CHAPTER 6

COMPARATIVE STUDY OF CHICKEN PLASMA PROTEIN AND SOME PROTEIN ADDITIVES ON PROTEOLYSIS AND GEL-FORMING ABILITY OF SARDINE (*SARDINELLA GIBBOSA*) SURIMI

6.1 Abstract

Effect of chicken plasma protein (CPP) and various protein additives including beef plasma protein (BPP), porcine plasma protein (PPP), egg white (EW) and soy protein isolate (SPI) on autolysis and gel-forming ability of sardine (*Sardinella gibbosa*) surimi was investigated. CPP and other protein additives showed the inhibitory activity toward autolysis of sardine surimi incubated at 70°C in a concentration-dependent manner. PPP and EW were more effective in proteolysis prevention than other protein additives as shown by greater percent inhibition ($P<0.05$) and more retained myosin heavy chain (MHC). Breaking force and deformation of both modori and kamaboko gels increased when CPP and other protein additives were added at levels up to 2% ($P<0.05$). Nevertheless, PPP and EW showed a higher gel strengthening effect than CPP and other protein additives ($P<0.05$). Addition of CPP and other plasma proteins resulted in decreased whiteness, especially when the concentration increased ($P<0.05$). However, no change in whiteness was observed with gels containing EW and SPI ($P>0.05$). Therefore, proteolysis of sardine surimi, associated with endogenous proteases, can be retarded by the addition of CPP and protein additives, leading to the increased gel-forming ability.

6.2 Introduction

Gel-forming ability of frozen surimi is the most important functional requirement imposing good quality on surimi-based products and it depends on both intrinsic and extrinsic factors (Benjakul *et al*., 2003b, c). Proteolytic disintegration of myofibrillar proteins has an adverse effect on gel-forming properties of surimi (An *et al*., 1996). The breakdown of myofibrillar proteins inhibits the development of three-dimensional gel network (Morrissey *et al*., 1993). In general, weakening of surimi gels

occurs at temperature ranges of 50-70°C. This phenomenon, so-called modori, is induced by endogenous heat activated proteases, which can degrade myosin (Jiang, 2000). Gel softening varies with species, but is generally caused by two major groups of protease, cathepsins and heat stable alkaline proteases (An *et al*., 1996). To alleviate the soft texture problem caused by endogenous proteases in surimi-based foods, some additives containing protease inhibitors such as beef plasma protein (BPP), egg white (EW) and whey proteins are commonly used (Akazawa *et al*., 1993; Morrissey *et al*., 1993; Reppond and Babbitt, 1993; Benjakul *et al*., 2004b). Benjakul *et al*. (2003c) also reported that proteolysis of lizardfish muscle or surimi can be retarded by the addition of BPP or EW, leading to the increased gel strength. Non-muscle proteins (e.g. sodium caseinate, wheat gluten and soy protein isolate (SPI)) and hydrocolloids (e.g. iota-carageenan, waxy corn starch) have also been used to improve the gel-forming capacity of sardine surimi (Gomez-Guillen and Montero, 1996; Gomez-Guillen *et al*., 1996, 1997; Alvarez *et al*., 1997). Wasson *et al*. (1992b) reported that plasma and EW were able to decrease gel strength value when added at levels higher than necessary to prevent proteolytic activity. Mackerel surimi gel increased to 2-fold and 1.7-fold in breaking force and deformation, respectively with addition of 1% porcine plasma protein (PPP) (Lee *et al*., 2000b). Apart from the main functions as protease inhibitor, enhancement of gel network development by cross-linking enzymes in plasma has been reported (Jiang and Lee, 1992; Benjakul *et al*., 2001a).

Sardine, a pelagic, dark muscle fish species, currently makes up 40-50% of the total fish catch in the world (Hultin and Kelleher, 2000). Sardine is a species particularly prone to gel softening at temperature of 60°C (Tsukamasa and Shimizu, 1989; Alvarez *et al*., 1999). A high proteolytic activity, sarcoplasmic proteins, and fat content, resulted in the poorer gelation characteristic and the high susceptibility to modori (Shimizu *et al*., 1981). Shimizu *et al*. (1992) reported that the poor gel-forming properties of muscle from dark-fleshed species is caused by the presence of heat-stable proteases, which are active in degrading myosin during heating at temperature range of 50-70°C. Leinot and Cheftel (1990) also reported that gelation of sardine mince sometimes present problems owing to intrinsic characteristics of the muscle. From our previous study, chicken plasma protein (CPP) was able to enhance the gel strength by acting as a filler and also protease inhibitor in surimi gels from bigeye snapper and lizardfish (Rawdkuen, *et al*., 2004a, b). Therefore, the addition of CPP or other protein additives possessing the protease inhibitory activity should pave the way for gel improvement of sardine surimi. The

objective of this study was to study the effect of CPP and some protein additives on autolysis and gel-forming ability of sardine (*Sardinella gibbosa*) surimi.

6.3 Materials and Methods

Chemicals and Surimi

Trisodium citrate and sodium chloride were purchased from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) was obtained from Fluka Chemika-BioChemika (Buchs, Switzerland). L-tyrosine was 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). BPP, SPI and EW were obtained from Food E.Q. Co., Ltd. (Bangkok, Thailand). Frozen surimi from sardine grade A (breaking force of 400-500 g; deformation of 8-10 mm) was purchased from Man A Frozen Food Co, Ltd. (Songkhla, Thailand).

Preparation of chicken plasma protein and porcine plasma protein

Chicken and porcine bloods were 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 to remove red blood cells using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The supernatant was then freeze-dried and kept at -18°C until used.

Autolysis study of sardine surimi

Autolytic activity assay was performed according to the method of Morrissey *et al*. (1993). Sardine surimi (3 g) was incubated at 50, 55, 60, 65, 70, 75 and 80°C for 10, 30 and 60 min. The reaction was terminated by addition of 27 ml of cold 5% (w/v) trichloroacetic acid (TCA). The concentration of the soluble peptides

released was measured using the Lowry method (Lowry *et al.*, 1951). The condition exhibiting the highest autolytic activity was chosen for further study.

