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Effect of microbial transglutaminase on autolysis and gelation of lizardfish surimi

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Abstract: In the absence of microbial transglutaminase (MTGase), the textural properties of lizardfish surimi (*Saurida* spp) improved when pre-incubated at 4 and 25 °C for 24 and 4 h, respectively. MTGase optimally catalyzed incorporation of monodansylcadaverine (MDC) into surimi at 40 °C. Addition of MTGase appeared to reduce autolytic activity at 25 and 40 °C, but had no effect on autolytic activity at 65 °C. Breaking force and deformation of lizardfish surimi significantly improved when 0.1 unit MTGase g^{-1} surimi (1.8 g kg^{-1}) was added and pre-incubated at either 25 or 40 °C. Textural properties improved concomitant with cross-linked polymers of myosin heavy chain and tropomyosin, but not actin. Addition of MTGase also improved the storage modulus (G'). The gel network of surimi mixed with MTGase and pre-incubated at 40 °C readily formed during the pre-incubation period, while formation of the gel network began at 48.1 °C in the absence of MTGase.

Keywords: lizardfish surimi; microbial transglutaminase; protein cross-linking; proteolysis

INTRODUCTION

Lizardfish (*Saurida* spp) surimi is considered a low value because of its poor gel-forming ability.[1] It also undergoes severe textural degradation due to endogenous proteinases.[2,3] Both serine and cysteine proteinases were found in lizardfish and contributed to proteolysis of muscle proteins.[2] Proteolytic activity of both lizardfish mince and surimi increased with temperature and reached the maximum at 65 °C at pH 6–7.[3] Myofibril-bound serine proteinase appeared to play a vital role in proteolysis of lizardfish surimi.[2,3] The myofibril-bound proteinase purified from lizardfish (*Saurida wanieso*) was classified as a trypsin-type serine proteinase and optimally hydrolyzed myosin heavy chain (MHC) at 55–60 °C.[4]

The problem of textural degradation induced by proteolysis of surimi has been mainly overcome by addition of food-grade proteinase inhibitors, namely egg white powder (EW), whey protein concentrate (WPC), beef plasma protein (BPP) and potato extract.[5–7] The effectiveness of each inhibitor varied with surimi species. EW and BPP increased shear stress of menhaden surimi to a similar extent,[5] but BPP was found to be a more effective inhibitor in Pacific whiting surimi.[6,7] EW at 10 g kg^{-1} addition improved gel-forming ability of lizardfish surimi to a greater extent than WPC.[3] However, addition of BPP has recently become unacceptable due to the outbreak of bovine spongiform encephalopathy or 'mad cow disease'. In addition, EW could result in a sulfurous odor in surimi seafood products. Consequently, alternative means of improving textural properties of lizardfish surimi are needed.

Transglutaminase (TGase; *R*-glutaminyl-peptide —amine γ-glutamyltransferase; EC 2.3.2.13) is an enzyme catalyzing acyl-transfer reactions, resulting in ε-(γ-glutamyl)lysine cross-links.[8] TGase is present in most mammalian tissue and body fluids and is also found in fish species. These TGases require Ca^{2+} for activation. Endogenous transglutaminase in fish muscle has been shown to be responsible for the 'setting' phenomenon, which resulted in more highly elastic gel.[9–12] In addition, microbial transglutaminase (MTGase) from *Streptoverticillium* sp which is a Ca^{2+}-independent enzyme, has also been reported to improve textural properties of various food protein gels, including fish protein.[13–15] However, application of MTGase in surimi with high proteolytic activity has not yet been elucidated. Proteolysis of muscle proteins could greatly affect cross-linking reaction of MTGase. Optimum conditions to minimize endogenous proteolytic activity and promote cross-linking would improve textural properties of lizardfish surimi. The objective of this study was to investigate the effect of MTGase on proteolytic degradation and gel-forming ability of lizardfish surimi.

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MATERIALS AND METHODS

Samples and chemicals

Frozen lizardfish surimi (*Saurida* spp) samples were obtained from the surimi plant at Samutsakorn, Thailand. Samples were packed in a polystyrene box, filled with ice, and immediately transported to the Suranaree University laboratory. Frozen surimi was cut into 1 kg blocks, vacuum-packed, and kept at −18 °C until use. The commercial MTGase derived from *Streptoverticillium* (Activa TG-K) was donated by Ajinomoto (Thailand) Inc (Bangkok, Thailand). Reagents used for gel electrophoresis were purchased from Bio-Rad (Hercules, CA, USA). All other chemicals were reagent-grade.

