CHAPTER 8

EFFECT OF CYSTEINE PROTEINASE INHIBITOR CONTAINING FRACTION FROM CHICKEN PLASMA ON AUTOLYSIS AND GELATION OF PACIFIC WHITING SURIMI

8.1 Abstract

The effects of cysteine proteinase inhibitor containing fraction (CPI fraction) from chicken plasma on autolysis inhibition and gelling properties of Pacific whiting surimi were investigated. The CPI fraction exhibited the inhibitory activity against autolysis of Pacific whiting surimi in a concentration dependent manner. SDS-PAGE pattern revealed that the CPI fraction effectively prevented the degradation of myosin heavy chain (MHC), tropomyosin and troponin-T in Pacific whiting surimi incubated at 55°C for 60 min as well as modori gel (55°C for 30 min/90°C for 20 min). The breaking force and deformation of modori gels increased as the concentration of CPI fraction increased up to 2% with a concomitant decrease in TCA-soluble peptides ($P<0.05$). However, a decrease in breaking force and deformation was noticeable with the modori gel added with 3% CPI fraction. The addition of CPI fraction had no detrimental effect on whiteness of surimi gels ($P>0.05$). The result suggested that CPI fraction would be an alternative additive for surimi containing the active cysteine proteinase causing the gel weakening.

8.2 Introduction

Surimi possesses the functionality including gelling, binding and emulsifying properties and can be used as a functional protein ingredient in several products (Lanier, 1986). The thermal aggregation of myosin molecule is a crucial process for developing the elastic gels that largely affect consumer preference and palatability of surimi-based products (Sano *et al.*, 1988). Gelling characteristics of surimi can be influenced by many factors causing the structural alteration of myosin (An *et al.*, 1996). The presence of endogenous proteolytic enzymes in surimi results in a decrease in gel

strength. This phenomenon, referred to as ″modori″, causes an irreversible destruction of the gel structure of surimi, especially at temperatures close to 60°C (An *et al*., 1996). Cathepsin L, a cysteine proteinase, has been reported to hydrolyze muscle proteins of Pacific whiting, arrowtooth flounder, mackerel and anchovy (Seymour *et al*., 1994; Aoki and Ueno, 1997; Heu *et al*., 1997; Visessanguan and An, 2000;). Serine proteinases were responsible for textural breakdown of threadfin bream, oval-filefish and Atlantic menhaden (Kinoshita *et al*., 1990; Toyohara *et al*., 1990b; Choi *et al*., 1999). Those proteinases caused the degradation of muscle protein, especially at the elevated temperature (55-65°C).

Protein additives have been widely used to enhance the gel strength via inhibition of proteolysis caused by endogenous proteinases (Benjakul *et al*., 2004b). Among those, beef plasma protein (BPP) has been known to be the most effective proteinase inhibitor and gel strengthening agent, compared to other cysteine proteinase inhibitors (Kang and Lanier, 1999). The addition of porcine plasma protein (PPP) increased the gel strength of surimi (Benjakul *et al*., 2001a). Benjakul and Visessanguan (2000) found that PPP effectively inhibited the activity of Pacific whiting muscle proteinases and autolytic activity of surimi. Protein with apparent molecular weight of 60-63 kDa in porcine plasma appeared to exhibit inhibitory activity against papain and trypsin (Benjakul and Visessanguan, 2000). From our previous study, chicken plasma protein (CPP) was able to enhance the gel strength by acting as filler in surimi gel matrix and also as proteinase inhibitor (Rawdkuen *et al*., 2004a, b). However, the addition of blood plasma to surimi or surimi products rendered the end product with off-color and off-flavor (Wasson *et al*., 1992b; Rawdkuen *et al*. 2004a, b). Therefore, the fractionation of selected component should be a promising means to concentrate the component of interest and to avoid the discoloration in the final products. Cysteine proteinase inhibitor (CPI) from chicken plasma was successfully fractionated by using polyethylene glycol (PEG-4000) (Rawdkuen *et al*., submitted). Thus, CPI containing fraction from chicken plasma can be used as a proteinase inhibitor in surimi without the problematic discoloration in the resulting surimi gel products. The purpose of this study was to investigate the preventive effects of CPI containing fraction from chicken plasma on autolysis and gelling properties of Pacific whiting surimi.

8.3 Materials and Methods

Chemicals and Surimi

Sodium chloride and trichloroacetic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). Bovine serum albumin (BSA) and *L*-tyrosine were procured from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Polyethylene glycol (PEG-4000) was obtained from Fluka Chemika-BioChemika (Buchs, Switzerland). Sodium dodecyl sulfate (SDS) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Novex pre-cast gels and other electrophoresis reagents were obtained from Invitrogen life technologies (Carlsbad, CA, USA).

Frozen surimi grade FA produced from Pacific whiting (*Merluccius productus*) was obtained from Trident Seafoods Corp. (Seattle, WA, USA) and kept at -25°C until used.

