1 mM, and EGTA at 5 mM. Samples were pre-incubated at either 25 or 40 °C for 10 min. The reaction was stopped by adding EDTA to achieve a final concentration of 20 mM and its fluorescence intensity was measured as previously described.

The inhibitory effect of the aforementioned compounds was also studied in a surimi system. Surimi was partially thawed and ground using a mortar and pestle. Salt and moisture contents were adjusted to 3% and 78%, respectively. PMSF, NEM, and IAA were added to the surimi paste at a final concentration of 1 μmol/g, whereas that of EGTA was 5 μmol/g. The mortar was kept in ice during grinding to eliminate temperature abuse during grinding. A control sample, without inhibitors, was also prepared. Five g of surimi paste were evenly spread in a beaker, and covered with parafilm and aluminum foil to minimize moisture loss during pre-incubation and heating. Samples were pre-incubated at 25 and 40 °C for 2 and 1 h, respectively. All samples were then heated at 90 °C for 30 min and chilled in ice for 15 min. Protein patterns were analyzed using SDS-PAGE. Autolytic activities of the paste were also measured as described by Yongsawatdigul and others (2000). Samples were incubated at 25 and 40 °C for 1 h. Trichloroacetic acid (TCA)-soluble oligopeptide contents were analyzed by Lowry's assay using tyrosine as a standard.

SDS-PAGE

The continuous SDS-PAGE was carried out according to the modified method of Weber and Osborn (1969). Surimi gel samples were cut into small pieces and 0.5 g was solubilized in 10 mL of solubilizing buffer containing 2% SDS, 8 M urea, 2% β-mercaptoethanol, and 20 mM Tris-HCl (pH 8.0). Samples were homogenized, heated for 3 min at 100 °C, and shaken for 24 h at room temperature (27 °C). Homogenates were centrifuged at 10000 × g for 30 min. Due to the presence of urea, the protein concentration of the supernatant was determined by the Bradford method (Bradford 1976) with Bio-Rad protein assay dye reagent using bovine serum albumin as a standard. Gels were 3% (w/v) acrylamide, 0.08% (w/v) bis-acrylamide, and 0.25% agarose. The amount of applied protein was 15 μg.

Degradation of muscle proteins was investigated according to the method described by Laemmli (1970). Samples (3 g) were solubilized in 5% hot SDS (80° C) and heated at 85 °C for 30 min. Homogenates were centrifuged as described above and protein content was determined by the Lowry method (Lowry and others 1951).

Surimi gel preparation

Frozen surimi was partially thawed and chopped in a Stephan vacuum cutter (Model UM5; Stephan Machinery Co., Columbus, Ohio, U.S.A.). Sodium chloride was added at 3% of the total weight. Ice was added to adjust the moisture content to 78%. The raw paste was stuffed into a 3 cm dia casing and pre-incubated at 4, 25, and 40 °C for various time periods prior to final heating at 90 °C for 30 min. A control sample heated at 90 °C without pre-incubation was also prepared. Surimi gels were chilled in ice water and kept refrigerated at 7 °C overnight for texture evaluation.

The effect of Ca^{2+} on the textural properties was determined as described above with the addition of 0, 0.07, 0.13, and 0.27% $CaCl_2$ $2H_2O$ corresponding to Ca^{2+} of 0, 0.05, 0.1, and 0.2%, respectively. Samples were incubated at 4, 25, and 40 °C for 24, 4, and 2 h, respectively, before heating at 90 °C for 30 min.

Texture evaluation

A puncture test was carried out using a Texture Analyzer (Stable Micro System, Surrey, England) equipped with a 5-mm spherical plunger at a test speed of 1 mm/s. Gel samples were cut into pieces 3.0 cm long. Breaking force (g) and deformation (mm) were recorded. For each treatment, at least 5 samples were measured. Sample temperature during measurement was 25 °C.

Statistical analyses

Analysis of variance (ANOVA) and the Duncan's range test were performed using the SAS system (SAS Institute Inc., Cary, N.C., U.S.A.).