MTGase activity

The dried powder of commercial MTGase was dissolved in deionized distilled water at concentration of 35 g l^{-1}. MTGase activity was assayed using the incorporation of monodansylcadaverine into lizardfish surimi according to Huang *et al*[16] with slight modifications. The solution (5 ml) contained 0.55 mM monodansylcadaverine, 2 g lizardfish surimi, 0.4 M NaCl in 13.33 mM Tris–HCl (pH 7.5), and 400 µl enzyme solution. The reaction mixture was incubated at 40 °C for 30 min; subsequently 5 ml of 100 g kg^{-1} trichloroacetic acid were added to terminate the reaction and precipitate surimi muscle proteins. The precipitate was washed four times with 20 ml diethyl ether and then dried in a vacuum oven at 30 °C for 1 h. The dried precipitate was dissolved in a solution containing 8 M urea, 10 g l^{-1} sodium dodecylsulfate (SDS) and 50 mM Tris–HCl (pH 8) at a ratio of 1:4. The fluorescence intensity of the solution was measured with excitation at 350 nm and emission at 480 nm. The blank samples were carried out using deionized distilled water to replace the enzyme solution. Monodansylcadaverine at concentrations of 0, 1 and 10 µM in the same buffer solution was used as the standard. The unit of activity was defined as nanomoles of monodansylcadaverine incorporated into surimi per minute.

Surimi gel preparation

Frozen surimi was thawed and chopped in a Stephan vacuum cutter (UM5, Stephan Machinery Co., Columbus, OH, USA). Sodium chloride was added at 20 g kg^{-1} total weight. The moisture content was adjusted to 780 g kg^{-1}. MTGase was added at 0.9, 1.8, 2.7 and 3.6 g kg^{-1} surimi, which was equivalent to 0.05, 0.1, 0.15 and 0.2 unit g^{-1} surimi, respectively. The raw paste was stuffed into a 3 cm-diameter casings and pre-incubated at 4 °C for 24 h, 25 °C for 4 h, and 40 and 65 °C for 1 h prior to heating at 90 °C for 30 min. The control sample was heated at 90 °C for 30 min without pre-incubation. Surimi gels were chilled in ice water and kept in a refrigerator (~5–8 °C) overnight before further analysis.

TCA-soluble oligopeptides

TCA-soluble oligopeptide contents were measured using the method described by Yongsawatdigul and Piyadhammaviboon.[3] The sample (3 g) was added 27 ml 5% cold trichloroacetic acid (TCA) solution, then the mixture was homogenized using an IKA homogenizer (IKA Works Asia, Bhd, Malaysia) and centrifuged at 8000 rpm• (Rotor PK 121R, ACCEL Co., Italy•) for 15 min at 4 °C. The supernatant was analyzed for oligopeptide content using Lowry's assay method[17] with tyrosine as a standard. TCA-soluble oligopeptide content was expressed as µmol tyrosine g^{-1}.

SDS–PAGE

Sample solubilization was carried out using 50 g kg^{-1} SDS solution as detailed by Yongsawatdigul *et al*.[18] Protein (30 µg) was loaded onto 10% (w/v) polyacrylamide gel according to the method of Laemmli.[19] Gels were run at a constant voltage setting of 120 V. Gels were stained with 0.125% Coomassie Brilliant Blue R-250 and destained in a solution containing 25% ethanol and 10% acetic acid.

A continuous SDS–PAGE at 50 g l^{-1} polyacrylamide was performed to evaluate cross-linked polymers according to the method of Yongsawatdigul and Piyadhammaviboon.[3] Protein (80 µg) was loaded. Gels were run at a constant current of 20 mA per gel. Staining and destaining were conducted as described above. Intensities of protein bands were analyzed using image analysis software (LabWorks version 4.0, Ultraviolet Product Inc, Upland, CA, USA). Retention of myosin heavy chain (MHC), actin (AC) and tropomyosin (TM) of samples was expressed as the ratio of the area to that of respective proteins of the unheated samples.

Texture evaluation

A texture analyzer (Stable Micro System, Surrey,• UK) was used to evaluate textural properties of the gels. Gel samples were cut into pieces of 3 cm length. Breaking force (g) and deformation (mm) were determined using a 5 mm spherical plunger probe at a test speed of 1 mm s^{-1}.

Oscillatory dynamic rheology

Lizardfish surimi pastes (with MTGase addition of 1.8 g kg^{-1} surimi and without MTGase) were subjected to dynamic rheological measurement using a CS-50 rheometer (Bohlin Instruments Inc, East Brunswick, NJ, USA) equipped with a 4° cone and plate. Samples were heated from 20 °C to either 25 or 40 °C at a rate of 1 °C/min and held for 2 and 1 h, respectively. Subsequently, samples were heated to 90 °C with the same heating rate. Pre-incubation at 25 °C was carried out for 2 h, instead of 4 h, to eliminate the drying effect caused by prolonged pre-incubation time. The oscillatory mode at a fixed frequency of 0.1 Hz and shear stress of 150–200 Pa, which was in linear viscoelastic range, was applied. A solvent trap equipped with a wet sponge was used to prevent moisture evaporation during measurement.