Effect of CPP and protein additives on autolytic activity of sardine surimi

Sardine surimi (3 g) was mixed with CPP or other protein additives including BPP, PPP, EW and SPI at levels of 1, 2 and 3% (w/w). The mixture was mixed thoroughly on ice for 2 min. Samples with and without protein additives were then incubated at 70°C for 60 min. TCA-soluble peptides and autolytic patterns of protein were determined. The inhibitory effect of CPP and other protein additives was expressed as % inhibition (Morrissey *et al.*, 1993) as follows:

$$\% \text{ Inhibition} = \frac{(A - B)}{A} \times 100$$

; Where A is tyrosine content in sample without protein additive.

B is tyrosine content in sample with protein additive.

Effect of CPP and protein additives on gel-forming ability of sardine surimi

Surimi gel preparation

Frozen surimi was partially thawed at 4°C for 2-3 h, cut into small pieces and chopped by a Moulinex Masterchef 350 mixer (Paris, France) for 4 min with 2.5% (w/w) NaCl. CPP and protein additives (BPP, PPP, EW and SPI) at different levels (1, 2 and 3%, w/w) were 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 paste was stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends were sealed tightly. The paste was incubated at 70°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". The kamaboko gel was prepared by incubating the surimi paste at 40°C for 30 min, followed by heating at 90°C for 20 min. A directly cooked gel was prepared by heating the surimi paste 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 diameter) were cut into the length of 2.5 cm. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyzer according to the method of Benjakul *et al*. (2003b). A spherical probe with a diameter of 5 mm was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed (60 mm/min) until puncture occurs. The force to puncture into the surimi gel (breaking force) and the distance at which the ball probe punctured into the surimi (breaking distance or deformation) were both recorded.

Determination of whiteness

Three samples from each treatment were subjected to whiteness measurement using a JP7100F colorimeter equipped with halogen lamp (Juki Corp, Tokyo, Japan). White standard plate was used for calibration prior to the measurement. 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 moisture

Expressible moisture was measured according to the method of Ng (1987). Three 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 (5 kg) was placed on the top of the sample for 2 min, and then the sample was removed from the papers and weighed again (Y). Expressible drip was calculated and expressed as percentage of sample weight as follow:

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

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 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 the Lowry method (Lowry *et al*., 1951) and expressed as μmole tyrosine/g sample.

SDS-Polyacrylamide gel electrophoresis

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 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. Quantitative analysis was performed using a Model GS-700 Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA, USA) with Molecular Analyst Software version 1.4 (Image Analysis Systems). The amount of myosin heavy chain (MHC) and actin in each sample was expressed relative to that of the control sample.

Statistical analysis

Completely randomized design and one-way analysis of variance (ANOVA) were used. Data obtained were subjected to statistical analysis using the SPSS

program for windows (SPSS version 10.0, SPSS Inc, Chicago, IL). Duncan's multiple-range test was used to compare the difference between means. The accepted level of significance for all comparisons was $P<0.05$. Experiments were conducted in duplicate and the analysis was run in three or five replications.

6.4 Results and Discussion

Autolysis study of sardine surimi

Autolytic activity of sardine surimi at different temperatures and times is shown in Figure 28. For all incubation time used, TCA-soluble peptides in sardine surimi increased as temperature increased and reached the maximum at 70°C. Subsequently, TCA-soluble peptides markedly decreased when incubated at 80°C. It was postulated that proteases were denatured at high temperature and lost their activity.

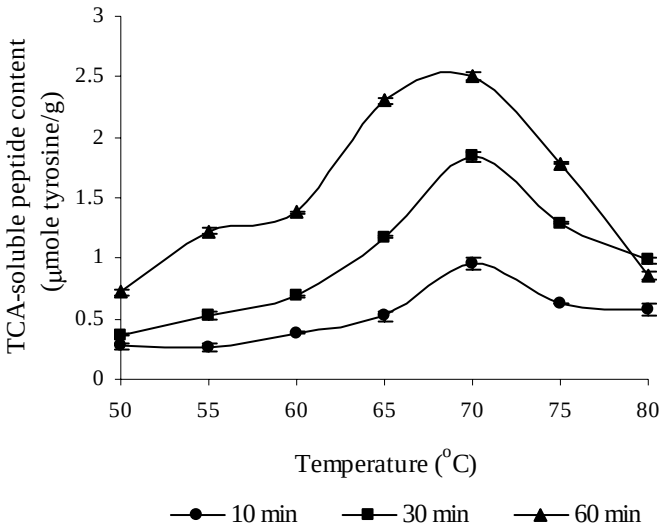

Figure 28. TCA-soluble peptide content in sardine surimi incubated at different temperatures and times. Bars represent the standard deviation from triplicate determinations.

Generally, greater TCA-soluble peptide content was observed with increasing incubation time, indicating more protein degradation. However, Benjakul *et al.* (2003d) reported that the highest autolysis of lizardfish mince and washed mince was

observed at 65 and 60°C, respectively. Modori phenomenon of sardine (*Sardinop melanosticta*) surimi occurred when the gels were incubated at temperatures in the region of 60°C (Tsukamasa and Shimizu, 1989). The differences in autolysis profile of surimi from different species might be due to the different types and amount of proteases present in surimi. The myofibril-associated proteases show a detrimental effect on gel-forming ability since they are still retained after washing process (Cao *et al.*, 1999). Those proteases played an essential role in protein degradation, particularly at elevated temperatures and were responsible for the softening of surimi gel from oval-filefish (Toyohara *et al.*, 1990b).

Effect of CPP on autolysis of sardine surimi

CPP and other protein additives showed inhibitory activity differently towards autolysis of sardine surimi incubated at 70°C (Figure 29). In general, higher inhibition was observed as the concentration of protein additives increased ($P<0.05$). At the same level tested, PPP showed the highest inhibitory activity on autolysis compared with other protein additives ($P<0.05$). Among all plasma proteins, CPP exhibited the lower inhibitory activity than PPP and BPP. At a level of 3% (w/w), inhibition of 69, 66 and 60% was observed for sardine surimi added with PPP, BPP and CPP, respectively ($P<0.05$). SPI had the lowest inhibitory activity at all levels tested ($P<0.05$). This result was in accordance with Benjakul and Visessanguan (2000) who reported that PPP showed higher inhibitory activity than BPP or EW against Pacific whiting protease. The ability of PPP to inhibit proteases increased proportionally with the increase in concentration (Benjakul *et al.*, 2001a, c). Visessanguan *et al.* (2000) also reported that PPP was effective in protecting the MHC of Pacific whiting natural actomyosin from proteolytic degradation. Whole plasma powder was a more effective inhibitor of proteolysis than whey protein concentrate or potato powder (Akazawa *et al.*, 1993). In general, mammalian plasma consisted of α_2-macroglobulin (α_2M) and kininogen, that none specifically traps all type of proteases and cysteine protease, respectively (Hamann *et al.*, 1990; Garcia-Carreno, 1996). Rawdkuen *et al.* (2004b) reported that autolysis in both mince and washed mince from bigeye snapper was reduced by addition of CPP, especially with increasing CPP concentration. Chicken blood plasma contains ornitho-kininogen, which has similar properties to mammalian high-molecular-weight kininogen (Kimura *et al.*, 1987).

