CHAPTER 3

RESULTS AND DISCUSSION

1. Gel properties of Pacific white shrimp meat

1.1 Effect of sodium chloride concentrations on gel properties of Pacific white shrimp meat

1.1.1 Breaking force and deformation

Breaking force and deformation of Pacific white shrimp gels with different sodium chloride (NaCl) concentrations are shown in Figure 16. Breaking force and deformation increased as the amount of NaCl increased up to 3.5% ($P<0.05$). The highest breaking force was found in sample containing 3.5 and 4% NaCl. No changes in deformation were found in Pacific white shrimp gel added with NaCl in the range of 2.5-4% ($P<0.05$). NaCl was found to dissolve myofibrillar proteins, thus facilitating the gelation (Kubota *et al*., 2006). Both myosin and actomyosin play an essential role in gelation of muscle (Niwa *et al*., 1980). NaCl interacts strongly with the positive charges on the muscle protein, however the sodium ion is only weakly bound (Puolanne and Terrell, 1983; Asghar *et al*., 1985).

The functional properties of protein, such as gelation, are often affected by protein solubility (Damodaran, 1996). Proteins generally show an increase in solubility (salting-in) with low ionic strength (0.3-1.0 M NaCl). Salting-in has been ascribed to nonspecific electrostatic interactions between charged proteins and the ionic environment (Damodaran and Kinsella, 1982). Salts that increase solubility of proteins also tend to denature them. Salts are either preferentially bound to proteins or react with proteins slightly. Sodium chloride with the range of 0.3 -1.0 M induces a salting-in effect of myofibrillar proteins and makes these proteins less stable. At high ionic strength, competition of ionic salts for water may result in a salting-out effect. Water interacting with protein is decreased, in turn, causing decreased solubility (Jittinandana, 2001). Salting-out is strongly dependent on the salt used. Salting out is generally ascribed to the loss of a stable hydrophilic surface, causing the exposed hydrophobic areas of proteins. Subsequently aggregate and precipitate can be formed (Damodaran and Kinsella, 1982).

Alvarez *et al* (1995) examined the contribution of NaCl concentration to textural properties of sardine surimi gels and found that gel strength was maximal when a gel contained 2.24% NaCl. The highest strength of walleye pollack surimi gel was obtained when a gel contained about 3%NaCl (Kubota *et al*., 2006). Roussel and Oheftel (1990) found that texture development of kamaboko gel from sardine surimi required a NaCl content of 1.7-3.5%. From the result, NaCl at levels of 3.5-4% might cause the sufficient dissociation of muscle proteins and free myosin could be released. As a result, the aggregation of myosin could take place and fine network could be formed.

1.1.2 Expressible moisture content

Expressible moisture content of Pacific white shrimp gels added with different levels of NaCl is shown in Table 6. Decrease in expressible moisture content was observed with increasing NaCl levels ($P<0.05$). Gel added with NaCl at levels higher than 3% had the lower expressible moisture content, compared with those added with 2 and 2.5% NaCl ($P<0.05$). This indicated the greater water holding capacity of the formers. NaCl solubilized muscle proteins, and this increased the number of reactive groups in the polypeptide chains capable of interacting during heating. This resulted in a stable, elastic and rigid protein gel matrix with good water- and fat-binding properties could be formed (Carballo *et al.*, 1995). Sodium chloride increases water binding and swelling of meat. Conformation stability and denaturation of myofibrillar proteins were greatly affected by the salt concentration in the muscle (Duerr and Dyer, 1952). At a low ionic strength (0.3-1.0 M NaCl), salts increase water binding capacity of proteins. However, at a high salt concentration, much of the existing water is strongly bound to salt ions. There are not enough water molecules available for protein salvation. Therefore, protein-protein interactions become more powerful than protein-water interactions and lead to aggregation followed by precipitation of protein molecules. This results in dehydration of the protein (Fennema, 1985). Weinberg *et al.* (1984) reported a decrease in expressible moisture of uncooked cod treated with increasing NaCl (0 and 0.25 M NaCl). The NaCl (2 and 4%) caused a rapid change in myosin structure, in which myosin was separated from actin and the uncoiled myosin structure underwent the greater interaction with water molecules (Holtzer *et al*., 1960). NaCl causes the myofibrillar

protein initially to swell, thereby increasing viscosity and water-holding capacity (Hamm 1975; Xiong and Blanchard, 1994).

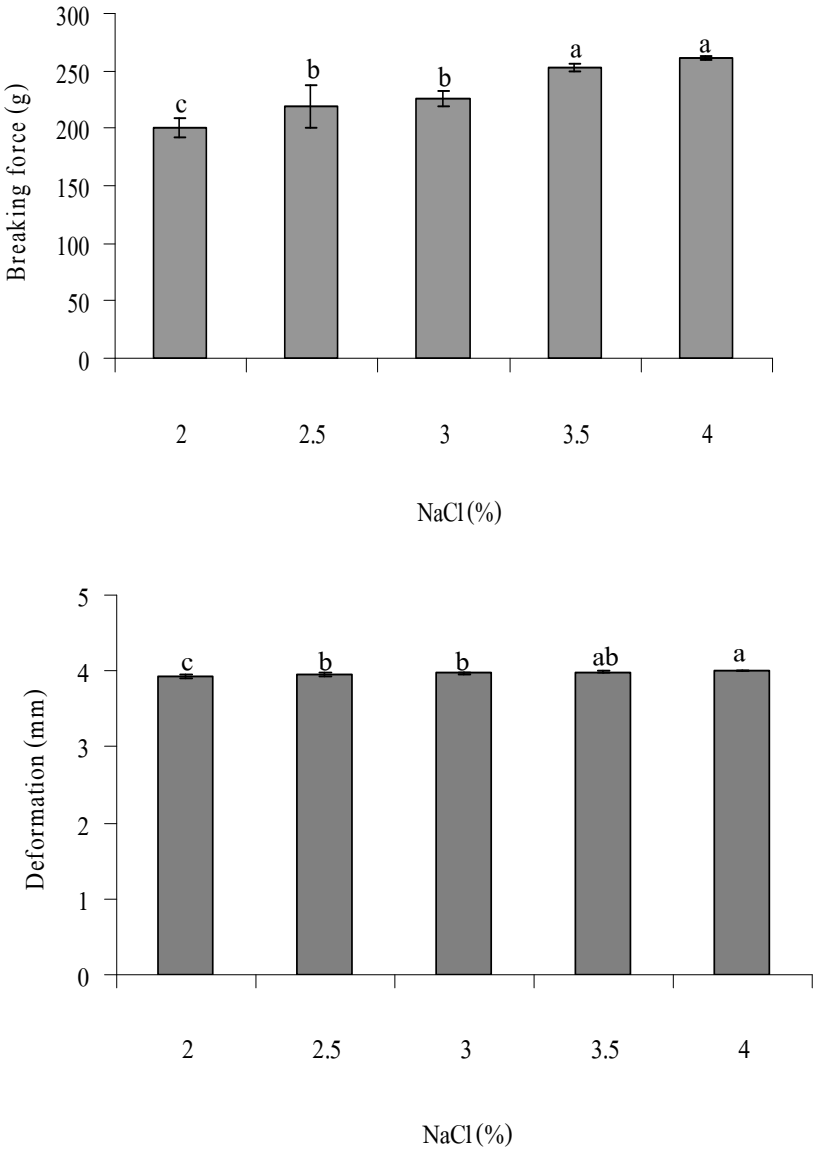

Figure 16 Breaking force and deformation of gels from Pacific white shrimp meat containing different NaCl levels (2-4%). Sols were heated at 90°C for 20 min, followed by cooling in iced water. Error bars represent the standard deviation for five determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

The lower water holding capacity in gels with lower NaCl content might be associated with a lower content of polar or charge residues. This would lead to the lower water-protein interaction (Ramirez *et al*., 2000). Therefore, salt level used for gel preparation of white shrimp meat affected the water holding capacity of gel.

1.1.3 Color

L, a** and *b**-values of Pacific white shrimp gels with different NaCl levels are shown in Table 6. The increases in *a** and *b** - values were found in gels added with NaCl greater than 2.5% NaCl ($P<0.05$). This was possibly due to the greater denaturation of shrimp muscle protein associated with pigment, such as astaxanthin. Carotenoids including astaxanthin and canthaxanthin are the major pigments in shrimp muscle (Simpson, 1982; Okada *et al*., 1998; Armenta-Lopez *et al.,* 2002). As a consequence, color of those pigments could become dominant with increasing NaCl. From the result, salt concentration had no marked impact on *L**-value of shrimp meat gel.

Table 6 Expressible moisture content and color of gels from Pacific white shrimp meat containing different NaCl levels (2-4%)

NaCl levels (%)	Expressible moisture content (%)	*L**	*a**	*b**
2	23.97±0.12a	79.30±0.39ab	15.80±0.35d	17.03±0.27a
2.5	22.64±0.15b	78.71±0.37ac	16.28±0.20c	18.63±0.25a
3	22.57±0.21bc	79.01±0.22bc	16.76±0.19b	18.23±0.24b
3.5	22.41±0.38bc	78.54±0.19a	16.99±0.19ab	18.14±0.12b
4	21.30±0.14c	79.44±0.35a	17.10±1.81a	18.18±0.18b

To prepare the gel, sols were heated at 90°C for 20 min, followed by cooling in iced water.

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

Mean ±SD from five determinations.

1.1.4 TCA-soluble peptide content

TCA-soluble peptide content in Pacific white shrimp gels indicated the occurrence of autolytic degradation of muscle protein (Figure 17). TCA-soluble peptide content of shrimp meat gels decreased as the amount of NaCl increased ($P<0.05$). NaCl might inhibit the autolysis in muscle due to the denaturation of proteases at high salt content. Salt can be a powerful inhibitor for most of the proteases, which release large fragments from protein, in particular cathepsin D and cathepsin H (Martin *et al*., 1997). Cathepsin activity in cured ham decreased upon salt addition (Sarraga *et al.,* 1989). Toldra *et al* (1992) found that salt (60 g/l) showed a powerful inhibitory effect in model cured meat systems, especially on cathepsin D and aminopeptidase activities where less than 13% of the original activity was obtained. Cathepsin D could have some activity in the lowest pH range prevailing postmortem in some fish (Kolodzieska and Sikorski, 1996). Generally, cathepsin has been regarded as most probably responsible for muscle autolysis and softening in chum salmon (Yamashita and Konagaya, 1990). Jiang *et al* (1997) reported that the loss of cathepsin B and L in mackerel surimi after grinding with 2.5% NaCl was due to the denaturation of enzymes during grinding. Gomez-Guillen and Batista (1997) reported that cathepsin D-like enzyme in sardine (*Sardina pilchardus*) had an optimum pH at 3.2 and its activity was strongly inhibited by 6% NaCl (~1 M). However, a thermostable proteinase in salted anchovy muscle was still active and was able to degrade myofibrillar proteins in commercial salted fillets containing 16-17% NaCl (Ishida *et al*., 1994). Furthermore, protease activity in the crude extract of the whole digestive tract from the brown shrimp *(Penneus californiensis)* had the maximum activity between 0 and 0.5 M NaCl but 50% activity was retained at 2 M NaCl (Vega-Villasante *et al*., 1995).

