CHAPTER 5

CHICKEN PLASMA PROTEIN: PROTEINASE INHIBITORY ACTIVITY AND ITS EFFECT ON SURIMI GEL PROPERTIES

5.1 Abstract

The inhibitory activity of chicken plasma protein (CPP) at different concentrations on sarcoplasmic proteinases and autolysis of mince and washed mince of bigeye snapper and lizardfish was investigated. CPP (0-2%, w/w) exhibited inhibitory activity toward sarcoplasmic proteinases and autolysis, especially when CPP concentration increased. Electrophoretic study revealed that CPP effectively prevented the degradation of myosin heavy chain (MHC) in mince and washed mince incubated at elevated temperature. The breaking force and deformation of modori gels from surimi of both fish species increased as CPP concentration increased ($P<0.05$) with a concomitant decrease in TCA-soluble peptides. MHC was retained more in the presence of CPP, especially when the CPP concentration increased. However, whiteness decreased with increasing CPP concentrations. Microstructure of modori gels with 2% (w/w) CPP added had an ordered fibrillar structure, indicating a preventive effect on hydrolysis of myofibrillar protein. This was accompanied by an increase in surimi gel strength.

5.2 Introduction

Animal blood is a by-product of slaughterhouses and contains a number of proteins. It can be used in both the feed and food industries owing to its good nutritional value and excellent functional properties (Tybor *et al.*, 1975). However, blood has been used only in limited quantities for direct human consumption because of its intense color and characteristic taste, as well as esthetic and religious objections. To improve the properties of blood for food use, plasma can be separated from the red cell fraction by centrifugation and then concentrated by ultrafiltration (Howell and Lawrie, 1983; Torres *et al.*, 2002). Plasma comprises a diverse range of proteins (at least 100 distinct protein components) with various properties. Therefore, fractionation of plasma protein has been

attempted to recover selective components for uses as functional ingredients in food (Cofrades *et al*., 2000; Tseng *et al*., 2000; Pares and Ledward, 2001; Silva *et al*., 2002). Additionally, plasma protein has been reported to exhibit proteinase inhibitory activity and gel strengthening ability during heat-induced gelation of surimi (Jiang and Lee, 1992; Seymour *et al*., 1997; Kang and Lanier, 1999; Benjakul and Visessanguan, 2000; Lee *et al*., 2000b; Visessanguan *et al*., 2000; Benjakul *et al*., 2001c).

The presence of endogenous proteolytic enzymes in fish mince or surimi results in a decrease in gel strength with a brittle and nonelastic gel, especially at temperatures close to 60°C. This phenomenon, referred to as "modori", causes an irreversible destruction of the gel structure of surimi (An *et al*., 1996; Alvarez *et al*., 1999). Proteases are found in the soluble sarcoplasmic component of muscle tissue, in association with cellular organelles, connective tissues and myofibrils, and in the interfiber space (Ashie and Simpson, 1997). Two major intracellular degradative pathways involve a lysosomal pathway, including cathepsin proteases, and/or a cytosolic calcium-dependent pathway with calpains (Zeece *et al*., 1992). Alkaline proteinases are also implicated in some fish species (Lin and Lanier, 1980; Boye and Lanier, 1988). Recently, Benjakul *et al*. (2003a) reported that heat-activated alkaline proteinase in bigeye snapper played an important role in degradation of muscle protein, especially at 60-65°C. The sarcoplasmic proteinase which contributes to poor gel forming ability of lizardfish (*Saurida tumbil*) muscle (Benjakul *et al*., 2003c) is also a heat-activated alkaline proteinase with an optimum pH and temperature of 8.0 and 65°C, respectively (Benjakul *et al*., 2003d).

To alleviate the problems associated with protein degradation caused by the endogenous proteinases, inhibitors and other additives have been used in surimi to improve the physical properties of surimi gels. Beef plasma protein (BPP), porcine plasma protein (PPP), egg white and potato powders can be used as food grade inhibitors in surimi (Morrissey *et al*., 1993; Reppond and Babbitt, 1993; Seymour *et al*., 1997; Kang and Lanier, 1999; Benjakul and Visessanguan, 2000; Lee *et al*., 2000b; Benjakul *et al*., 2001c). These are often used to improve gelling characteristics of surimi having a high activity of endogenous heat-activated proteinase, such as from arrowtooth flounder (Wasson *et al*., 1992a), Pacific whiting (Akazawa *et al*., 1993), and even Alaska pollock (Liu *et al*., 1996). Recently, the addition of chicken plasma protein (CPP) was found to increase the strength of surimi gels from bigeye snapper (Rawdkuen *et al*., 2004a). Such gel strengthening was attributed to its proteinase inhibitory activity. No information

regarding the inhibitory activity of CPP on fish proteinases has previously been reported. The purpose of this study was to investigate the preventive effects of CPP on the hydrolysis of myofibrillar protein by endogenous proteinases in surimi made from bigeye snapper and lizardfish, and to study the associated effects on gelling properties of these surimis.

5.3 Materials and Methods

Chemicals

Trisodium citrate and sodium chloride were purchased from Merck (Darmstadt, Germany). Casein from bovine milk and L-tyrosine were obtained from Sigma Chemical Co. (St Louis, MO, USA). Sodium dodecyl sulfate (SDS), *N, N, N′, N′* tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were obtained from Bio-Rad Laboratories (Hercules, CA, USA).

