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

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ABSTRACT

The effect of chicken plasma protein (CPP) and various protein additives on autolysis and gel-forming ability of sardine (Sardinella gibbosa) surimi was investigated. CPP and other protein additives showed inhibitory activity toward autolysis of sardine surimi incubated at 70°C in a concentration-dependent manner. Porcine plasma protein (PPP) and egg white (EW) were more effective in proteolysis prevention than CPP and other protein additives. 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 greater gel-strengthening effect than CPP and other protein additives (P < 0.05). Addition of CPP and other plasma proteins resulted in decreased whiteness, especially with increasing amount (P < 0.05). However, no change in whiteness was observed with gels containing EW and soy protein isolate (SPI) (P > 0.05). Proteolysis of sardine surimi can be retarded by the addition of CPP and protein additives, leading to increased gel-forming ability.

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**PRACTICAL APPLICATIONS**

Chicken plasma protein (CPP) at the appropriate amount could retard the autolysis of sardine surimi and increased the breaking force and deformation of the gel. Therefore, CPP could be used as the alternative protein additive in surimi, not only those from dark-fleshed fish but also from lean fish, commonly used in surimi industry. Additionally, the full utilization of chicken blood can be achieved and the value added product can be produced.

**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. 2003a,b). 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 a 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 proteases, 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. 2004). Benjakul et al. (2003b) also reported that proteolysis of lizardfish muscle or surimi can be retarded by the addition of BPP or EW, leading to increased gel strength. Nonmuscle proteins (e.g., sodium caseinate, wheat gluten and soy protein isolate [SPI]) and hydrocolloids (e.g., iota-carrageenan, waxy cornstarch) have also been used to improve the gel-forming capacity of sardine surimi (Gomez-Guillen et al. 1996, 1997; Gomez-Guillen and Montero 1996; Alvarez et al. 1997). Wasson et al. (1992) 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 2-fold and 1.7-fold in breaking force and deformation, respectively, with the addition of 1% porcine plasma protein (PPP) (Lee et al. 2000a). Apart from the main function 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 60°C (Tsukamasa and Shimizu 1989; Alvarez et al. 1999). The presence of high proteolytic activity, sarcoplasmic proteins and fat content resulted in poorer gelation characteristic and 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 are caused by the presence of heat-stable proteases, which are active in degrading myosin during heating at temperature range of 50–70°C. The differences in gel-forming ability between dark and ordinary muscles were apparently from the differences in the unfolding ability and thermal stability of myosin (Lo et al. 1991). From our previous study, chicken plasma protein (CPP) was able to enhance the gel strength by acting as 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.

MATERIALS AND METHODS

Chemicals and Surimi

Trisodium citrate and sodium chloride were purchased from Merck (Darmstadt, Germany). Bovine serum albumin was obtained from Fluka Chemika–BioChemika (Buchs, Switzerland). L-tyrosine was obtained from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate (SDS), \(N,N,N',N'-\text{tetramethyl ethylene diamine and Coomassie Blue R-250 were obtained from Bio-Rad Laboratories (Hercules, CA). BPP, SPI and EW were obtained from Food EQ Co., Ltd. (Bangkok, Thailand). Two different lots of frozen surimi from sardine grade A (breaking force of 400–500 g; deformation of 8–10 mm) were purchased from Man A Frozen Food Co., Ltd. (Songkhla, Thailand) and used as the composite samples.}

Preparation of CPP and PPP

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 the red blood cells using a Sorvall Model RC-B Plus centrifuge (Newtown, CT). 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 the 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:

\[
\text{% Inhibition} = \left(\frac{(A - B)}{A}\right) \times 100
\]

where A is tyrosine content in the sample without protein additive, and B is tyrosine content in the 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 4–5 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 polyvinylidene 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, England). Gels were equilibrated at room temperature (28–30°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.* (2003a). 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 occurred. 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):

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

**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 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. Expressible drip was calculated and expressed as percentage of sample weight as follows:

\[
\text{Expressible drip (\%)} = \left[ (X - Y)/X \right] \times 100
\]
where $X$ is the weight of the original sample and $Y$ is the weight of the sample after being pressed.

**Determination of Autolysis in Surimi Gel.** To 2 g of finely chopped gel samples, 18 mL of 5% TCA was added and homogenized for 2 min using an IKA homogenizer (Selangor, Malaysia) 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 x 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 of tyrosine per gram of sample.