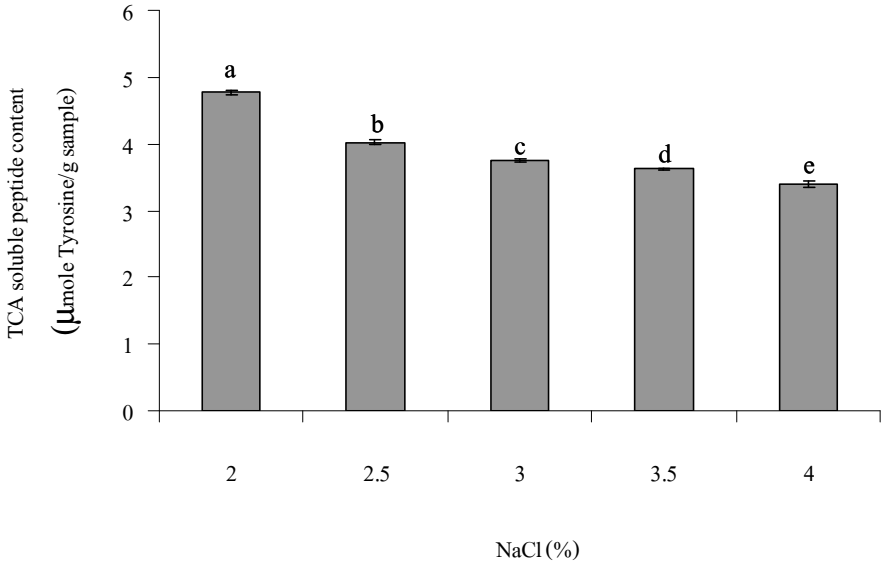

Figure 17 TCA-soluble peptide content of gels from Pacific white shrimp meat containing different NaCl levels (2-4%). Sols were heated at 90°C for 20 min, followed by cooling in iced water. Error bars represent the standard deviation for triplicate determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

1.1.5 Protein patterns

Protein patterns of Pacific white shrimp gels are shown in Figure 18. Myosin heavy chain (MHC) band intensity increased slightly when the levels of NaCl increased. No marked differences in MHC band intensity were observed in gels added with 3 and 3.5% NaCl. However, similar actin band intensity was observed in gels with all NaCl levels used. MHC appears to be responsible for the gelling properties of myofibrillar protein from rabbit and Alaska pollack (Ishioroshi *et al*., 1980; Namakura *et al*., 1985). Solubilization of muscle proteins under high ionic strength involves two events: the depolymerization of the thick filament backbone, and the subsequent dissociation of the myosin heads from the actin filaments (Parsons and Knight 1990). From the result, salt at high concentration (3.5-4%) might inactivated endogenous TGase at some extents, leading to less polymerization. On the other hand, high salt content possibly inactivate endogenous proteases at some degree, resulting in the lower degradation of MHC. Therefore, salt at 2.5% would be an appropriate level for preparation of Pacific white shrimp gel

with high gel strength. The increase in MHC band intensity was in agreement with the lowered TCA-soluble peptide content found in gel added with higher salt content.

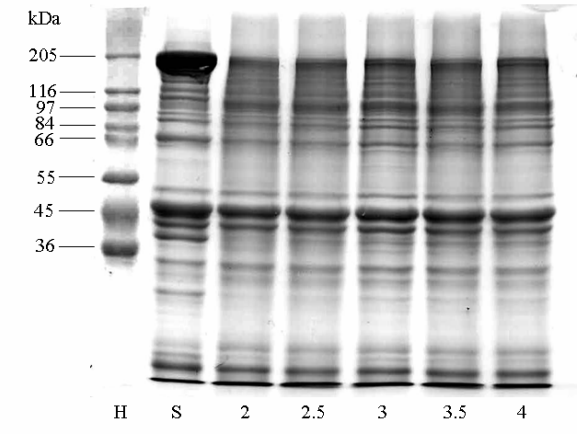

Figure 18 Protein patterns of gels from Pacific white shrimp meat at different NaCl levels (2-4%). Sols were heated at 90°C for 20 min, followed by cooling in iced water. M: High molecular weight marker; S: shrimp mince. Numbers designate NaCl level (%).

1.1.6 Microstructure

Microstructures of Pacific white shrimp gels containing different NaCl levels are illustrated in Figure 19. Gels added with NaCl at all levels had the fine fibrous network. Kubota *et al* (2006) studied on the internal structure of walleye pollack surimi gel containing various salt concentrations (2-5%) and found that porous structures were observed in gel containing 1 to 2%, but the structure became smoother with fewer and smaller pores with increasing salt concentrations. Gomez-Guillen and Montero (1996) found that the apparent matrix of sardine mince gel made with lowered salt concentration (1.5% NaCl) was more irregular than that with high salt concentration (2.5%). With sufficient amount of salt, the extensive solubilization of myofibrillar proteins could favor the monomer form of myosin (Burgarella wt al., 1985). Min and Lee (2004) reported that the unfolding network structure of surimi produced from mechanically debone chicken meat began to appear at NaCl concentration greater than 2%. From the results, gel network might be formed predominantly between solubilized and unfolded MHC in the presence of NaCl. Subsequent aggregation attained after heating at 90°C appears to result from additional S-S bonds and hydrophobic interaction (Roussel and Chelftel, 1990). Since gel

containing 2.5% NaCl was found to render the finest and most ordered network as well as yield the gel with the highest gel strength, it was used for further study. Additionally, gel containing higher salt content became more salty.

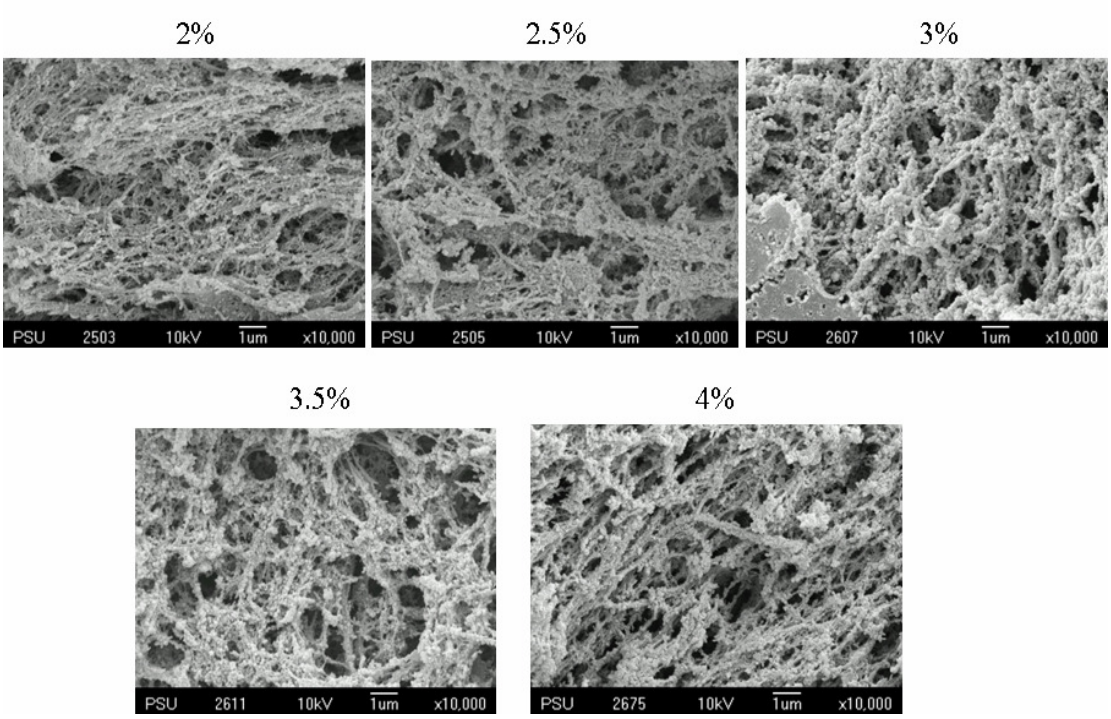

Figure 19 Microstructures of gels from Pacific white shrimp meat containing different NaCl levels. Sols were heated at 90°C for 20 min, followed by cooling in iced water. Numbers designate NaCl level (%).

1.2. Effect of pyrophosphate in combination with magnesium chloride and/or calcium chloride on gel properties of Pacific white shrimp meat

1.2.1 Breaking force and deformation

Breaking force and deformation of Pacific white shrimp gels with different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$ were depicted in Figure 20. With the addition of 150 mmole$CaCl_2$/kg, breaking force of Pacific white shrimp gel increased by 29.82%**,** compared with the control gel (added with 2.5%NaCl). $CaCl_2$ might enhance the gelation of protein by the formation of Ca^{2+}- bridge between protein molecules. The possible electrostatic depression by Ca^{2+} would facilitate hydrophobic interaction of protein molecules through non-polar amino acids (Xiong and Brekke, 1990). In the absence of $MgCl_2$, Pacific white shrimp gels

added with 5 mmolePP/kg had the increase in breaking force by 113.24%, compared with the control (gel added with 2.5% NaCl). However, the decrease in breaking force was observed when PP at 10 mmol/kg was used, compared with PP at 5 mmol/kg. PP might form complex with calcium ions to a greater extent with increasing amount added. As a consequence, it could impede the activity of endogenous TGase, a Ca^{2+}- dependent enzyme. PP caused the actomyosin complex to be dissociated into actin and myosin (Tarigai and Konno, 1996). When phosphate at optimal concentration was added, actomyosin was dissociated and strong gel network was formed (Ellinger, 1975). Myosin is the most responsible protein for heat-induce gel forming ability of muscle protein (Yasui *et al*., 1980; Samejima *et al.,* 1981). It is widely believed that myosin becomes stable upon binding to F-actin, or that F-actin protects myosin from denaturation. Myosin in myofibrils would be a typical case of the most stabilized form (Yoshioka *et al*, 2002). The actin-myosin complex (actomyosin) specifically dissociates with ATP, pyrophosphate and a few polyanions (Ociai and Chow, 2000). ATP is a strong dissociating reagent (Yoshioka *et al*, 2002). A greater efficacy of PP in dissociation of kuruma prawn actomyosin was observed when compared with ATP (Benjakul *et al.*, 2007). Accelerated inactivation of Mg-ATPase upon the addition of PP indicated that PP diminished the protective effect of F-actin by dissociating myosin from F-actin. This accelerating effect of PP on myofibril denaturation was reported with fish myofibrils (Matsukawa and Arai, 1991). Offer and Knight (1988) explained the action of phosphates on myofibrils and water holding capacity of meat in 3 ways. Firstly, phosphates are good buffers, which may assist in the depolymerization of thick filaments and increase water uptake and retention. Secondly, in the presence of Mg^{2+}, pyrophosphate and triphosphate bind to the myosin molecule. Pyrophosphate acts as an analog to ATP and binds to the myosin heads, thus promoting the dissociation of actomyosin. Thirdly, polyphosphate can bind to the myosin tails and promote dissociation of the myosin filaments to myosin molecules. However, the excessive charge modification of proteins by phosphates might induce the protein repulsion, leading to the less aggregation with poor gel properties at the higher levels used (10mmolePP/kg). Julavittayanukul *et al* (2006) reported that PP at a level of 0.025% would be sufficient to dissociate the actomyosin, and a sufficient amount of $CaCl_2$ (50 mmol/kg) was used for TGase activity. Calcium ion remaining after complexing with PP was most likely involved in the setting induced by TGase.

From the result, breaking force of the gels decreased continuously when $MgCl_2$ concentration increased at both PP levels used ($P<0.05$). It was reported that Mg^{2+} is required for dissociations of actin-myosin complex (Ochiai and Chow, 2000). However, dicationic salts negatively affect the gel texture at high ionic-strength (Seman *et al.,* 1980; Hand *et al*., 1982). Replacing 2.8% NaCl with $MgCl_2$ at an equal ionic strength decreased firmness of mechanically deboned turkey frankfurters (Hand *et al*., 1982). Generally, myosin is rich in negatively charged amino acids (glutamine and aspartic acids) and poor in positive charged ones (histidine, lysine and arginine) and thus is negatively charged under physiological ionic strength and pH. The isoelectric point in the absence of divalent cations (Mg^{2+}, Ca^{2+}) is 5.4 , but in their presence, the isoelectric point shifts to an alkaline side as a result of their binding to the molecule (Ochi and Chow, 2000). Magnesium chloride was added to shift the isoelectric point of myosin to make it more soluble at neural pH (Chang *et al*., 2001). However, magnesium ions inhibited myosin solubility unless the muscle was first exposed to the pyrophosphate and sodium chloride. From the result, magnesium ions at higher levels might inhibit the solubilization of actomyosin to a greater extent. Its effect was also minimized in the presence of greater content of PP (10 mmol/kg). Therefore, $MgCl_2$ was shown to exhibit the detrimental effect on gel-forming ability of Pacific white shrimp. It is possible that magnesium ions are inhibitory to solubilization of actomyosin. Actomyosin must be dissociated into its component proteins before magnesium becomes an effective solubilizing agent at that concentration (Chang *et al*., 2001).