Chicken plasma protein preparation

Chicken blood was obtained from a slaughterhouse in Hat Yai, Thailand. During collection, one-tenth volume of 3.8% (w/v) trisodium citrate was added to prevent coagulation. The blood was centrifuged twice at 1,500*g* for 15 min at 4°C using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The supernatant was then freeze-dried and kept at -18°C until used. CPP powder contained 55.83% protein (dry basis) as determined by Kjeldahl method (A.O.A.C, 1999).

Fish preparation

Bigeye snapper (*Priacanthus macracanthus*) and lizardfish (*Saurida tumbil*) caught off the Songkhla Coast along the Gulf of Thailand were purchased from a dock in Songkhla, Thailand. Fish were stored in ice with a fish/ice ratio of 1:2 (w/w) and transported to Department of Food Technology, Prince of Songkla University within 1 h. Fish were headed, gutted and washed. Some fish were filleted and kept at -18°C for

sarcoplasmic fluid and washed mince preparation. Another portion was immediately used for surimi preparation.

Frozen fillets were thawed in running water (25-26°C) until the core temperature reached 0-2°C. The fillets were then chopped into small pieces, followed by centrifugation at 5000*g* for 30 min at 4°C. The supernatant obtained was referred to as "sarcoplasmic fluid". Washed mince was prepared according to the method of Toyohara *et al*. (1990a). Finely chopped fish meat was homogenized with 5 volumes of 50 mM NaCl. The homogenate was centrifuged at 10,000*g* for 10 min at 4°C. The washing was repeated twice. The final precipitate was designated as "washed mince".

Inhibitory activity assay

Inhibitory activity against sarcoplasmic proteinases was tested using casein as a substrate according to Benjakul and Visessanguan (2000). The substrate solution consisted of 0.1 ml of 2% (w/v) casein solution, 0.7 ml of buffer (0.1 M NaH_2PO_4, 0.05 M $Na_2B_4O_7$) pH 8.5 for bigeye snapper proteinase; and pH 8.0 for lizardfish proteinase. Distilled water was used to adjust the final volume to 1.0 ml. CPP solution (200 μl) at different concentrations (0, 5, 10, 20 and 40 mg/ml) was mixed thoroughly with the enzyme solution (200 μl) to obtain final concentrations of 0, 2.5, 5, 10 and 20 mg/ml, respectively. The enzyme-CPP mixture was incubated at room temperature (25°C) for 20 min. To initiate reaction, 1 ml of substrate solution pre-warmed to the reaction temperature (60°C) was added. The reaction was conducted at 60°C for precisely 40 min and was terminated with 200 μl of 50% TCA. The supernatant containing soluble oligopeptides was obtained by centrifuging the reaction mixture for 5 min at 8,000*g* (Eppendorf Micro Centrifuge, Model 5415C, Brinkmann, New York, USA). TCA-soluble peptides were determined by the Lowry assay (Lowry *et al*., 1951). Inhibitory activity was expressed as percent of proteinase activity inhibited by CPP in comparison with initial activity (without CPP addition).

The inhibitory activity of CPP against mince and washed mince autolysis was measured according to the method of Morrissey *et al*. (1993). CPP at levels of 0, 0.3, 0.5, 1 and 2% (w/w) were added to 3 g of mince and washed mince. The mixture was then immediately incubated in a water bath (Memmert, Schwabach, Germany) at

60°C for 1 h for bigeye snapper mince and washed mince and 2 h at 60°C and 65°C for lizardfish washed mince and mince, respectively. Autolysis was stopped by addition of 27 ml of 5% cold TCA solution, then the sample was homogenized for 2 min and kept on ice for 1 h. The mixture was centrifuged at 8,000*g* for 5 min using an Eppendorf Micro Centrifuge. TCA-soluble peptides in the supernatant were analyzed using the Lowry assay (Lowry *et al*., 1951). Inhibition of autolysis was expressed as percent of autolytic activity inhibited, compared to that in the control (without CPP addition).

Surimi preparation

After deskinning and deboning, fish mince was subjected to washing using a meat/water ratio of 1:3 (w/v) at temperature below 10°C. Washing was repeated 3 times, followed by dewatering using a model CE 21 K basket centrifuge (Grandiumpiant, Belluno, Italy) until the moisture content of surimi was around 78-80%. Surimi was mixed with the cryoprotectant, 4% sucrose and 4% sorbitol, using a paddle-type mixer (T.D Chemical Trader, Birmingham, UK) and then packed into polyethylene bags (600g), frozen at -18°C using an air-blast freezer for 24 h. Frozen surimi was stored at -18°C until used.

Surimi gel preparation

Frozen surimi (200g) was partially thawed at 4°C for 2-3 h, cut into small pieces and chopped in a Moulinex Masterchef 350 mixer (Paris, France) for 4 min with 2.5% (w/w) NaCl. CPP at different levels (0, 0.3, 0.5, 1 and 2%, w/w) was added. Prior to mixing, the mixing bowl was placed in ice to lower the temperature and the mixing process was conducted in a walk-in cold room (4°C). The temperature was maintained below 10°C during chopping. The paste was stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends were sealed tightly. The paste was incubated at 60°C for 30 min, followed by heating at 90°C for 20 min in a water bath (Memmert, Schwabach, Germany). This sample was referred to as "modori gel", since 60°C is near the optimum temperature for proteinase activity (An *et al*., 1996). The kamaboko gel was prepared by incubating the paste at 40°C for 30 min and heated for 20 min at 90°C. A

directly heated gel was heated at 90°C for 20 min. After heating, all gels were immediately cooled in iced water for 30 min and stored at 4°C overnight prior to analysis.