**SDS–Polyacrylamide Gel Electrophoresis (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 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 x 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[hydroxymethyl]aminomethane [Tris]–HCl, pH 6.8 containing 4% SDS, 20% glycerol and 10% β-mercaptoethanol) and boiled for 3 min in the test tubes. The samples (20 μg protein) were loaded onto 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). 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 of protein band intensity was performed using a Model GS-700 Imaging Densitometer (Bio-Rad Laboratories) 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 (without protein additive).

**Statistical Analysis**

Completely randomized design and one-way analysis of variance 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 $P < 0.05$. The analyses were run in three or five replications.
RESULTS AND DISCUSSION

Autolysis Study of Sardine Surimi

The autolytic activity of sardine surimi at different temperatures and times is shown in Fig. 1. For all incubation times 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. Generally, greater TCA-soluble peptide content was observed with increasing incubation time, indicating more protein degradation. However, Benjakul et al. (2003c) reported that the highest autolysis of lizardfish mince and washed mince was observed at 65 and 60°C, respectively. Modori phenomenon of sardine (Sardinop melanos-ticta) 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 caused by the different types and amount of proteases present in surimi. The myofibril-associated proteases show a detrimental effect on gel-forming ability because they are still retained after the washing process (Cao et al. 1999). Those proteases played an essential role in protein degradation, particularly at elevated tem-

![Graph showing TCA-soluble peptide content in sardine surimi incubated at different temperatures and times.](image)

**FIG. 1. TRICHLOROACETIC ACID (TCA)-SOLUBLE PEPTIDE CONTENT IN SARDINE SURIMI INCUBATED AT DIFFERENT TEMPERATURES AND TIMES**

Bars represent the standard deviation from triplicate determinations.
perature, and were responsible for the softening of surimi gel from oval filefish (Toyohara et al. 1990).

**Effect of CPP on Autolysis of Sardine Surimi**

CPP and other protein additives showed inhibitory activity differently toward autolysis of sardine surimi incubated at 70C (Fig. 2). In general, greater inhibition was observed as the concentration of protein additives increased ($P < 0.05$). At the same level tested, PPP showed the greatest inhibitory activity on autolysis compared with other protein additives ($P < 0.05$). Among all plasma proteins, CPP exhibited less inhibition 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 least inhibition at all concentrations tested ($P < 0.05$). This result was in accordance with Benjakul and Visessanguan (2000) who reported that PPP showed greater 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,b). Visessanguan et al. (2000) also reported that PPP was effective in protecting 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 consists of $\alpha_2$M and kininogen, that none specifically traps all types 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 the addition of CPP, especially with increasing CPP concentration. Chicken blood plasma contains ornitho-kininogen, which has similar properties to mammalian high-molecular weight (HMW) kininogen (Kimura et al. 1987). Kos et al. (1992) reported that HMW kininogen is present in chicken plasma and strongly inhibited chicken cathepsin L and papain, but is a much weaker inhibitor of chicken cathepsin B. EW showed inhibitory activity because of 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 Hernandez-Cortes 2000). Garcia-Carreno (1996) also reported that EW contains a specific competitive inhibitor, namely ovomucoid.

The 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 Fig. 3. MHC degradation was markedly observed in the control 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) (lane 1). The result indicated that MHC in sardine surimi was prone to proteolysis at high temperature. It has been reported that MHC, $\beta$-tropomyosin and troponin-T were more susceptible to degradation than actin (An et al. 1994). Benjakul et al. (2003c) 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 a MW of 60 kDa hydrolyzed MHC in lizardfish muscle at 55–65°C (Cao et al. 2000). From the result, the efficacy of autolysis inhibition varied, depending on the protein additives used. At all levels of protein additives tested, MHC of surimi tended to have the greatest intensity with the 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 (Fig. 2). 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 the 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. However, the degree of inhibition varied with protein additives as evidenced by the different band intensities of MHC retained. 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 the 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.

FIG. 3. AUTOLYSIS PATTERN OF SARDINE SURIMI ADDED WITH CHICKEN PLASMA PROTEIN (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, beef plasma protein; PPP, porcine plasma protein; EW, egg white; SPI, soy protein isolate; MHC, myosin heavy chain; AC, actin.
Effect of CPP on Gel-forming Ability of Sardine Surimi

Effect of CPP on Textural Properties of Sardine Surimi Gels. The breaking force and deformation of sardine surimi gels added with CPP or other protein additives at different levels are shown in Figs. 4 and 5. The type of protein and concentration used affected both breaking force and deformation.