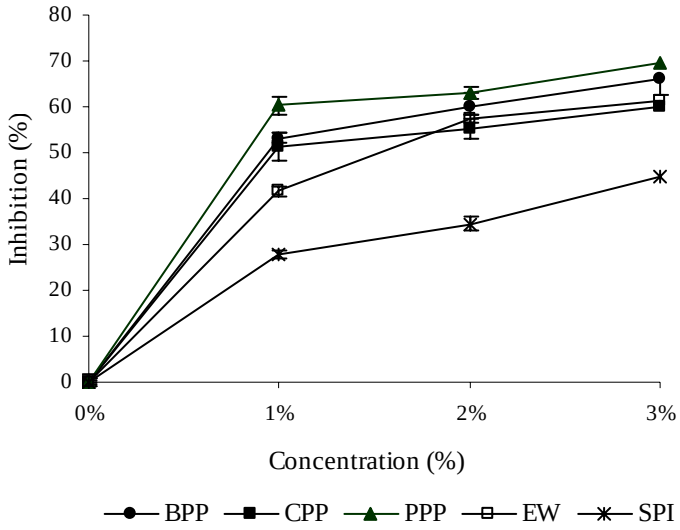

Figure 29. Effect of CPP or other protein additives at different concentrations on inhibition of autolysis in sardine surimi at 70°C for 60 min. Bars represent the standard deviation from triplicate determinations.

Kos *et al.* (1992) reported that high-molecular-weight kininogen was present in chicken plasma and strongly inhibited chicken cathepsin L and papain, but was a much weaker inhibitor of chicken cathepsin B. EW showed inhibitory activity due to the presence of some protease inhibitors, such as cystatin, ovoinhibitor and ovomacroglobin, which are specific to cysteine protease, serine protease and aspartic protease, respectively (Garcia-Carreno and Hermandez-Cortes, 2000). Garcia-Carreon (1996) also reported that EW contains specific competitive inhibitor namely ovomucoid.

Autolytic patterns of sardine surimi incubated at 70°C for 60 min in the absence and presence of CPP and other protein additives at different levels are shown in Figure 30. Myosin heavy chain (MHC) degradation was markedly observed in the sample without the addition of CPP or other protein additives (lane 2) as indicated by the low MHC band intensity retained (14.9% of that of starting surimi) (lane1). The result indicated that MHC in sardine surimi was prone to proteolysis at high temperature. It has been reported that MHC, β-tropomyosin and troponin-T were more susceptible to degradation than actin (An *et al.*, 1994b). Benjakul *et al.* (2003d) reported that degradation of muscle proteins, especially MHC, in lizardfish mince and washed mince was found at temperatures ranging from 60 to 65°C. Myofibril-associated serine protease with

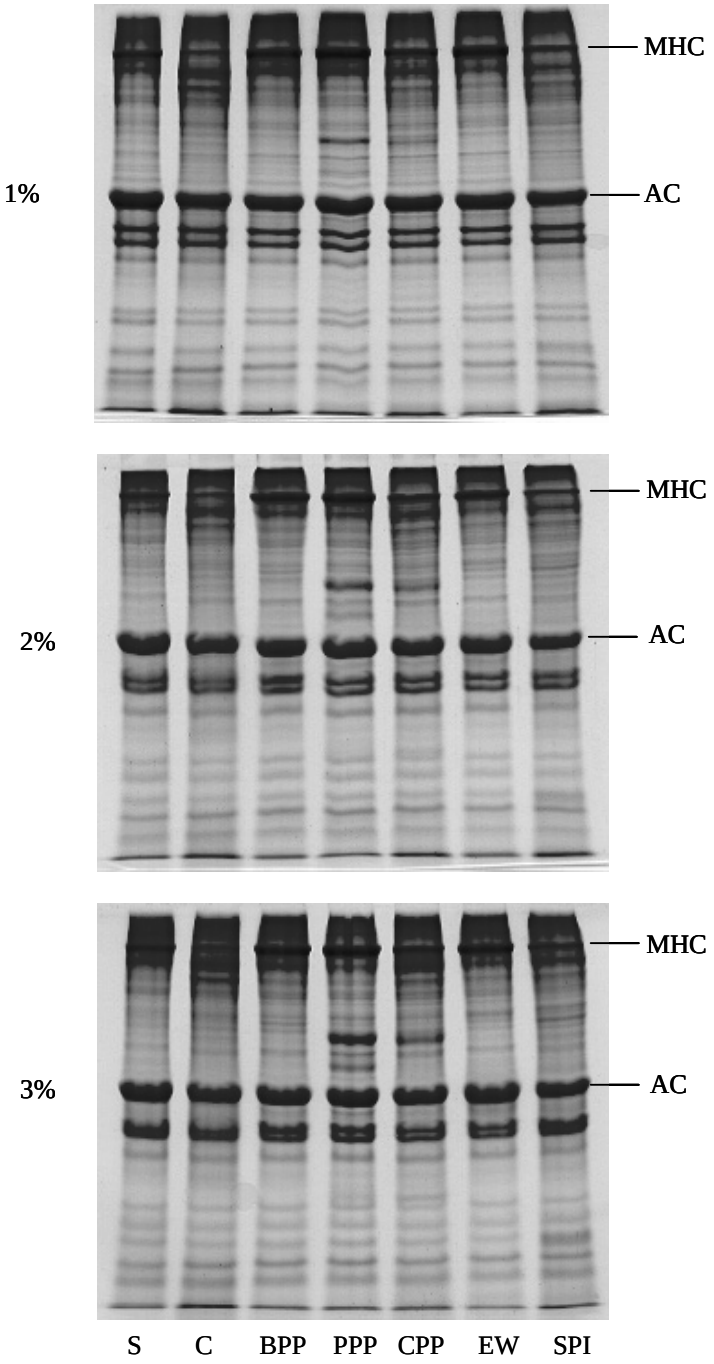

Figure 30. Autolysis pattern of sardine surimi added with CPP or other protein additives at different concentrations. Samples were incubated at 70°C for 60 min. S: sol; C: surimi without protein additives; BPP: surimi with beef plasma protein; CPP: surimi with chicken plasma protein; PPP: surimi with porcine plasma protein; EW: surimi with egg white; SPI: surimi with soy protein isolate. MHC: myosin heavy chain, AC: actin.