Texture analysis

Texture analysis of surimi gels was carried out using a Model TA-XT2 texture analyzer (Stable Micro System, Surrey, UK). Gels were equilibrated at room temperature (25°C) before analysis. Five cylindrical samples (2.5 cm in length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyzer equipped with a spherical probe (5 mm diameter, 60 mm/min depression speed).

Whiteness measurement

Five samples from each treatment were subjected to whiteness measurement using a JP7100F colorimeter (Juki Corp, Tokyo, Japan). CIE L*, a* and b* values were measured. Whiteness was calculated using the following equation (Park, 1994).

$$\text{Whiteness} = L^* - 3b^*$$

Determination of expressible moisture

Expressible moisture was measured according to the method of Ng (1987). Cylindrical gel samples were cut to a thickness of 5 mm, weighed (X) and placed between two pieces of Whatman paper No.1 at the bottom and one piece of paper on the top. A standard weight (5kg) was placed on the top of the sample for 2 min, then the sample was removed from the papers and weighed again (Y). Expressible drip was calculated with the following equation and expressed as percentage of sample weight.

$$\text{Expressible drip (\%)} = 100 \times \{(X-Y)/X\}$$

Determination of autolysis in surimi gel

To 2 g of finely chopped gel samples, 18 ml of 5% TCA were added and homogenized for 2 min using an IKA homogenizer at a speed of 11,000 rpm. The

homogenate was incubated at 4°C for 1 h and centrifuged at 8,000*g* for 5 min. TCA-soluble peptides in the supernatant were measured according to the Lowry method (Lowry *et al*., 1951) and expressed as μmole tyrosine/g sample (Morrissey *et al*., 1993).

SDS-PAGE

SDS-PAGE analysis was performed according to the method of Laemmli (1970). To 2 g of sample, 18 ml of 5% (w/v) SDS solution was added. The mixture was then homogenized using an IKA Labortechnik homogenizer at a speed of 11,000 rpm for 1 min. The homogenates were incubated at 85°C in a water bath for 1 h to dissolve total proteins. The sample was centrifuged at 10,000*g* for 5 min to remove undissolved debris. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8 containing 4% SDS, 20% glycerol and 10% βME) and boiled for 3 min. The samples (20 μg protein) were loaded into the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protean II unit (Bio-Rad Laboratories, Inc, Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

Scanning Electron Microscopy (SEM)

Microstructure of surimi gels was determined using SEM. Modori gels from bigeye snapper and lizardfish surimi without and with 2% (w/w) CPP with a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with a serial concentration of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 10 kV.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Analysis was performed using a SPSS package (SPSS 10.0 for windows, SPSS Inc, Chicago, IL).

5.4 Results and Discussion

Inhibitory activity of CPP on sarcoplasmic proteinases

At a level of 2.5 mg/ml, percent inhibitions of 76.8 and 40.9 were found for bigeye snapper and lizardfish proteinases, respectively (Table 12). Higher inhibition was observed with both fish species as the CPP concentration increased ($P<0.05$). Difference in inhibitory activity of CPP toward the proteinases from the two different species is likely due to differences in the type(s) and amount(s) of proteinase(s) predominantly found in the sarcoplasmic fluid. Benjakul *et al.* (2003a) reported that major sarcoplasmic proteinase in bigeye snapper was heat-activated alkaline proteinase, classified as a serine proteinase. It had an optimum temperature at 60°C. However, sarcoplasmic proteinase in lizardfish muscle was characterized to be heat-activated alkaline cysteine proteinase, exhibiting the highest activity at 65°C (Benjakul *et al.*, 2003d). Su *et al.* (1981) reported that alkaline proteinase activity was found in the sarcoplasmic fraction of several tissues of Atlantic croaker, including the muscle, kidney, liver, alimentary canal, and skin. These enzymes contributed to degradation of fish gel texture at 60°C. Our results thus demonstrate that the degradation of muscle caused by sarcoplasmic proteinases in fish mince could be minimized by addition of CPP. Chicken blood plasma contains ornitho-kininogen with a molecular mass of 74 kDa, which has similar properties to mammalian high-molecular-weight kininogen (Kimura *et al.*, 1987). Kos *et al.* (1992) also reported that high-molecular-weight kininogen was present in chicken plasma and was a strong inhibitor of chicken cathepsin L and papain, but was a much weaker inhibitor of chicken cathepsin B. Benjakul *et al.* (2001c) reported that PPP inhibited papain, trypsin and fish muscle and viscera proteinases effectively.

Table 12. Inhibitory activity of chicken plasma protein at different concentrations on sarcoplasmic proteinases from bigeye snapper and lizardfish muscle.

CPP concentration (mg/ml)	% Inhibition	
	Bigeye snapper	Lizardfish
0	0.0 ± 0.0*[a**]	0.0 ± 0.0[a]
2.5	76.8 ± 0.9[b]	40.9 ± 0.1[b]
5	78.9 ± 0.2[c]	41.8 ± 0.1[c]
10	81.2 ± 1.0[d]	42.9 ± 0.9[d]
20	83.0 ± 0.8[e]	47.1 ± 0.1[e]

*Values are given as mean ± SD from triplicate determinations.

