CHAPTER 2

CHICKEN PLASMA PROTEIN AFFECTS GELATION OF SURIMI FROM BIGEYE SNAPPER (*PRIACANTHUS TAYENUS*)

2.1 Abstract

Effect of chicken plasma protein (CPP) at different concentrations on gel properties of grade SA and A bigeye snapper surimi was investigated. Addition of 0.5% (w/w) CPP in combination with setting at 40°C for 30 min prior to heating at 90°C for 20 min resulted in the highest breaking force and deformation ($P<0.05$). However, whiteness decreased to some extent. CPP was able to prevent the degradation of surimi proteins as indicated by the decrease in TCA-soluble peptides ($P<0.05$). Electrophoretic studies revealed that myosin heavy chain (MHC) underwent polymerization to a lower extent as CPP concentration increased. Therefore, CPP worked as protease inhibitor rather than protein cross-linker. Microstructure of kamaboko gels, added with 0.5% (w/w) CPP, had less linkage between protein strands with a coarser fibrillar structure, indicating the interfering effect of CPP on cross-linking of myofibrillar proteins. Thus, at an appropriate amount, CPP possibly worked as filler in the surimi gel matrix, resulting in gel strengthening.

2.2 Introduction

Gelation of surimi proteins is a process, which involves protein unfolding and aggregation. During heating, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds. When sufficient bonding occurs, a three-dimensional network is formed, resulting in a gel (Lanier, 2000). Setting is a common practice for gel quality improvement in surimi manufacture. Setting phenomenon (pre-incubation below 40°C) is attributed to the polymerization of myosin heavy chain (MHC) through the formation of ε(γ-glutamyl) lysine linkage by endogenous transglutaminase (TGase) (Seki *et al*., 1990).

Furthermore, additives are commonly used to improve or modify textural properties. These additives act physically and/or chemically, producing structural changes in the protein matrix which depend on their composition, distribution, physical state, volume fraction and interaction with the continuous protein matrix (Montero *et al*., 2000). Addition of non-meat proteins such as whey proteins to formulate meat products has been used extensively as fillers, binders, and extender in meat system (Pietrasik and Li-Chan, 2002). The filler particles can be distinguished into two types, active fillers and inactive fillers. For active fillers, strong interactions exist between individual filler particles and the gel matrix, and the elastic modulus increase with the increasing volume fraction of filler. For inactive fillers, there is little or no interaction between filler particles and gel matrix and the modulus decreases with increasing volume fraction of the filler (Lanier, 1991). Protein additives, such as beef plasma protein (BPP), egg white and potato extract, have also been widely used in surimi (Morrissey *et al*., 1993). Foegeding *et al*. (1986) reported that the fibrinogen-rich fraction of beef plasma enhanced the strength of heat-induced myosin gels. Kang and Lanier (1997) reported that the addition of 1% BPP to Pacific whiting surimi increased strength of gels due not only to its protease inhibitory components, but also to other gel-enhancing components such as plasma TGase and thiol-containing gelling proteins. Porcine plasma protein (PPP) containing both proteinase inhibitor and TGase also effectively enhanced surimi gel strength (Jiang and Lee, 1992; Benjakul and Visessanguan, 2000; Benjakul *et al*., 2001a, b, c).

Plasma contains at least 100 different protein components (McCullough, 1995) and can be used as functional ingredients in food (Cofrades *et al*., 2000; Tseng *et al*., 2000; Pares and Ledward, 2001; Silva *et al*., 2002). Among the functional properties of protein, the capacity to form a gel on heating and emulsifying properties are important in food processing (Pares *et al*., 1998; Pares and Ledward, 2001). Several of them contribute to increase surimi gel strength, such as factor XIII (Jiang and Lee, 1992), kininogen (Lee *et al*., 2000a) and α_2-macroglobulin (α_2M) (Sareevaravitikul *et al*., 1996).

Consumption of chicken meat has been increasing in Thailand. Chicken production is approximately 78.49% of total animal production in all parts of Thailand (Dept. Livestock Development, 1996). During slaughtering process, blood is produced. Some blood has been used as coagulated blood for local consumption. However, most blood is discarded, causing the cost for disposal or treatment. Due to its intense color and

characteristic taste, separation or fractionation of plasma should maximize its utilization, especially as protein additive in surimi. Understanding the functional behavior of chicken plasma protein (CPP) in surimi is important for quality optimization of product. However, no information regarding the effect of CPP on surimi gel properties has been reported. The objective of this study is to determine the effect of CPP at different levels on the gel properties of surimi from bigeye snapper.

2.3 Materials and Methods

Chemicals

β-mercaptoethanol (βME), protein marker and trisodium citrate were purchased from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany), respectively. 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% trisodium citrate was added to prevent coagulation. The blood was centrifuged twice at 1,500*g* for 15 min at 4°C using Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The supernatant was then freeze-dried and kept at -18°C until used.

Surimi preparation

Frozen surimi from bigeye snapper grade SA (breaking force of 600-800 g; deformation of 12-14 mm) and grade A (breaking force of 400-500 g; deformation of 8-10 mm) was purchased from Man A Frozen Food Co., Ltd. (Songkhla, Thailand). Frozen surimi was stored at -18°C until used.