Statistical analyses

Two different lots of surimi were used. The experiment was analyzed as a split–split plot. The five levels of MTGase activity (including no added MTGase) were assigned as a split plot factor and the five heating treatments as a split–split plot factor. In each treatment, at least five gel specimens were measured to obtain average values of breaking force and deformation. Autolytic analyses were repeated twice in each treatment. Duncan's multiple range test (DMRT) was used to determine differences between means at $p < 0.05$. Statistical analysis was performed using Statistical Analysis System (SAS) software version 6.08 (SAS Institute Inc, Cary, NC, USA).

RESULTS AND DISCUSSION

Incorporation of MDC to lizardfish surimi

The commercial MTGase used in this study exhibited MDC-incorporation activity of 50.3 unit g^{-1} powder at 40 °C. MTGase exhibited minimal catalytic reaction towards muscle proteins at 25 °C (Fig 1), but the activity increased with time and reached a maximum at 2 h. This suggested stability of the enzyme at 25 °C. The activity of MTGase at 65 °C appeared to be higher than that at 40 °C during the first 30 min, but declined after prolonged incubation time (Fig 1). Muscle proteins of lizardfish surimi could unfold to a greater extent at 65 °C, exposing more reactive sites, namely glutamine and lysine residues, for MTGase catalytic reaction. As a result, a higher rate of MDC incorporation was observed during the initial stage (30 min) of incubation at 65 °C. However, prolonged incubation time at 65 °C resulted in decreased MDC incorporation. Since lizardfish surimi contained endogenous proteinases that optimally hydrolyzed muscle proteins at 65 °C,[3] prolonged incubation at 65 °C (beyond 30 min) promoted degradation of muscle proteins as evidenced by relatively high TCA-soluble oligopeptide content of 7.1 μmol g^{-1}. Smaller protein fragments resulting from proteolysis might not serve as substrates of MTGase. In addition, MTGase could be thermally inactivated at 65 °C, as previously reported by Ando *et al.*[20] It should be noted that the amount of incorporated MDC also decreased after incubation at 40 °C for longer than 60 min. This could also be due to proteolysis of lizardfish surimi, as indicated by TCA-soluble oligopeptide contents of 5.6 μmol g^{-1}. Therefore, endogenous proteolytic activity must be taken into consideration in determining the optimum condition for MTGase in lizardfish surimi.

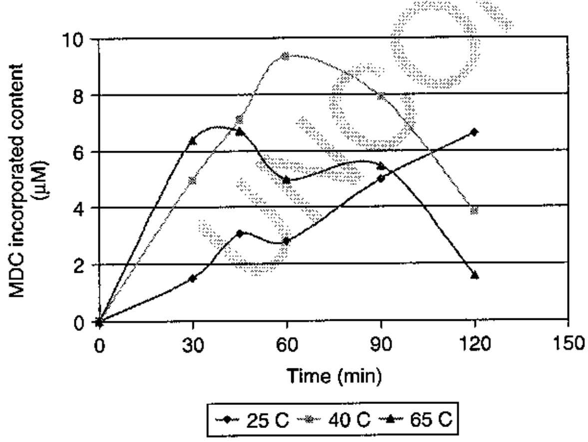

Figure 1. Incorporation of monodansylcadaverine (MDC) into lizardfish surimi by MTGase.

Effect of MTGase on textural properties

In the absence of MTGase, the breaking force of lizardfish surimi was lowest when pre-incubated at either 40 or 65 °C for 1 h [$p < 0.05$; Fig 2(a, b)]. The gel strength was increased when surimi was pre-incubated at 25 °C for 4 h. Several species of tropical fish muscle proteins were reported to show a setting effect at 40 °C.[3,9,12,21,22] However, pre-incubation of lizardfish surimi at 40 and 65 °C deteriorated its textural properties. This was probably due to an increased proteolytic activity of endogenous proteinases at higher temperature.[3]

Addition of MTGase did not increase breaking force and deformation of surimi gels pre-incubated at 65 °C at all studied levels ($p > 0.05$). However, the gel-enhancing effect of MTGase was significant when pre-incubated at 4, 25, and 40 °C and without pre-incubation (90 °C; $p < 0.05$). Addition of 0.1 unit g^{-1} surimi and pre-incubation at 25 °C resulted in the highest breaking force ($p < 0.05$). Addition of MTGase at higher level did not further improve textural properties at 25 °C. Textural properties of surimi with MTGase pre-incubated at 4 and 40 °C showed a similar trend to those at 25 °C at all studied levels ($p > 0.05$). Unlike samples without MTGase, surimi with MTGase showed an increase in gel strength when pre-incubated at 40 °C. Since MTGase showed high activity with MDC incorporation at 40 °C (Fig 1), protein cross-linking catalyzed by MTGase at 40 °C could occur at a faster rate than proteolysis induced by endogenous proteinases. Addition of MTGase also slightly improved the textural properties of lizardfish surimi heated at 90 °C [$p < 0.05$; Fig 2(a, b)]. Owing to the slow heat transfer in the 3 cm-diameter casing, the catalytic reaction of MTGase took place in a limited time at 90 °C before thermal inactivation of MTGase occurred.