**Different superscripts in the same column indicate significant differences ($P<0.05$).

Inhibitory activity of CPP on the autolysis of mince and washed mince

Marked inhibition of autolysis of mince and washed mince from the two species was observed when CPP was added at 0.3% (w/w) ($P<0.05$) (Figure 23). In the presence of 0.3% (w/w) CPP, autolysis of bigeye snapper mince and washed mince was inhibited by 88.9 and 88.8%, respectively, and autolysis in lizardfish mince and washed mince was inhibited by 51.1 and 38.5%, respectively. No marked increases in inhibition were observed in any samples as CPP concentrations were increased above 0.3% (w/w) ($P<0.05$). When comparing the inhibitory activity of CPP on autolysis of mince versus washed mince, higher activity was found in mince. Fish mince contains both sarcoplasmic proteinases and myofibril-associated proteinases. As a consequence, higher autolysis generally occurs in mince, compared to washed mince in which sarcoplasmic proteinase has been removed (Benjakul *et al.*, 2003a). Since sarcoplasmic proteinases were inhibited effectively by CPP, especially at a higher concentration (Table 12), autolysis caused by sarcoplasmic proteinases in mince was reduced. However, autolysis of washed mince was also inhibited by CPP to some extent, indicating an inhibitory activity of CPP toward myofibril-associated proteinase(s) in both species. The results indicate that autolysis in both mince and washed mince, especially from bigeye snapper, can be reduced by CPP.

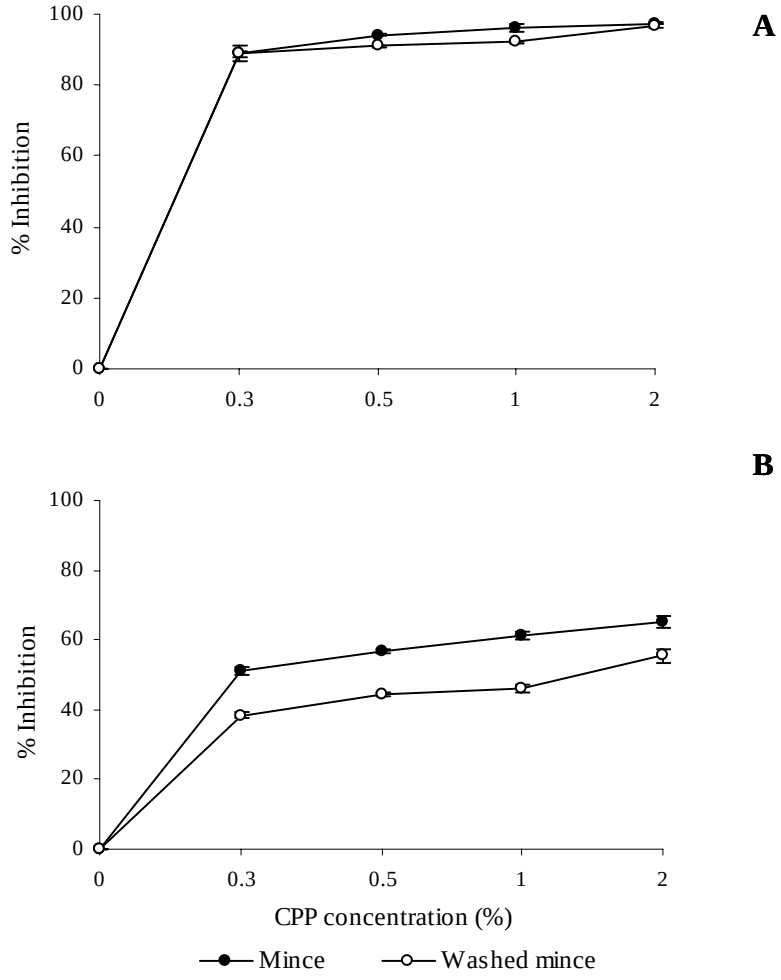

Figure 23. Inhibitory activity of chicken plasma protein at different concentrations on the autolysis of mince and washed mince from bigeye snapper (A) and lizardfish (B).

Toyohara *et al.* (1990a) reported that proteolytic degradation of fish gels could be classified into two types according to the localization of the gel degradation-inducing factor (GIF) in muscle cells. One type was induced by sarcoplasmic GIF, which could be easily washed out from the myofibrils. The other type was induced by myofibrillar GIF, which is tightly associated with myofibrils. The former type was shown to be responsible for gel degradation in threadfin bream, while the latter was shown to be active in oval-file fish (Toyohara *et al.*, 1990b).

Autolytic patterns of mince and washed mince from bigeye snapper and lizardfish, which contained various levels of CPP, are shown in Figure 24. For mince and washed mince without CPP addition (lane 2), MHC band intensity decreased considerably

after incubation at elevated temperature. The degradation was attributed to endogenous proteinases, which are activated at high temperature normally ranging from 60 to 65°C (Benjakul *et al*., 2003a). Cao *et al*. (2000) reported that a serine myofibril-bound proteinase in lizardfish, with a molecular mass of 29 kDa, hydrolyzed MHC at 55-60°C. Suwansakornkul *et al*. (1993) also reported that the modori-inducing (at 40°C) factors in washed mince of three lizardfish species were serine and cysteine proteinases.