Proximate analysis

Moisture, protein, fat and ash in surimi and chicken plasma protein were determined according to the method of AOAC (1999).

pH determination

pH of surimi and chicken plasma protein was measured as described by Benjakul *et al*. (1997a). Surimi and chicken plasma protein was homogenized in 10 ml volumes water (w/v), and pH was measured using bench pH meter (Cyberscan 500, Singapore).

Surimi gel preparation

Frozen surimi was partially thawed at 4°C for 2 h, cut into small pieces and chopped by a Moulinex Masterchef 350 mixer (Paris, France). CPP at different levels (0, 0.25, 0.5, 0.75, 1 and 2% w/w) were added. The moisture of the mixture was adjusted to 80% with iced water and NaCl (2.5%) was added. The mixture was chopped for 4 min. The temperature was maintained below 10°C during chopping. The surimi sol was stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends were sealed tightly. The sol was incubated at 40°C for 30 min, followed by heating at 90°C for 20 min in a temperature controlled water bath (Memmert, Schwabach, Germany). These gels were referred to as 'kamaboko gel'. For directly heated gel, surimi sol was subjected to heating at 90°C for 20 min. After heating, all gels were cooled in iced water for 30 min and stored at 4°C overnight prior to analysis.

Texture analysis

Texture analysis of surimi gel 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 placed in the texture analyzer equipped with a spherical probe (5 mm diameter, 60 mm/min depression speed). Breaking force and deformation indicate strength and cohesiveness, respectively.

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).

$$\text{Whiteness} = 100-[(100 - L^*)^2+a^{*2}+b^{*2}]^{1/2}$$

Determination of expressible drip

Expressible drip was measured according to the method of Ng (1987). Gel samples were cut into a thickness of 5 mm, weighed and placed between two pieces of Whatman paper No.1 at the bottom and one piece of paper on the top. The standard weight (5kg) was placed on the top of the sample for 2 min, then the sample was removed and weighed again. Expressible drip was calculated and expressed as percentage of sample weight.

SDS-PAGE

SDS-PAGE analysis was performed according to the method of Laemmli (1970). To 2 g of gel sample, 18 ml of 5% SDS solution was added. The mixture was then homogenized using an IKA Labortechnik homogenizer (Selangor, Malaysia) for 1 min. The homogenates was incubated at 85°C in a temperature-controlled waterbath 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. The high molecular weight protein standard [myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), fructose-6-phosphate kinase (84 kDa), albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalbumin (45 kDa) and glyceraldehyde-3-phosphate dehydrogenase (36 kDa)] and low molecular weight protein standard [albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14.2 kDa) and aprotinin (6.5 kDa)] were used as reference.

Protein solubility

Finely chopped gel sample (1g) was solubilized with a 20 ml of 20 mM Tris-HCl, pH 8.0 containing 1% SDS, 2% βME and 8 M urea. The mixture was homogenized for 1 min, boiled for 2 min and stirred for 4 h at room temperature (25°C) using a magnetic stirrer (IKA-Werke, Staufen, Germany). The mixture was centrifuged at 10,000*g* for 30 min. A 2 ml of cold 50% trichloroacetic acid (TCA) was added to 10 ml of supernatant. The mixture was kept at 4°C for 18 h prior to centrifugation at 10,000*g* for 20 min. The precipitate was washed with 10% TCA, and solubilized in 0.5 M NaOH. Protein concentration was determined by the Biuret method (Robinson and Hodgen, 1940).

TCA-soluble peptides determination

To 2 g of finely chopped gel sample, 18 ml of 5% TCA were added and homogenized for 2 min. 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 Lowry's method (Lowry *et al.*, 1958) and expressed as μmole tyrosine/g sample (Morrissey *et al.*, 1993).

Scanning Electron Microscopy (SEM)

Microstructure of surimi gels was determined using SEM. Kamaboko gels without and with 0.5% CPP as well as directly heated gels from surimi grade SA and A with a thickness of 2-3 mm were fixed with 2.5% 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 a grade ethanol series 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)

2.4 Results and Discussion

Characterization of surimi and chicken plasma protein

The chemical compositions of grade SA and A surimi and CPP are shown in Table 7. Surimi grade SA and A contained high moisture content (75.83 and 76.97%). Protein content in surimi grade SA was slightly higher than surimi grade A. Very low fat content was observed in both surimi. During washing process, fish proteins mainly myofibrillar proteins were concentrated with a concomitant removal of fat, blood as well as other flavoring compounds. As a result, fat content was very low (Hall and Ahmad, 1997). This result was in accordance with Ching and Leinot (1993) who reported that surimi contains 18-21% protein, 75-78% moisture, 0-2% fat, 4% sucrose, 4% sorbitol and polyphosphates (0.2-0.3%). Ash in surimi possibly represented inorganinc matter, mainly from phosphate salt added in commercially produced surimi to enhance water binding capacity as well as to prevent protein denaturation during frozen storage. TCA-

soluble peptides were found in both surimi grade A and SA. Surimi grade A had slightly higher TCA-soluble peptides than surimi grade SA. This suggested that the myofibrillar proteins of surimi grade A with an inferior gel forming ability underwent proteolytic degradation to higher extent, compared to surimi grade SA. The surimi had pH of 7.24 and 6.97 for grade SA and A, respectively. pH has been found to affect the gel forming ability of surimi (Lee, 1984).