Preparation of chicken plasma protein

Chicken blood was obtained from a slaughterhouse in Hat Yai, Thailand. During collection, one-tenth volume of 3.8% (w/v) trisodium citrate was added to prevent coagulation. The blood was centrifuged twice at 1,500*g* for 15 min at 4°C to remove red blood cells using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The supernatant obtained was referred to as "chicken plasma".

Fractionation of CPI from chicken plasma

Fractionation of CPI was carried out using PEG-4000 according to the method of Hao *et al*. (1980) with a slight modification. All operations were conducted in the cold room (4°C). Initially, solid PEG was added into chicken plasma (200 g/L) with gentle stirring. After the complete solubilization of PEG, the mixture was allowed to stand for 2 h at 4°C. The supernatant was obtained by centrifugation (7000*g*, 15 min, 4°C) and the precipitated protein was discarded. An additional PEG (200 g) was added into the supernatant obtained with gentle stirring. When the PEG was completely solubilized, the

mixture was equilibrated as mentioned previously. The protein pellet was collected in the same manner and referred to as "cysteine proteinase inhibitor containing fraction; *CPI fraction*". CPI fraction was then dialyzed against 50 volumes of 10 mM sodium phosphate buffer, pH 7.4 containing 0.9 mM $CaCl_2$ and 0.05 mM $MgCl_2 6H_2O$ overnight at 4°C to remove the residual PEG. The dialysis buffer was changed for 4 times every 3 h. The CPI fraction was freeze-dried and kept at -18°C until used.

Effect of CPI fraction on autolysis of Pacific whiting surimi

Pacific whiting surimi (3 g) were placed in a beaker and incubated in a water bath at 55°C for different times (0, 10, 20, 30, 60, 90, 120 and 180 min). At time designated, autolysis was terminated by addition of 27 ml of 5% SDS solution (85°C). After 1 h, the solubilized samples were analyzed for protein content by the Biuret method (Robinson and Hodgen, 1940). Autolytic patterns were determined using SDS-PAGE according to the method of Laemmli (1970). Autolysis of surimi in the presence of CPI fraction at the level of 1% was also monitored during the incubation at 55°C.

The inhibitory activity of CPI fraction against autolysis of Pacific whiting surimi was measured according to the method of Morrissey *et al*. (1993). CPI fraction at levels of 0, 0.3, 0.5, 1, 2 and 3% were added to 3 g of surimi. The mixture was mixed thoroughly and then incubated in a water bath at 55°C for 1 h. Autolysis was terminated by addition of 27 ml of 5% cold TCA solution. The mixture was then homogenized using a Sorvall OMNI-MIXER (Ivan Sorvall Inc, Norwalk, CONN, USA) at a speed of 1 for 1 min and kept on ice for 1 h. The homogenate was centrifuged at 5,000*g* for 5 min using a Marathon microA centrifuge (Fisher Scientific, Pittsburgh, PA, USA). TCA-soluble peptides in the supernatant were analyzed using the Lowry method (Lowry *et al*., 1951). The autolysis inhibition was calculated as follows:

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

; where A is TCA-soluble peptide content in sample without CPI fraction

B is TCA-soluble peptide content in sample with CPI fraction

To study the autolytic pattern of Pacific whiting surimi in the presence of different levels of CPI fraction, samples whose autolysis was terminated using 5% SDS solution (85°C) were subjected to SDS-PAGE analysis after being completely solubilized as previously described.

Surimi gel preparation

To prepare the gel, frozen surimi was partially thawed in a cold room (4°C) for 2-3 h. The surimi was cut into small pieces with an approximate thickness of 1 cm and then placed in the mixer (Cuisinart original food processor, East Windsor, NJ, USA). The moisture content of samples was then adjusted to 80% and salt (2.5%, w/w) was added. CPI fraction at different levels (0, 0.3, 0.5, 1, 2 and 3% of total weight) was then added. The mixture was chopped for 4 min at 4°C. The paste was stuffed into polyvinylidine chloride casing with a diameter of 3 cm and both ends were sealed tightly. The paste was incubated at 55°C for 30 min, followed by heating at 90°C for 20 min in a water bath. This sample was referred to as "modori gel". A directly heated gel was prepared by heating the 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 length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyzer equipped with a spherical probe (5 mm diameter; 60 mm/min depression speed).

Oscillartory dynamic testing

Rheological changes (storage modulus: G', loss modulus: G'' and phase angle) of surimi pastes during gelation were continuously measured using a ATS

Rheosystem Stree Tech rheometer (Stresstech, Rheologica instruments AB, Lund, Sweden). The rheometer was equipped with 40-mm, 4 degrees slope cone and plate geometry. An oscillation of 0.1 Hz with a resistance stress of 35 Pa was used for testing. This condition was determined to give a linear response in the viscoelastic region. Temperature sweeps were recorded from 20 to 90°C at a rate of 1°C/min. To avoid evaporation of the sample during heating, a lubricant and cover were used. The elastic modulus (G'), viscous modulus (G'') and phase angle were recorded for samples. Testing for each sample was carried out in duplicate.

