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Inhibition of autolytic activity of lizardfish surimi by proteinase inhibitors

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Abstract

Optimum autolytic activities of lizardfish (*Saurida tumbil*) mince and surimi were at pH 6 and 7, respectively, with optimum temperature at 65 °C. Autolysis of surimi was mainly inhibited by phenylmethanesulfonyl fluoride and *p*-tosyl-L-phenylalanyl-chloromethylketone, indicating the involvement of myofibrillar-associated serine proteinase. Myosin heavy chain (MHC) and tropomyosin were preferentially hydrolyzed, resulting in poor textural properties. Based on TCA-soluble oligopeptide assay, egg white powder (EW) and whey protein concentrate (WPC) showed 77% and 96% inhibition, respectively. However, a significant loss of MHC was found. At any pre-incubation condition (25 °C/4 h, 40 °C/1 h and 65 °C/1 h), EW improved gel-forming ability of lizardfish surimi to a greater extent than WPC. Addition of 1% EW and pre-incubation at 25 °C resulted in an increase of higher molecular weight cross-linked proteins, corresponding to a twofold increase in the breaking force.

Keywords: Lizardfish; Proteolysis; Egg white; Whey protein concentrate; Surimi

1. Introduction

Lizardfish (*Saurida* spp.) is one of the most important species for surimi production in the southeast Asian region. Lizardfish surimi is considered to be of low value because of its poor gel-forming ability (Morrissey & Tan, 2000). Formaldehyde is rapidly accumulated during storage of whole fish, resulting in an inferior quality for surimi production (Kurokawa, 1979). In addition, lizardfish surimi typically undergoes textural degradation by endogenous proteinases (Suwansakornkul, Itoh, Hara, & Obatake, 1993). To improve gel quality of lizardfish surimi, some means of inhibiting proteolytic activity must be sought.

Proteolysis of fish muscle induced by endogenous proteinase has been widely studied. Cathepsin L, a cysteine proteinase, has been reported to hydrolyze muscle proteins of Pacific whiting (Seymour, Morrissey, Peters, & An, 1994), mackerel (Aoki & Ueno, 1997), and anchovy (Heu, Kim, Cho, Godber, & Pyeun, 1997). Serine proteinases were found to be responsible for textural breakdown of threadfin bream (*Nemipterus bathybius*) (Kinoshita, Toyohara, & Shimizu, 1990), oval-filefish (*Navodon modestus*) (Toyohara, Sakata, Yamashita, Kinoshita, & Shimizu, 1992), *Tilapia* (*Oreochromis niloticus*) (Yongsawatdigul, Park, Virulhakul, & Viratchakul, 2000), and Mexican flounder (*Cyclopsetta chittendeni*) (Ramirez, Garcia-Carreno, Morales, & Sanchez, 2002). Choi, Cho, and Lanier (1999) purified two trypsin-like serine proteinases from Atlantic menhaden (*Brevoortia tyrannus*). In addition, heat-stable alkaline proteinases, exhibiting high proteolytic activity at pH 8.5–9.0 at 60–65 °C, were found in white croaker flesh (*Micropogon opercularis*) (Folco, Busconi, Martone, Trucco, & Sanchez, 1984) and in viscera of anchovy (*Engraulis japonica*) (Heu, Pyeun, Kim, & Godber, 1991). Makinodan, Toyohara, and Ikeda (1984) demonstrated that the sarcoplasmic fluid of lizardfish contained acid, neutral, and alkaline proteinases. Both serine and cysteine proteinases were later found in lizardfish, depending on species (Suwansakornkul et al., 1993). However, an effective means of inhibiting endogenous proteinases and of improving

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textural properties of lizardfish surimi has not yet been found.

Food-grade proteinase inhibitors, commercially used in surimi, include egg white powder (EW), whey protein concentrate (WPC), beef plasma protein (BPP), and potato extract (Hamann, Amato, Wu, & Foegeding, 1990; Kang & Lanier, 1999; Morrissey, Wu, & An, 1993). The effectiveness of each inhibitor varies with surimi species. EW and BPP increases the shear stress of menhaden surimi to similar extents (Hamann et al., 1990), but BPP was found to be a more effective inhibitor in Pacific whiting surimi (Kang & Lanier, 1999; Morrissey et al., 1993). However, the application of BPP in surimi has recently been limited by the outbreak of bovine spongiform encephalopathy (BSE) or "mad cow disease". Food grade inhibitors derived from sources other than cattle are preferred. Various legume seed extracts were found to inhibit serine proteinase in fish muscle (Ramirez et al., 2002). In addition, porcine plasma protein containing L-kininogen inhibited the degradation of mackerel surimi (Lee, Tzeng, Wu, & Jiang, 2000). However, legume seed extract and porcine plasma protein are commercially unavailable at present. EW and WPC are the inhibitors of choice regarding their commercial availability. But, the effectiveness of both inhibitors on lizardfish surimi needs to be investigated. Our objective was to characterize autolytic degradation of lizardfish mince and surimi and to evaluate the inhibitory effect of EW and WPC on the proteolysis of lizardfish surimi.