Table 7. Proximate composition of surimi and dried chicken plasma protein

Compositions	CPP	Surimi	
		grade SA	grade A
Moisture (%)	7.31±0.22*	75.83±0.03	76.97±0.06
Protein (%)	55.83±0.49	17.40±0.29	16.74±0.33
Fat (%)	0.54±0.04	0.15±0.02	0.17±0.01
Ash (%)	17.44±0.03	0.59±0.04	0.56±0.05
pH	9.35±0.01	7.24±0.02	6.97±0.00
TCA-soluble peptides (μmole/g sample)	10.54±0.27	0.69±0.01	0.78±0.02

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

Protein patterns of surimi determined using SDS-PAGE are shown in Figure 1. MHC was found to constitute as a major muscle protein, which appeared at MW of 200 kDa. Actin was found to be the second abudant protein in surimi with a MW of 45 kDa. Tropomyosin (molecular weight of 36 kDa) was also found in both grades of surimi. Pomeranz (1991) reported that the myofibrillar proteins of skeletal muscle are composed of 50-58% myosin, 15-20% actin, 5-8% tropomyosin, 5-8% troponin, 2-3% α-actinin, 0.5-1% β-actinin, 2-3% component C and 3-5% M-line proteins.

Proximate compositions of CPP are present in Table 7. CPP powder contained high protein content (55.83%) followed by ash (17.44%) and moisture content (7.31%), respectively. Sodium citrate added to the whole blood at the time of collection

would be a source of inorganic matter in plasma. Howell and Lawrie (1983) reported that the chemical compositions of dried plasma in various animals were different. The variation in the composition is probably due to the differing methods employed in separation of plasma. Commercial dried porcine plasma and bovine plasma had 67-70% protein, 8.9-9.4% moisture, 10.3-11.8% ash, 1.5-2% of lipid (Howell and Lawrie, 1983). Delaney (1975) reported that porcine blood plasma powder consisted of 65.1% crude protein, 1.2% fat, 7% moisture and 17% ash. Higher content of TCA-soluble peptide was found in the CPP when compare with the surimi. This indicated the presence of low MW peptides or protein in plasma.

CPP was further characterized by SDS-PAGE as shown in Figure 1. Two major bands with MW of 61 and 23 kDa were found. These two major bands were possibly albumin and globulin, respectively. Protein bands with MW of 56, 51 and 37 kDa were also obtained on SDS-PAGE. Porcine plasma contained a wide range of proteins, including albumin (65-69 kDa), fibrinogen (40-70 kDa), γ-globulins (33-58 kDa), α-globulin (56-60 kDa) and β-globulins (11-87 kDa) (Howell and Lawrie, 1983). Morrissey *et al*. (1991) reported that the major protein in plasma fraction, which accounts for ~50% of total plasma protein, was albumin. Ockernan and Hansen (2000) reported that liquid blood plasma contained 6-8% protein, consisting of 50% albumin fraction, 23-27% globulin fraction and 17-23% fibrinogen. Gautier *et al*. (1983) compared proteins and other haematological parameters of closely related species by revealing different protein patterns. Different protein patterns were also formed with American kestrel from 3 different groups (laying and non-laying females, and males) (Gautier *et al*., 1983). Kuryl and Gasparska (1985) also reported that the patterns of protein in blood plasma of chicken, quail and goose were considerably different between laying and non-laying in four regions of plasma proteins: pre-albumin, pre-transferrin, post-transferrin and γ-globulin. Proteins vary significantly with age, health as well as sex of the birds' and season of sampling (Gautier *et al*., 1983). Electrophoretic study revealed that CPP are highly complex entities differing in MW. Plasma proteins are able to form strong gels (Howell and Lawrie, 1984).