Determination of whiteness

Five gel samples from each treatment were subjected to whiteness measurement using a Chroma Meter CR-300 (Minolta Camera Co., Ltd., Osaka, Japan). CIE L^* (lightness, 0-100), a^* (red/green; "+" being toward the red and "-" being toward the green) and b^* (yellow/blue; "+" being toward the yellow and "-" being toward the blue) 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 autolysis in surimi gel

The autolysis of surimi gel added with CPI fraction at different levels was determined according to the method of Morrissey *et al.* (1993). Gel sample (3 g) was mixed and homogenized with 27 ml of 5% TCA. The peptides in supernatant were measured as previously described and expressed as μmole tyrosine/g sample.

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis

SDS-PAGE analysis was performed according to the method of Laemmli (1970). The sample solubilized using 5% SDS solution (85°C) was centrifuged at 5,000*g* for 5 min to remove undissolved debris. Solubilized samples were mixed at 1:1 (v/v) ratio with the Novex tris-glycine SDS sample buffer containing reducing agent and boiled for 3 min. The samples (20 μg protein) were loaded into Novex 10% tris-glycine

pre-cast gel and subjected to electrophoresis at a current constant at 30 mA/gel using a *Xcell SureLock*™ Mini-Cell (Novex, San Diego, CA, USA). After separation, the proteins were stained with SimplyBlue™ SafeStain for 3 h and destained with water. The gel was dried overnight with Gel-Dry™ drying solution.

Statistical analysis

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

8.4 Results and Discussion

Autolysis profile of Pacific whiting surimi

The protein degradation of Pacific whiting surimi was monitored during the incubation of surimi paste at 55°C for up to 180 min (Figure 44A). After incubation at 55°C for 10 min, a substantial decrease in myosin heavy chain (MHC) with the concomitant formation of degradation products was observed. MHC and actin are the main components of myofibrillar proteins in surimi, followed by troponin and tropomyosin, respectively. Generally, the degradation of all myofibrillar proteins, especially MHC, in surimi increased with time. The MHC band intensity decreased as the increasing incubation time increased. MHC band disappeared within 30 min. The bands of degradation products (97-200 kDa) were found in the first 30 min and completely disappeared when the incubation time was longer than 60 min. Those products might be hydrolyzed to smaller peptides with the sufficient time. An *et al.* (1995) reported that after cooking Pacific whiting surimi for 30 min at 60°C, MHC was completely hydrolyzed. Cathepsin L is the major proteolytic enzyme of Pacific whiting surimi since cathepsin B, H and other proteinases are removed by washing process, while cathepsin L is not completely removed (An *et al.*, 1994b).

The troponin-T degradation was found in the first 30 min of incubation and completely disappeared within 60 min. Tropomyosin was also hydrolyzed, especially

with increasing incubation time. Nevertheless, the rate of degradation was much lower than that of MHC and troponin-T. Only slight changes in actin band intensity were noticeable during incubation of 180 min. It is well known that actin was more resistant to hydrolysis caused by proteolytic enzymes than other myofibrillar proteins. Benjakul *et al.* (1997a) reported that no degradation of actin was observed in Pacific whiting muscle, while myosin was prone to proteolysis during extended iced storage.