2. Materials and methods

2.1. General

Fresh lizardfish (*S. tumbil*) and surimi 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. Fish and surimi were vacuum-packed, and kept at −18 °C until use. EW was obtained from Igrecha (France). WPC manufactured to be used as inhibitor in surimi was a gift from Arla Food Ingredient (Tokyo, Japan).

Phenylmethanesulfonyl fluoride (PMSF), *p*-tosyl-L-phenylalanyl-chloromethylketone (TPCK), *trans*-epoxysuccinyl-L-leucylamido(4-guanidino)-butane (E-64), soybean trypsin inhibitor (STI), leupeptin, pepstatin A, bovine serum albumin (BSA), and β-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylenediaminetetraacetic disodium salt (EDTA) was purchased from Fluka (Buchs, Switzerland). Reagents used for gel electrophoresis were purchased from Bio-Rad (Hercules, CA, USA). All other chemicals were of reagent grade.

2.2. Autolytic assays

Autolytic activity was measured using TCA-soluble oligopeptides (Yongswatdigul, Worratao, & Park, 2000). Three grams of sample were incubated at 0, 25, 40, 55, 60, 65, 70, 80 and 90 °C for 1 h. Autolysis was stopped by adding 27 ml of 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. For the pH profile, 3 g of lizardfish sample were added to 9 ml of McIlvaine buffer (0.2 M sodium phosphate, 0.1 M sodium citrate) at pHs 2, 4, 5, 5.5, 6, 7, 8, respectively and Tris–HCl buffer (0.1 M Tris) at pHs 8, 8.5, 9, respectively and incubated at 65 °C for 1 h. The reaction was stopped by adding 18 ml 7.5% cold TCA solution. The mixture was then homogenized using an IKA homogenizer and centrifuged at 8000 rpm (Rotor PK 121R, ACCEL Co., Italy) for 15 min at 4 °C. The sample blanks were kept in ice and were treated in the same manner as the samples. Supernatant was analyzed for oligopeptide content using Lowry's assay (Lowry, Rosebrough, Farr, & Randall, 1951). The samples were solubilized in 5% hot sodium dodecylsulfate (SDS) solution (1:9) and determined for total soluble protein. Autolytic activity was expressed as μmole of tyrosine/mg protein/h.

2.3. Effect of proteinase inhibitors

Two grams of sample were mixed with 93 nmole E-64/g sample, 60 μmole EDTA/g sample, 1.05 μmole leupeptin/g sample, 300 nmole pepstatin A/g sample, 500 μg STI/g sample, 300 μmole PMSF/g sample, 40 μmole TPCK/g sample. Mince and surimi samples were incubated at pH 6 and 7, which were the optimum pHs found from the autolytic assay for each sample, respectively. The mixtures were incubated at 65 °C for 1 h. The reaction was stopped by adding 12 ml of 7.5% cold TCA. The mixture was then homogenized, centrifuged, and assayed for oligopeptide content as described above. The control was the sample incubated without inhibitors. Percent inhibition was calculated as follows:

$$\%\text{Inhibition} = \frac{(\text{TC} - \text{TC}_\text{b}) - (\text{TI} - \text{TI}_\text{b})}{\text{TC} - \text{TC}_\text{b}} \times 100,$$

where, TC = tyrosine of control (without inhibitor) incubated at 65 °C, TC_b = tyrosine of control incubated at 0 °C, TI = tyrosine of sample (with inhibitor) incubated at 65 °C, TI_b = tyrosine of sample incubated at 0 °C.

The effectiveness of various inhibitors on proteolysis of myosin heavy chain (MHC) was monitored. Two grams of sample were incubated with either 300 μmole PMSF/g surimi, 2% EW or 2% WPC, at 65 °C for 1 h.

Then, 18 ml hot 5% SDS solution were added and homogenized. The degradation of protein was observed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE).

2.4. SDS–PAGE

Three grams of sample were added to 27 ml hot 5% SDS solution and then homogenized using an IKA homogenizer. The homogenates were incubated in a 90 °C water bath for 30 min and centrifuged at 10,000×*g* (Centrifuge 5415 D, Eppendorf, Hamburg, Germany) for 20 min at room temperature. The supernatants were mixed with treatment buffer in a 1:1 ratio and boiled for 3 min. Thirty micrograms of protein were loaded onto 10% (w/v) polyacrylamide gel according to the method of Laemmli (1970). 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 5% (w/v) was performed according to the method of Huff-Lonergan, Mitsuhashi, Parrish, Jr, and Robson (1996) with a slight modification. A gel prepared from 30% acrylamide (acrylamide: *N*,*N*′-bis methylene acrylamide = 100:1), 0.375 M Tris–HCl (pH 8.8), 2 mM EDTA, 1% SDS, 0.1% ammonium persulfate, and 0.67% TEMED, was used. Protein supernatants were mixed with treatment buffer (20 mM Tris–HCl pH 8.8, 2 mM EDTA, 2% SDS, 20% glycerol, 2% β-mercaptoethanol) at a ratio of 1:1 (v/v). Eighty micrograms of protein were loaded. Tank buffer contained 25 mM Tris, 192 mM glycine, 2 mM EDTA, and 0.1% SDS (pH 8.3). Gels were run at constant current of 20 mA per gel. Staining and destaining were conducted as described above.