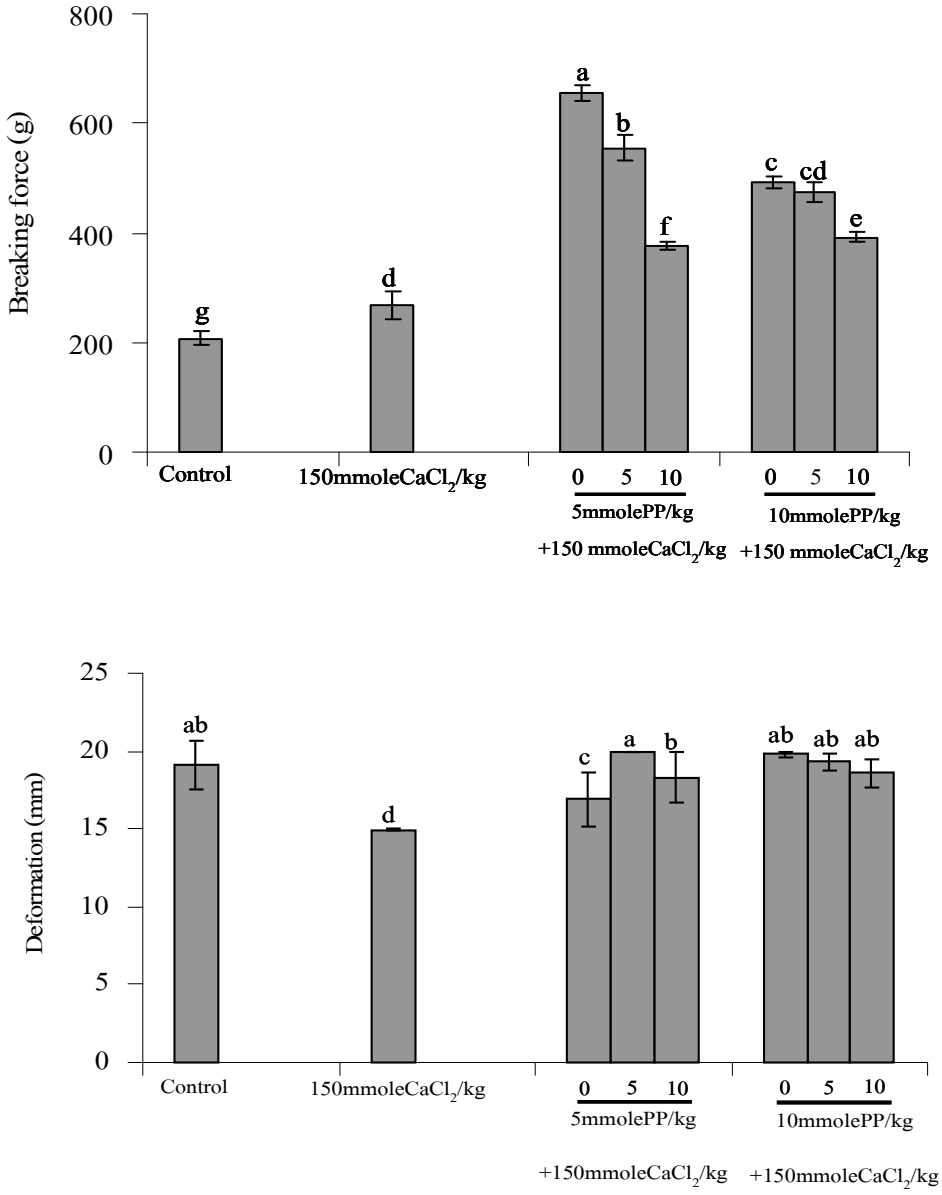

Figure 20 Breaking force and deformation of gels from Pacific white shrimp meat at different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$. Sols containing 2.5% NaCl without and with different additives were heated at 90°C for 20 min, followed by cooling in iced water. Control: gel without additives. Error bars represent the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$). Numbers designate $MgCl_2$ concentration (mmol/kg).

1.2.2 Expressible moisture content

Expressible moisture content of Pacific white shrimp gels added with different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$ are shown in Table 7. The lowest expressible moisture content of Pacific white shrimp gel was found when 5 mmolePP/kg was added, suggesting the high water holding capacity of gel matrix. Phosphate anion act as polyelectrolytic to increase ionic strength, resulting in increased water holding capacity by direct binding of water to the phosphate anion and the repulsion of protein groups due to the predominance of negative charges on the protein groups. This repulsing effect opens up protein structure, and increases the number of binding sites available for water, which allows for more water to be contained in the meat (Xiong, 2005). The effectiveness of phosphates on water retention properties of meat products depends on the type of phosphate, the amount used, and the specific food product. It has also been concluded that a combination of phosphates and NaCl increases moisture retention in meat more than NaCl alone (Lindsay, 1996). When PP at the levels of 5 and 10 mmol/kg was used, the increase in $MgCl_2$ levels had no effect on the expressible moisture contents ($P<0.05$). However, with the addition of 10 mmolePP/kg**,** the slight increase in expressible moisture content was observed. The higher PP content might lower Ca^{2+} requires for TGase activity. This led to the lower cross-linking of proteins, in which the order network can be formed and imbibe more water. In the presence of divalent ions, both $CaCl_2$ or $MgCl_2$, the expressible moisture content increased. This might be associated with increased ionic strength, leading to the 'salting out' effect. The higher expressible moisture content was in agreement with the lower breaking force (Figure 20).

1.2.3 Color

L, a** and *b**-values of Pacific white shrimp gels added with different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$ are shown in Tables 7. *L**- value increased as $CaCl_2$ or PP were added. On the other hand, *a** and *b**- values decreased with the addition of PP, $MgCl_2$ or $CaCl_2$ ($P<0.05$). Generally, the lowered *a** and *b**- values were in accordance with the increase in *L**-values.

Table 7 Expressible moisture content and color of gels from Pacific white shrimp meat at different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$

Treatment	Expressible moisture content (%)	Color		
		*L**	*a**	*b**
2.5%NaCl	24.72±0.13a	68.41±0.32e	25.49±0.34a	21.84±0.25a
150 mmole$CaCl_2$/kg	23.53±0.78ab	76.54± 0.14c	21.38±0.06b	19.29± 0.25b
5 mmolePP/kg+150mmole$CaCl_2$/kg	20.23±1.06d	75.53±1.03d	20.07±0.40c	18.77±0.64bc
5 mmolePP/kg +5 mmole$MgCl_2$ /kg+150mmole$CaCl_2$/kg	21.58±0.99c	77.24±0.37b	19.55±0.66d	18.69±0.72c
5 mmolePP/kg +10 mmole$MgCl_2$/kg+150mmole$CaCl_2$/kg	21.57±0.71c	77.36±0.49b	18.79±0.27e	17.69±0.21d
10 mmolePP/kg+150 mmole$CaCl_2$/kg+150mmole$CaCl_2$/kg	23.34±0.93ab	76.78±0.48bc	19.55±0.40d	18.92±0.33bc
10 mmolePP/kg +5 mmole$MgCl_2$/kg+150mmole$CaCl_2$/kg	23.91±0.76ab	78.87±0.18a	17.99±0.27f	17.74±0.27d
10 mmolePP/kg +10 mmole$MgCl_2$/kg+150mmole$CaCl_2$/kg	22.04±1.30bc	79.23±0.26a	17.75±0.24f	17.54±0.27d

To prepare the gel, sols containing 2.5% NaCl without and with different additives were heated at 90°C for 20 min followed by cooling in iced water.

Control: gel without additives.

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

Mean ± SD from five determinations.

1.2.4 TCA-soluble peptide content

TCA-soluble peptide content in Pacific white shrimp gels added with different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$ are depicted in Figure 21. Regardless of $MgCl_2$ concentration, TCA-soluble peptide content of Pacific white shrimp gels decreased when PP was added. It was presumed that PP might chelate the ions required for endogenous proteinases, leading to the lower autolysis. Various phosphates are the most popular chelating agent used in food (Lindsey, 1996). The polyphosphates are excellent sequestering agent for calcium, magnesium and iron (Ellinger, 1997). Most metalloproteinases have zinc as the active metal in the catalytic site (Garcia-Carreno and Hernandez-Cortes, 2000). Metalloproteinases are inhibited by metal chelators that remove the metal ion from the active site (Garcia-Carreno and Hernandez-Cortes, 2000). Konno and Fukazawa (1993) found that the addition of PP along with other inhibitors can prevent autolysis in the processing of squid muscle. Bracho and Haard (1995) reported two metalloproteinases (47 and 98 kDa) in the rockfish muscle. The activity of these enzymes was activated by calcium and inhibited by calcium chelators. Furthermore, the proteolytic activity of calpain, calcium activated proteinase, is manifested generally in hydrolysis of fish muscle. The multiple forms of calpain isolated from American lobster are capable of hydrolyzing all myofibrillar proteins (Mykles and Skinner, 1986). The m-calpain from grass shrimp muscle was composed of two identical subunits of 80 kDa. (Wang *et al*., 1993). From the result, slightly lower TCA-soluble peptide content was found in gel added with 150 mmole$CaCl_2$/kg. Sufficient amount of calcium ions might induce the cross-links of protein mediated by endogenous TGase. The cross-links formed might be more resistant to hydrolysis by the endogenous proteases. The impact of $MgCl_2$ on TCA-soluble peptide content varied with the PP level used.

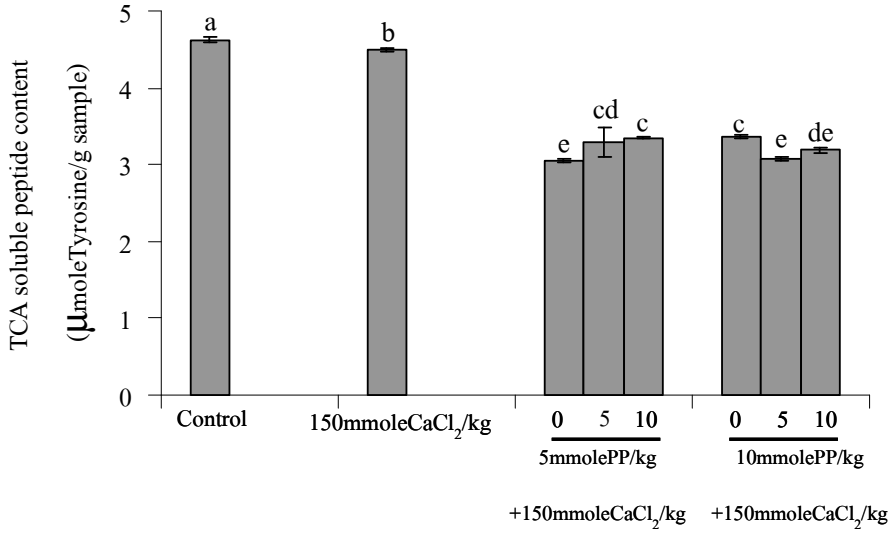

Figure 21 TCA-soluble peptide content of gels from Pacific white shrimp meat at different concentrations of PP in combination with $MgCl_2$ and/or $CaCl_2$. Sols containing 2.5% NaCl without and with different additives were heated at 90°C for 20 min, followed by cooling in iced water. Control: gel without additives. Error bars represent the standard deviation from triplicate determinations. The different letters on the bars indicate the significant differences ($P<0.05$). Numbers designate $MgCl_2$ concentration (mmol/kg).