The MHC band in washed mince decreased to a lesser extent compared with that in mince (lane 2). This result suggests that proteinases in muscle, especially water-soluble proteinases, were largely removed during the washing process. Chang-Lee *et al*. (1989) reported that the protease activity in mechanically deboned Pacific whiting flesh was reduced to 56.3% by washing and refining processes. An *et al*. (1994b) reported that washing during Pacific whiting surimi processing removed cathepsin B and H, but not cathepsin L. More than 80% of the residual activity of calpains, cathepsins B, L and L-like remained in frozen mackerel surimi (Jiang *et al*., 1996). Additionally, Su *et al*. (1981) found that protease activity was still retained in minced Atlantic croaker after washing. The presence of these residual proteinases affects the texture of surimi-based products (Masaki *et al*., 1993). With increasing addition of CPP, MHC was increasingly retained (lanes 3-6). At 2% (w/w) CPP, MHC band intensity of bigeye snapper mince and washed mince was similar to that of the starting material (lane 1). However, a slightly lower MHC band intensity was observed for mince and washed mince of lizardfish in the presence of 2% (w/w) CPP. This indicated that CPP only partially inhibited proteolysis in lizardfish such that remaining active proteinases could hydrolyze the muscle proteins. This result was in accordance with the lower inhibitory activity of CPP on autolysis noted for this species (Figure 23). No changes in actin, tropomyosin or troponin were observed for any samples. Aranishi *et al*. (1998) reported that MHC was completely degraded by carp cathepsin L within 30 min at pH 5.5 and 37°C and underwent further degradation during subsequent incubation. Tropomyosin and troponin were also completely degraded within 24 h by this endogenous enzyme, during which a small amount of actin and a large amount of α-actinin remained undegraded. An *et al*. (1994b) reported that myofibrillar proteins of Pacific whiting underwent extreme textural softening upon heating due to an endogenous heat stable proteinase which primarily hydrolyzed myosin. Ladrat *et al*. (2000) found that m-calpain was able to degrade MHC, α-actinin and desmin but not actin and

tropomyosin. Cathepsin B, L and D degraded MHC and α-actinin of white muscle of sea bass, while tropomyosin and actin were only susceptible to the action of cathepsins B and L. (Ladrat *et al*., 2003).

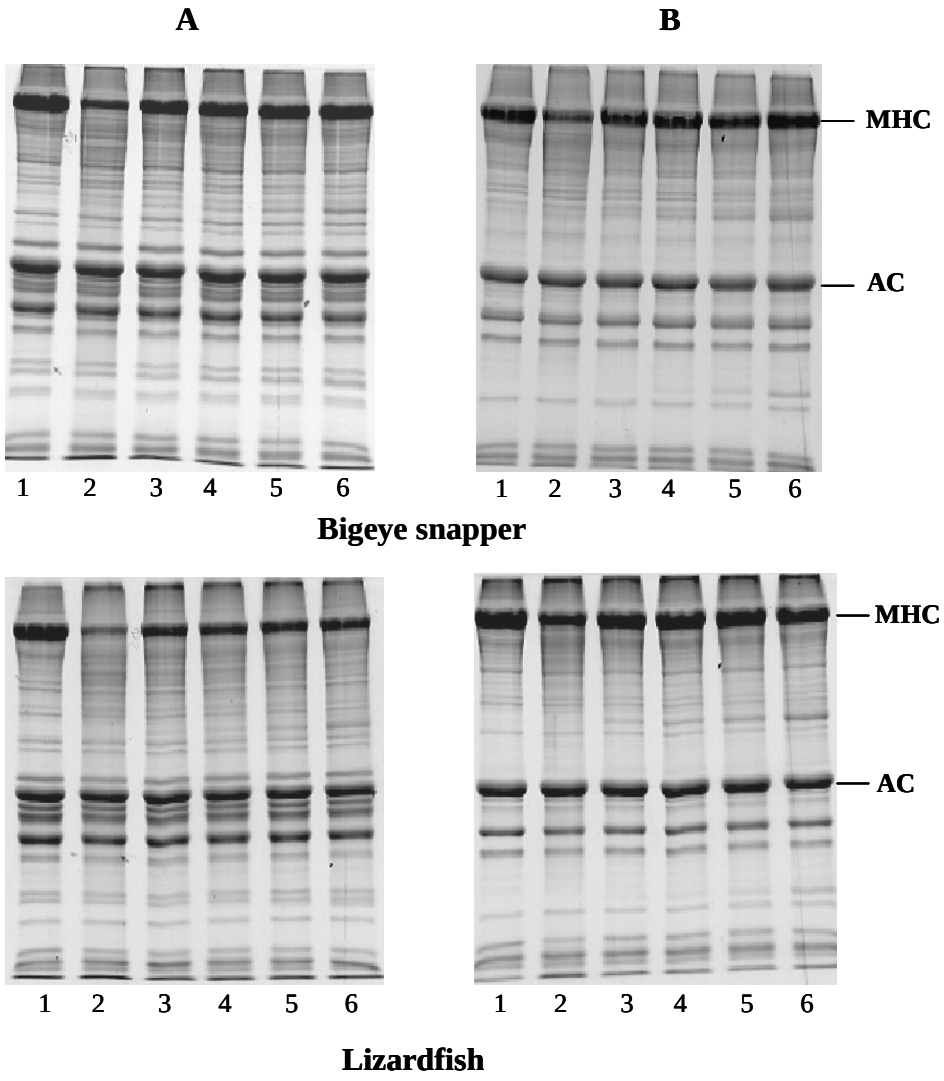

Figure 24. Protein patterns of mince (A) and washed mince (B) added with different concentrations of chicken plasma protein. Lane 1, mince or washed mince (control/without heating); lane 2-6, mince or washed mince added with 0, 0.3, 0.5, 1 and 2% (w/w) CPP, respectively. Bigeye snapper mince and washed mince were incubated at 60°C for 1 h, and lizardfish mince and washed mince were incubated at 65°C for 2 h. MHC: myosin heavy chain; AC: actin. Protein (20μg) was loaded into each lane.