2.5. Surimi gel preparation

Frozen surimi was thawed and chopped in a Stephan vacuum silent cutter (model UM5; Stephan Machinery Co., Columbus, OH, USA). Sodium chloride was added at 2% of the total weight. The moisture content was adjusted to 78%. EW and WPC were added at various concentrations (0%, 1%, 2%, 3% and 4%) of total weight. The raw paste was stuffed into a 3 cm-diameter casing and pre-incubated at 25 °C for 4 h, 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 texture evaluation.

2.6. Texture evaluation

A texture analyzer (Stable Micro System, Surrey, England) 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.

2.7. Statistical method

The experiment was analyzed as a split–split plot. The five levels of inhibitor (including no added inhibitor) were assigned as a split plot factor and the four heating treatments as a split–split plot factor. Duncan's multiple range test (DMRT) was used to determine differences between mean at $p < 0.05$ (SAS, 1996).

3. Results and discussion

3.1. Autolytic activity of lizardfish mince and surimi

Proteolytic activity of both mince and surimi increased with temperature and reached the maximum at 65 °C (Fig. 1), indicating that heat-stable proteinase(s) was responsible for muscle protein degradation of lizardfish. Optimum pH of mince was at pH 6, while that of surimi was at pH 7 (Fig. 2). Since the pH of surimi-based seafoods is around 7, lizardfish surimi would be vulnerable to proteolysis when subjected to a high temperature (65 °C) for a longer period of time during

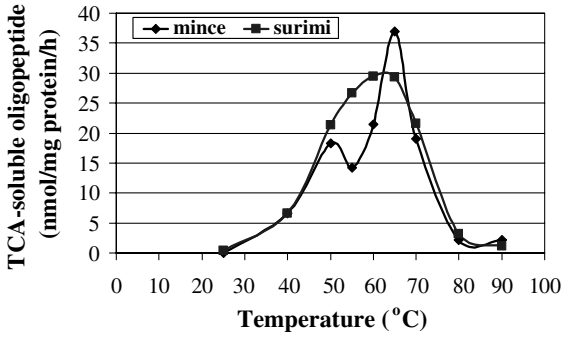

Fig. 1. Temperature profiles of lizardfish mince and surimi.

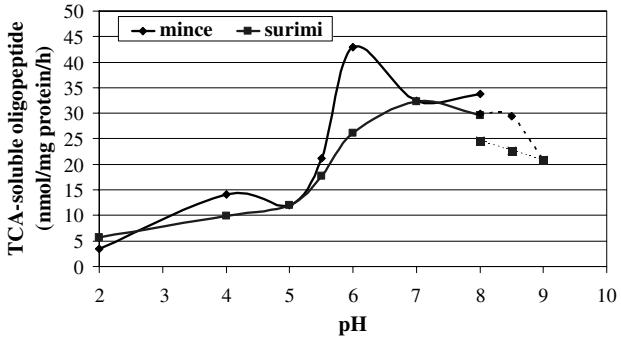

Fig. 2. pH profiles of lizardfish mince and surimi incubated at 65 °C. Buffers used were MaIlvaine buffer (–) and 0.1 M Tris–HCl (···).

Table 1
Effect of various inhibitors on autolysis activity at 65 °C

Inhibitors	Concentration	Degree inhibitition (%)	
		Mince	Surimi
Control		–	–
E-64	93 nmole/g sample	0	2.1 ± 3.0
EDTA	60 μmole/g sample	9.8 ± 13.9	43.5 ± 9.5
Leupeptin	1.05 μmole/g sample	53.3 ± 5.8	48.8 ± 7.1
Pepstatin A	300 nmole/g sample	0	63.9 ± 3.3
PMSF	300 μmole/g sample	27.0 ± 4.2	91.9 ± 0.4
STI	500 μg/g sample	33.3 ± 11.5	64.8 ± 3.9
TPCK	40 μmole/g sample	0	77.8 ± 5.0

processing. Pacific whiting (*Merluccius productus*) surimi exhibited severe muscle degradation at 55 °C, pH 5.5 (An, Seymour, Wu, & Morrissey, 1994).