Changes of deformation were in a similar pattern to those of breaking force [Fig 2(b)]. Deformation values of surimi with MTGase were higher than those of the control (no MTGase; $p < 0.05$). An increase in MTGase to 0.2 unit g^{-1} surimi did not increase deformation values. Pre-incubation of surimi paste with addition of MTGase at 4, 25 and 40 °C resulted in greater deformation values than at 65 °C and without

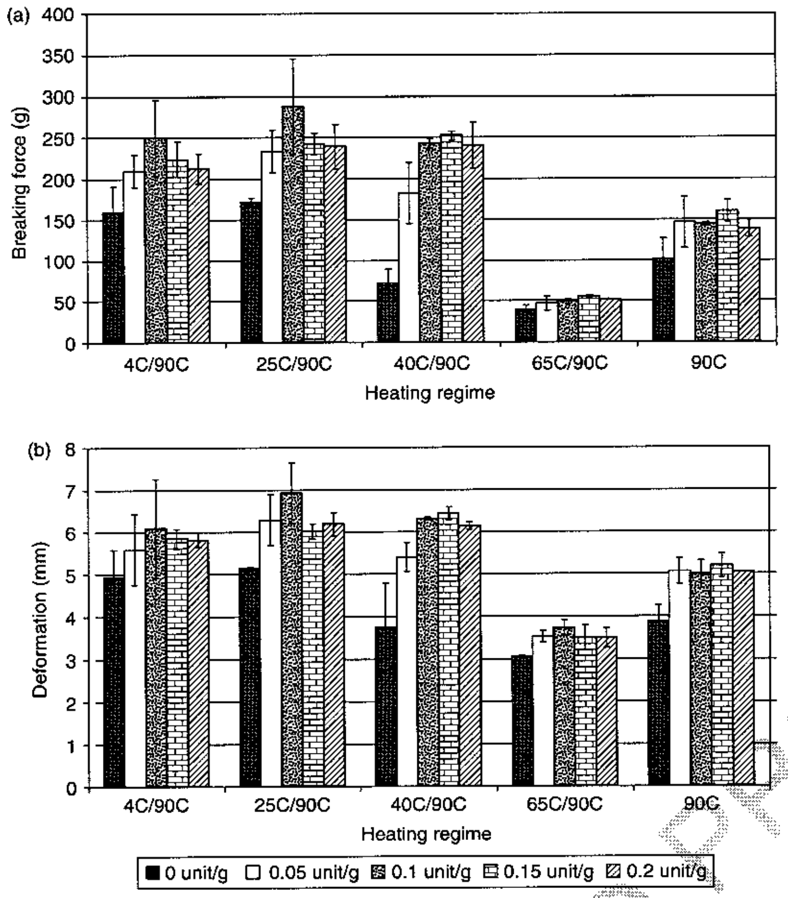

Figure 2. Breaking force (a) and deformation (b) of lizardfish surimi at various addition levels of MTGase and pre-incubated at various temperatures. Bars indicate standard deviation.

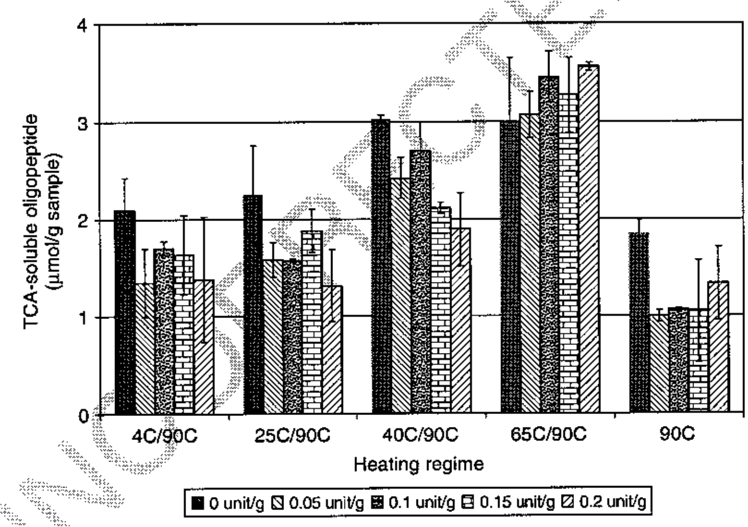

Figure 3. TCA-soluble oligopeptides of lizardfish surimi gels with various addition levels of MTGase and pre-incubated at various temperatures. The bars indicate standard deviation.

pre-incubation (90 °C). The highest deformation of 6.9 mm was found in lizardfish surimi with addition of 0.1 unit g^{-1} surimi and pre-incubation at 25 °C for 4 h. This was almost 2-fold greater than that of the control (without MTGase and with no pre-incubation).