Results and Discussion

Changes of TGase activity during surimi processing

Residual TGase activity of the first washed mince (1st WM) was lower than that of mince (M) (Figure 1), indicating that the initial washing cycle efficiently removed water soluble sarcoplasmic proteins including the enzyme. The second washed mince (2nd WM) contained higher residual activity than the 1st WM. This might be
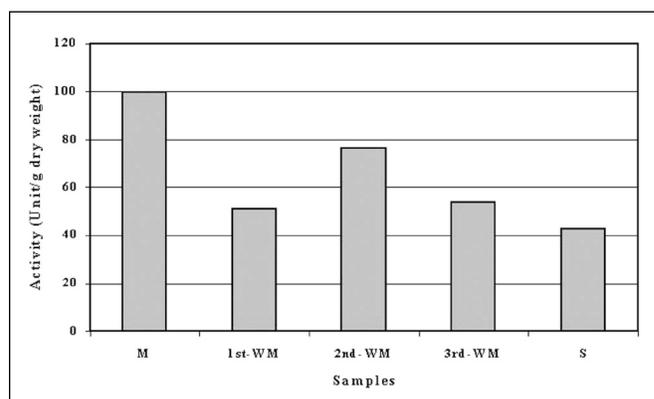
Figure 1—Changes of TGase activity during threadfin bream surimi processing. M = minced fish, 1st-WM = first washed mince, 2nd-WM = second washed mince, 3rd-WM = third washed mince, S = screw-pressed sample

contributed from the presence of TGase in the cytosolic fraction of muscle cell, which can only be removed by extensive washing or by detergent, such as Triton (Kishi and others 1991). In addition, the 2nd WM contained lower amount of dry matter than the 1st WM as sarcoplasmic proteins were subsequently leached out from the 1st washing cycle (7.2 mg/mL) and the 2nd washing cycle (1.7 mg/mL). As a result, activity based on the dry matter of the 2nd WM was higher than that of the 1st WM. TGase appeared to be removed to the greater extent in the 3rd washing cycle, resulting in a decreased residual TGase in the 3rd washed mince (3rd MW). Screw-pressed dewatering did not seem to further remove the enzyme. Approximately 44% of TGase activity was retained in the final surimi, indicating that TGase was not completely removed during surimi processing.

Araki and Seki (1993) found a slight change in TGase from pollock mince (0.41 unit/g) to pollock surimi (0.33 unit/g). It should be noted that activity reported by Araki and Seki (1993) was smaller than ours. This could be because their assay was conducted at 25 °C, rather than at 37 °C. In addition, such differences could arise from inherent differences between the 2 species.

Optimum temperature of crude TGase from threadfin bream was 55 °C (Figure 2). Activity at 55 °C was almost 2 times greater than that at the typical temperature used in TGase activity, 37 °C. The optimum temperature of TGase activity, however, varies with the fishery resource. Yasueda and others (1994) reported that the optimum temperature of TGase purified from red sea bream (*Pagrus major*) was 55 °C. Purified TGase from Japanese oyster showed optimum activity at 25 and 40 °C (Kumazawa and others 1997), while optimum activity for walleye pollock liver was at 50° C (Kumazawa and others 1996).

Although the optimum temperature of the crude enzyme was determined to be 55 °C, our preliminary results showed that incubation of surimi at 55 °C induced proteolysis of the muscle proteins with TCA-soluble oligopeptides of 7.66 nmol/mg/hr and reached a maximum of 10.11 nmol/mg/hr at 60 °C. Therefore, pre-incubation at 40 °C was chosen as the highest temperature for surimi setting in this study.

Effect of temperature on textural properties

Textural properties of surimi gels pre-incubated at various temperatures are shown in Figures 3a and 3b, respectively. Gel strength (breaking force) of surimi gels pre-incubated at 4 and 25 °C increased as the incubation time increased ($p < 0.05$). However, deformation of surimi at 25 °C decreased after pre-incubation for 6 h. At 40 °C, gel strength increased significantly after 2 h, reaching values

3 times higher than the control sample. However, gel strength values rapidly decreased after 3 h. A similar trend was also observed for deformation. Breaking force of surimi gels pre-incubated at 25 °C for 4 h was not different from those pre-incubated at 40 °C for 2 h ($p > 0.05$).

These results revealed that setting of threadfin bream could be induced at both 25 and 40 °C, but setting at 25 °C took longer time (4 h) than at 40 °C (2 h). However, Yongsawatdigul and Park (2001) demonstrated that actomyosin of threadfin bream exhibited minimal conformational changes at 25 °C. Unfolding of threadfin bream actomyosin began at > 35 °C. Therefore, thermal unfolding of protein might not be the sole prerequisite for the setting phenomenon of threadfin bream surimi. Partial unfolding of actomyosin induced by NaCl appeared to be sufficient for TGase binding at reactive residuals. Longer times required for setting at 25 °C was probably due to lower TGase activity at 25 °C than at 40 °C (Figure 2).