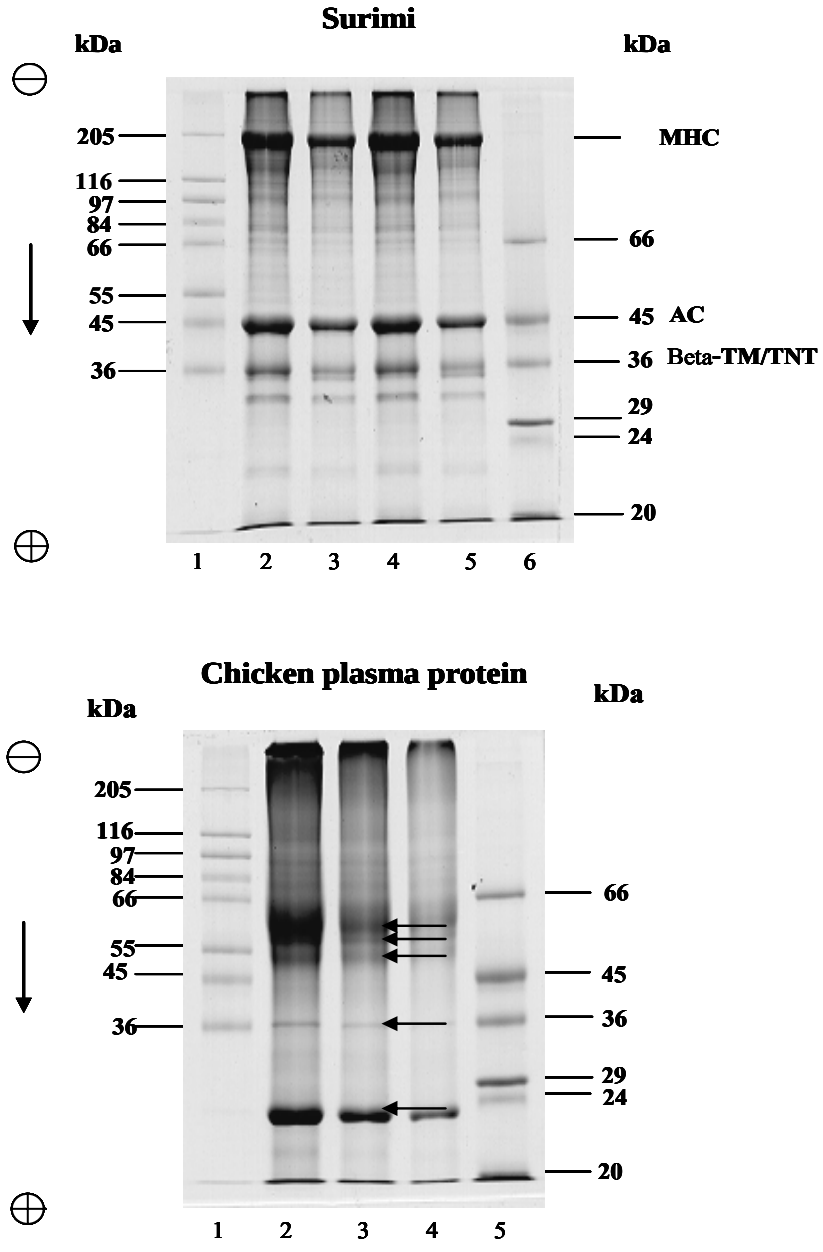

Figure 1. Protein pattern of surimi from bigeye snapper and chicken plasma protein. For surimi; lane 1 and 6, protein marker; lane 2-5, 20 and 10 μg of protein from grade SA and A, respectively. For CPP; lane 1 and 5, protein marker, lane 2-4, 20, 10, 4 μg of protein from CPP.

Effect of CPP on textural properties of surimi

The breaking force and deformation of kamaboko gel from both grades of bigeye snapper surimi increased as the CPP concentration increased up to 0.5% ($P<0.05$) (Figure 2). From the result, addition of 0.5% CPP increased breaking force of kamaboko gel of surimi grade SA and grade A by 158.7 and 204.3%, respectively, compared with directly heated gel and by 23.6 and 63.3%, respectively, compared with kamaboko gel without CPP. Deformation of kamaboko gels of surimi grade SA and grade A increased by 38.6 and 67.5%, respectively, compared with directly heated gel and by 6.6 and 21.8%, respectively, compared with kamaboko gel without CPP. However, the decreases in breaking force and deformation were observed with the addition of CPP at a level higher than 0.5%. The higher decrease was generally found with the sample added with a higher amount of CPP.

When comparing breaking force and deformation between kamaboko gel and directly heated gel without CPP addition, it was found that breaking force of the former was 109.3 and 86.4% higher than the latter for surimi grade SA and A, respectively. Deformation of kamaboko gel of surimi grade SA and grade A was also higher than that of directly heated gel. This indicated the essential role of setting in gel strengthening. Endogenous TGase has been reported to induce the formation of ε-(γ-glutamyl) lysine linkage during setting (Wan *et al*., 1994; Tsukamasa *et al*., 2002).

For kamaboko gels, addition of CPP at an appropriate level resulted in the increased breaking force and deformation. Addition of 0.5% CPP could strengthen the gel matrix. This was possibly because CPP worked as a binder, which was able to interact with protein matrix. CPP-myofibrils interactions led to the strengthened gel network. The result was in accordance with Benjakul *et al*. (2001c) who reported that breaking force and deformation of surimi gel from bigeye snapper increased with an addition of 0.5% (w/w) PPP. Foegeding *et al*. (1986) found that myosin and fibrinogen interacted to form a gel, which was stronger than the gel produced by the individual proteins (fibrinogen or myosin) at the same heating rate. Moreover, active components in CPP were also presumed to contribute to the gel strength enhancement. Kang and Lanier (1999) concluded that 1.0% BPP resulted in enhanced gelation of Pacific whiting surimi by inhibition of fish protease and also by other gel-enhancing factors in the plasma. Seymour *et al*. (1997) reported that beef plasma has gel enhancing activity from both plasma TGase and α_2M. This contributed

to strengthening of the gel by myosin and/or fibrinogen cross-linking and could also reduce the availability of myosin as a substrate for protease action (Wan *et al.*, 1994). Nevertheless, the larger amount of CPP could inhibit the gel network formation due to the dilution of myofibrillar protein or interference of myofibrillar protein cross-linking. Visessanguan *et al.* (2000) also reported that at the higher concentration of PPP added, interfering effect with the formation of actomyosin gel was observed in lower gel modulus.