Effect of CPP on textural properties of surimi gel

The breaking force of modori gels from surimi of both fish species increased as the CPP concentration increased up to 2% (w/w) ($P<0.05$) (Figure 25). Gel deformation of modori gels from lizardfish surimi also increased when CPP concentration increased. However, deformation of bigeye snapper modori gels did not change as CPP concentration increased ($P<0.05$). When 2% (w/w) CPP was added, breaking force of modori gel from bigeye snapper and lizardfish surimi increased by 87.7 and 128.8%, respectively. Karlsrud *et al.* (1996) reported that porcine blood plasma consists of several types of proteinase inhibitors. Reppond and Babbitt (1993) showed that addition of 2% (w/w) bovine plasma increased the strength of arrowtooth flounder gels. Morrissey *et al.* (1993) also reported that BPP at a concentration as low as 1% (w/w) showed strongest inhibition of proteolysis in Pacific whiting surimi when measured by autolysis, gel electrophoresis and torsion. Dried hydrolysate beef plasma prevented modori (increased stress and strain values) in surimi gels precooked at 60°C (90°C final cook) (Hamann *et al.*, 1990). Comparing breaking force between directly heated gels and kamaboko gels without CPP addition, the latter were 34.6% and 58.6% higher for surimi from bigeye snapper and lizardfish, respectively. Deformation of kamaboko gels of surimi from both species was also higher than that of the directly heated gels. Transglutaminase (TGase) has been reported to induce the formation of ε-(γ-glutamyl) lysine linkage during setting (Tsukamasa *et al.*, 2002; Wan *et al.*, 1994). Addition of CPP to prevent proteolysis, combined with a setting preincubation, is thus an effective means to improve the gel-forming ability of surimi from both bigeye snapper and lizardfish.

Effect of CPP on whiteness and expressible moisture of surimi gels

The whiteness of modori gels decreased when CPP was added, especially at higher CPP concentrations ($P<0.05$) (Table 13). With the addition of 2% (w/w) CPP, whiteness of modori gel of surimi from bigeye snapper and lizardfish decreased by 60.1 and 66.0%, respectively, compared to that of modori gels without CPP. This result was in accordance with Benjakul *et al.* (2001c) who reported that increased addition of PPP resulted in decrease in whiteness of surimi gels. The whiteness of directly heated gels was higher than that of kamaboko gels or modori gels ($P<0.05$). The plasma fraction retains

some hemoglobin as well as other pigments with a pale straw color (Benjakul *et al*., 2001c).

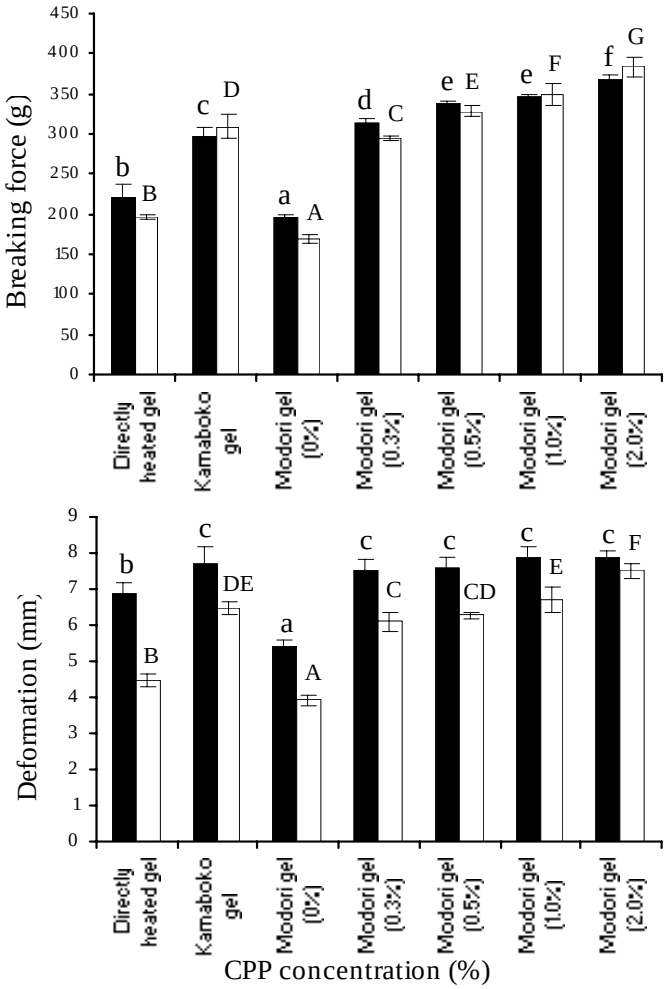

Figure 25. Breaking force and deformation of surimi gels added with different concentrations of chicken plasma protein. Directly heated gel: heated at 90°C for 20 min; Kamaboko gel: incubated at 40°C for 30 min, followed by heating at 90°C for 20 min; Modori gel: incubated at 60°C for 30 min, followed by heating at 90°C for 20 min. *Bars represent the standard deviation from five determinations. **Different letters in the same species indicate significant differences ($P<0.05$). ■ Bigeye snapper, □ Lizardfish.

Table 13. Whiteness and expressible moisture of surimi gels added with different concentrations of chicken plasma protein.

Samples	Whiteness*		Expressible moisture **(%)	
	Bigeye snapper	Lizardfish	Bigeye snapper	Lizardfish
Directly heated gel	48.9±0.5^{f***}	54.6±0.5^{f}	5.7±0.1^{d}	5.4±0.1^{e}
Kamaboko gel	48.7±0.6^{f}	53.3±0.2^{e}	4.7±0.2^{b}	4.1±0.1^{b}
Modori gel without CPP	47.3±0.4^{e}	52.7±0.5^{e}	6.6±0.3^{e}	6.7±0.1^{f}
Modori gel with 0.3% CPP	43.1±0.4^{d}	46.9±1.1^{d}	5.2±0.3^{c}	5.0±0.2^{d}
Modori gel with 0.5% CPP	39.4±0.2^{c}	42.9±0.4^{c}	5.2±0.2^{c}	4.5±0.1^{c}
Modori gel with 1% CPP	31.4±0.9^{b}	33.5±0.6^{b}	4.7±0.1^{b}	4.3±0.1^{c}
Modori gel with 2% CPP	18.9±0.2^{a}	17.9±0.7^{a}	4.2±0.1^{a}	3.8±0.0^{a}

*Values are given as mean ± SD from five determinations.