Effect of temperature on electrophoretic patterns of proteins

According to SDS-PAGE patterns, MHC slightly decreased as pre-incubation time increased at 4 °C (Figure 4a). Cross-linked proteins (CP), which did not travel through 3% polyacrylamide gel, apparently increased with prolonged incubation time. A significant decrease in MHC was obvious in samples pre-incubated at 25 °C for 4 h (Figure 4b). MHC completely disappeared after pre-incubation at 25 °C for 6 h. A decrease of MHC was observed when samples were pre-incubated at 40 °C for 30 min (Figure 5a). At 2 h pre-incubation at 40 °C, MHC disappeared concomitantly with a noticeable
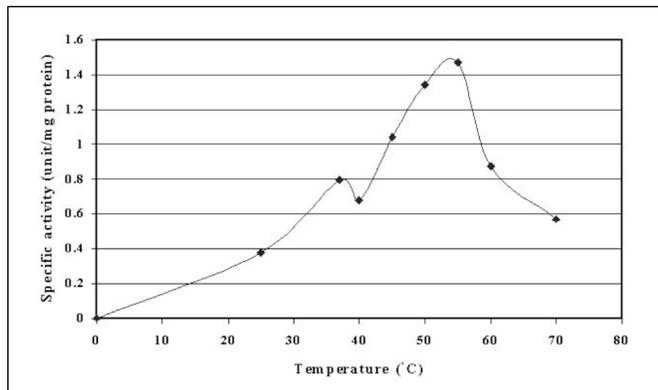
Figure 2—Temperature profile of crude TGase extracted from minced threadfin bream
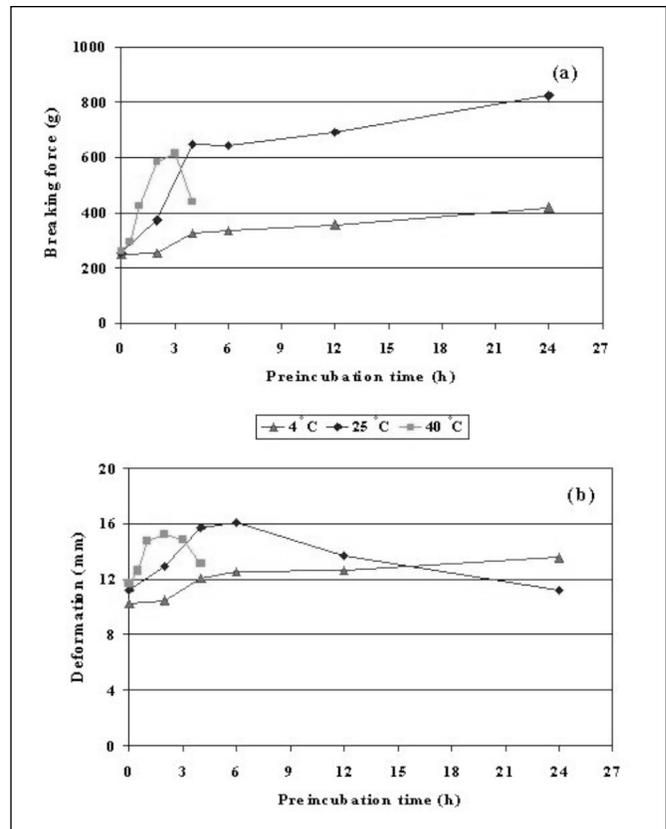
Figure 3—Effect of pre-incubation time and temperature on breaking fore (a) and deformation (b) of threadfin bream surimi

intensity of CP at the top of the gel. Formation of CP corresponded with an increase of gel strength observed at 4, 25, and 40 °C. (Figures 3a and 3b). Disappearance of MHC in conjunction with the formation of cross-link polymers is known to be catalyzed by endogenous TGase. Covalent cross-linking of ϵ-(γ-glutamyl)-lysine strengthens the gel structure, resulting in high shear stress (Joseph and others 1994). Our results also revealed that MHC appeared to be a preferred substrate for the cross-linking induced by endogenous TGase. Actin did not participate in the cross-linking as its intensity was unchanged at all studied temperatures.