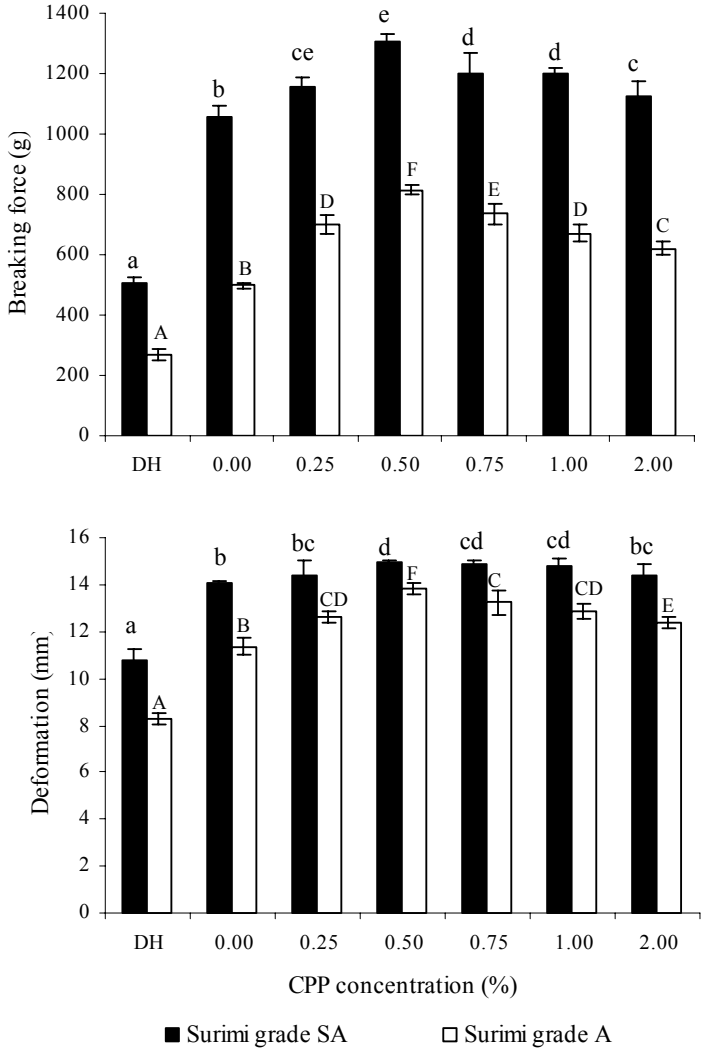

Figure 2. Breaking force and deformation of surimi gels added with various concentrations of chicken plasma protein. The surimi gels were prepared by incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min (kamaboko gel) or by direct heating at 90°C for 20 min (DH). Bars represent the standard deviation from five determinations. Different letters in the same grade surimi indicate significant differences ($P<0.05$).

Effect of CPP on whiteness and expressible drip of surimi gels

The whiteness of kamaboko gel decreased when CPP was added, especially when CPP concentration increased ($P<0.05$) (Figure 3). The whiteness of directly heated gel was higher than that of kamaboko gels ($P<0.05$). This was possibly because the reactions associated with discoloration occurred to a higher extent with the additional setting at 40°C for kamaboko gels. Some hemoglobin remained in plasma fraction as well as other pigments exhibited pale straw color. These pigments possibly reduced the whiteness of surimi gels. As a consequence, the decrease in whiteness was proportional to the amount of CPP added. With the addition of 0.5% CPP, whiteness of kamaboko gel of surimi grade SA and A decreased by 2.3 and 2.5%, respectively, compared to that of kamaboko gel without CPP. The result was in accordance with Benjakul *et al.* (2001c) who reported that the increased amount of PPP resulted in the decrease in whiteness of surimi gels.

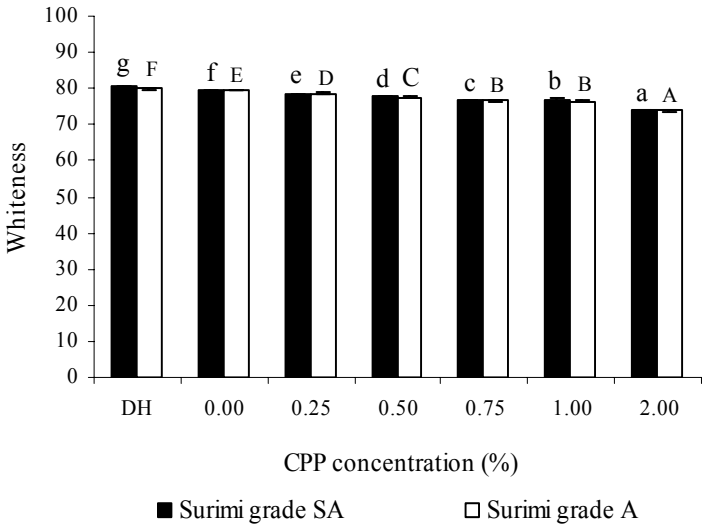

Figure 3. Whiteness of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein. Bars represent the standard deviation from five determinations. Different letters in the same grade surimi indicate significant differences ($P<0.05$).