1.2.5 Protein pattern

The protein patterns of Pacific white shrimp gels without and with addition of PP in combination with $MgCl_2$ and/or $CaCl_2$ are depicted in Figure 22. No marked differences in MHC band intensity were noticeable among all gels with and without $CaCl_2$ addition (Figure 22). The band intensity was decreased in gel, compared to that found in shrimp mince. This indicated the polymerization of MHC, particularly via non-disulfide covalent bond formation took place during gelling process. Furthermore, no changes in actin band intensity were observed in all gels. The increases in MHC band intensity were observed when a higher level of PP (10mmol/kg) were used, regardless of $MgCl_2$ added, compared with 5 mmolePP/kg. Phosphates might chelate the calcium ions required for endogenous TGase, resulting in lower amount of calcium ion. As a consequence, polymerization of MHC added with high PP content was reduced as shown by more retained MHC band intensity (Julavittayanukul *et al*., 2006). The result was concomitant with

lowered breaking force and deformation when the gel was added with 10 mmolePP/kg (Figure 20). $MgCl_2$ concentrations had negligible impact on protein patterns of the resulting gels. PP and $MgCl_2$ at the appropriate concentrations could enhance the dissociations of actomyosin complex effectively as evidenced by the increased band intensity of soluble denatured actin from kuruma prawn muscle (Benjakul *et al.*, 2007). However, PP is strongly bound with myosin, which increases the negative charge on the protein. The legend-induced electrostatic alteration of myosin results in the increased electrostatic repulsion between protein molecules, thereby reducing protein – protein interactions (Nauss *et al*., 1969).

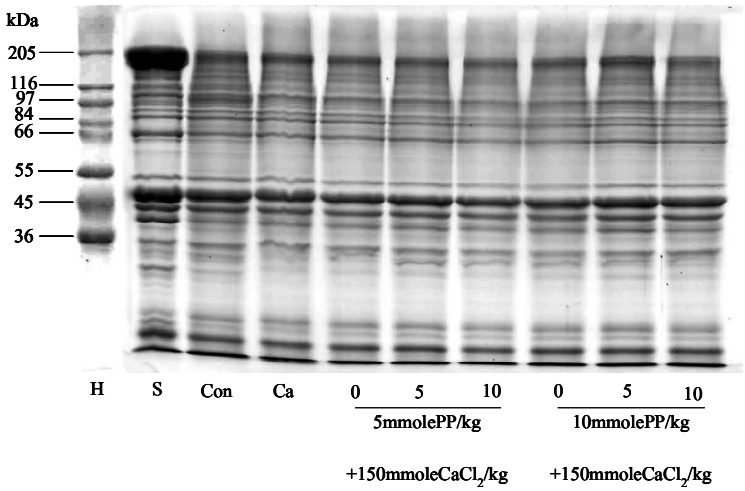

Figure 22 Protein patterns of gels from Pacific white shrimp meat at different concentrations of PP in combination with $MgCl_2$ and /or $CaCl_2$. Sols containing 2.5% NaCl without and with different additives were heated at 90°C for 20 min, followed by cooling in iced water. H: High molecular weight marker; S: shrimp mince; Con: 2.5% NaCl; Ca: 2.5%NaCl+150mmole$CaCl_2$/kg. Numbers designate concentration of $MgCl_2$ (mmol/kg).

1.2.6 Microstructure

Microstructures of Pacific white shrimp gels without and with addition of PP in combination with $MgCl_2$ and/or $CaCl_2$ are shown in Figure 23. Gel with a finer network was formed when added with 5 mmolePP/kg and 5 mmole$MgCl_2$/kg together with 150 mmole$CaCl_2$/kg. The gel with the largest filaments with the large void was formed when $CaCl_2$ at

150 mmol/kg was added. The finer filaments with small voids were obtained when PP at 5 mmol/kg was added incombination with 150 mmole$CaCl_2$/kg. From the result, it indicated that the addition PP in combination with $MgCl_2$ together with 150 mmole$CaCl_2$/kg yielded Pacific white shrimp gel with ordered and fine network.

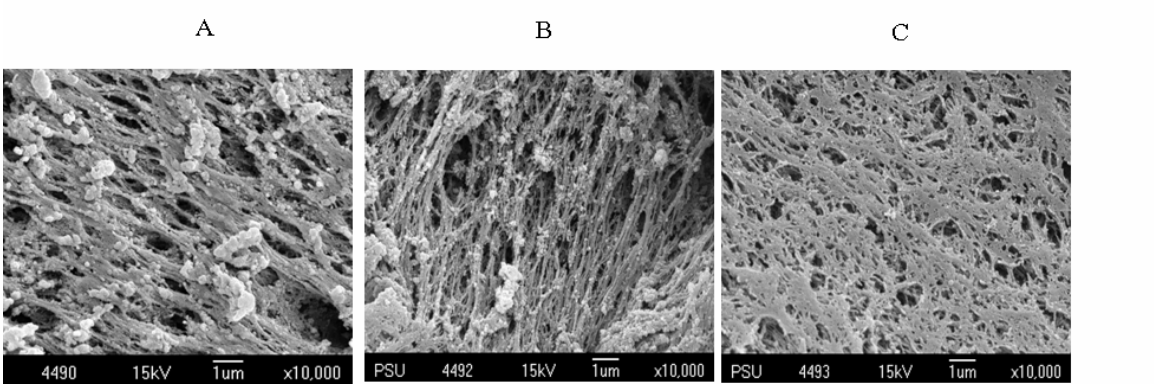

Figure 23 Microstructures of gels from Pacific white shrimp meat containing 2.5% NaCl added with 150mmole$CaCl_2$/kg (a) 150 mmole$CaCl_2$/kg + 5 mmolePP/kg (b) and 150 mmole$CaCl_2$/kg + 5 mmolePP/kg + 5 mmole$MgCl_2$/kg (c). Sols were heated at 90°C for 20 min, followed by cooling in iced water.

2. Effect of some protein additives on gel properties of Pacific white shrimp meat

2.1 Breaking force and deformation

Effects of EW, WPC and BPP at various concentrations (0, 0.5, 1, 2 and 3% (w/w)) on properties of Pacific white shrimp gels prepared by one-step and two-step heating are depicted in Figures 24 and 25, respectively. Generally, two-step heated gel showed the lower breaking force than did one-step heated counterpart. This confirmed that "modori" occurred at 40°C with Pacific white shrimp gel reported by Thammatinna *et al* (2007) Modori for fish surimi such as Pacific whiting, Atlantic menhaden, and Alaska pollock occurs at 60-65°C (Chang-Lee *et al*., 1990; Lanier 1986; Lee, 1986). Although two-step cooking is widely used to enhance the gelation of surimi from some fish species, pre-incubation at 40°C directly induced the weakening of Pacific white shrimp gel. Gel weakening has been ascribed to the degradation of myosin by endogenous proteases (An *et al*., 1994; Jiang *et al*., 1996). Therefore, the degradation of

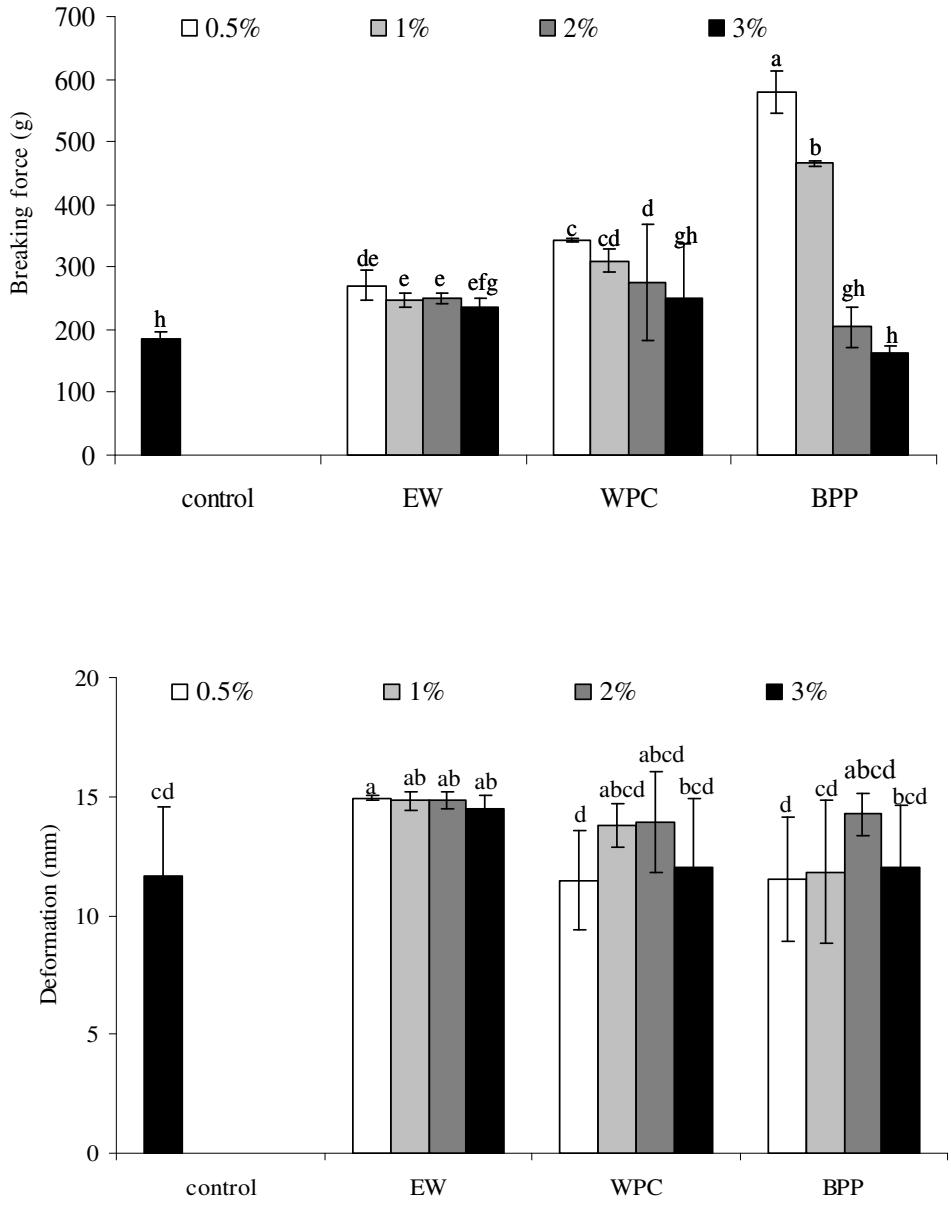

Figure 24 Breaking force and deformation of one-step heated gels from Pacific white shrimp meat added with different types and concentrations of protein additives. Error bars represent the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