The intensity of protein with a molecular weight of 105 and 127 kDa, increased with prolonged incubation time at 40 °C (Figure 5b). Loss of troponin was also noticed when the surimi was pre-incubated for 3 h. An increase of these smaller molecular weight proteins corresponded with a decrease of breaking force and deformation (Figures 3a and 3b). Degradation of MHC and troponin were possibly induced by the endogenous proteolytic activity in threadfin bream surimi (Toyohara and Shimizu 1988). Our results indicated that proteolysis of threadfin bream surimi took place when subjected to 40 °C beyond 2 h, yielding poor gel texture and loss of MHC. It confirms the contradictory behaviors (gel enhancing by TGase

and gel softening by proteases) occurring in threadfin bream at 40 °C, depending on incubation time.

The effect of endogenous TGase on MHC cross-linking was confirmed (Figures 6a and 6b). MHC intensity of samples mixed with PMSF, NEM, IAA, and EGTA set at 25 and 40 °C was greater than the control. MHC cross-linking at 25 °C was effectively inhibited by IAA and EGTA, while PMSF and NEM showed slight inhibition (Figure 6a). However, IAA, NEM, and EGTA inhibited TGase activity assayed by MDC and DMC at 25 °C to a similar extent (Figure 7). NEM and IAA were known to react with SH group at the active site of TGase (Kumazawa and others 1996, 1997), therefore they showed an inhibitory effect towards the enzyme. EGTA inhibits TGase activity through chelating Ca^{2+}, a divalent cation required for the activation of tissue TGase (Folk 1980).