Based on the inhibitor study, proteolysis of mince was inhibited by about 53% in the presence of leupeptin, while PMSF and soybean trypsin inhibitor inhibited proteolytic activity by about 30% (Table 1). Leupeptin is a reversible inhibitor that mimics the tetrahedral intermediate formed during peptide bond hydrolysis (Salvesen & Nagase, 2001). Leupeptin reacts equally well with both serine and cysteine proteinase. PMSF irreversibly inhibits serine proteinase and reacts reversibly with some cysteine proteinases (Salvesen & Nagase, 2001). E-64, a specific cysteine proteinase inhibitor, did not inhibit the proteolytic activity of mince. These results indicated that serine-like proteinases were responsible for proteolysis of lizardfish mince. Since EDTA and pepstatin A exhibited no inhibitory effect on proteolysis of mince, metallo- and acid proteinase were not involved in protein degradation of lizardfish mince. Suwansakornkul et al. (1993) reported the inhibitory effect of leupeptin on proteolytic acitivities of lizardfish mince at 60 °C. Kinoshita et al. (1990) also reported a serine proteinase in threadfin bream muscle that was inhibited by soybean trypsin inhibitor, leupeptin, and antipain.

PMSF was the most effective proteinase inhibitor in lizardfish surimi (Table 1). In addition, inhibitory effects of soybean trypsin inhibitor and TPCK were relatively high (65–78%). These results suggested that serine-like proteinase(s) was mainly responsible for muscle protein degradation of lizardfish surimi. Furthermore, pepstatin A and EDTA inhibited proteolysis of lizardfish surimi to a lesser extent. Jiang, Wang, Gau, and Chen (1990) reported that pepstatin-sensitive proteinases were responsible for the degradation of *Tilapia* myofibrils at pH 5.5 and 6.0. Their activity was not found at higher pH. Since pH of lizardfish surimi was about 7, it was unlikely that acid proteinases would contribute to proteolysis of lizardfish surimi. EDTA inhibits metallo-proteinases and calpains. It may also destabilize the structure of some serine proteinases (North & Beynon, 2001). Jiang, Wang, and Chen (1991) purified calpain, Ca^{2+}-activated neutral proteinase, from *Tilapia* and speculated that it could play a role in post-mortem tenderization of *Tilapia* muscle. The inhibitory effect of EDTA could imply the involvement of metallo neutral proteinases in the proteolysis of lizardfish surimi.

Surimi production involves both washing and dewatering processes. Sarcoplasmic proteins, including enzymes, are removed during washing. As a result, proteolytic activity of surimi is typically lower than that of mince. However, the autolytic activities of lizardfish mince and surimi were comparable (Figs. 1 and 2), suggesting the presence of myofibril-bound proteinase(s) that were not removed during washing. The proteinase was postulated to be a serine proteinase since its activity was mainly inhibited by PMSF, TPCK, and soybean trypsin inhibitor. Suwansakornkul et al. (1993) reported that both serine and cysteine proteinase(s) were responsible for MHC degradation of washed lizardfish mince, varying with species. In addition, Cao, Osatomi, Hara, and Ishihara (2000) purified the myofibril-bound proteinase in lizardfish (*S. wanieso*) and classified it as a trypsin-type serine proteinase. The purified enzyme showed optimum temperature toward a synthetic substrate (*t*-butyloxycarbonyl-Phe-Ser-Arg-4-methylcoumaryl-7-amide) at 50 °C and at pH 7–8. The enzyme optimally hydrolyzed MHC at 55–60 °C. The purified enzyme was also inhibited by soybean trypsin inhibitor, leupeptin, and TPCK.

Myofibril-bound proteinases contribute to proteolysis of muscle proteins of various fish species. Osatomi, Sasai, Cao, Hara, and Ishihara (1997) purified myofibril-bound serine proteinase from carp (*Cyprinus carpio*) muscle. The purified enzyme optimally hydrolyzed MHC in myofibrils and surimi gel at 55 and 60 °C (Cao, Hara, Osatomi, Tachibana, & Ishihara, 1999). Myofibril-bound proteinases are problematic for surimi production. Effective means to inhibit proteinase activity in these species are absolutely required to improve textural properties.