Based on the breaking force and deformation, the optimum MTGase activity required to improve the textural properties of lizardfish surimi was 0.1 unit g^{-1} surimi, which was equivalent to 1.8 $g\,kg^{-1}$. The optimum amount of MTGase reported to improve the textural properties of fish proteins varied with fish species and type of MTGase. The breaking force of Alaska pollock surimi increased when MTGase was added up to 0.3 $g\,kg^{-1}$ and with pre-incubation

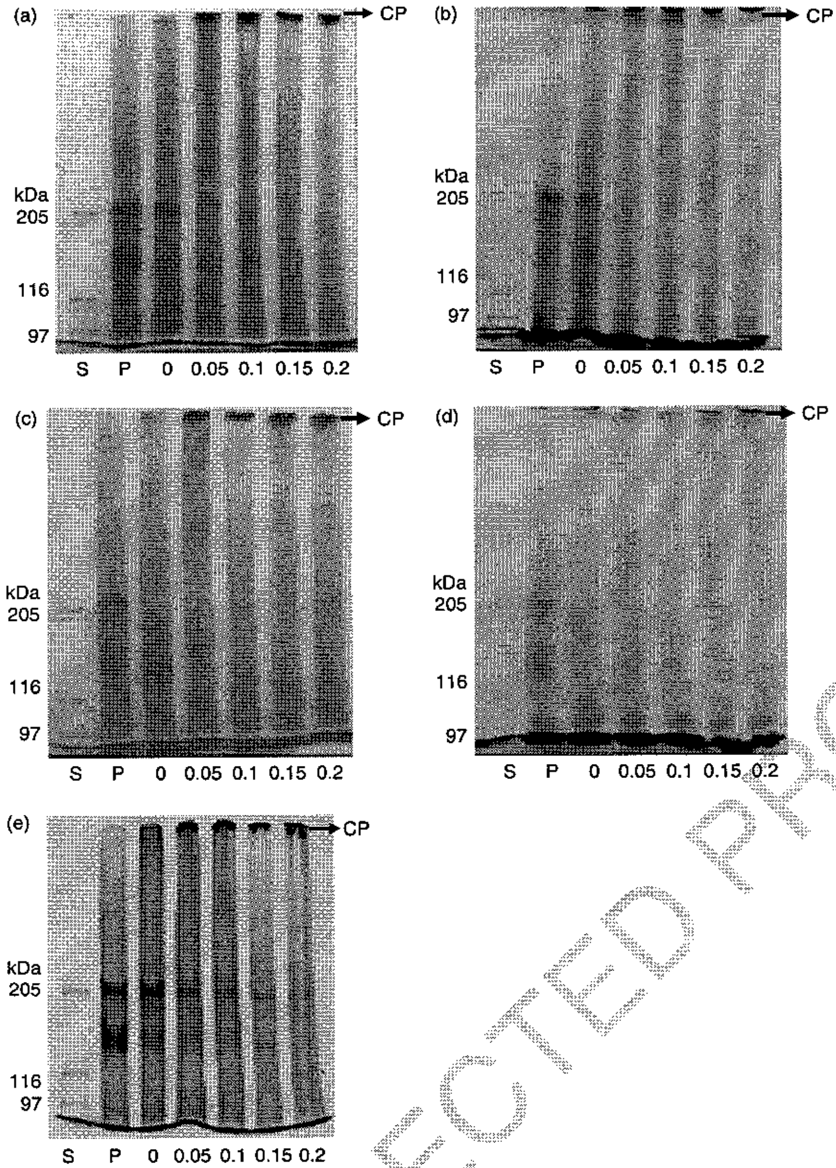

Figure 4. SDS–PAGE patterns on 50 g L^{-1} polyacrylamide gel of lizardfish surimi with various addition levels of MTGase and pre-incubated at 4 °C for 24 h (a), 25 °C for 4 h (b), 40 °C for 1 h (c), 65 °C for 1 h (d), without pre-incubation (e), followed by heating at 90 °C for 30 min. The numbers indicate the units, MTGase g^{-1} surimi. (S) Standard molecular weight; (P) raw paste; (CP) cross-linked polymers.

at either 10 or 45 °C.[14] The optimum condition for silver carp (*Hypophthalmichthys molitrix*) surimi was predicted to be 8.8 g kg^{-1} with pre-incubation at 39.6 °C for 1 h.[22] However, MTGase from *S ladakanum* alone at 0.6 unit g^{-1} surimi improved the textural properties of hairtail surimi, but these were still not commercially acceptable.[23] Our study demonstrated that addition of MTGase increased both breaking force and deformation of lizardfish surimi when pre-incubated at either 25 or 40 °C.