The extent of TGase inhibition at 40 °C could not be interpreted based on the disappearance of MHC (Figure 6b) because proteolytic degradation of MHC also occurred as evident from the degraded protein band (Figure 6b). TCA-soluble oligopeptide content confirmed that proteolysis was greater at 40 °C than at 25 °C (Figure 8). PMSF appeared to be an effective proteinase inhibitor at 40 °C. NEM, IAA, and EGTA had no effect on proteolysis as compared to the control. PMSF inhibits serine proteinase, whereas IAA and NEM are cysteine proteinase inhibitors (Salvesen and Nagase 1990). We postulated that serine proteinase(s) played a major role in autolysis and MHC degradation of threadfin bream surimi. Kinoshita and
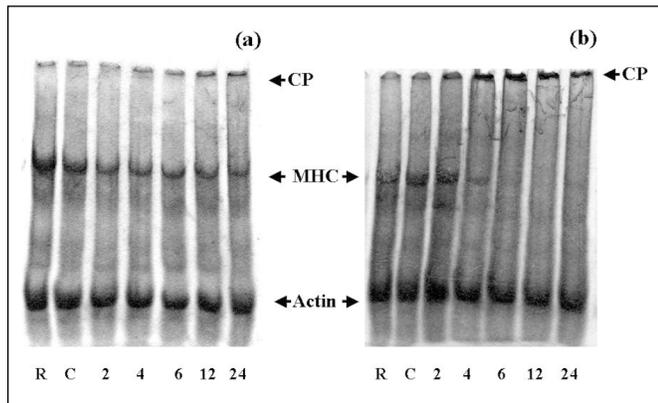
Figure 4—SDS-PAGE pattern (3% acrylamide + 0.25% agarose) of threadfin bream surimi pre-incubated at 4 °C (a) and 25 °C (b) for various time before heating to 90 °C for 30 min. R = raw surimi, C = control (no pre-incubation), 2 to 4 e-incubation time in h, CP = cross-linked protein, MHC = myosin heavy chain
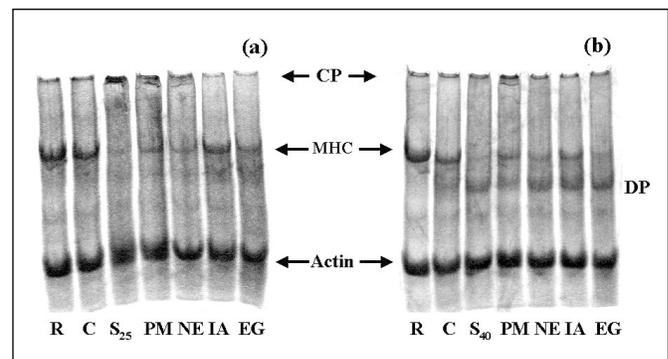
Figure 6—SDS-PAGE pattern (3% acrylamide + 0.25% agarose) of threadfin bream surimi pre-incubated at 25 °C for 4 h (a) and 40 °C for 1 h (b) in the presence of various inhibitors. R = raw surimi, C = control (no pre-incubation), S_{25} and S_{40} = set at 25 and 40 °C without inhibitor, respectively, PM = PMSF, NE = NEM, IA = IAA, EG = EGTA, CP = cross-linked proteins, MHC = myosin heavy chain, DP = degraded proteins
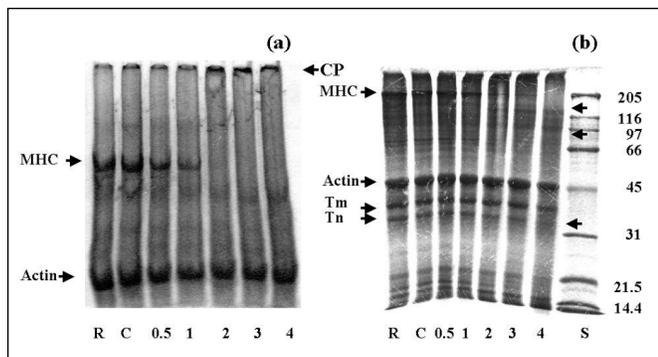
Figure 5—SDS-PAGE pattern of threadfin bream surimi pre-incubated at 40 °C on 3% acrylamide + 0.25% agarose (a) and 10% acrylamide (b). R = raw surimi, C = control (no pre-incubation), 0.5-4 = pre-incubation time in h, S = standard molecular weight (kDa), CP = cross-linked protein, MHC = myosin heavy chain, Tm = tropomyosin, Tn = troponin.
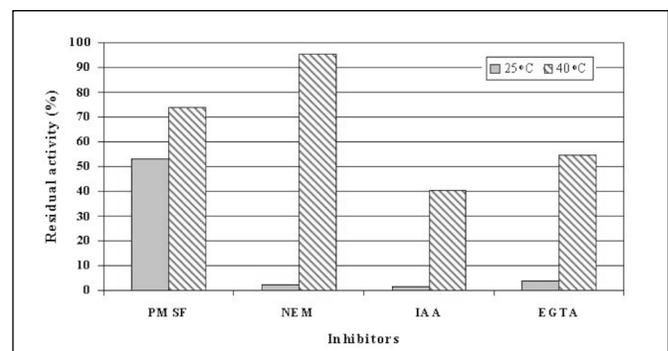
Figure 7—Residual activity of crude TGase in the presence of various inhibitors at 25 and 40 °C

others (1990) also reported that serine proteinase was responsible for the textural degradation of threadfin bream muscle in the presence of NaCl at pH 7.0 and at 60 °C.

PMSF only inhibited crude TGase activity at 40 °C about 30% (Figure 7), but effectively inhibited autolysis (Figure 8). Therefore, higher retention of MHC seen in the PMSF-mixed sample might have resulted from proteolytic inhibition. The degree of inhibition by IAA towards crude TGase was greater than that by NEM at 40 °C when MDC and DMC were used as substrates (Figure 7). In addition, retention of MHC in surimi gels mixed with IAA was greater than that mixed with NEM, although the degree of proteolysis was similar (Figure 8). Therefore, IAA also inhibited endogenous TGase more effectively than NEM when MHC was used as a substrate.

Since EGTA activated threadfin bream autolysis (Figure 8) and inhibited crude TGase activity (Figure 7), the disappearance of MHC (Figure 6b) was more likely due to proteolytic degradation than the formation of covalent cross-linking. This study revealed that endogenous TGase mediated cross-linking of threadfin bream MHC at both 25 and 40 °C. It should be noted that cross-linking and proteolysis occurred simultaneously at 40 °C. Long term pre-incubation at 40 °C promoted endogenous proteinase(s), which negatively affects gel texture. Gel strength of threadfin bream surimi, therefore, can be improved by minimizing proteolytic activity while enhancing TGase activity.