![Graph showing breaking force and deformation of sardine surimi gels with added CPP or other protein additives at different concentrations.]

**FIG. 4. BREAKING FORCE AND DEFORMATION OF SARDINE MODORI GELS WITH ADDED CHICKEN PLASMA PROTEIN (CPP) OR OTHER PROTEIN ADDITIVES AT DIFFERENT CONCENTRATIONS**

Bars represent the standard deviation from five determinations. DH, directly heated gel.
of surimi gel. For modori gels (Fig. 4), the gel with added CPP showed the higher breaking force and deformation, compared with the control gel (without protein additives) \( (P < 0.05) \). The 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 (Fig. 2). Although CPP could prevent the decrease in 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 greatest 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. (2000b) 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 toward autolysis of sardine surimi (Fig. 2). Chang-Lee et al. (1990) reported that varying concentrations of SPI (1–5%) in batters did not affect gel hardness produced from Pacific whiting surimi. The slight increases in breaking force and deformation of modori gel with SPI were possibly caused by the simple filler effect or forming interpenetrating network of nonmuscle protein (Ziegler and Foegeding 1991). Soy proteins could be formulated into food as binders (Meyer and Williams 1976), and they have less adhesive strength in the processing of restructured meat, compared to myofibrillar proteins, gluten, blood plasma and EW albumin (Lu and Chen 1999). At a concentration of 3%, samples with all protein additives except BPP had the lowered breaking force and deformation, compared with samples added with the protein additives at a concentration of 2% \( (P < 0.05) \). This was probably because these nonmuscle 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 nonmuscle protein additives could be caused by “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, the 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 proteins including MHC. This was associated with the lower gel strength of the control gel.

For kamaboko gels (Fig. 5), 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 concentration of 2% (w/w), all samples had the greatest breaking force and deformation, except those added with BPP \( (P < 0.05) \). The addition of 2% CPP resulted in an increase in
breaking force and deformation by 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. PPP still showed the greatest 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 cogelling 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 greater 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 nondisulfide covalent bonds (Benjakul and Visessanguan 2003). Transglutaminase is known to play an important role in setting of surimi, resulting in improved gel quality (Benjakul et al. 2003a).

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 (Fig. 2), it might act as a cogelling 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 1. 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 the increasing EW and SPI concentration slightly increased whiteness. Wasson et al. (1992) reported that a noticeable increase in off-white tones of arrowtooth flounder cooked gels resulted from the addition of plasma powder and EW. 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 greater than that of kamaboko gel or modori gel. Benjakul et al. (2001b) found that the lower whiteness of gel was observed with plasma protein addition, because some hemoglobin as well as other pigments with a pale straw color were retained in the plasma.

The expressible moisture of kamaboko and modori gels added with CPP at different concentrations is shown in Table 1. 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 ($P < 0.05$). The addition of CPP or other protein additives resulted in less 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 the addition of hydro-

### Table 1.

<table>
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<tr>
<th>Sample</th>
<th>Whiteness</th>
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<th>Expressible moisture (%)</th>
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<td>Kamaboko gel</td>
<td>Modori gel</td>
<td>Kamaboko gel</td>
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<td>$67.9 \pm 0.8f$</td>
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<td>$64.0 \pm 0.3b$</td>
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<td>$64.9 \pm 0.5b$</td>
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<td>PPP 1%</td>
<td>$66.9 \pm 0.2efg$</td>
<td>$66.3 \pm 0.2cde$</td>
<td>$3.2 \pm 0.2b$</td>
<td>$3.4 \pm 0.1bc$</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>$66.5 \pm 0.1ef$</td>
<td>$66.0 \pm 0.2cd$</td>
<td>$3.1 \pm 0.04b$</td>
<td>$3.3 \pm 0.1b$</td>
<td></td>
</tr>
<tr>
<td>3%</td>
<td>$66.3 \pm 0.5de$</td>
<td>$65.8 \pm 0.5c$</td>
<td>$2.9 \pm 0.1a$</td>
<td>$3.0 \pm 0.1a$</td>
<td></td>
</tr>
<tr>
<td>EW 1%</td>
<td>$67.6 \pm 0.4h$</td>
<td>$66.2 \pm 0.2de$</td>
<td>$3.7 \pm 0.1fg$</td>
<td>$3.9 \pm 0.03f$</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>$67.8 \pm 0.6h$</td>
<td>$66.4 \pm 0.6cde$</td>
<td>$3.7 \pm 0.03fg$</td>
<td>$3.7 \pm 0.02e$</td>
<td></td>
</tr>
<tr>
<td>3%</td>
<td>$67.9 \pm 0.1h$</td>
<td>$67.1 \pm 0.5ef$</td>
<td>$3.6 \pm 0.1efg$</td>
<td>$3.6 \pm 0.1de$</td>
<td></td>
</tr>
<tr>
<td>SPI 1%</td>
<td>$66.8 \pm 0.5ef$</td>
<td>$66.5 \pm 0.3cde$</td>
<td>$4.0 \pm 0.02h$</td>
<td>$3.6 \pm 0.02de$</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>$67.2 \pm 0.5fgh$</td>
<td>$66.8 \pm 0.1de$</td>
<td>$3.5 \pm 0.1de$</td>
<td>$3.6 \pm 0.1de$</td>
<td></td>
</tr>
<tr>
<td>3%</td>
<td>$67.6 \pm 0.1h$</td>
<td>$67.0 \pm 0.4ef$</td>
<td>$3.4 \pm 0.1cd$</td>
<td>$3.4 \pm 0.1b$</td>
<td></td>
</tr>
</tbody>
</table>