a molecular weight of 60 kDa hydrolyzed MHC in lizardfish muscle at 55-65°C (Cao *et al.*, 2000). From the result, efficacy of autolysis inhibition varied, depending on protein additives used. At all levels of protein additives tested, MHC of surimi tended to have the greatest intensity with addition of 3% PPP. The amount of MHC was 334.2% relative to that of the control (without protein additives), suggesting the high effectiveness in proteolysis inhibition. The result was in agreement with the highest inhibitory activity (Figure 29). CPP showed the lower inhibition toward autolysis, compared with BPP and EW. The lowest MHC band intensity was observed with sample added with SPI compared with that found in surimi added with other protein additives.

The amount of MHC observed in sample added with 1% SPI was 4.91% relative to that of the control (without protein additive). The result revealed that PPP, BPP, EW and also CPP functioned as effective inhibitors in sardine surimi. For the same protein additives tested, the relative amount of MHC slightly increased as the level added increased from 1 to 3% (data not shown). The amount of MHC increased from 69.55% to 123.12% relative to that of the control (without protein additive) when CPP amount increased from 1% to 3%. Therefore, CPP could retard the autolysis of sardine surimi, leading to the more retained MHC which has been shown to contribute to gelation of surimi.

Effect of CPP on textural properties of sardine surimi gels

Breaking force and deformation of sardine surimi gels added with CPP or other protein additives at different levels are shown in Figures 31-32. The type of protein and concentration used affected both breaking force and deformation of surimi gel. For modori gels (Figure 31), the gel added with CPP showed the higher breaking force and deformation, compared with the control gel (without protein additives) ($P<0.05$). Addition of CPP up to 2% resulted in the increase in breaking force, however CPP at a level of 3% caused a slight decrease in breaking force and deformation ($P<0.05$). CPP retarded the degradation of proteins in modori gel caused by heat-activated proteases (Figure 29).

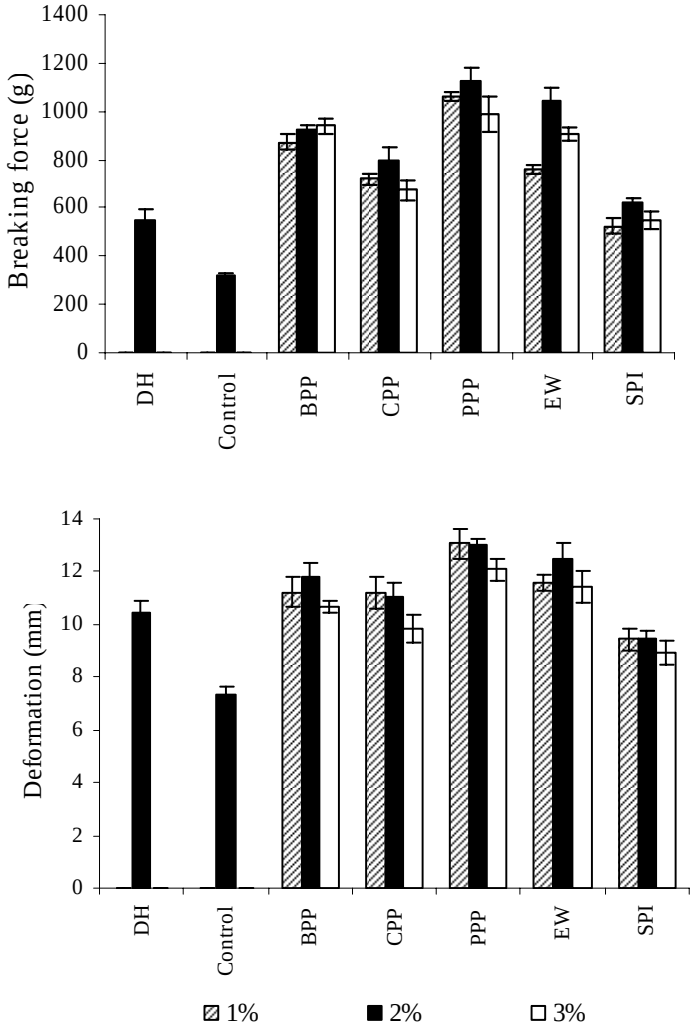

Figure 31. Breaking force and deformation of sardine modori gels added with CPP or other protein additives at different concentrations. Bars represent the standard deviation from five determinations.

Although CPP could increase the breaking force and deformation of surimi gel, PPP, BPP and EW rendered the higher efficiency in enhancing gel strength. Similar to CPP, all protein additives showed the highest enhancing effect of modori gel at 2% (w/w) ($P<0.05$). CPP was reported to consist of ornitho-kininogen, protease inhibitor for cathepsin L and papain (Kimura *et al.*, 1987; Kos *et al.*, 1992). PPP contained cysteine and serine protease inhibitor with MW of 60-63 kDa (Benjakul and Visessanguan, 2000). Lee *et al.* (2000a) also reported the presence of kininogen in PPP. From the result, modori gel with SPI showed the lowest breaking force and deformation compared to