Optimal pre-incubation time at 40 °C was dependent on sample geometry and shape. Differences in sample size and geometry resulted in varied heat transfer and temperature distribution. There was no evidence of MHC proteolysis when surimi was pre-incubated at 40 °C for 2 h in a 3-cm-dia casing. With the same surimi paste, degradation of MHC was noticed at 1 h pre-incubation in the small sample (5 g) used in the inhibitor study.

Effect of CaCl$_2$ on textural properties

The addition of CaCl$_2$ and pre-incubation conditions significantly affected the textural properties of threadfin bream surimi gels (Figure 9a and 9b). Pre-incubation time at each temperature was selected based on the textural properties shown in Figure 3. Setting condition affected breaking force ($p < 0.05$), but had no effect on deformation ($p > 0.05$). At any added Ca^{2+} concentration, the breaking force of samples set at high temperature (40 °C) were higher than those set at low temperature (4 and 25 °C) and without setting ($p < 0.05$).

Breaking force of threadfin bream surimi gel was highest when 0.2% Ca^{2+} was added and set at 40 °C for 2 h. Effect of Ca^{2+} was more pronounced at 40 °C than at 4 and 25 °C because activity of TGase was greater at 40 °C. Lee and Park (1998) found that addition of

0.2% calcium compounds improved shear stress of Pacific whiting, whereas the lower concentrations (0.05 to 0.1%) effectively increased gel texture of Alaska pollock. Species-dependence of optimum CaCl$_2$ was attributed to the inherent, varied concentrations of Ca^{2+} in muscle among species (Lee and Park, 1998).

Effect of CaCl$_2$ on electrophoretic patterns of proteins

Calcium concentration and pre-incubation conditions (Figures
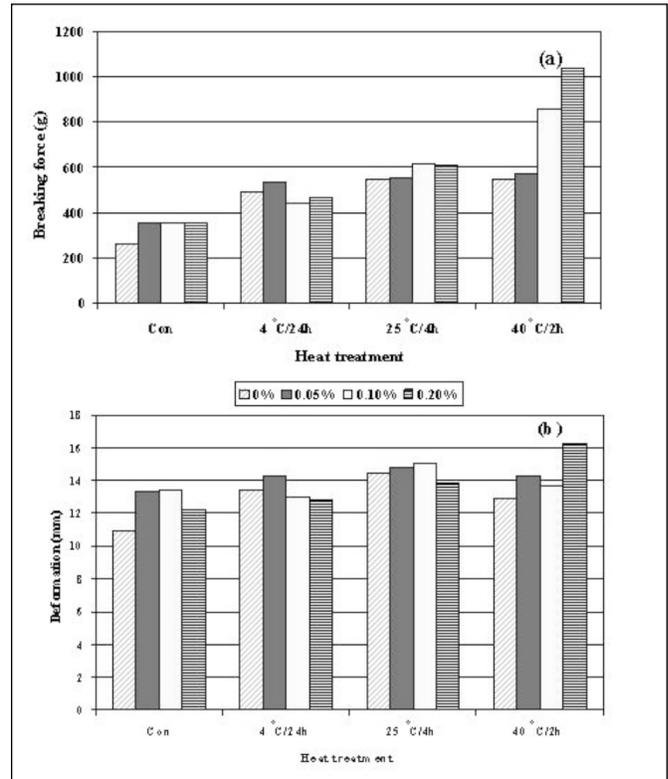
Figure 9—Breaking force (a) and deformation (b) of threadfin bream surimi gels with various Ca^{2+} concentrations. Con = no pre-incubation; 4 °C/24h, 25 °C/4h, 40 °C/2h = pre-incubation at 4 °C for 24 h, 25 °C for 4 h, and 40 °C for 2 h
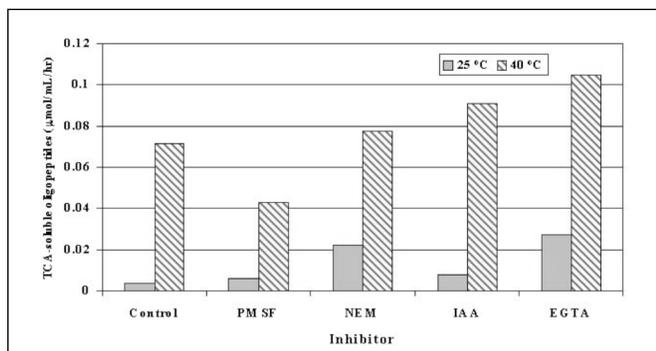
Figure 8—Autolytic activity of threadfin bream surimi at 25 and 40 °C in the presence of various inhibitors
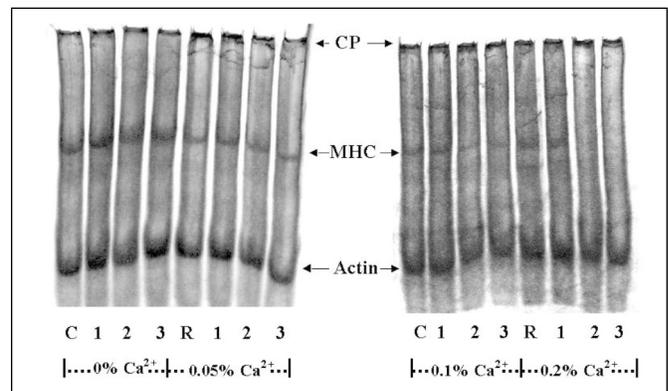
Figure 10—SDS-PAGE patterns (3%acrylamide + 0.25% agarose) of threadfin bream surimi mixed with 0, 0.05% Ca^{2+} (a) and 0.1, 0.2% Ca^{2+} (b) and pre-incubated at various conditions. C = control (no pre-incubation); R = raw surimi paste; 1, 2, and 3 = pre-incubated at 4, 25, and 40 °C for 24, 4, and 2 h, respectively.