** Values are given as mean ± SD from triplicate determinations.

***Different superscripts in the same column indicate significant differences ($P<0.05$).

The expressible moisture of surimi gels from both species decreased when higher amounts of CPP was added ($P<0.05$) (Table 13). Highest expressible moisture was found in modori gels without CPP addition. Modori gels to which 2% (w/w) CPP was added exhibited expressible moisture 57.1 and 76.3% lower than those without CPP for bigeye snapper and lizardfish surimi, respectively. Expressible moisture for kamaboko gels without CPP was 10.6 and 7.3% higher than modori gels containing 2% (w/w) CPP, for bigeye snapper and lizardfish surimi respectively. These data indicate that dried CPP was able to absorb and retain water effectively. The lower expressible moisture content obtained in the samples added with CPP is indicative for the higher water binding property of protein gel matrix. During thermal gelation, protein matrix was formed and water was imbibed regularly throughout gel network. In modori gel without CPP, the highest expressible moisture was observed, indicating the poor gel matrix with low water holding capacity. Therefore, CPP could prevent the degradation of muscle proteins, leading to the well-ordered network with high water holding capacity.

Effect of CPP on protein degradation in surimi gels

Protein degradation was monitored as TCA-soluble peptides in different surimi gels (Table 14). The highest TCA-soluble peptides were observed for modori gels without CPP addition, indicating the highest degradation by proteinase of muscle proteins. TCA-soluble peptides decreased as added CPP concentration increased ($P<0.05$). However, TCA-soluble peptides in modori gels from bigeye snapper were not significantly decreased when CPP above 0.5% (w/w) was added. Thus 0.5% (w/w) CPP addition might be enough to inhibit modori-inducing proteinases in bigeye snapper surimi gels, and gains in textural parameters at levels above this can be attributed to gel-enhancing ability besides that of proteinase inhibition. Gels from lizardfish surimi evidenced greater degradation by this index than those from bigeye snapper surimi ($P<0.05$).

Table 14. TCA-soluble peptides of surimi gels added with different concentrations of chicken plasma protein.

Samples	Tyrosine (μmoles/g sample)	
	Bigeye snapper	Lizardfish
Directly heated gel	1.13±0.01*^{c**}	1.22±0.01^{e}
Kamaboko gel	1.22±0.01^{d}	1.59±0.01^{f}
Modori gel without CPP	1.34±0.01^{e}	1.81±0.04^{g}
Modori gel with 0.3% CPP	0.42±0.04^{b}	0.80±0.01^{d}
Modori gel with 0.5% CPP	0.36±0.01^{a}	0.66±0.01^{c}
Modori gel with 1% CPP	0.35±0.00^{a}	0.51±0.02^{b}
Modori gel with 2% CPP	0.34±0.01^{a}	0.44±0.02^{a}

*Values are given as mean ± SD from triplicate determinations.

**Different superscripts in the same column indicate significant differences ($P<0.05$).

It is possible that myofibrillar proteins of surimi from lizardfish were possibly more labile to proteolytic degradation than those from bigeye snapper, or that proteinase activity is greater in the former species. In both species, upon addition of 2% (w/w) CPP, TCA-soluble peptides decreased by 300% in modori gels. CPP therefore

clearly had inhibitory activity towards proteinase degradation of proteins at 60°C. Directly heated gels exhibited lower TCA-soluble peptide production as compared to kamaboko gels. Degradation probably occurred during setting at high temperature (40°C), which is close to the optimum temperature for most heat-activated fish proteinases (50-60°C) (Kang and Lanier, 2000).