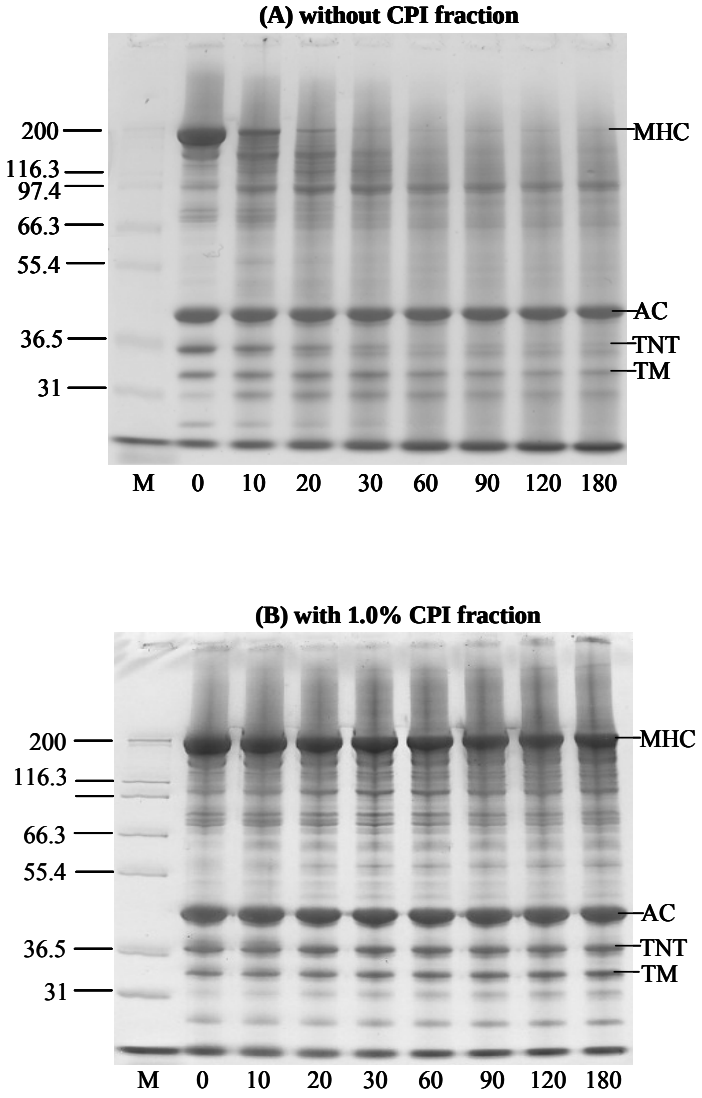

Figure 44. Electrophoresis pattern of Pacific whiting surimi without (A) and with 1% CPI fraction from chicken plasma (B) incubated at 55°C for 0-180 min. M: protein marker, MHC: myosin heavy chain, AC: actin, TNT: troponin-T, TM: tropomyosin. Numbers designate the incubation time (min) at 55°C.

In the presence of CPI fraction at the level of 1%, autolysis of Pacific whiting surimi incubated at 55°C was markedly inhibited as evidenced by more retaining proteins band intensity. The MHC still remained after incubation for 180 min in the presence of 1% CPI fraction. No substantial changes in MHC band intensity were observed during the first 30 min of incubation. However, a slight decrease was noticeable with a coincidental formation of degradation peptides having MW ranges of 100-200 kDa, as the incubation time increased. This result suggested that the CPI fraction at the level of 1% had high effectiveness in inhibiting proteolysis in Pacific whiting surimi. Benjakul *et al.* (2001c) reported that fraction IV-1 from porcine plasma at a level of 0.5% effectively prevented the degradation of MHC in bigeye snapper muscle. From the result, no changes in actin band intensity were observed in all samples added with 1% CPI fraction when compared with that found in surimi. The addition of 1% CPI fraction also prevented the degradation of both troponin-T and tropomyosin effectively. From the result, cysteine proteinase inhibitors in the fraction could inhibit cathepsin L effectively as indicated by the remaining myofibrillar proteins of Pacific whiting surimi subjected to incubation at 55°C for a long time.

Autolysis inhibition of Pacific whiting surimi by CPI fraction

CPI fraction inhibited the autolysis of Pacific whiting surimi at different degrees, depending on the level of fraction used (Figure 45). Generally, the greater percent inhibition was observed with increasing fraction levels added. In the presence of 0.3% CPI fraction, autolysis of Pacific whiting surimi at 55°C was inhibited more than 80%. When the concentration of CPI fraction was greater than 1%, no differences in autolysis inhibition were found ($P>0.05$). It was suggested that autolysis of surimi could be inhibited efficiently by CPI fraction at a level of 1%. From the result, CPI fraction could inhibit the proteinase in surimi, especially those associated with myofibrillar proteins. Washing process could remove some endogenous proteinases which play an important role in the degradation of MHC. Chang-Lee *et al.* (1989) reported that the proteinase activity in mechanically deboned Pacific whiting flesh was reduced to 56.3% by washing and refining processes. Cathepsin L was identified as the predominant proteinase in surimi wash water (Benjakul *et al.*, 1996, 1997a, b, 1998).

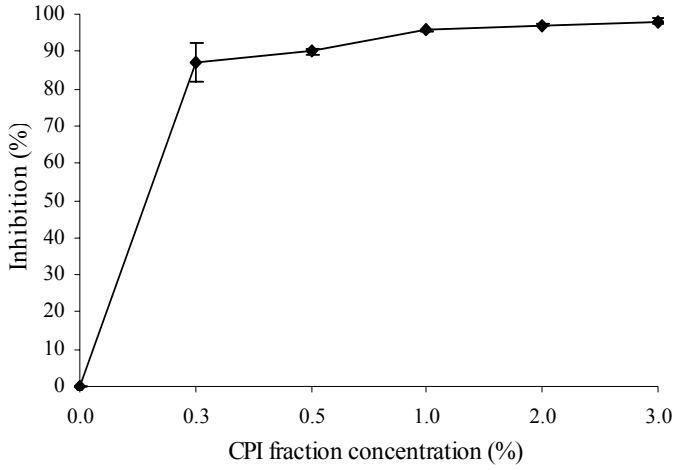

Figure 45. Inhibitory activity of CPI fraction from chicken plasma at different concentrations on the autolysis of Pacific whiting surimi at 55°C for 60 min. Bars represent the standard deviation from three determinations.