The expressible drip of surimi gel from both grades decreased when CPP was added ($P<0.05$) (Figure 4). Expressible drip decreased to a higher extent as higher amount of CPP was added. This indicated that CPP in dried form was able to absorb water

effectively. As a result, more expressible drip was found in the gels without CPP. The expressible drip is indicative for the water binding properties. During thermal gelation, protein matrix was formed and water was imbibed regularly throughout the network. Though CPP did not enhance the gel strength when added at a level higher than 0.5%, the expressible drip decreased with an increasing amount of CPP. This suggested that a larger portion of water was absorbed into CPP, resulting in the lower amount of water in gel matrix. Thus, the balance between water-protein and protein-protein interaction was possibly changed, resulting in the inferior gel-forming ability.

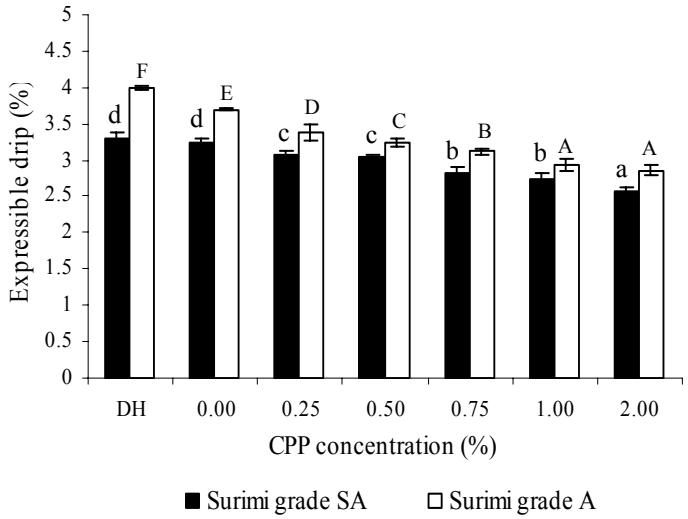

Figure 4. Expressible drip of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein. Bars represent the standard deviation from triplicate determinations. Different letters in the same grade surimi indicate significant differences ($P<0.05$).

Effect of CPP on protein solubility of surimi gels

No changes in solubility were observed in kamaboko gel added with CPP at a level up to 0.75% ($P<0.05$) (Table 8). Thereafter, increased solubility was observed, especially with increasing CPP concentrations. When comparing the solubility between directly heated gel and kamaboko gels, it was found that the former had higher solubility than the latter. This result indicated that setting at 40°C for 30 min, followed by 90°C for

20 min induced a large number of protein-protein interactions stabilized by non-disulfide. When CPP concentration increased from 0.75 to 2%, the solubility continuously increased. This was coincidental with the decrease in breaking force and deformation.

The result suggested that a large amount of CPP interfered the formation of non-disulfide covalent bond, possibly due to the dilution effect on myofibrillar proteins, which mainly contributed to gel matrix formation. Mixture containing sodium dodecyl sulfate, urea and βME were used to solubilize protein via destroying all bonds, except non-disulfide covalent bond (Roussel and Cheftel, 1990). Therefore, the low solubility indicated the presence of such a linkage, which was formed to a higher extent when CPP at an appropriate amount was added. From this result, non-disulfide covalent bond was presumed to be another contributor for strengthening the gel matrix, which was associated with the low solubility.

Table 8. Solubility of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein.

Samples	Solubility (%)	
	Surimi grade SA	Surimi grade A
Control (NaOH)	100	100
Direct heating	75.83±5.13*^{bc**}	79.93±3.42^{bc}
0% CPP	71.15±0.82^{ab}	71.92±1.78^{a}
0.25% CPP	70.92±0.39^{ab}	71.09±2.94^{a}
0.50% CPP	68.01±0.52^{a}	70.98±2.86^{a}
0.75% CPP	73.55±3.14^{ab}	75.65±0.61^{ab}
1% CPP	81.29±4.69^{c}	82.02±3.88^{cd}
2% CPP	82.60±6.65^{c}	85.89±2.88^{d}

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

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

Effect of CPP on TCA-soluble peptides of surimi gels

TCA-soluble peptides of different surimi gels are presented in Table 9. For kamaboko gels from both grades of surimi, TCA-soluble peptides decreased as CPP concentration increased ($P<0.05$). This result suggested that CPP had inhibitory activity towards degradation of protein. BPP consists of α_2M known as a proteinase inhibitor, which exhibit the most effective inhibitory activity toward all proteinases (Seymour *et al.*, 1997). The degradation occurred during heat-induced gelation is considered to result from the action of indigenous proteases (An *et al.*, 1996).

Table 9. TCA-soluble peptide of surimi gel from bigeye snapper added with various concentrations of chicken plasma protein.