TCA-soluble oligopeptide content

Low-temperature pre-incubation (4 and 25 °C) resulted in lower TCA-soluble oligopeptide content than high temperature settings (40 and 65 °C; $p < 0.05$; Fig 3). The maximum TCA-soluble oligopeptide content was found at 65 °C ($p < 0.05$; Fig 3). Severe proteolysis at 65 °C explained poor textural properties of surimi gel pre-incubated at this temperature [Fig 2(a, b)]. Although MTGase catalyzes the formation of ε-(γ-glutamyl)lysyl isopeptides, which are resistant to proteolysis,[14] proteolysis of lizardfish surimi at 65 °C was not inhibited by addition of MTGase at the studied level (Fig 3). However, the inhibitory effect of MTGase was noted in the samples pre-incubated at 40 °C. High catalytic activity of MTGase at 40 °C could result in more ε-(γ-glutamyl)lysyl isopeptides, which were less susceptible to proteolytic degradation. The TCA-soluble oligopeptide contents of samples heated at 90 °C were lower than for those heated at 40 and 65 °C ($p < 0.05$; Fig 3). This resulted in higher breaking force and deformation values than for those incubated at 40 and 65 °C [$p < 0.05$; Fig 2(a,b)]. Heating at 90 °C without pre-incubation reduced proteolysis in lizardfish surimi because endogenous proteinases were thermally inactivated more rapidly.

SDS–PAGE patterns

Samples with addition of MTGase contained cross-linked polymers (CP) with molecular weight too large to travel into 50 g L^{-1} acylamide gel as noticed on the top of the gel (Fig 4). A high intensity of CP was observed in samples pre-incubated at 4 and 25 °C, and in those without pre-incubation (90 °C) at all addition levels of MTGase [Fig 4(a, b, e)]. Retention of MHC and TM decreased as MTGase concentration increased in these three heating conditions [Fig 5(a, b)]. Since TCA-soluble oligopeptide content of samples heated by these three heating regimes decreased with increased MTGase levels (Fig 3), the reduction of MHC and TM observed was more likely to be caused by the cross-linking reaction catalyzed by MTGase than by proteolysis. Among the myofibrils, myosin was reported as a preferred substrate for MTGase and endogenous TGase extracted from fish muscle.[16,24] Our study also indicated that TM was cross-linked by MTGase, but to a lesser extent than with MHC.

Significant losses of MHC and TM were observed when samples were pre-incubated at 40 °C at all addition levels of MTGase [Fig 5(a, b)]. Reductions in MHC and TM at 40 °C resulted from both the cross-linking reaction and proteolysis. High TCA-soluble oligopeptide content along with low retention of MHC (30%) and TM (37%) in surimi gels pre-incubated at 40 °C without MTGase indicated evidence of endogenous proteolysis [Figs 3 and 5(a, b)]. Yongsawatdigul and Piyadhammaviboon[3] reported that both MHC and TM were preferred substrates of endogenous proteinases in lizardfish surimi. However, the formation of CP in samples pre-incubated at 40 °C indicated the occurrence of cross-linking [Fig 4(c)]. Proteolysis and cross-linking reactions appeared to occur simultaneously at 40 °C. However, the extent of cross-linking could be more pronounced at 40 °C because textural properties of surimi with MTGase were improved as compared with those without MTGase [Fig 2(a, b)].

Complete disappearance of MHC and TM at 65 °C was mainly caused by proteolysis (Fig 5). Severe degradation of muscle proteins resulted in highly TCA-soluble oligopeptide content at 65 °C (Fig 3). Formation of CP at 65 °C occurred to the least extent compared with other heating regimes (Fig 4). This was in agreement with the MDC-incorporation activity (Fig 1). Degradation of muscle proteins to smaller molecules could limit the formation of high-molecular-weight polymers. In addition, MTGase exhibited optimum temperature at 50 °C and minimal activity at 65 °C.[20] Therefore, pre-incubation at high temperature (65 °C) for 1 h could thermally inactivate MTGase, resulting in limited protein cross-linking. Consequently, textural properties of lizardfish surimi with addition of MTGase were not improved when pre-incubated at 65 °C.