3.2. Inhibitory effect of EW and WPC

Lizardfish surimi without an inhibitor underwent severe textural degradation at 65 °C as is evident from the highest TCA-soluble oligopeptide content (Fig. 3(a) and (b)) and disappearance of MHC and tropomyosin (TM) (Fig. 4). Proteolysis also occurred at 25 and 40 °C, but to a lesser extent than at 65 °C (Figs. 3 and 4). Rapid heating to 90 °C reduced the TCA-soluble oligopeptide content and the degradation of MHC. TCA-soluble oligopeptide contents of samples pre-incubated at 40 and 65 °C decreased with the addition of EW and WPC (Fig. 3(a) and (b)). EW exhibited about 40% inhibition at 1–3% addition at 40 °C whereas its degree of inhibition increased with concentration up to 77% at 65 °C (Table 2). The inhibitory effect of EW at 1% addition

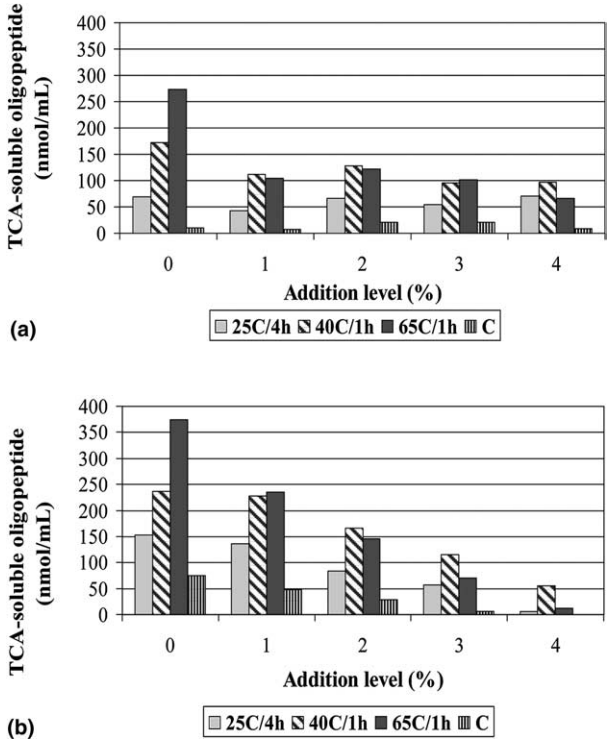

Fig. 3. TCA-soluble oligopeptide contents of lizardfish surimi mixed with EW (a) and WPC (b) and subjected to various heating conditions: 25, 40, and 65C indicate pre-incubation temperature in °C; 1 and 4 h indicate pre-incubation time in h; (C) control sample heated at 90 °C for 30 min.

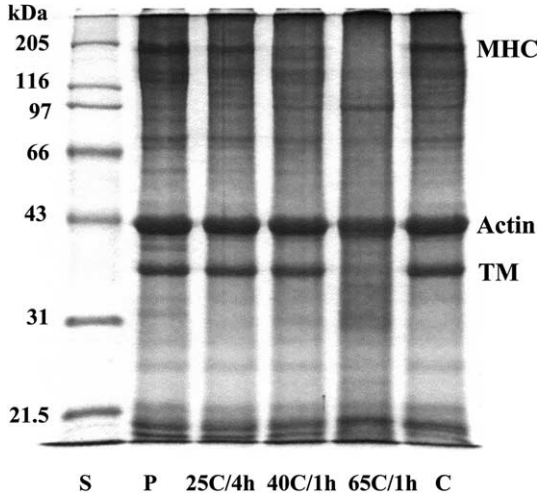

Fig. 4. SDS–PAGE pattern of lizardfish surimi heated at various conditions: (S) standard molecular weight; (P) raw paste; (25 °C/4 h)-(C) same as indicated in Fig. 3.

was higher than that of WPC. EW contains several proteinase inhibitors, namely ovomucoid, ovoinhibitor, ovomacroglobulin, which exhibit inhibitory activity against serine proteinase (Nakamura & Doi, 2000). A cysteine proteinase inhibitor, cystatin, was also found in egg white (Nakamura & Doi, 2000). These inhibitors,

Table 2
Inhibitory effect of EW and WPC on autolytic activity of lizardfish surimi at 40 and 65 °C

Addition level (%)	Degree inhibition (%)			
	40 °C		65 °C	
	EW	WPC	EW	WPC
1	36.6 ± 15.1	3.5 ± 5.2	57.4 ± 17.7	29.9 ± 23.5
2	24.9 ± 4.0	25.4 ± 16.3	52.1 ± 13.6	55.3 ± 18.1
3	44.4 ± 1.2	45.4 ± 21.3	60.1 ± 11.4	78.7 ± 8.5
4	44.4 ± 6.9	73.7 ± 10.5	77.0 ± 4.7	96.0 ± 2.0

however, did not inhibit proteolysis of MHC at 65 °C (Fig. 5(a)).