Autolytic patterns of Pacific whiting surimi incubated at 55°C for 60 min in the absence and presence of CPI fraction at different levels are shown in Figure 46. Without CPI fraction addition, MHC, troponin-T and tropomyosin were almost completely hydrolyzed. Band intensity of actin decreased slightly. From the result, the degradation of MHC was successfully inhibited, particularly when CPI fraction concentration increased. MHC band was totally recovered in the presence of 1% CPI fraction, suggesting the inhibitory activity of the fraction towards autolysis of MHC. This result was in agreement with that shown in Figure 44 in which MHC band was totally retained with the addition of 1% CPI fraction. Actin, troponin-T and tropomyosin were completely recovered in all samples added with CPI fraction. The result suggested that CPI fraction could prevent the degradation not only MHC in Pacific whiting surimi, but also tropomyosin and troponin.

Effect of CPI fraction on textural properties

The breaking force and deformation of modori gels from Pacific whiting surimi increased as the concentration of CPI fraction increased ($P<0.05$) (Figure 47). Breaking force and deformation reached the maximal values when 2% CPI fraction was used.

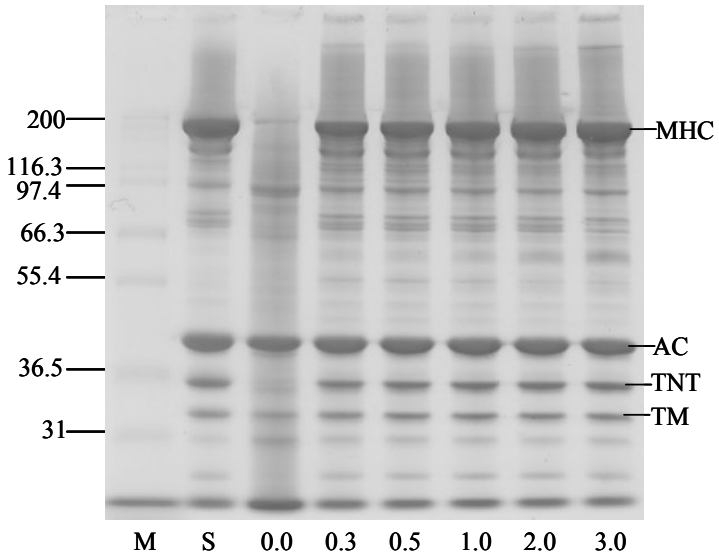

Figure 46. Autolytic pattern of Pacific whiting surimi added with CPI fraction from chicken plasma at different concentrations. Samples were incubated for 60 min at 55°C. M: protein marker; MHC: myosin heavy chain; AC: actin; TNT: troponin-T, TM: tropomyosin. Numbers designate the concentration of CPI fraction (%).

However, the decreases in both breaking force and deformation were observed with the gel added with 3% CPI fraction. Without addition of CPI fraction, the modori gel showed the lowest breaking force and deformation, compared with other samples with CPI fraction addition. It has been reported that cathepsin L, heat-activated endogenous proteinase, hydrolyzes the muscle protein at 55-60°C, leading to the weakened gel network of modori gel (An *et al.*, 1996; Alvarez *et al.*, 1999). At the levels of 2% CPI fraction, the breaking force of modori gel increased by 49.4%, and the deformation increased by 26.2%, compared with those of the control (without CPI fraction). Directly heated gel showed the higher breaking force and deformation, compared with the control modori gel. Benjakul *et al.* (2001a) reported that the addition of fraction IV-I from PPP into bigeye snapper surimi resulted in the substantial increases in breaking force and deformation of resulting gels. The increase in breaking force and deformation might be owing to the inhibitory activity toward endogenous proteinase in surimi. Additionally, protein components in CPI fraction possibly functioned as the filler in the gel matrix. However, at the CPI level higher than 2% addition, both breaking force and deformation of modori gel decreased.