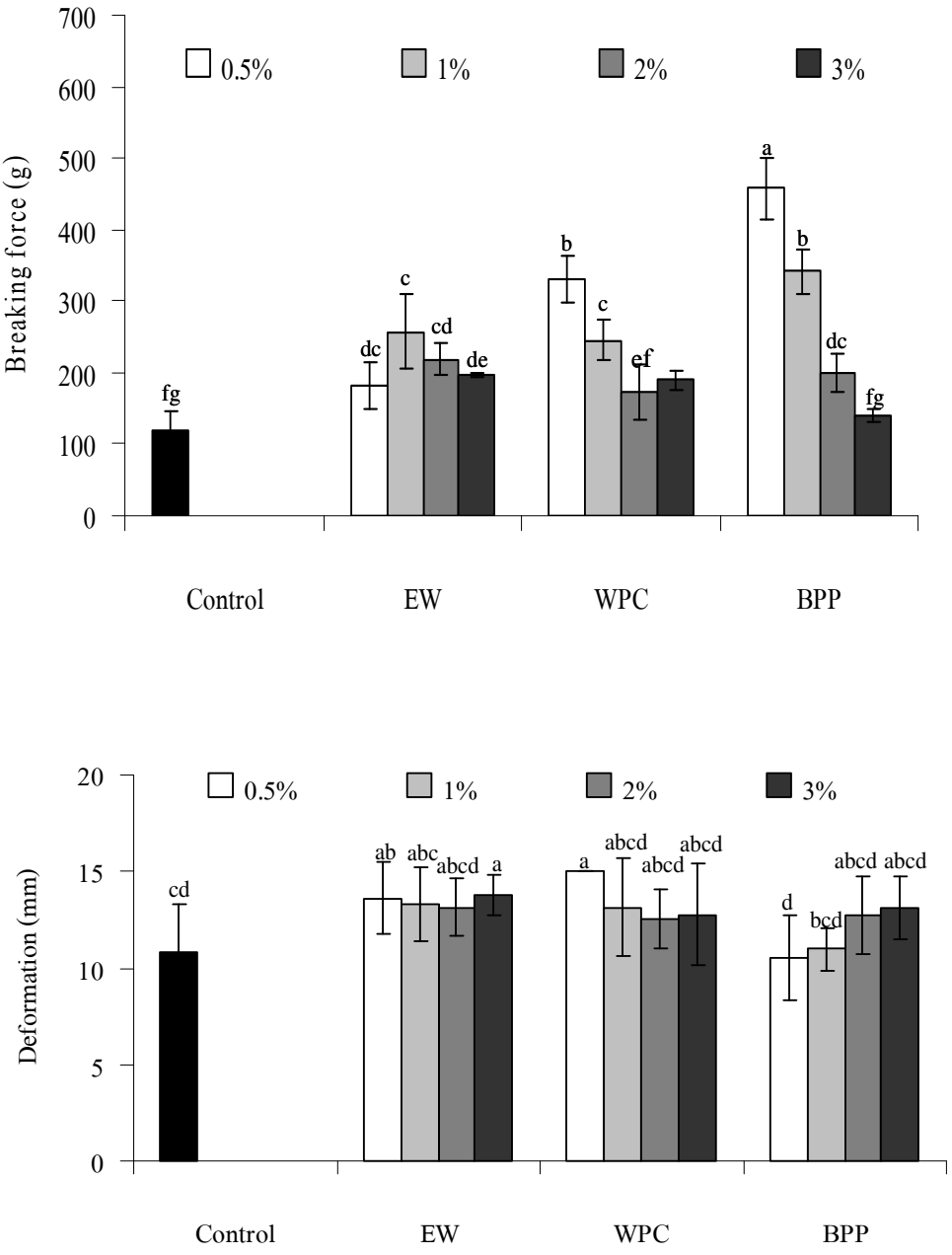

Figure 25 Breaking force and deformation of two-step heated gels from Pacific white shrimp meat added with different types and concentrations of protein additives. Error bars represent the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

myofibrillar proteins was most likely responsible for the gel-weakening of Pacific white shrimp gel pre-incubated at this temperature. Lou *et al* (2000) also reported that pre-incubation of paddlefish (*Polyodon spathula*) surimi at 40°C for 30 min produced gels with much lower strength compared to the control (cooked at 90°C for 30 min).

At the same level used, BPP was more effective in increasing breaking force of Pacific white shrimp gel than were WPC and EW, regardless of heating conditions. In general, breaking force decreased as the levels of all protein additives increased ($P<0.05$). For one-step heated gel, breaking force of gel added with EW, WPC and BPP at 0.5% increased by 45.85%, 85.09% and 211.95%, respectively compared with the control gel. The marked decreases in breaking force with increasing amounts added were possibly due to the diluted myofibrillar protein and the interference of these non-fish proteins in myofibrillar gelation (Chung and Lee, 1990, 1991). However, amounts of protein additive had no impact on deformation of the resulting gels ($P>0.05$). Two-step heated gel showed similar results, except for gel added with EW, in which the highest breaking force was found when EW at 1% was added. The addition of BPP and WPC at the level of 0.5% increased breaking force of two-step heated gel by 283.26% and 176.81%, respectively. The addition of EW, WPC and BPP also had no impact on deformation of gels prepared with one-step heating ($P>0.05$).

The effectiveness of BPP may be attributed to its proteinase inhibitors including α_2-macroglobulin (α_2M) and kininogen (Hamann *et al*., 1990; Morrissey *et al*., 1993). Plasma also has protein cross-linking activity from both pig plasma transglutaminase (PTGase) and α_2M (Seymour *et al*., 1997). This could contribute to the strengthening of gel by myosin and/or fibrinogen cross-linking and could also reduce the availability of myosin as a substrate for proteinase action (Lorand, 1983; Seki *et al*., 1990; Kimura *et al*., 1991; Sakamoto *et al*., 1995). Additionally, fibrinogen and serum albumin underwent aggregation synergistically with myosin during heating (Foegeding *et a*l., 1986). EW contains several proteinase inhibitors, namely ovomucoid, ovoinhibitor, ovomacroglobulin, which exhibit inhibitory activity against serine proteinase (Nakamure and Doi, 2000). Cystatin, a cysteine proteinase inhibitor, was also found in egg white (Anastasi *et al*., 1983). WPC was more effective in inhibiting cysteine proteinase rather than serine proteinase (Weerasinghe *et al.*, 1996). Benjakul *et al* (2004) observed that porcine plasma protein (PPP) was effective in increasing breaking force and deformation of kamaboko

gels set at 40°C for 30 min and heated at 90°C for 20 min. Chen (2000) reported that the addition of plasma protein concentrate increased gel strength of kamaboko gel from horse mackerel surimi when the addition level was 2%. Benjakul *et al* (2004) reported that the addition of BPP and EW up to 3% increased gelling properties of lizard surimi regardless of heating condition. Lee *et al* (2000) reported that the egg white displayed protease inhibitory effects for preventing gel softening of surimi gels. However, egg white forms the soft gel after heating. The over uses of egg white may destroy the original protein network of surimi and result in the undesirable texture in heated surimi products. These results indicated that non muscle protein, BPP WPC and EW, should be added at an appropriate level to avoid the interfering effect on the gelation of myofibrillar proteins.

2.2 Expressible moisture content

Expressible moisture content of Pacific white shrimp gels added with EW, WPC and BPP at various concentrations (0, 0.5, 1, 2 and 3%) and prepared by one-step and two-step heating are shown in Tables 7 and 8, respectively. For one-step heated gel, expressible moisture content decreased as the concentration of protein additives increased ($P<0.05$). For-two step heated gel, expressible moisture content decreased when the concentration of BPP increase ($P<0.05$). Levels of EW and WPC had no effect on expressible content of resulting gels ($P>0.05$). Decrease in expressible moisture content indicated an increase in water-holding capacity of the gel. 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 (Niwa *et al*., 1988). Gomez-Guillen and Montero (1996) also concluded that the addition of egg white and soy protein at a level of 2% increased water-holding capacity of sardine mince gel.

2.3 Color

L, a** and *b**- values of Pacific white shrimp gels added with EW, WPC and BPP at various concentrations (0, 0.5, 1, 2 and 3%) and prepared by one-step and two-step heating are shown in Tables 8 and 9, respectively. The addition of all additives resulted in the changes in color values differently, depending on types of protein additives and heating

conditions. For one-step heated gels, all protein additives resulted in the increase in *b**-value, indicating the increases in yellowness. No much changes in *a**- value were found in the gel added with EW and WPC at all levels used ($P>0.05$). However, the decrease in *a**- value was found with the gel added with BPP as the concentration increased. The increase in *L**-value was observed when all protein additives were added, regardless of levels. Nevertheless, Benjakul *et al*. (2001) found that the lower whiteness of gel from bigeye snapper surimi was observed with plasma protein addition, because some hemoglobin as well as other pigments with a pale straw color were retained in the plasma. For the shrimp meat, protein additives might lower the pigment concentration in shrimp muscle. As a result, the increased whiteness was found in shrimp meat gel added with protein additives. Reppon and Babbitt (1993) also found that *L**-value of arrowtooth flounder surimi decreased with addition of BPP. Chen (2000) reported that addition of liquid egg white to kamaboko of horse mackerel surimi increased lightness (*L**) and whiteness but reduced greenness (-*a**) and yellowness (+*b**). Similar result was found with two-step heated gel. In the absence of protein additives, greater *L**, *a** and *b** - values were observed in two-step heated gel, compared with one-step heated gel. Two-step heating might cause the greater denaturation of proteins, leading to the whiter gels. Also, proteins associated with the pigments might undergo denaturation, resulting in more exposure of pigment. This was evidenced by the increase in *a** and *b**-values.

Table 8 Expressible moisture content of one-step heated gels from Pacific white shrimp meat added with different types and concentrations of protein additives

Protein additives	Level (%)	Expressible moisture conten (%)	color		
			*L**	*a**	*b**
EW	0	33.70±0.64a	74.83±0.23d	18.34±0.58b	18.11±0.68b
	0.5	29.76±0.23ab	80.31±0.33ab	19.65±0.09a	21.75±0.14a
	1	29.75±0.23ab	80.17±0.08b	19.57±0.17a	21.74±0.41a
	2	28.22±4.84b	80.61±0.31a	19.45±0.33a	21.53±0.45a
	3	27.05±0.29b	80.47±0.24ab	19.23±0.09a	21.45±0.20a
WPC	0	33.70±1.81ab	74.83±0.23c	18.34±0.58a	18.11±0.68b
	0.5	35.51±0.35ab	78.90±0.73b	19.44±0.56b	18.86±0.86a
	1	38.11±0.35a	79.21±0.66b	20.18±0.58a	22.06±1.01a
	2	32.50±3.57b	79.98±0.53a	19.30±0.36b	22.15±0.26a
	3	25.80±4.18c	80.10±0.29a	19.11±0.20b	21.49±0.15a
BPP	0	33.70±1.8a	74.83±0.23d	18.34±0.58a	18.11±0.68c
	0.5	30.67±2.50a	77.79±0.83a	18.24±0.84a	20.32±1.93b
	1	31.94±0.84a	78.21±0.30a	16.42±0.59c	21.98±1.14b
	2	23.42±3.77b	78.19±0.93a	16.25±0.38c	23.96±0.18a
	3	24.95±0.71b	76.67±0.60b	16.12±0.39c	24.63±0.40a

Different letters in the same column under the same protein additive indicate significant difference ($P<0.05$).

Mean ± SD from five determinations.

Table 9 Expressible moisture content of two-step heated gels from Pacific white shrimp meat added with different types and concentrations of protein additives

Protein additives	Level (%)	Expressible moisture (%)	Color		
			L^*	a^*	b^*
EW	0	29.95±0.45	80.06±0.51c	19.94±0.46a	21.2±0.17a
	0.5	30.45±1.68	80.12±0.46c	19.40±0.46b	20.66±0.20b
	1	30.58±1.89	80.78±0.44b	18.73±0.34c	20.46±0.19bc
	2	30.74±2.70	81.00±0.25b	18.79±0.15ce	20.24±0.17c
	3	26.65±0.44	82.23±0.21a	18.01±0.19d	19.51±0.13d
WPC	0	29.95±0.45	80.06±0.51a	19.94±0.46b	21.20±0.170c
	0.5	28.987±3.50	79.03±0.26b	20.66±0.23a	21.93±0.20a
	1	31.31±1.189	79.83±0.12a	19.69±0.16b	20.79±0.22d
	2	31.93±1.57	79.74±0.15a	19.08±0.36c	21.52±0.31b
	3	31.20±2.77	79.92±0.17a	18.78±0.27d	20.728±0.17d
BPP	0	29.95±0.45a	80.06±0.51a	19.93±0.46a	21.20±0.17d
	0.5	32.505±2.94a	79.57±0.15a	18.16±0.06b	21.914±0.08c
	1	25.48±0.84a	78.40±0.21b	17.76±0.16c	21.37±0.14d
	2	22.23±1.88b	77.08±0.37c	17.15±0.20d	22.36±0.33b
	3	30.10±1.87b	76.60±0.10d	15.55±0.06e	23.53±0.16a

Different letters in the same column under the same protein additive indicate significant difference ($P<0.05$).