those with other protein additives. This result was in accordance with the lowest inhibitory activity towards autolysis of sardine surimi (Figure 29). Chang-Lee *et al*. (1990) reported that varying concentrations of SPI (1 to 5%) in batters did not affect gel hardness produced from Pacific whiting. The slight increases in breaking force and deformation of modori gel with SPI were possibly due to the simple filler effect or forming interpenetrating network of non-muscle protein (Ziegler and Foegeding, 1991). Soy proteins could be formulated into food binders (Meyer and Williams, 1976) and they have less adhesive strength in the processing of restructure meat, compared to myofibrillar proteins, gluten, blood plasma and egg white albumin (Lu and Chen, 1999). At a level of 3%, samples with all protein additives except BPP had the lowered breaking force and deformation ($P<0.05$). This was probably because these non-muscle proteins interfered with gel formation by preventing actomyosin cross-linking (Chung and Lee, 1991). Burgarella *et al*. (1985) suggested that loss of gel strength with non-muscle protein additives could be due to "dilution" of myofibrillar protein. At a level of 2%, the breaking force of modori gel added with PPP, EW, BPP, CPP and SPI increased by 254, 229, 191, 151 and 96%, respectively, and deformation increased by 77, 71, 61, 50 and 29%, respectively, compared with the control. From these results, breaking force and deformation of the control gels were much lower than those of directly heated gel. Direct heating at 90°C shortened the time surimi sol was exposed to the temperature suitable for heat-activated proteolysis (modori). On the other hand, the incubation at 70°C prior to heating at 90°C maximized the activity of heat-activated proteases, resulting in the greater degradation of muscle protein. This was associated with the lower gel strength in the control gel.

For kamaboko gels (Figure 32), similar results were observed, compared with those of modori gels. However, greater breaking force and deformation in all samples were observed with kamaboko gels, compared with modori gels ($P<0.05$). The higher breaking force and deformation were observed when CPP or other protein additives were added. At a level of 2% (w/w), all samples had the highest breaking force and deformation, except those added with BPP ($P<0.05$). Addition of 2% CPP resulted in an increase in breaking force and deformation of 66 and 16%, respectively, compared with the control kamaboko gel. At a level of 2%, the breaking force of kamaboko gel with PPP, EW, BPP and SPI increased by 98, 94, 76, and 61%, respectively, and deformation increased by 22, 17, 13, and 17%, respectively, compared with the control.

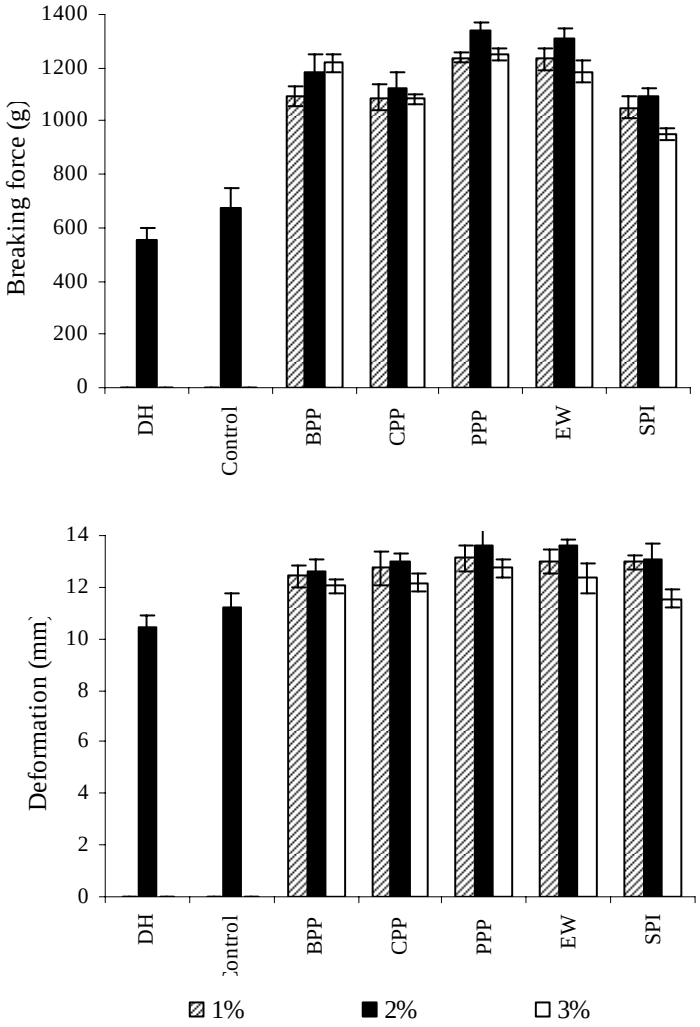

Figure 32. Breaking force and deformation of sardine kamaboko gels added with CPP or other protein additives at different concentrations. Bars represent the standard deviation from five determinations.

PPP still showed the highest efficiency in increasing breaking force and deformation when compared with other additives ($P<0.05$). Protein additives, which have gelling ability, might form interpenetrating networks and a co-gelling role has even been postulated between the fibrinogen in blood plasma or certain fractions of soy proteins and myofibrillar proteins (Lanier, 1990). Kamaboko gel without additives (control) showed the higher breaking force and deformation than directly heated gel. This result was in according with Niwa (1985) who reported that the quality of directly cooked gels was poorer than those

with prior setting. Setting has been reported to play an important role in gel strengthening via the induction of non-disulfide covalent bond (Benjakul and Visessanguan, 2003). Transglutaminase (TGase) has been known to play an important role in setting of surimi, resulting in the improved gel quality (Benjakul *et al*., 2003b). Markedly increased breaking force and deformation were found in kamaboko gel with SPI, when compared with modori gels with SPI. Although SPI had low inhibitory activity against gel softening (Figure 29), but it might act as a co-gelling agent or filler. This led to the increased gel strength. Park (2000) reported that conglycinin and glycinin play a major role in the gel formation of soy proteins, which form aggregates or gels at 85°C in the presence of salt (0.2 M). Kang and Lanier (1999) concluded that plasma contributed to the enhanced gelation of Pacific whiting surimi by inhibition of fish protease and also by other gel-enhancing factors in the plasma. No significant differences in deformation of the samples with BPP, SPI, CPP and EW were observed ($P>0.05$). These results revealed that CPP and protein additives were effective in improving the hardness of kamaboko gel, but not cohesiveness.

Effect of CPP on whiteness and expressible moisture of sardine surimi gel.