The degree of inhibition by WPC increased with concentration at both 40 and 65 °C (Table 2). WPC inhibited about 96% autolytic activity at 65 °C at 4% level, but significant loss of MHC was evident (Fig. 5(b)). WPC was a more effective inhibitor for papain, a cysteine proteinase, than trypsin, a serine proteinase (Weerasinghe, Morrissey, Chung, & An, 1996). Our results show that WPC did not effectively reduce proteolysis of MHC, but it inhibited the degradation of actin and TM. WPC slightly inhibited autolytic activity of Pacific whiting surimi at 0.2% addition (Akazawa, Miyauchi, Sakurada, Wasson, & Reppond, 1993). The degree inhibition by WPC was as high as 100% at 4% addition in Pacific whiting surimi (Piyachomkwan & Penner, 1995; Weerasinghe et al., 1996). Despite a high degree inhibition by WPC, loss of MHC was observed in these studies, especially at 1 h incubation (Akazawa et al., 1993; Piyachomkwan & Penner, 1995). Our results corresponded with these findings. Autolytic assay measures only TCA-soluble oligopeptides resulting from proteolytic activity. It was possible that some proteolytic degradation did not produce TCA-soluble products, but resulted in insoluble smaller molecular weight fragments. For this reason, the discrepancy between TCA-soluble oligopeptides and retention of MHC observed on SDS–PAGE was evident.

The inhibitory effects of 2% EW and WPC on the proteolysis of MHC was compared with that of PMSF (Fig. 6). Although PMSF showed about 90% inhibition (Table 1), severe degradation of MHC was observed and was comparable to those of EW and WPC. This confirmed the discrepancy between the results obtained from TCA-soluble oligopeptide content and those shown on SDS–PAGE. PMSF exhibited an inhibitory effect against actin and TM that was greater than EW and WPC. Cao et al. (2000) reported that proteinase purified from lizardfish preferably hydrolyzed MHC, but not α-actinin and actin. A difference in substrate specificity between our results and theirs could be because of a difference between enzyme in situ and the purified form.

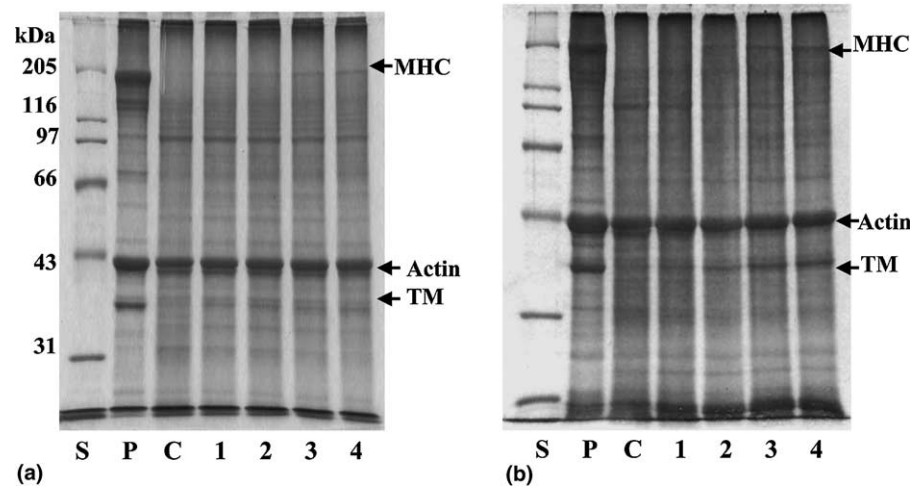

Fig. 5. SDS–PAGE pattern of lizardfish surimi mixed with EW (a) and WPC (b) at 1–4% and pre-incubated at 65 °C for 1 h followed by heating at 90 °C for 30 min: 1–4 indicate addition level in %; (S) standard molecular weight; (P) raw paste; (C) no inhibitor.

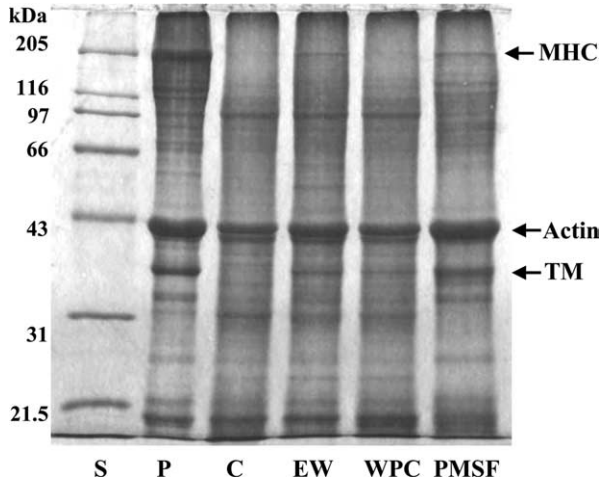

Fig. 6. SDS–PAGE pattern of lizardfish surimi mixed with various inhibitors and pre-incubated at 65 °C for 1 h.