Mean ± SD from five determinations.

2.4 TCA-soluble peptide content

TCA-soluble peptide content in Pacific white shrimp gels can be used as an indicator of autolytic degradation of shrimp muscle protein (Thammatinna *et al*., 2007). The autolysis was inhibited to a greater extent in one-step heated gel as protein additives levels increased ($P<0.05$) (Figure 26). Nevertheless, no differences in autolysis inhibition were observed in two-step heated gel when protein additives were used at levels ranging from 0.5 to 3%. However, the lower TCA-soluble peptide content was observed with the addition of protein additives, compared with the control. Ayensa *et al* (2002) reported that batters of squid muscle containing BPP exhibited less proteolytic activity than the control, incubated at either 40°C or 65°C. This inhibition was more pronounced when 2% BPP was used. However, with concentrations of more than 2%, it can produce an off-taste. Morrissey *et al* (1993) found that BPP inhibited proteolytic activity better than egg white or potato powder in surimi from Pacific hake (*Merluccius productus*). Rawdkuen and Benajkul (2008) reported that the addition of WPC at a level of 3% might be effective enough to prevent proteolysis in surimi gel of most fish species tested, bigeye snapper (*Priacanthus tayenus*), goatfish (*Mulloidichthys vanicolensis*) and threadfin bream (*Nemipterus bleekeri*), except for the gel from lizardfish (*Saurida tumbil*) surimi. Proteolysis in surimi from some tropical fish can occur during setting at 40°C (Benjakul *et al*., 2003, 2004). From these results, the highest TCA-soluble peptide content was observed in the sample without any protein additives. This was in agreement with the lowest breaking force and deformation of the gels without protein additives (Figures 24 and 25). These reconfirmed that the improved gel strength of Pacific white shrimp gel by the addition of BPP, WPC and EW was associated with the lowered proteolysis caused by endogenous proteinases.

2.5 Protein pattern

Protein patterns of Pacific white shrimp gels added with different types and levels of protein additives and prepared by one-step and two-step heating are shown in Figure 27. The lowest intensity of MHC band was observed in Pacific white shrimp gels without protein additives, regardless of heating condition. However, no changes in actin were observed in all treatments. For both heating conditions, MHC was more retained as the concentration of protein additives increased. MHC band intensity became increased with increasing amount of protein

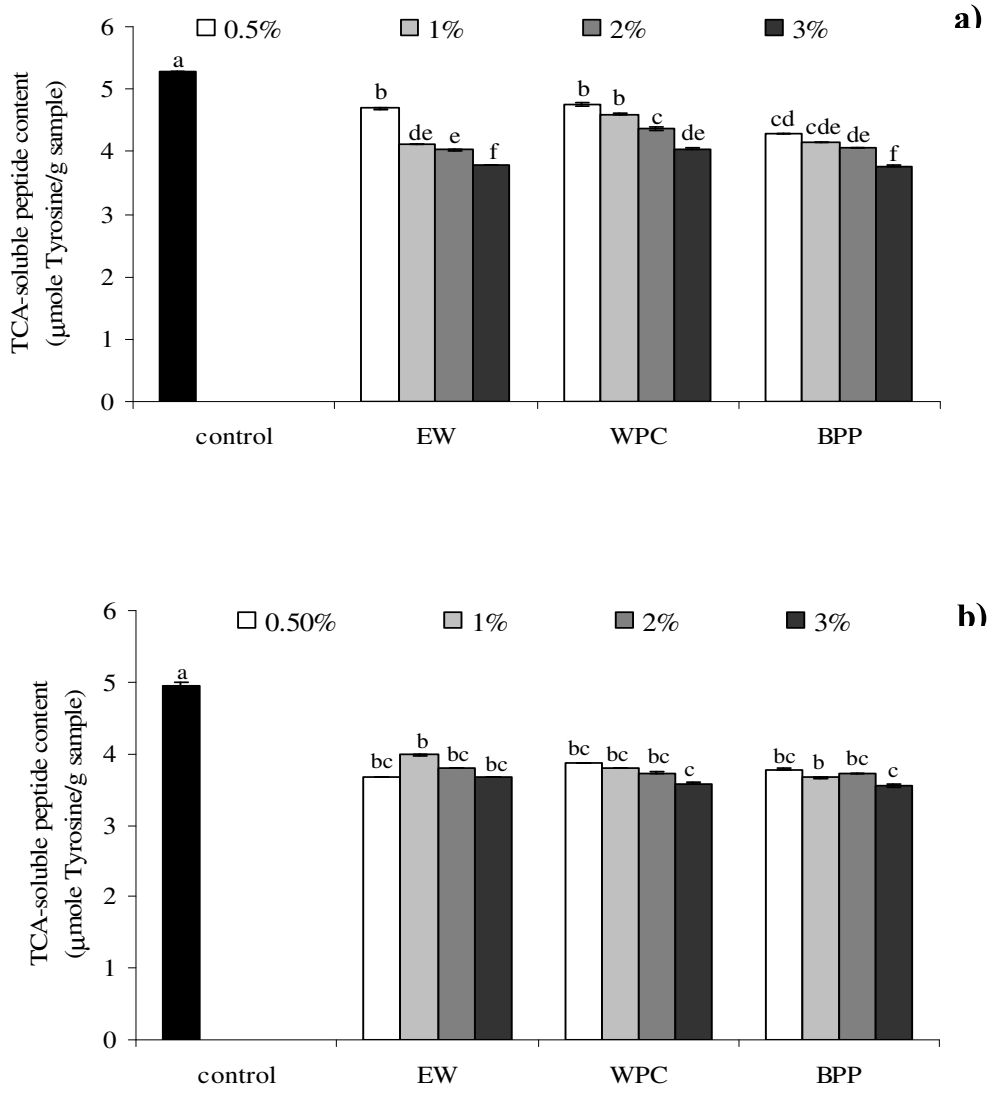

Figure 26 TCA-soluble peptide content of one-step (a) and two-step (b) heated gels from Pacific white shrimp meat added with different types and concentrations of protein additives. Error bars represent the standard deviation from triplicate determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

additives. This might be caused by the inhibitory effect of protein additives on muscle protein degradation. The result indicated that BPP, WPC and EW could inhibit the degradation of MHC to some extent, as evidenced by the more retained MHC. Nevertheless, the efficacy in preventing the degradation of MHC varied with kind of protein additives. Lou *et al* (2000) reported that BPP suppressed the degradation of MHC in paddlefish surimi during incubation at 40°C. BPP enhances gelation mainly by inhibiting endogenous proteases responsible for the degradation of myofibrillar proteins, particularly myosin (Weerasinghe *et al*., 1996). However, they did not exclude the possibility for BPP acting as a gel-forming component, because BPP contains multiple polypeptides which may facilitate the gelation of surimi proteins. BPP contains active transglutaminase which catalyzes the formation of covalent bonds and hence cross-linked proteins might be resistant to proteolysis. Rawdkuen *et al* (2007) reported that chicken plasma protein (CPP) can prevent the degradation of MHC of modori gel (incubated at 70°C for 30 min followed by heating at 90°C for 20 min) from sardine (*Sardinella Gibbosa*) surimi. Rawdkuen and Benjakul (2008) reported that MHC of surimi form bigeye snapper, goatfish, threadfin bream and lizardfish was more retained in the presence of WPC (0-3%). From the result, it was noted that gel added with 0.5% BPP showed the highest breaking force (Figure 24 and 25) but the decrease in breaking force was observed with increasing amount of BPP added. The addition of BPP at higher level might impede the cross-linking between MHC as evidenced by more retained MHC band, though it could inhibit proteolysis at high degree. Therefore, the balance of proteolysis and cross-linking of MHC most likely governed the property of gel.

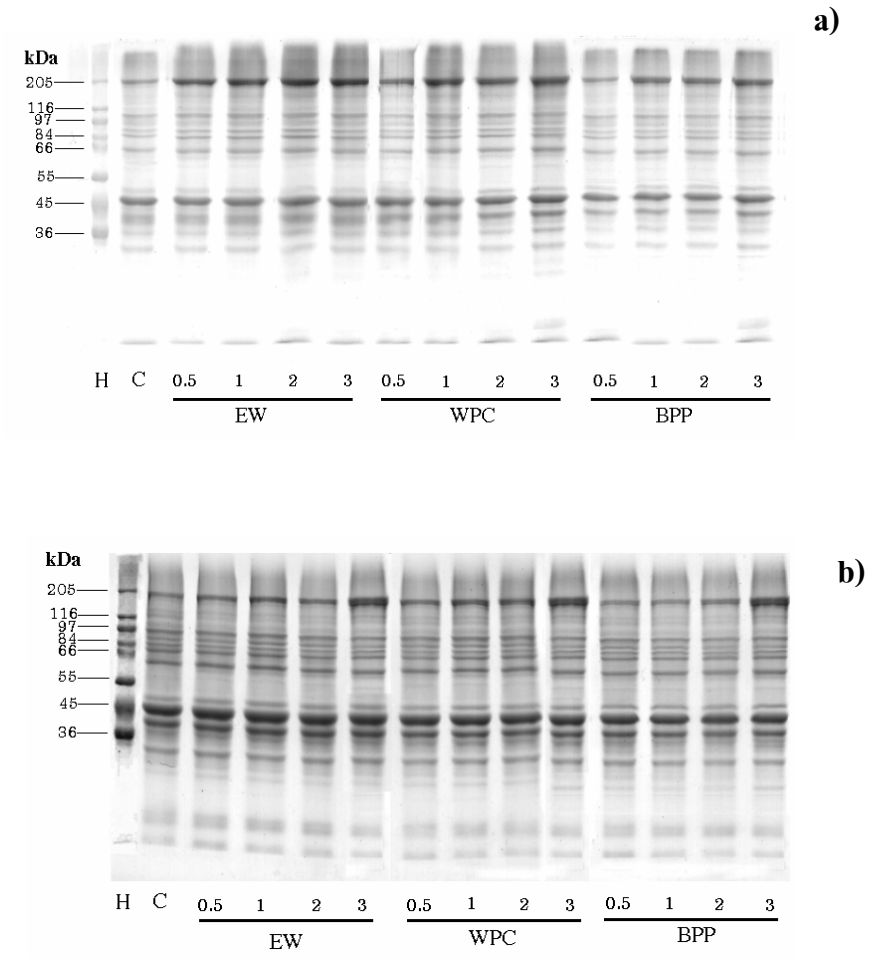

Figure 27 Protein patterns of one-step (a) and two-step (b) heated gels from Pacific white shrimp meat added with different types and concentrations of protein additives. H: high molecular weight maker C: Control (without protein additive); EW: egg white; WPC: whey protein concentrate; BPP: bovine plasma protein. Numbers designate protein additive concentrations (%).

2.6 Microstructure

Microstructures of Pacific white shrimp gels without and with 0.5% WPC or 0.5% BPP, which showed the higher textural properties than that with 0.5% EW, are illustrated in Figure 28. Gel network with finer structure were observed for two-step heated gel, compared with one-step counterpart. However, the protein network of Pacific white shrimp gel containing 0.5% BPP or WPC seemed to be more compact with smaller voids than that of the control gel. These observations suggested that BPP and WPC might distribute uniformly as the filler in the ordered network. Also, they could function as proteinase inhibitor to prevent the degradation of MHC. The regularly order and more fibrous structure of Pacific white shrimp gel was likely responsible for the higher breaking force and deformation.