The whiteness of modori and kamaboko gels with CPP at different levels is shown in Table 15. The whiteness of modori gels with CPP and other plasma proteins (BPP, PPP) decreased to some extent compared with the control gel ($P<0.05$). No changes in whiteness of gel samples added with EW and SPI were observed compared with the control gel ($P>0.05$). The increasing concentration of plasma protein resulted in decreased whiteness, whereas increasing EW and SPI concentration slightly increased whiteness. Wasson *et al*. (1992b) reported that a noticeable increase in off-white tones of arrowtooth flounder cooked gels resulted from the addition of plasma powder and egg white. In this study, a slightly lower whiteness value was observed in kamaboko gels when compared with modori gels. The whiteness of directly heated gel was higher than that of kamaboko gel or modori gel. Benjakul *et al*. (2001c) found that the lower whiteness of gel was observed with plasma protein addition, because of some hemoglobin as well as other pigments with a pale straw color were retained in the plasma.

Table 15. Effect of CPP on whiteness and expressible moisture of modori and kamaboko gel of sardine surimi

Sample		Whiteness		Expressible moisture (%)	
		Modori gel	Kamaboko gel	Modori gel	Kamaboko gel
DH		67.9±0.8*h**	67.9±0.8f	3.7±0.1efg	3.7±0.1de
Control		67.5±0.8gh	66.5±0.2cde	4.1±0.2i	3.6±0.04de
CPP	1%	65.7±0.1cd	64.0±0.6b	3.8±0.01g	3.7±0.1de
	2%	63.9±0.3b	64.0±0.3b	3.7±0.02fg	3.6±0.1de
	3%	63.0±0.1a	62.4±0.9a	3.5±0.02d	3.5±0.1de
BPP	1%	66.4±0.2de	64.9±0.5b	3.7±0.1fg	3.7±0.02e
	2%	65.3±0.4c	64.7±0.5b	3.5±0.02d	3.7±0.1de
	3%	64.4±0.3b	64.4±0.5b	3.3±0.1bc	3.5±0.1cd
PPP	1%	66.9±0.2efg	66.3±0.2cde	3.2±0.2b	3.4±0.1bc
	2%	66.5±0.1ef	66.0±0.2cd	3.1±0.04b	3.3±0.1b
	3%	66.3±0.5de	65.8±0.5c	2.9±0.1a	3.0±0.1a
EW	1%	67.6±0.4h	66.2±0.2de	3.7±0.1fg	3.9±0.03f
	2%	67.8±0.6h	66.4±0.6cde	3.7±0.03fg	3.7±0.02e
	3%	67.9±0.1h	67.1±0.5ef	3.6±0.1def	3.6±0.1de
SPI	1%	66.8±0.5ef	66.5±0.3cde	4.0±0.02h	3.6±0.02de
	2%	67.2±0.5fgh	66.8±0.1de	3.5±0.1de	3.6±0.1de
	3%	67.6±0.1h	67.0±0.4ef	3.4±0.1cd	3.4±0.1b

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

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

The expressible moisture of kamaboko and modori gels added with CPP at different levels is shown in Table 15. The highest expressible moisture was found in modori gel without CPP or other protein additives ($P<0.05$), indicating a poor gel matrix with low water holding capacity. In the modori gel, the lowest expressible moisture was found in the sample with 3% CPP, compared with those added with 1 or 2% CPP

($P<0.05$). Addition of CPP or other protein additives resulted in lower expressible moisture in modori gel, but caused no marked change in kamaboko gel except those with PPP. The high water holding capacity of protein additives causes them to swell and augment elasticity by reducing the moisture content of the mixtures and increasing the density of surrounding protein matrix (Iso *et al*., 1985; Niwa *et al*., 1988). Gomez-Guillen and Montero (1996) also concluded that addition of hydrocolloids increased the water holding capacity considerably irrespective of the quality of the muscle protein. The expressible moisture of the control kamaboko gel was lower than the control modori gel. Thus, the kamaboko gel had higher water holding capacity than modori gel. However, expressible moisture of the modori gel was lowered with the addition of dried proteins, which were able to absorb and retain water effectively.

Effect of CPP on degradation of sardine surimi gel

TCA-soluble peptide content in both modori and kamaboko gels with different levels of CPP and other protein additives is shown in Figure 33. The TCA-soluble peptides of modori and kamaboko gels decreased as the concentration of CPP and other protein additives increased ($P<0.05$). A slight decrease of TCA-soluble peptide in all samples was observed with addition of CPP or other protein additives above 2%, except for modori gel added with 3% BPP. These results indicated that the addition of protein additives at levels higher than 2% might not be necessary for prevention of proteolysis. The highest TCA-soluble peptides in both modori and kamaboko gels were observed in the sample with SPI ($P<0.05$). The result was in agreement with the low inhibitory activity toward autolysis of SPI (Figure 29). For modori and kamaboko gels, TCA-soluble peptide content in the gels with CPP was similar to those found in gels with BPP, PPP and EW. Generally, TCA-soluble peptide content in the modori gel was higher than those of kamaboko gel. This suggests that greater degradation of surimi proteins occurs in modori gels as compared with kamaboko. However, it could be reduced by addition of CPP or other protein additives.