3.3. Effect of EW and WPC on textural properties

The breaking force and deformation of gels incubated at 65 °C without inhibitors was too soft and mushy for texture evaluation (Fig. 7(a)–(d)). A gel network could not be formed due to a significant loss of MHC (Fig. 4). Low temperature setting (25 °C for 4 h) without inhibitor did not enhance textural properties ($p > 0.05$). A lower breaking force, accompanying significant loss of MHC, was observed at high temperature setting (40 °C). At any pre-incubation treatments, EW increased the breaking force and deformation at 1% addition ($p < 0.05$). A further increase in EW concentration did not significantly improve the textural properties ($p > 0.05$). Addition of WPC did not increase breaking force of surimi gel but it improved deformation at 1% addition ($p < 0.05$). EW enhanced textural properties of lizard-

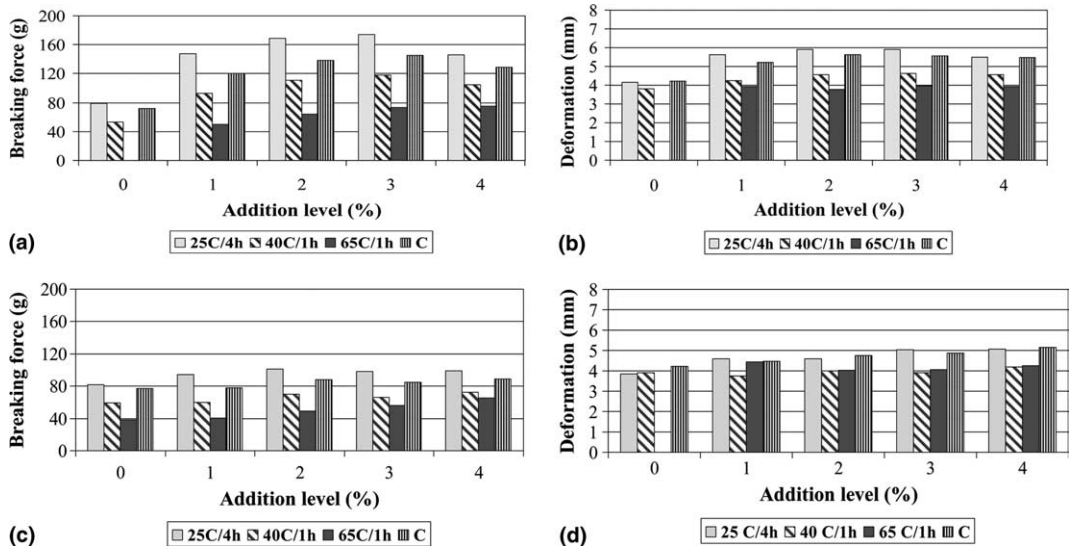

Fig. 7. Textural properties of surimi gels mixed with EW (a–b) and WPC (c–d) and subjected to various heating conditions as described in Fig. 3.

fish surimi gel to a greater extent than WPC although it showed lower inhibitory activity (Table 2). The gel enhancing effect of EW appeared to play a more important role in improving textural properties of lizardfish surimi than its inhibitory effect. EW was also found to increase the shear stresses of Alaska pollock and menhaden surimi pre-incubated at 60 °C (Hamann et al., 1990).

Low temperature setting improved the textural properties of surimi with the addition of EW and WPC ($p < 0.05$). The breaking force of surimi gels, with 1–3% EW addition and pre-incubated at 25 °C, increased about twofold, compared to the control (no inhibitor) (Fig. 7(a)). Yongswatdigul et al. (2002) demonstrated that high temperature setting at 40 °C was a more effective means of increasing textural properties of threadfin bream surimi. Endogenous transglutaminase in threadfin bream optimally catalyzed covalent cross-linking between glutamine and lysine residues of muscle proteins, resulting in increased ε-(γ-glutamyl) lysyl isopeptides and elasticity of gel. In addition, threadfin beam surimi exhibited no autolytic degradation when incubated at 40 °C for 1 h (Yongswatdigul et al., 2002). High temperature setting accelerated proteolytic activity in lizardfish surimi and subsequently retarded gel network formation. Textural properties of lizardfish surimi gel could be improved through the addition of as little as 1% EW and pre-incubation at 25 °C for 4 h.

3.4. Effect of low temperature setting and inhibitors on SDS–PAGE patterns

The TCA-soluble oligopeptide content was minimal at 25 °C (Fig. 3(a)), but pre-incubation without an inhibitor for a long time (4 h) induced degradation of MHC and TM (Fig. 8(a)). The addition of either EW or WPC at the 1% level effectively reduced proteolysis of