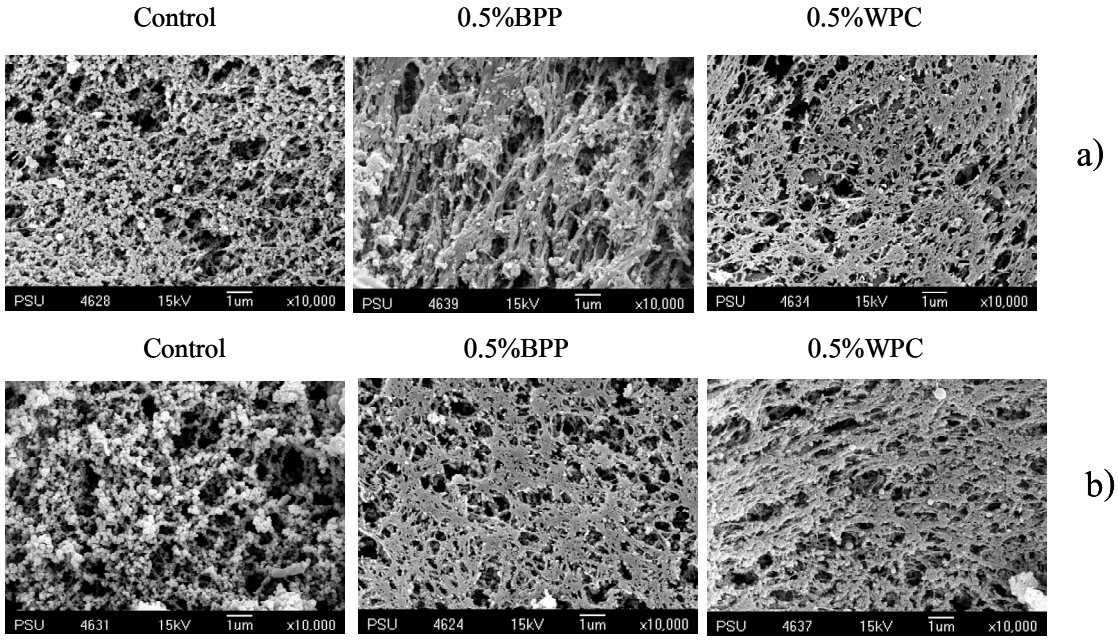

Figure 28 Microstructures of gels from Pacific white shrimp meat added with different types and concentrations of protein additives. Sols containing 2.5% NaCl and different protein additives were incubated at 40°C for 60 min before heating at 90°C for 20 min (a) or were directly heated at 90°C for 60 min (b).

3. Autolysis of Pacific white shrimp meat

3.1 Effect of temperatures on autolysis of Pacific white shrimp mince

Autolysis of Pacific white shrimp mince in the presence and in the absence of 2.5%NaCl at different temperatures (30-70°C) with various incubation times (30 and 60 min) is shown in Figure 29. Autolytic activity was highest at 35°C and 40°C in the absence and presence of 2.5% NaCl, respectively ($P<0.05$). The activity decreased sharply at temperature above 40°C, probably due to the thermal denaturation of the endogenous proteinases. No differences in autolytic activity were observed in the temperature range of 45-70°C ($P>0.05$). Konno and Fukazawa (1993) reported the increase in autolysis of squid mantle muscle with increasing temperature up to 40°C, followed by an abrupt decrease above 40°C. No activity was observed at incubation temperature above 50°C. Jiang (2000) found that proteolytic enzyme in mackerel and milkfish muscle had an optimum temperature of 45 and 50°C, respectively. From the result, the difference in optimal temperatures for Pacific white shrimp autolysis was governed by salt. It was found that the activity peak was noticeable at 40°C in the presence of 2.5% NaCl, whereas optimal temperature of 35°C was found in Pacific white shrimp mince without NaCl addition. NaCl might activate some proteinases, which had the optimal temperature of 40°C. Additionally, the autolytic activity of Pacific white shrimp in the presence of 2.5% NaCl was higher than that observed in the absence of 2.5% NaCl. Salt might induce the disassembly of myosin filament into the monomeric form, which was much more susceptible to hydrolysis (Weeds and Pope 1977). Konno and Fukazawa (1993) also reported the increased autolysis in mantle of squid (*Todarodes pacificus*) with increasing NaCl concentration. Myofibril associated proteinases from lizardfish were still active in the presence of NaCl at a level of 2–3%, which is commonly used in surimi processing (Benjakul *et al*., 2003). From the result, a longer incubation time resulted in the higher TCA - soluble peptide content ($P<0.05$), suggesting that the proteins or peptides underwent autolysis caused by endogenous proteinases to a larger extent. Sufficient incubation time therefore increased the hydrolysis of muscle proteins.

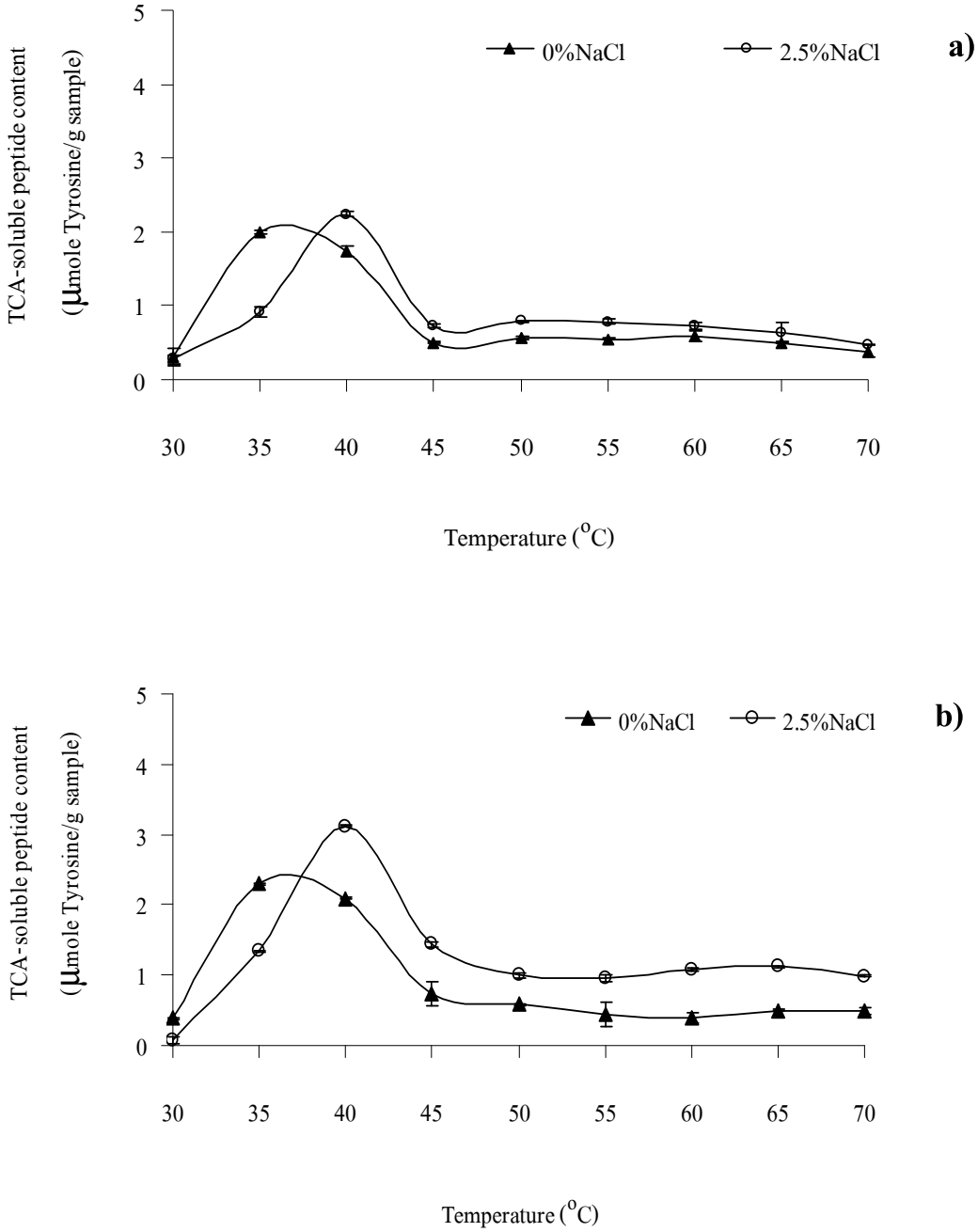

Figure 29 TCA-soluble peptide content of gels from Pacific white shrimp mince incubated at different temperatures for 30 (a) and 60 min (b) in the presence and in the absence of 2.5% NaCl. Error bars represent the standard deviation for triplicate determinations.

Protein patterns of Pacific white shrimp autolyzed in the absence and in the presence of 2.5% NaCl at the optimal temperature are shown in Figure 30. The intensity of MHC band decreased with increasing incubation time. This result was in agreement with the increased TCA-soluble peptide content with longer incubation times (Figure 29). Protein with MW of 65 kDa was also degraded when the incubation time increased. However, actin band intensity decreased to a lower extent, compared to MHC. Thus MHC was more susceptible to hydrolysis by endogenous proteinase in shrimp muscle. Benjakul *et al* (1997) reported that MHC in Pacific whiting was more susceptible to degradation than actin. From the result, the degradation took place to a higher extent in the presence of 2.5% NaCl, compare with that found in shrimp mince without NaCl, when the same incubation time was used. This result was in accordance with the higher TCA-soluble peptide content in shrimp mince autolyzed in the presence of 2.5% NaCl (Figure 29). NaCl might increase the solubilization of muscle protein via dissociation of myofilaments (Thawornchinsombut and Park, 2005). As a consequence, those proteins could be cleaved more effectively by the proteinases, which distributed uniformly in the shrimp paste. When NaCl was added to proteins, the Na ion and Cl ions act as counter ions toward negatively and positively charged group, respectively, disturbing the native conformation of protein (Sikorski *et al*., 1994).

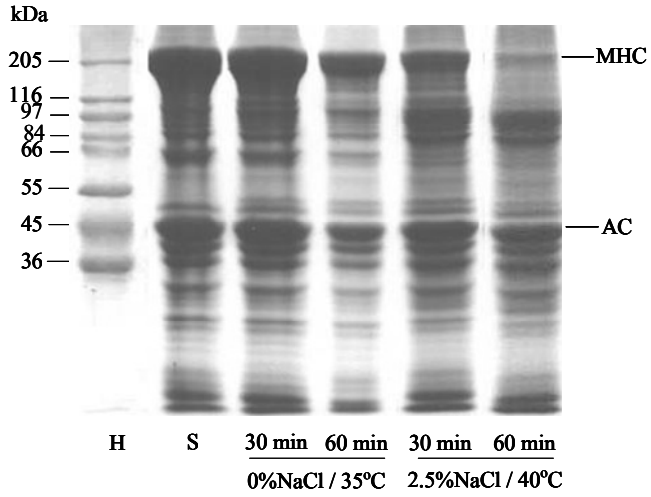

Figure 30 Protein patterns of Pacific white shrimp mince incubated at different temperatures for 30 and 60 min in the presence and absence of 2.5% NaCl. MHC: myosin heavy chain; AC: actin; H: high molecular weight marker; S: shrimp mince.

NaCl increased the activity of protease with optimal temperature at 40°C in octopus muscle by 33 to 38% (Hurtado *et al*., 2002). The increase in activity induced by NaCl in the octopus muscle was presumably due to the action of the Cl ion rather than the cations. Cathepsin C in muscle of squid (*Illex illecebrosus*) was activated by Cl ion and not by the action of cations like K ion or Na ion (Hameed and Haard 1985). The result suggested that shrimp paste or gel, which NaCl was present, might be prone to autolysis. This might lead to the poor gel quality.