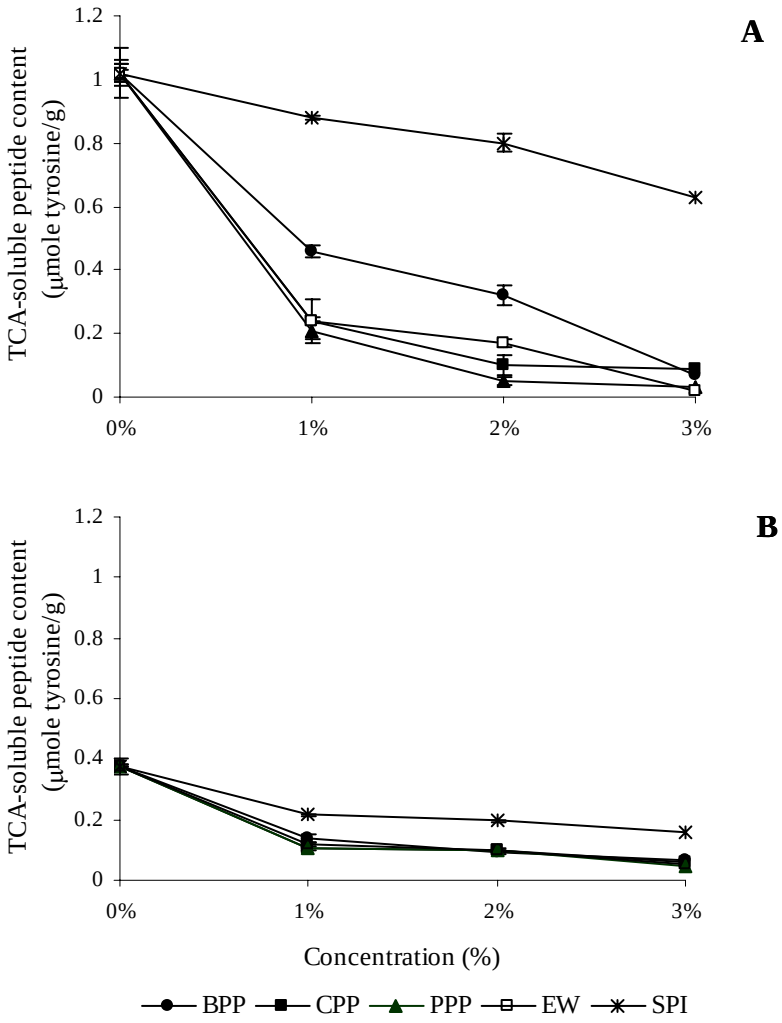

Figure 33. TCA-soluble peptide content in sardine modori (A) and kamaboko (B) gels added with CPP or other protein additives at different concentrations. Bars represent the standard deviation from triplicate determinations.

Effect of CPP on protein pattern of sardine surimi gel

The protein patterns of modori and kamaboko gels with CPP at 1, 2 and 3% in comparison with other protein additives are shown in Figure 34-35. The amount of MHC in the control modori gel (Figure 34: lane 2) decreased by 74.75% relative to that of the starting surimi (lane 1). Conversely, the increase in relative amount of MHC in all samples added with protein additives were observed as the concentration of protein additives

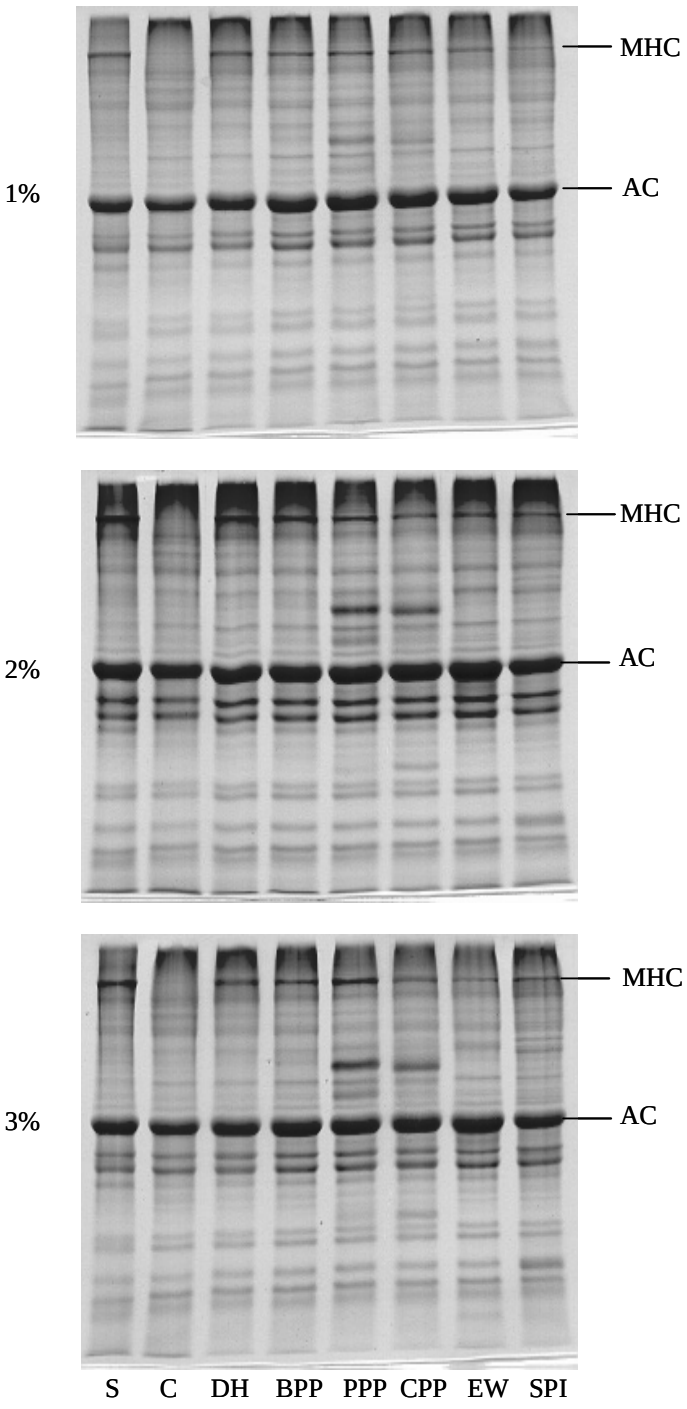

Figure 34. Protein pattern of modori gels added with CPP or other protein additives at different concentrations. S: sol; C: surimi without protein additives; DH: directly heated gel; BPP: gel with beef plasma protein; CPP: gel with chicken plasma protein; PPP: gel with porcine plasma protein; EW: gel with egg white; SPI: gel with soy protein isolate. MHC: myosin heavy chain, AC: actin.

increased. At the same level of additive, modori gels with CPP showed the lower relative amount of MHC (200.26%) than those added with BPP (214.68%) or PPP (235.49%). The result was in accordance with the lower breaking force, deformation and percent inhibition of autolysis of CPP, compared with BPP and PPP. The result was also in accordance with our previous work that showed CPP to have no cross-linking activity by TGase (data not shown), while BPP or PPP contained both protease inhibitor and cross-linking enzyme (Seymour *et al.*, 1997; Jiang and Lee, 1992). Remained MHC band of modori gel with CPP or other protein additives indicated that CPP or other proteins could prevent proteolysis in modori gel to some extent, while SPI had a lower inhibitory activity toward degradation of proteins in modori gel. Actin bands were clearly visible in all gel samples with CPP or protein additives. However, the amount of actin in modori gel without additive (lane 2) decreased by 21.16%, compared with that of the initial surimi. Actin could be a substrate for proteases in sardine surimi when no MHC was available.