Samples	Tyrosine (μmole/g gel)	
	Surimi grade SA	Surimi grade A
Direct heating	0.13±0.03*[a**]	0.64±0.01[c]
0% CPP	0.29±0.00[e]	0.80±0.01[e]
0.25% CPP	0.26±0.03[d]	0.73±0.01[d]
0.50% CPP	0.25±0.01[cd]	0.65±0.01[c]
0.75% CPP	0.22±0.01[c]	0.64±0.00[c]
1% CPP	0.16±0.02[b]	0.60±0.01[b]
2% CPP	0.13±0.02[a]	0.47±0.01[a]

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

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

When comparing the degradation between directly heated gel and kamaboko gels, the former contained the lower TCA-soluble peptides than the latter. Degradation probably occurred during setting at high temperature (40°C), which was close to the temperature of gel weakening (50-60°C) (Kang and Lanier, 2000). From the result, CPP effectively reduced the degradation during setting in kamaboko gels as observed by the decrease in TCA-soluble peptides in kamaboko gels added with a high amount of CPP.

Between two grades surimi tested, directly heated gel and kamaboko gels of surimi grade A contained higher amount of TCA-soluble peptides than surimi grade SA. This indicated that myofibrillar proteins of surimi grade A were more prone to proteolytic degradation than surimi grade SA. Generally, denatured proteins are preferable substrate for proteolysis. As a result, proteins in surimi grade A with less integrity were possibly degraded more easily than those in surimi grade SA.

Effect of CPP on protein pattern of surimi gels

Protein patterns of different surimi gels from both grades are depicted in Figure 5. MHC was more retained as the concentration of CPP increased. Among all samples tested, kamaboko gel without CPP (lane 4) showed the lowest MHC intensity. However, no changes in actin and tropomyosin were observed in all samples. Without CPP added, it was postulated that polymerization of myosin was formed during setting. However, myosin polymerization was decreased in the presence of CPP. This was probably due to the interfering effect of CPP on MHC cross-linking. The result was coincidental with the decreased gel strength when CPP at a higher amount added.

From SDS-PAGE, no marked degraded protein bands were observed, though some degradation was monitored by increased TCA-soluble peptides. Therefore, it was presumed that degradation was not a major cause of decrease in MHC. Conversely, polymerization was suggested to cause such a decrease. From the result, polymerization of MHC was negatively affected by CPP addition. At a level of 0.5% CPP, which rendered the highest breaking force and deformation, the lowest MHC intensity was not observed. Therefore, the increase in gel strength and elasticity was possibly caused by the filler or binder effect of CPP with myofibrillar protein in association with proteinase inhibitory effect, instead of enhancing of polymerization.

SDS-PAGE result was in accordance with the solubility studies (Table 2). No changes in solubility were observed with the addition of CPP in the range of 0.25-0.75%. However, a significant increase in solubility was noted when CPP at levels of 1-2% was added, which was incoincidental with the less polymerization as shown by the higher MHC retained.

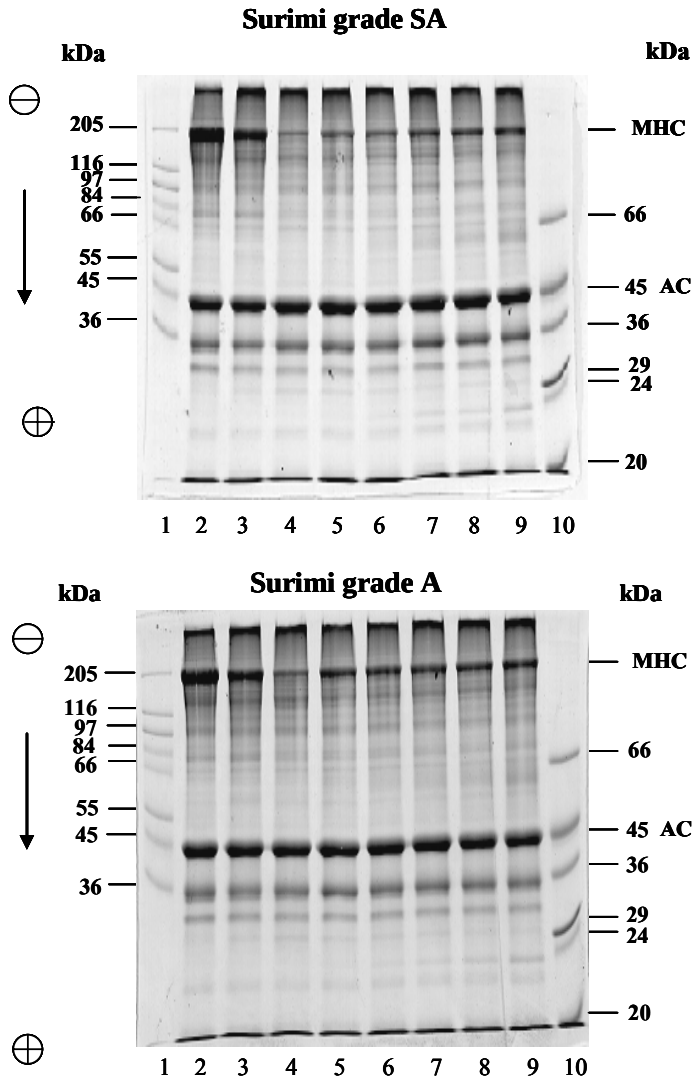

Figure 5. SDS-PAGE protein pattern of surimi gels from bigeye snapper added with various concentrations of chicken plasma protien. Lane1 and 10, protein marker; lane 2, bigeye snapper surimi sol without CPP; lane 3, directly heated gel without CPP; lane 4, kamaboko gel without CPP; lane 5-9, kamoboko gel added with 0.25, 0.5, 0.75, 1 and 2% CPP, respectively.