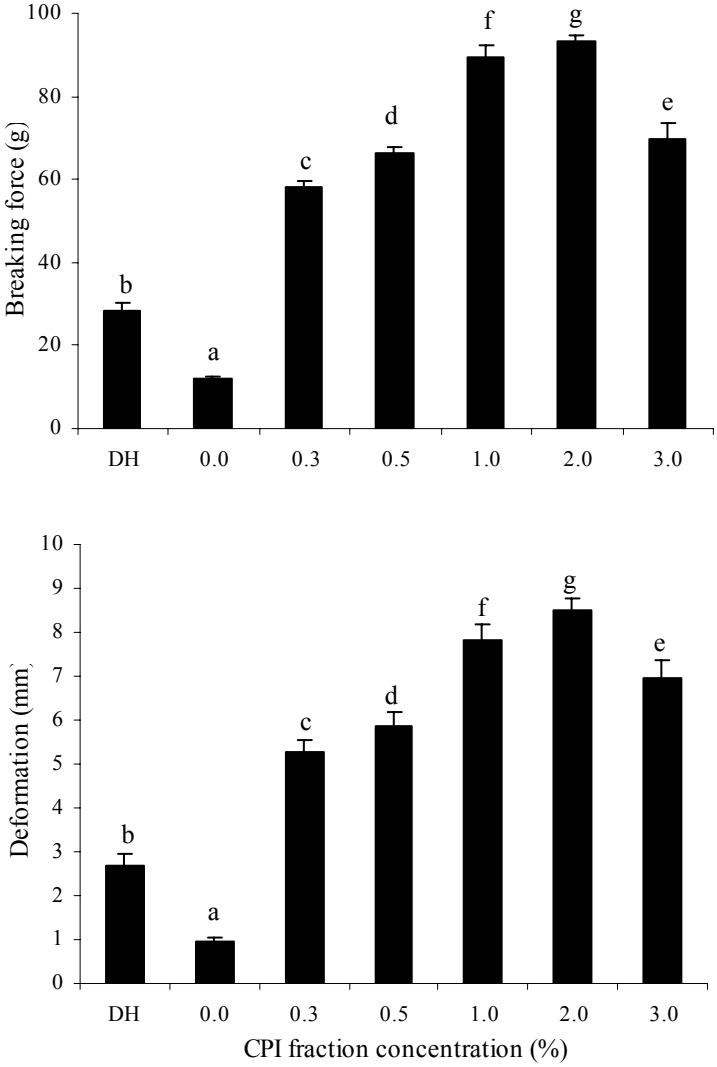

Figure 47. Breaking force and deformation of surimi gels added with CPI fraction from chicken plasma at different concentrations. DH: directly heated gel. Bars represent the standard deviation from five determinations.

This might be caused by the dilution effect of MHC, the main protein components for gel formation in surimi. These results were similar to our previous study in bigeye snapper and lizardfish surimi, in which CPP addition at levels greater than 2% showed the decrease in breaking force and deformation of the gels (Rawdkuen *et al.* 2004b). Morrissey *et al.* (1993) also reported that the highest strain value was obtained in modori gel from Pacific whiting added with 2% BPP and slightly decreased at higher concentrations. BPP has been reported as the most effective protein additive for strengthening the gel of Pacific whiting

surimi (Seymour *et al*., 1997; Kang and Lanier, 1999). From the result, CPI fraction exhibited the gel enhancing effect in Pacific whiting surimi and can be used as the promising alternative for BPP.

Effect of CPI fraction on dynamic viscoelastic properties

Changes in G′ of Pacific whiting surimi paste without and with CPI fraction at different levels were monitored during gelation with the temperature ranging from 20 to 90°C at a heating rate of 1°C/min (Figure 48). G′ initially increased at about 30°C and reached the maximal value at 33°C. Thereafter, the continuous decrease in G′ was found until the temperature reached 42°C. The sharp increase in G′ at the first transition suggested that myofibrillar proteins in surimi started to form a gel network and was referred to as "gel setting". Ziegler and Acton (1984) reported that heating natural actomyosin at temperature less than 40°C generally resulted in dissociation of some myofibrillar components. The magnitude of G′ at the temperature ranges of 30-42°C was not different between surimi paste without and with 0.3-1% CPI fraction. However, the greater decrease in G′ values in the temperature ranges of 30-42°C were obtained in the sample added with 2-3% CPI fraction. For the control, G′ increased slightly during heating at 42-46°C with the subsequent decrease and reached the minimal value at 55°C. The rate of decrease was greater in the control, compared with samples with CPI fraction. This result suggested that the addition of CPI fraction could prevent the abrupt loss of G′ upon heating around 55°C caused by the intensive degradation of myofibrillar proteins. The degradation was more pronounced in the surimi paste containing no CPI fraction after rheological study was carried out (data not shown). The sharpest decrease in G′ value in surimi paste at the temperature of 55°C of the control was most likely due to the modori phenomenon caused by proteolytic activity in Pacific whiting (An *et al*. 1994b). Egelandsdal *et al*. (1986) reported that the decline in G′ between 50 and 60°C was caused by the denaturation of light meromyosin, resulting in a less strong and more viscous gel.

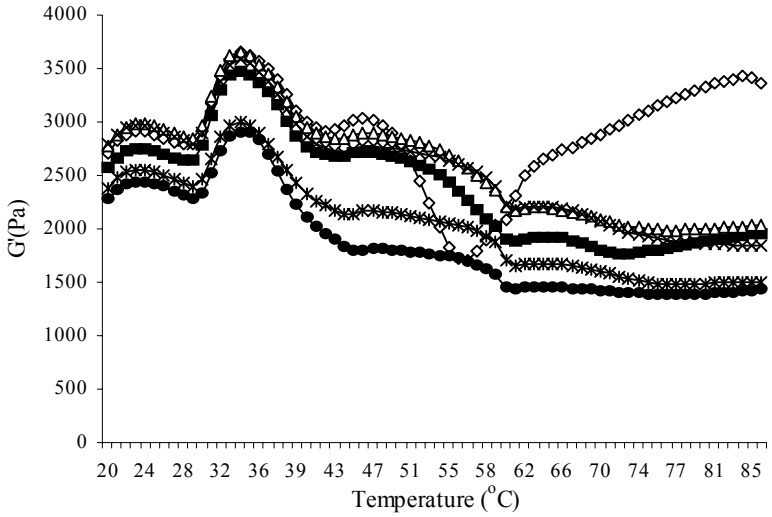

Figure 48. Effect of CPI fraction from chicken plasma on storage modulus (G′) of Pacific whiting surimi paste heated from 20 to 90°C at a rate of 1°C/min. 0 (◇), 0.3 (■), 0.5 (△), 1 (×), 2 (∗), 3% (●) CPI fraction.