For kamaboko gel (Figure 35), the amount of MHC in control kamaboko gel (lane 2) decreased about 79.24% compared with that of starting surimi (lane 1), while no changes in actin were observed. From the result, no differences in the amount of MHC were observed among all kamaboko gels with and without CPP or other protein additives (data not shown). Disappearance of MHC in kamaboko gels either with or without protein additives was most likely due to the polymerization, which possibly resulting from both endogenous and plasma TGase activity during setting process (Kurth, 1983). Careche *et al.* (1995) reported that endogenous TGase from sardine surimi had the optimum temperature at around 35-40°C. Montero and Gomez-Guillen (1996) found that kamaboko gel of sardine (preset at 35°C and then cooked at 90°C) had no MHC remaining as a consequence of setting, in which more covalent bonds could be formed. Thus, proteolysis of MHC could be retarded in modori gel in which the temperature was optimized for heat activated proteases. At setting temperature (40°C), proteolysis still occurred at a much lower extent, compared with 70°C. In presence of CPP or other protein additives, degradation of protein caused by less active proteases could be minimized effectively as evidenced by very low TCA-soluble peptides in kamaboko gels (Figure 33). Also, activity of endogenous TGase as well as plasma TGase could be maximized and the formation of non-disulfide covalent bond was induced as shown by the absence of MHC band in SDS-PAGE. Careche *et al.* (1995) reported that no MHC was observed in suwari gels heat-set at 35 and 40°C of sardine surimi gel.

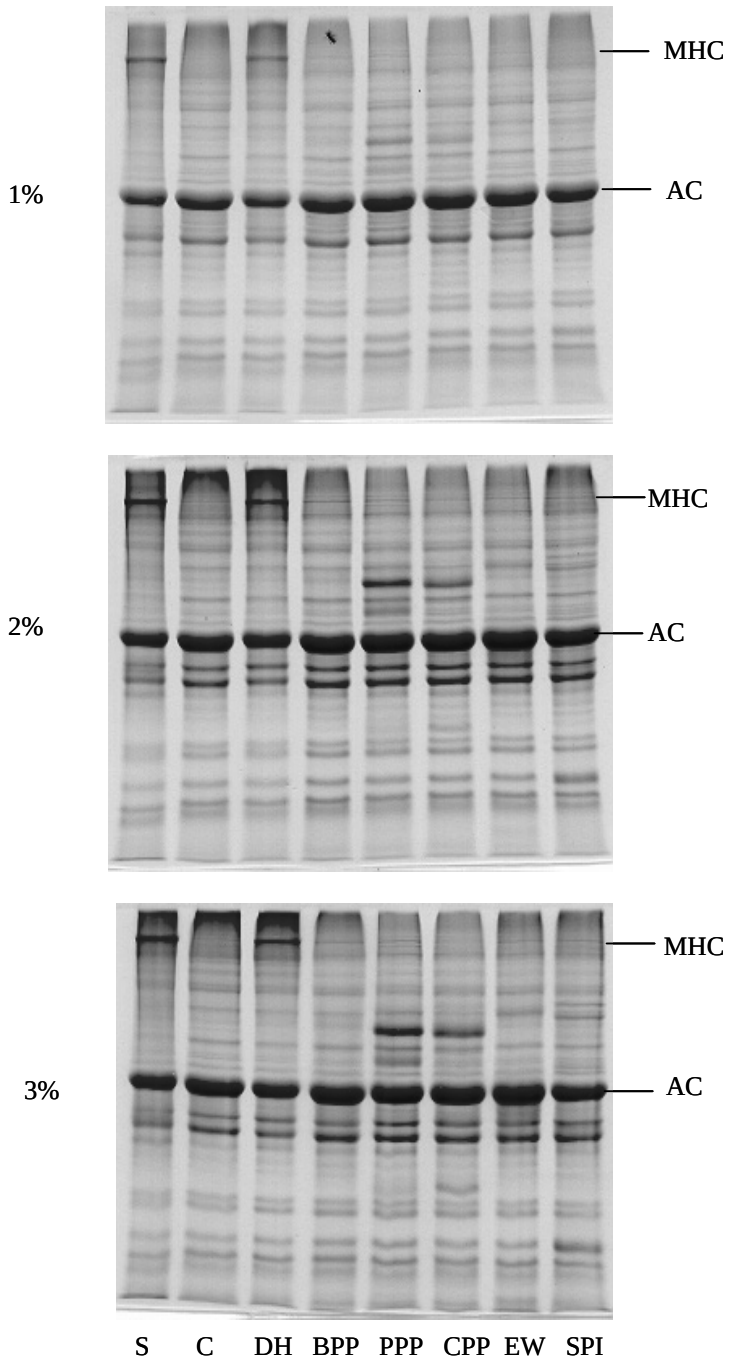

Figure 35. Protein pattern of kamaboko gels added with CPP or other protein additives at different concentrations. S: sol; C: surimi without protein additives; DH: directly heated gel; BPP: gel with beef plasma protein; CPP: gel with chicken plasma protein; PPP: gel with porcine plasma protein; EW: gel with egg white; SPI: gel with soy protein isolate. MHC: myosin heavy chain, AC: actin.

As a result, the higher gel strength was observed in kamaboko gel, especially those added with CPP or other protein additives, when compared with modori gel. Benjakul and Visessanguan (2003) reported the increased formation of non-disulfide covalent bonds, which coincided with increased gel strength and the decrease in MHC polypeptide in two species of bigeye snapper induced by endogenous TGase. The increased gel strength of surimi was associated with the increased cross-linking of MHC and ε-(γ-glutamyl)lysine isopeptide formed (Kumazawa *et al*., 1995).

6.5 Conclusion

Autolysis of sardine surimi caused by heat-activated proteases could be partially inhibited by addition of CPP. The addition of CPP up to 2% increased gelling properties of sardine surimi regardless of heating condition. However, CPP addition causes decreased whiteness, especially with increasing levels of protein.