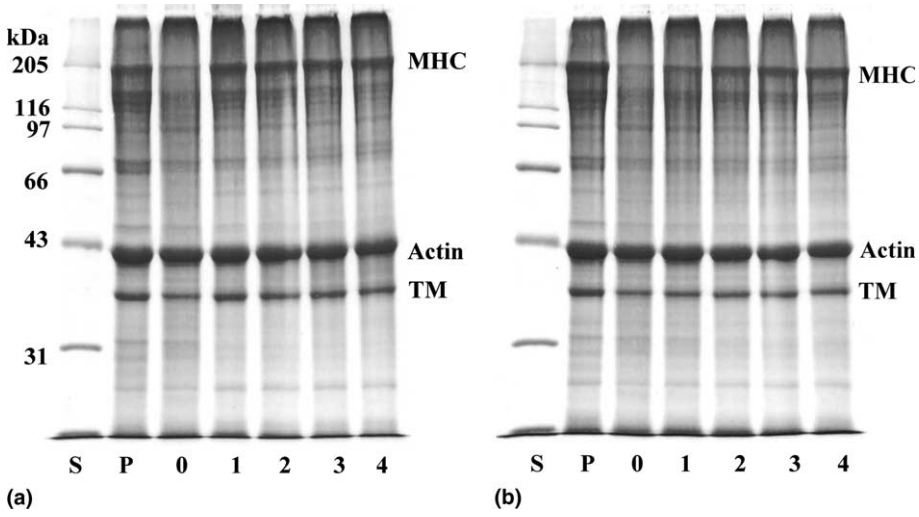

Fig. 8. SDS–PAGE patterns on 10% polyacrylamide gel of lizardfish surimi mixed with EW (a) and WPC (b) at 1–4% and pre-incubated at 25 °C for 4 h followed by heating at 90 °C for 30 min: abbreviations are the same as described in Fig. 3.

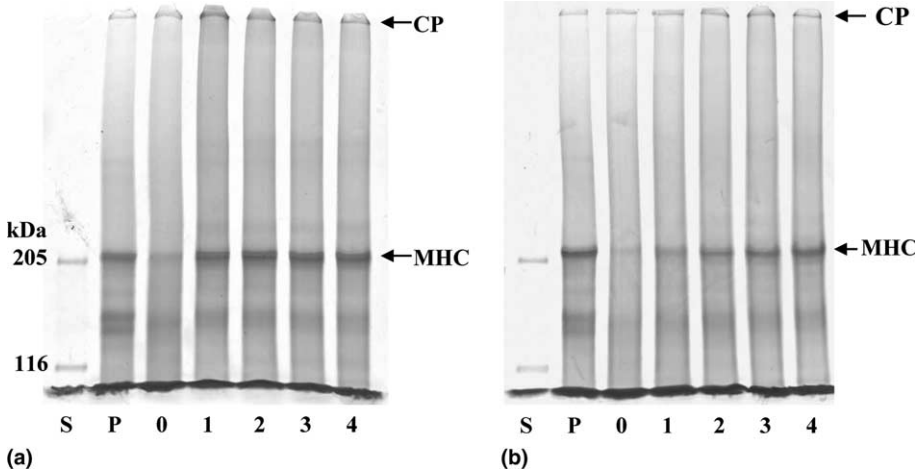

Fig. 9. SDS–PAGE patterns on 5% polyacrylamide gel of lizardfish surimi mixed with EW (a) and WPC (b) at 1–4% and pre-incubated at 25 °C for 4 h followed by heating at 90 °C for 30 min: (CP) cross-linked proteins; abbreviations are the same as described in Fig. 3.

both MHC and TM at 25 °C (Fig. 8(a) and (b)). Furthermore, protein bands with molecular weights higher than MHC appeared in samples with EW addition (Fig. 9(a) and (b)). Occurrence of higher molecular weight proteins coincided with higher breaking force and deformation of surimi gel (Fig. 7(a) and (b)). Non-disulfide covalent bonds were involved in cross-linked proteins because surimi gel samples were solubilized by SDS and BME, that mainly disrupted hydrophobic interactions and disulfide linkages, respectively. It is well established that covalent cross-linking of surimi gel is mediated by transglutaminase (TGase) activity (Yongswatdigul et al., 2002). The enzyme catalyzes the formation of an isopeptide bond between γ-carboxyamide groups of peptide-bound glutamine residues and ε-amino group of lysine (Folk, 1980). The inhibitory effect of EW could result in more available intact MHC for the TGase catalytic reaction. The sample without EW underwent severe proteolysis, which prevented the formation of ε-(γ-glutamyl)lysyl bonds. Formation of higher molecular weight proteins was less obvious in samples with the addition of WPC, corresponding to a lower breaking force (Fig. 7(c) and (d)). The retention of MHC in WPC-treated samples was slightly less than that of EW-treated samples. Therefore, EW was a more effective inhibitor for lizardfish surimi at 25 °C. Pre-incubation of surimi mixed with EW at 25 °C accelerated the formation of cross-linked proteins, improving breaking force and deformation.

4. Conclusions

Lizardfish surimi undergoes a severe textural degradation, due to serine proteinase(s), that is not removed by washing. MHC and TM are preferentially hydrolyzed at 65 °C. Both EW and WPC showed high inhibitory activity based on TCA-soluble oligopeptide content, but degradation of MHC was noted. Addition of EW, in conjuction with setting at 25 °C, increased the breaking force of surimi and high molecular weight cross-linked proteins.

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