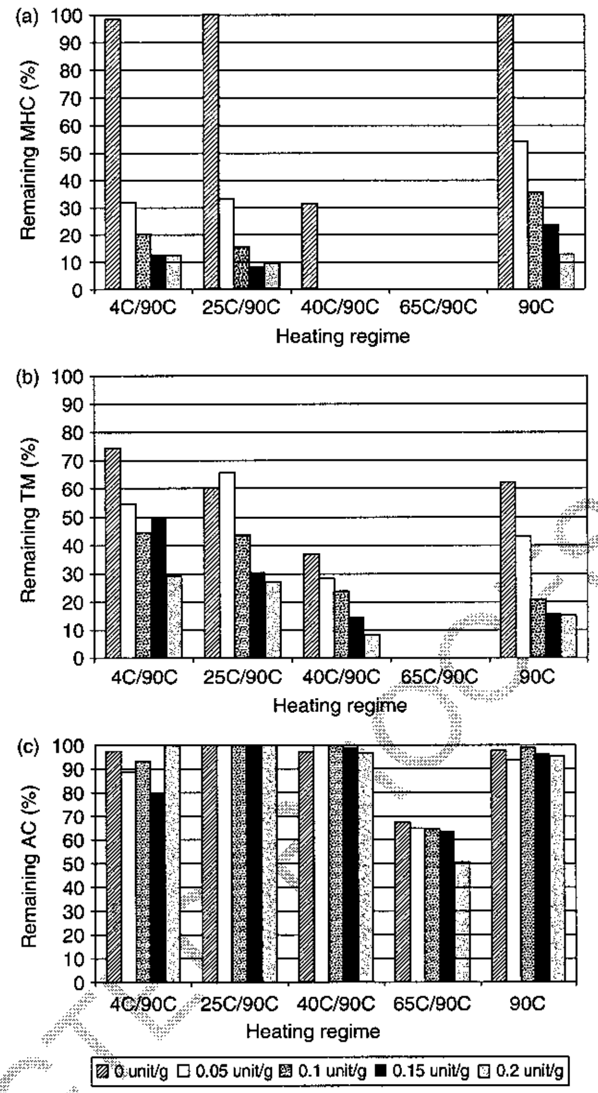

Figure 5. Effect of addition level of MTGase and pre-incubation condition on retention of myosin heavy chain (a), tropomyosin (b) and actin (c).

Intensity of AC was not affected by pre-incubation at 4, 25, 40 and 90 °C, but it decreased to about 50% when pre-incubated at 65 °C for 1 h [Fig 5(c)]. These results indicated that AC was not a preferred substrate for MTGase, but it was hydrolyzed by endogenous proteinase(s) associated with lizardfish surimi. Huang *et al*[16] also reported that AC was not cross-linked by MTGase.

The cross-linking reaction also occurred even in the samples without pre-incubation [90 °C; Fig. 4(e)]. MTGase appeared to catalyze the reaction during the heating period before the enzyme was thermally inactivated at >65 °C. A decrease in MHC and TM intensity confirmed the occurrence of cross-linking during the heating period at 90 °C. Catalytic reaction time was relatively short because more retention of MHC was observed [Fig 5(a)]. This explained the slight improvement in textural properties of samples without pre-incubation [Fig 2(a, b)].

Our results demonstrated that addition of MTGase would significantly enhance the textural properties of lizardfish surimi only when a proper heating regime was applied. Pre-incubation at 25 °C minimized the degree of proteolysis but required a longer time (4 h) to induce CP formation due to limited MTGase activity at 25 °C. Pre-incubation of lizardfish surimi at 40 °C in the presence of MTGase appeared to accelerate the cross-linking reaction to a greater extent than proteolysis of MHC, resulting in comparable breaking force and deformation to those pre-incubated at 25 °C.

Dynamic rheological changes

The storage modulus (G') of lizardfish surimi without MTGase initially increased at about 32 °C, showed a peak at 41 °C, and declined to the minimum at 48 °C before continuously reaching the maximum at about 75 °C [Fig 6(a)]. Similar patterns with higher G' values were also observed in the sample mixed with MTGase and pre-incubated at 25 °C for 2 h. The initial increase in G' was attributed to the aggregation of partially unfolded actomyosin, while the decline of G' resulted from dissociation of myosin and actin from actomyosin complex and denaturation of myosin, leading to increased fluidity.[25] The second increase in G' observed at 48 °C was attributed to formation of permanent myosin gel networks. When lizardfish surimi was pre-incubated at 25 °C for 2 h in the presence or absence of MTGase, the initial G' values were higher than those without pre-incubation [Fig 6(a, b)]. These results suggested that pre-incubation at 25 °C promoted aggregation of lizardfish myofibrillar proteins. In addition, the catalytic reaction of MTGase occurred even at 25 °C, resulting in higher G' during 2 h pre-incubation at 25 °C [Fig 6(d)]. It has been generally accepted that muscle proteins from tropical fish do not undergo extensive aggregation for the 'setting' effect at low temperature (4, 25 °C) due to the higher denaturation temperature of fish proteins.[26] However, our study demonstrated that muscle proteins of lizardfish, a tropical fish species, aggregated to a certain extent, and such aggregation could be promoted by addition of MTGase. G' continually increased from 20 to 90 °C without decline after the samples were pre-incubated at 40 °C for 1 h [Fig 6(c)]. Lizardfish myosin underwent thermal denaturation when pre-incubated at 40 °C for 1 h. The unfolded proteins subsequently aggregated to form gel networks. However, pre-incubation at 40 °C without MTGase appeared to have a detrimental effect on gel network formation as G' gradually decreased throughout the 1 h incubation period [Fig 6(d)]. This was due to proteolytic degradation as supported by relatively high TCA-soluble oligopeptide content. Addition of MTGase extensively enhanced protein cross-linking and aggregation, resulting in higher G' values [Fig 6(c, d)]. Furthermore, the onset of gel network development (the second increase in G') of samples with added MTGase began at a lower temperature (40 °C) than that of the samples without MTGase (48.1 °C). Therefore, the catalytic reaction of MTGase promoted the extensive cross-linking of MHC at 40 °C, which subsequently induced the early