* Values are given as mean ± SD from triplicate determinations.
† Different letters in the same column indicate significant differences ($P < 0.05$).
DH, directly heated gel; BPP, beef plasma protein; PPP, porcine plasma protein; EW, egg white; SPI, soy protein isolate.
colloids 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 that of the control modori gel. Thus, the kamaboko gel had higher water-holding capacity than the modori gel. However, the 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. The TCA-soluble peptide content in both modori and kamaboko gels with different levels of CPP and other protein additives is shown in Fig. 6. 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 the 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 greater than 2% might not be necessary for the prevention of proteolysis. The greatest TCA-soluble peptide contents 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 (Fig. 2). For both modori and kamaboko gels, the TCA-soluble peptide content in the gels with CPP was similar to those found in gels with BPP, PPP and EW. Generally, the TCA-soluble peptide content in the modori gel was greater than those of the kamaboko gel. This suggests that greater degradation of surimi proteins occurs in modori gels as compared with kamaboko. However, protein degradation could be reduced by the addition of CPP or other protein additives.

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 Figs. 7 and 8. The amount of MHC in the control modori gel (Fig. 7: lane 2) decreased by 74.75% relative to that of the starting surimi (lane 1). Conversely, a slight increase in the relative amount of MHC in all samples added with protein additives was observed as the concentration of protein additives increased. At the same level of additive (2%), 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 (Jiang and Lee 1992; Seymour et al. 1997). The remaining 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. It was postulated that actin could be a substrate for proteases in sardine surimi when no MHC was available.

For kamaboko gel (Fig. 8), the amount of MHC in the control kamaboko gel (lane 2) decreased to about 79.24% compared with that of the starting surimi.
FIG. 7. PROTEIN PATTERN OF MODORI GELS ADDED WITH CHICKEN PLASMA PROTEIN (CPP) OR OTHER PROTEIN ADDITIVES AT DIFFERENT CONCENTRATIONS S, sol; C, surimi without protein additives; DH, directly heated gel; BPP, beef plasma protein; CPP, chicken plasma protein; PPP, porcine plasma protein; EW, egg white; SPI, soy protein isolate; MHC, myosin heavy chain; AC, actin.
FIG. 8. PROTEIN PATTERN OF KAMABOKO GELS ADDED WITH CHICKEN PLASMA PROTEIN (CPP) OR OTHER PROTEIN ADDITIVES AT DIFFERENT CONCENTRATIONS
S, sol; C, surimi without protein additives; DH, directly heated gel; BPP, beef plasma protein; CPP, chicken plasma protein; PPP, porcine plasma protein; EW, egg white; SPI, soy protein isolate; MHC, myosin heavy chain; AC, actin.
(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). The disappearance of MHC in kamaboko gels either with or without protein additives was most likely because of the polymerization, which possibly resulted from both endogenous and plasma TGase activity during the 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 the presence of CPP or other protein additives, the degradation of protein caused by less active proteases could be minimized effectively as evidenced by very low TCA-soluble peptides in kamaboko gels (Fig. 6). Also, the 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. As a result, the greater 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).

**CONCLUSION**

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

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