The G′ value of the control sample increased sharply with the temperature above 55°C and reached a maximal value at the end of testing. Lou *et al*. (2000) reported that the increase in G′ after 47°C probably attributed to the formation of irreversible gel network. Surprisingly, the samples added with CPI fraction had no increase in G′ after 55°C as observed in the control sample. In addition, the lower G′ values were obtained at the end of testing when compared with the control, especially at the level of 3% addition. The result obtained might reflect the dilution effect of muscle proteins by the CPI fraction added. In this study, CPI fraction was added into surimi paste as the substitute of the myofibrillar proteins to obtain the constant protein content. Therefore, the myofibrillar proteins were reduced with increasing CPI fraction amount added. This might be associated with the lower G′ of samples added with CPI fraction. However, the developments in G′ were not in agreement with the gel strength (Figure 47). Liu and Xiong (1997) mentioned that the gel strength did not necessarily agree with the final G′ in dynamic rheological measurements, probably because the final G′ was the measurement of elasticity of myofibrillar gels when the sample still hot, while gel strength was measured after the formed gels were cooled down. Also, dynamic rheological measurements are non-

destructive, whereas gel penetration force measurement is destructive. They represent different aspects of the rheological profile of protein gels and hence, may not be simply related (Liu and Xiong, 1997).

Effect of CPI fraction on whiteness of surimi gels

The whiteness of modori gels in the presence of various levels of CPI fraction is shown in Figure 49. The result showed that no differences in whiteness between all samples tested were observed ($P>0.05$). Benjakul *et al.* (2001a, c) reported that no significant change in whiteness of bigeye snapper surimi gel was observed with the addition of fraction IV-I from porcine plasma. Since CPI fraction prepared by PEG precipitation could remove a large amount of color pigments in plasma (Rawdkuen *et al.*, 2005), the higher amount of CPI fraction could be added without adverse effect on the whiteness of surimi gels. The result suggested that CPI fraction could be used in surimi seafood without affecting the color of finished products.

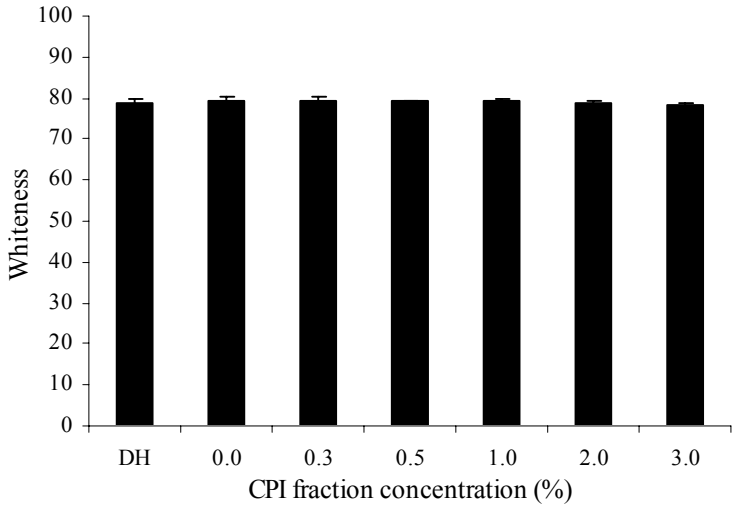

Figure 49. Whiteness of Pacific whiting surimi gels added with different concentrations of CPI fraction from chicken plasma. Bars represent the standard deviation from five determinations.

Effect of CPI fraction on TCA-soluble peptides of surimi gels

Protein degradation in modori gels without and with CPI fraction was monitored as TCA-soluble peptides content (Figure 50). The highest TCA-soluble peptide content was observed in modori gels without CPI fraction addition, indicating the greatest degradation caused by endogenous proteinases. This result was in accordance with the lowest gel strength in the control modori gel (without CPI fraction). The degradation occurred during heat-induced gelation is considered to result from the action of indigenous proteases, especially cathepsin L (An *et al*. 1996). These enzymes are highly active in both post-mortem muscle condition and during heat- induced gelation and thus can degrade the myofibrillar proteins and cause gel degradation in surimi (Visessanguan *et al*., 2001). TCA-soluble peptide content of modori gels decreased as the concentration of CPI fraction increased ($P<0.05$). At the level of 2% CPI fraction addition, TCA-soluble peptide content in modori gel was 20.6%, relative to that found in the control sample.