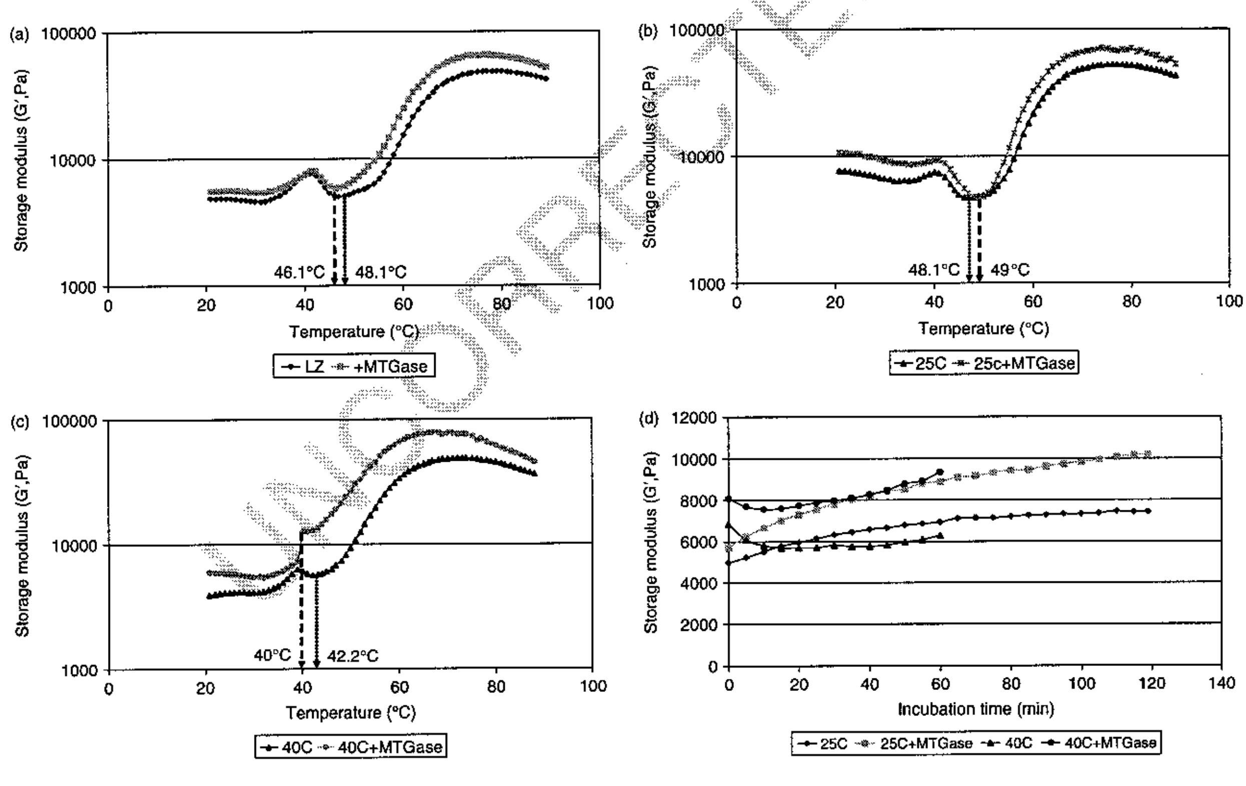

Figure 6. Dynamic rheograms of lizardfish surimi with and without addition of MTGase heated from 20 to 90 °C without pre-incubation (a), with pre-incubation at 25 °C for 2 h (b), with pre-incubation at 40 °C for 1 h (c), and during the pre-incubation period (d).

gel network development and resulted in a higher elastic gel.

CONCLUSIONS

MTGase can be used to improve textural properties of lizardfish surimi, which exhibited severe proteolytic degradation. This would be beneficial to surimi seafood products requiring pre-incubation or a 'setting' process, such as kamaboko or imitation scallop. Addition of 0.1 unit MTGase g^{-1} surimi ($1.8\,g\,kg^{-1}$) and pre-incubation at either 25 °C for up to 4 h or 40 °C for up to 1 h significantly increased both breaking force and deformation. MTGase catalyzes covalent cross-linking of MHC and TM, resulting in more elastic gel networks. It should be noted that the gel-enhancing effect of MTGase will be minimal when surimi is subjected to a rapid heating process. Therefore, addition of MTGase is not necessary in rapidly cooked surimi seafoods.

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