Effect of CPP in microstructure of surimi gels

The microstructure of bigeye snapper surimi gels prepared with different conditions including directly heated gel, kamaboko gel without and with 0.5% CPP were visualized by scanning electron microscopy as shown in Figure 6.

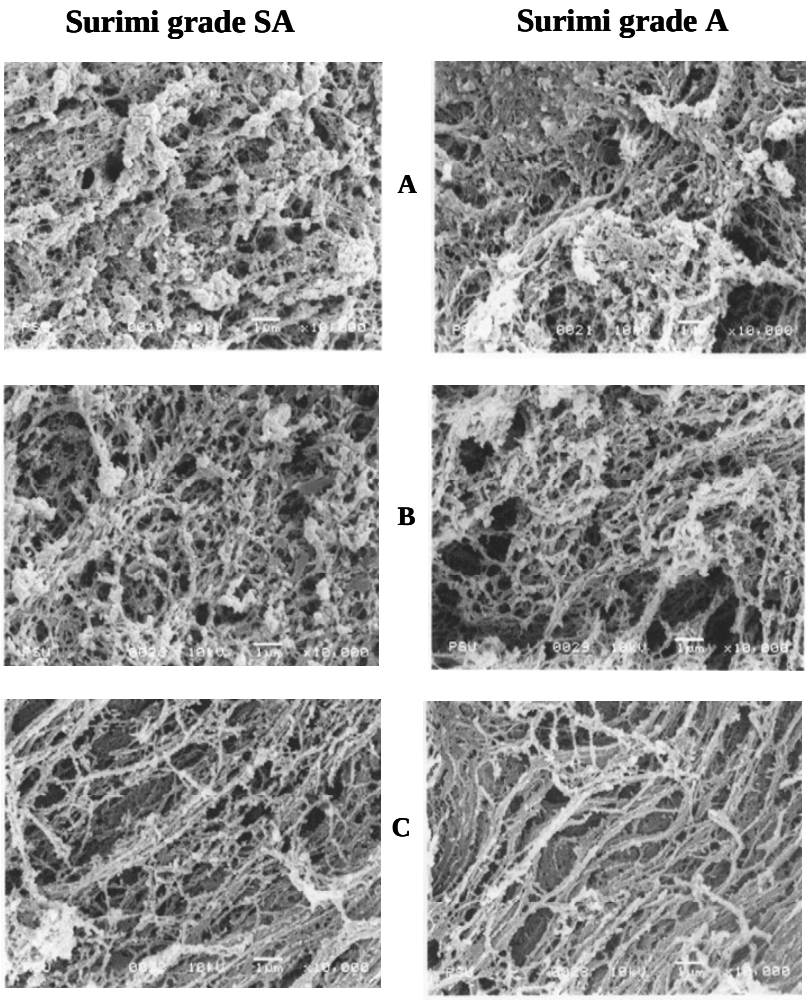

Figure 6. Electron microscopic image of surimi gels from bigeye snapper (Magnification: 10,000X.) A: directly heated gel; B: kamaboko gel; C: kamaboko gel added with 0.5% CPP.

Directly heated gel (Figure 6A) showed a regular structure formed by aggregates of densely packed spherical proteins, arranged in clusters. A coarse cross-link structure with more globular appearance on the surface was observed. Without prior setting, the ordered fibrillar structure was lacking, but some fibrillar structure and more globular aggregation were observed. The micrograph of kamaboko gel without CPP shows a well-structured matrix with a highly interconnected network of strands (Figure 6B). That may cause more resistance to applied stress and greater water-holding capacity. The kamaboko gel without CPP has the fine and ordered cross-link structure with a small globular

appearance on the surface. Consequently, it was suggested that this network structure might be formed through intermolecular ε-(γ-glutamyl) lysine cross-links induced by endogenous TGase as observed by the decrease in solubility.

The structure of kamaboko gel with 0.5% CPP seemed somewhat discontinuous with less linkage between protein strands (Figure 6C). The gel had the coarser fibrillar structure, compared with kamoboko gel without CPP addition. This might be the presence of CPP, non-muscle protein, leading to be less interconnected fibrils. However, fibers and fibers bundles network structure might be formed through the intermolecular ε-(γ-glutamyl) lysine crosslinks induced by plasma TGase in co-operation with endogenous TGase. The protein network of kamaboko gels without CPP seems to be more compact, with smaller clusters and more regular pores as compared with the microstructure of kamaboko with 0.5% CPP. These observations suggested that CPP impeded the formation of an ordered structure, though an increase in gel the breaking force and deformation was obtained.

2.5 Conclusion

Chicken plasma protein at a level of 0.5% in combination with setting at 40°C increased the breaking force and deformation of gels from both grade SA and grade A bigeye snapper surimi. However, CPP addition resulted in the decrease in whiteness. CPP mainly exhibited the proteinase inhibitory activity as well as the filler effect on surimi gel strengthening.