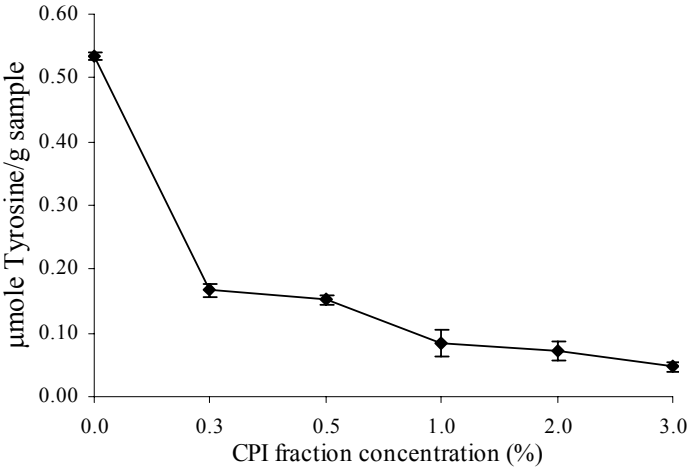

Figure 50. TCA-soluble peptide content of Pacific whiting modori gels added with CPI fraction from chicken plasma at different concentrations. Bars represent the standard deviation from three determinations.

From the result, TCA-soluble peptide formation was greatly inhibited by CPI fraction, especially with increasing amount of CPI fraction. This result was similar to our previous study in bigeye snapper and lizardfish surimi, in which TCA-soluble peptide

content decreased by 300% with the addition of 2% CPP into modori gels (Rawdkuen *et al.*, 2004). The result reconfirmed that CPI fraction therefore had inhibitory activity towards proteolytic degradation of myofibrillar proteins in Pacific whiting surimi at 55°C.

Effect of CPI fraction on protein pattern of surimi gels

The protein patterns of modori gels added with CPI fraction at the level of 0-3% are shown in Figure 51. The lowest MHC band intensity was found in modori gel without CPI fraction addition and MHC band was more retained with increasing CPI fraction levels. For directly heated gel, small band of MHC was retained and the degradation products were formed. Direct heating could reduce the degradation caused by endogenous proteinase to some extent. However, when the temperature reached the optimum temperature (55°C), the intensive hydrolysis occurred as observed by the less MHC band retained.

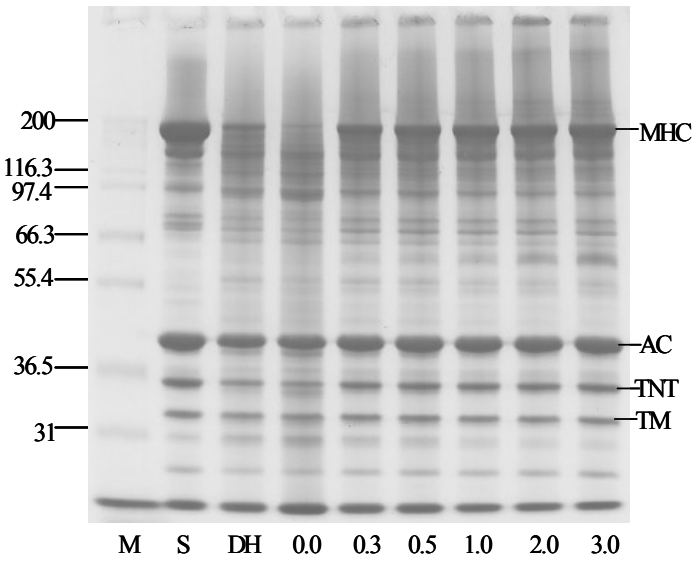

Figure 51. SDS-PAGE pattern of Pacific whiting surimi gels added with different concentrations of CPI fraction from chicken plasma. M: protein markers, S: Pacific whiting surimi, DH: directly heated gel without CPI fraction addition, MHC: myosin heavy chain, AC: actin, TNT: troponin-T, TM: tropomyosin. Numbers designate the concentration of CPI fraction (%).

Increased MHC band intensity was coincidental with the decrease in TCA-soluble peptide content of modori gels (Figure 50) and the increase in breaking force and deformation (Figure 47). However, with the addition of CPI fraction above 1%, no changes in MHC band intensity were observed, suggesting the sufficient amount of CPI fraction in inhibiting the proteolytic activity in modori gel from Pacific whiting surimi. The remained MHC of modori gels added with CPI fraction indicated that CPI fraction could prevent the degradation of myofibrillar proteins in surimi gel effectively. The changes in actin, troponin and tropomyosin were not observed in all samples tested.

8.5 Conclusion

Autolysis of Pacific whiting surimi and surimi gel caused by proteolytic enzymes could be successfully inhibited by CPI fraction. The addition of CPI fraction up to 2% increased gelling properties of modori gel and no adverse effect on the whiteness of the gels was observed with addition of CPI fraction up to 3%.