10a and 10b) significantly affected protein patterns on SDS-PAGE of surimi. MHC decreased with an increased concentration of Ca^{2+}. At 0 and 0.05% Ca^{2+} (Figure 10a), MHC intensity at various setting conditions was comparable. When 0.1% Ca^{2+} was added, MHC intensity of gels pre-incubated at 25 °C for 4 h and 40 °C for 2 h were less than those of the control and those pre-incubated at 4 °C (Figure 10b). MHC completely disappeared when 0.2% Ca^{2+} was added and pre-incubated at 25 °C for 4 h and 40 °C for 2 h (Figure 10b). Ca^{2+} had a more pronounced effect on the disappearance of MHC when pre-incubated at 25 °C for 4 h and 40 °C for 2 h.

Changes of MHC at various conditions corresponded well with breaking force of surimi gels (Figure 9a). An increased breaking force at higher Ca^{2+} concentration was due to an increase in the catalytic activity of endogenous TGase. Concentration dependency of Ca^{2+} for TGase also varied with species. Several researchers have reported on the Ca^{2+}-dependent catalytic reaction of TGase from hoki (Kimura and others 1991), red sea bream (Yasueda and others 1994), the egg envelop (chorion) of rainbow trout (Ha and Iuchi 1997), Japanese oyster (Kumazawa and others, 1997), and scallop (Nozawa and others 1999; Nozawa and Seki 2001). Reduction of MHC reached 90% of its original content when 0.06% $CaCl_2$ was added into hoki surimi incubated with crude hoki TGase (Kimura and others 1991). Cross-linked MHC of Alaska pollock surimi was maximally formed in the presence of 0.02% Ca^{2+}, and a further increase in Ca^{2+} concentration did not increase MHC cross-links (Wan and others 1994).

It should be noted that MHC of the control also decreased with added Ca^{2+} concentration. This result indicated endogenous TGase catalyzed cross-linking of MHC even without incubation. Slow temperature increase in a 3 cm dia casing allows the catalytic activity of endogenous TGase to progress before thermal inactivation occurs. Yongsawatdigul and Park (1996) also reported that slow ohmic heating 1 °C/min induced MHC cross-linking of Alaska pollock surimi.

Conclusions

ENDOGENOUS TGASE WAS RETAINED IN THREADFIN BREAM SURIMI after 3 washing steps. The enzyme significantly contributed to MHC cross-linking and enhanced breaking force at 25 and 40 °C setting. Optimal pre-incubation time at 25 and 40 °C was 4 and 2 h, respectively, in a 3 cm dia casing. Proteolytic degradation of MHC was noticed when pre-incubation at 40 °C was extended beyond the optimal time. IAA effectively inhibited MHC cross-linking while PMSF reduced proteolysis of MHC at 40 °C. Addition of 0.2% Ca^{2+} improved gel strength through enhancing the covalent cross-linking reaction of MHC.

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