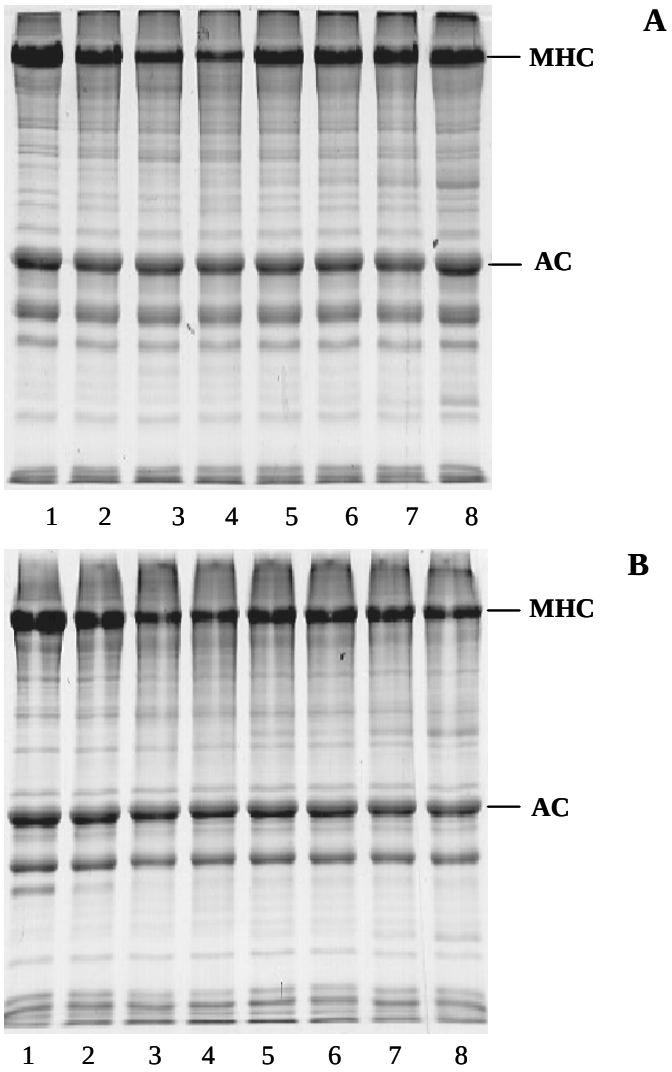

Figure 26. Protein patterns of bigeye snapper (A) and lizardfish (B) gels prepared under different conditions. Lane 1, surimi sol without chicken plasma protein, lane 2 directly heated gel, lane 3 kamaboko gel, lane 4 - 8 modori gel added with 0, 0.3, 0.5, 1 and 2% (w/w) CPP, respectively. MHC: myosin heavy chain; AC: actin. Protein (20μg) was loaded into each lane.

The lowest intensity of MHC band in SDS-PAGE was measured from modori gels without CPP addition (Figure 26: lane 4). MHC was retained more as the concentration of CPP increased (Figure 26; lanes 5-8). This result was in agreement with the lower TCA-soluble peptides observed in modori gels containing higher levels of CPP. However, no changes in actin, troponin and tropomyosin were observed in any samples. Higher intensity of MHC bands from bigeye snapper modori gels were observed with 2% (w/w) CPP addition, being almost equal to that from the unheated surimi sol (Figure 26; lane 1). A lower MHC band intensity was found with the addition of 2% (w/w) CPP to the lizardfish modori gel, compared to that of the unheated surimi sol. This indicated that CPP more effectively inhibited the proteolytic activity in bigeye snapper than lizardfish surimi.

In kamaboko gels, decreases in the MHC band were most likely also caused by polymerization of MHC, especially during setting (Benjakul and Visessanguan, 2003). Conversely, decreased MHC in modori gels resulted only from proteolysis (Benjakul *et al*., 2003a). The changes in MHC, either polymerization or degradation, which were found in modori and kamaboko gels, were mirrored by increases or decreases in gel strength, respectively. Thus, addition of CPP could prevent the degradation of proteins in surimi, which in turn allowed a stronger three-dimensional gel network to be formed.

Microstructure of surimi gels

Modori gels without CPP addition (Figure 27A) from both bigeye snapper and lizardfish showed a structure with aggregates of sparse packed spherical proteins, arranged in clusters. These evidenced a disordered cross-linked structure with a small globular appearance on the surface. This was caused by the cross-linking or aggregation of protein fragments resulting from activity of proteinases. Large cavities were observed at the surface of the gel structure, likely caused by the haphazard globule aggregation. When 2% (w/w) CPP was added (Figure 27B), the network fibers became more clearly defined. The regularly ordered and fine fibrillar structures in such surimi gels likely are responsible for the higher breaking force and deformation.

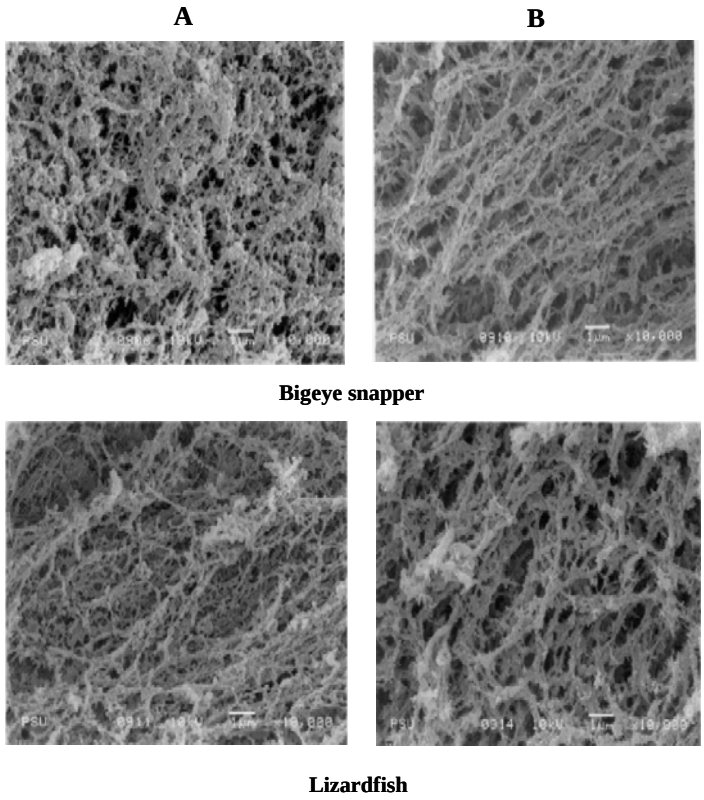

Figure 27. Scanning electron microscopic image of modori gel without (A) and with 2% (w/w) chicken plasma protein (B). Magnification: 10,000X.

5.5 Conclusion

CPP at a level of 2% (w/w) showed the highest inhibitory activity toward sarcoplasmic proteinases and autolysis of mince and washed mince from both bigeye snapper and lizardfish. The increased breaking force and deformation of surimi gel with higher water holding capacity was obtained when the CPP concentration increased, but the higher amount of CPP added resulted in the decrease in whiteness.