3.2 Effect of pH on autolysis of Pacific white shrimp mince

The pH profiles for Pacific white shrimp autolysis conducted at 35°C in the absence of 2.5% NaCl or at 40°C in the presence of 2.5% NaCl are depicted in Figure 31. The activity peak was found at pH 3 when 2.5% NaCl was present. For shrimp mince without NaCl, activity decreased rapidly with an increase in pH. The high activity was observed in the pH range of 2-4. Small activity peak was found at pH values of 8 and 9 for shrimp mince added without and with 2.5% NaCl, respectively. The result suggested that acidic proteinases were the major proteinases and alkaline proteinases were found at a low level. Additionally, the pH values below or above the isoelectric point (pI) resulted in a net positive and negative charge of proteins, respectively (Vojdani 1996). The repulsion between charged residues of protein molecules might be associated with dissociation or solubilization of protein substrates. Solubilized proteins could be more hydrolyzed by proteinases, especially acidic proteinase.

The degradation of proteins in Pacific white shrimp mince at various pH values determined by SDS-PAGE was compared (Figure 32). MHC was almost degraded at pH values of 2–4 with a concomitant formation of low-molecular-weight peptides. This result was in agreement with the greater TCA-soluble peptide content of shrimp mince autolyzed in this acidic pH ranges (Figure 31). The higher band intensity of MHC at pH above 4 indicated the lower hydrolysis caused by endogenous proteinases. Optimal pH for the autolysis of Pacific white shrimp mince was similar to those reported by Makinodan *et al*. (1982) who found that carp muscle proteinase showed the high activity at pH 4.0. It is well known that cathepsin D in fish muscle has the optimum pH in acidic pH range (Barret 1997). At acidic pH, actin band intensity decreased to some extent, suggesting that actin was also degraded in addition to MHC.

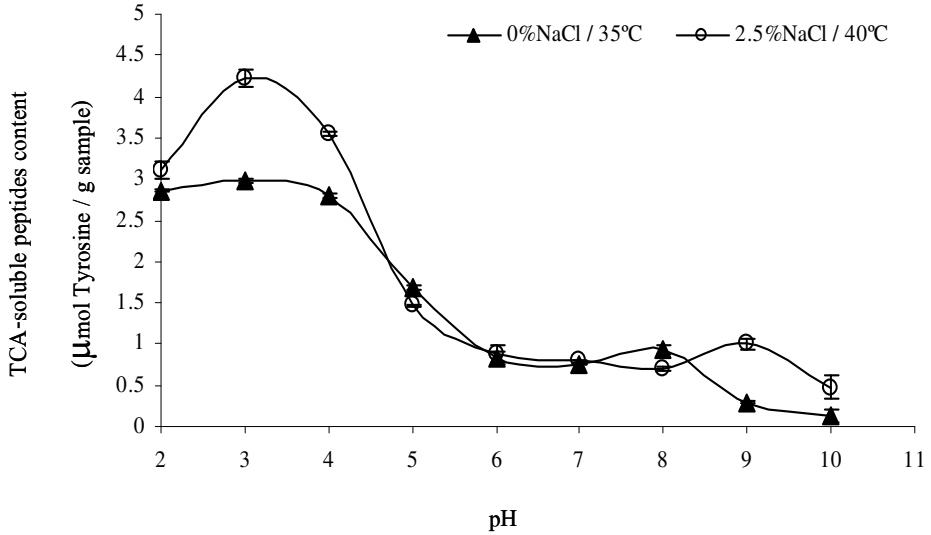

Figure 31 TCA-soluble peptide content of Pacific white shrimp mince incubated at different pH values (2-10) at 40°C for 60 min in the presence of 2.5% NaCl and 35°C for 60 min in the absence of 2.5% NaCl. Error bars represent the standard deviation for triplicate determinations.

Furthermore, in the alkaline pH ranges, the lowest band intensity of MHC was noticeable at pH values of 9 and 8 for shrimp mince added with and without 2.5% NaCl, respectively. The result was coincidental with the highest TCA - soluble peptide content found at those pH values (Figure 31). In general, the degradation was more pronounced in the presence of 2.5% NaCl. Therefore, shrimp gel, in which NaCl is used to solubilize proteins, might be prone to degradation during heating process applied to achieve the gelation process. From the result, the activity was also found at the neutral pH values, regardless of NaCl addition.

3.3 Effect of inhibitors on autolytic activity of Pacific white shrimp mince

The effect of different proteinase inhibitors towards the autolytic activity of Pacific white shrimp mince at different pH values in the absence and in the presence of 2.5% NaCl at 35°C and 40°C for 60 min, respectively, is shown in Table 10. In the absence of NaCl, pepstatin A, an aspartic proteinase inhibitor, showed the highest inhibition (51.5%) when tested at pH 3, while SBTI, a serine proteinase inhibitor, exhibited the high inhibition at pH 7 (68.5%) and

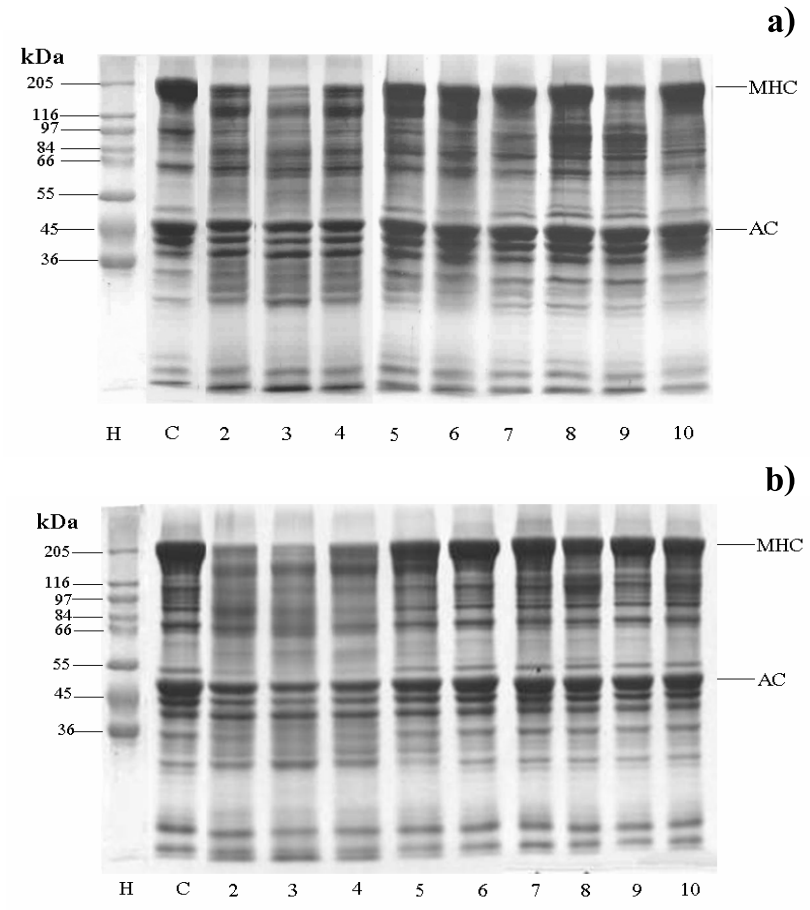

Figure 32 Protein patterns of Pacific white shrimp mince incubated at different pH values at 40°C for 60 min in the presence of 2.5% NaCl (a) and 35°C for 60 min in the absence of 2.5% NaCl (b). MHC: myosin heavy chain; AC: actin; H: high molecular weight marker and S: shrimp mince, Numbers designate different pH values (2-10).

pH 9 (64.8%). However, EDTA, a chelator, also showed the inhibition against shrimp autolysis at pH values of 7 and 9. Some metal ion might be required for proteinase activity at neutral and alkaline pH values. In the presence of 2.5% NaCl, pepstatin A also showed the highest inhibition toward autolytic activity at pH 3. However, at pH 7, pepstatin A showed the higher inhibition toward autolysis in the absence of NaCl than in the presence of 2.5% NaCl. Furthermore, %inhibition of 40.4% was found with EDTA. However, SBTI significantly inhibited the autolytic activity of Pacific white shrimp at pH values of 7 and 9 by 42.7% and 70.6%, respectively. EDTA and EGTA, a chelator specific to Ca ion, also showed the high inhibition at pH 9. From the result, E-64, a cysteine proteinase inhibitor, had a little effect on inhibition at all conditions tested. The

result indicated that cathepsins might be minor proteinases in Pacific white shrimp. Lugo-Sanchez *et al* (1996) found that sarcoplasmic fluid of Monterey sardine (*Sardinops sagax caerulea)* contained cathepsin D-like enzyme, which showed high activity at pH 3. From the result, aspartic proteinase most likely cathepsin D was the dominant proteinase, which was active at pH values of 2-4. Alkaline proteinase was also another proteinase present in shrimp meat but constituted at a lower extent. Doke and Ninjoor (1980) found an alkaline protease and exopeptidase in shrimp (*Penaeus indicus*) muscle. Serine proteinase in hapatopancreas of white shrimp (*Penaeus vannamei*) was acid labile and exhibited maximal activity at pH 8 (Cortes *et al*., 1997).

Table 10 Effect of various protease inhibitors on Pacific white shrimp meat autolysis in the presence and in the absence of 2.5% NaCl

Inhibitors	Concentrations	% Inhibition					
		0%NaCl			2.5%NaCl		
		pH 3	pH 7	pH 9	pH 3	pH 7	pH 9
Pepstatin A	2 μmole/kg	51.5±1.4	19.2±2.4	15.2±1.4	62.4±2.8	17.0±2.2	35.2±3.4
EGTA	10 mmole/kg	8.4±2.2	20.8±2.4	18.6±1.4	24.9±0.5	20.8±1.7	69.4±1.9
E-64	0.1 mmole/kg	9.4±1.4	24.8±2.3	19.7±0.8	27.5±2.6	20.0±1.6	33.1±0.9
SBTI	0.1 mmole/kg	8.1±0.8	68.5±3.9	64.8±1.3	21.4±4.4	42.7±1.0	70.6±1.8
EDTA	20 mmole/kg	7.4±3.4	28.7±2.3	56.4±0.8	40.4±3.9	35.4±1.6	64.9±0.9

Values are means ± standard deviation from triplicate determinations

The effect of different inhibitors on protein patterns of Pacific white shrimp mince autolyzed in the presence and in the absence of 2.5% NaCl is depicted in Figures 33 and 34, respectively. Without proteinase inhibitors added, no MHC remained, irrespective of NaCl addition. MHC was more retained in the presence of pepstatin A at pH 3. At pH values of 7 and 9, MHC was more retained when SBTI and EDTA were added. The increases in band intensity of MHC in samples added with appropriate inhibitor were in agreement with the increases in %inhibition (Table10). This reconfirmed that aspartic and serine proteinases were the dominant proteinases in Pacific white shrimp meat. Nevertheless, MHC was also retained as E-64, cysteine

proteinase inhibitors, was added, particularly at pH 7. Therefore, cysteine proteinase such as cathepsin might also involve in the degradation of shrimp protein. Cathepsin D was found in the muscle of grass prawn (Jiang *et al*., 1991).

In the presence of 2.5% NaCl, similar patterns of inhibition towards autolysis by various inhibitors were observed, compared to those found in shrimp without 2.5% NaCl addition. However, it was noted that band intensity of shrimp mince added with pepstatin A in the presence of 2.5% NaCl was more retained, when compared with that of the counterpart without NaCl. The solubilization of muscle protein by salt might enhance the distribution of pepstatin A throughout the sample. As a result, the inhibition efficacy could be enhanced. Therefore, Pacific white shrimp meat contained a variety of proteinases, which had different optimal pH values and characteristics.

3.4 Effect of some protein additives on autolysis of Pacific white shrimp mince

Effects of various protein additives at different concentrations on autolysis inhibition of Pacific white shrimp mince incubated at 40°C for 60 min in the presence of 2.5% NaCl are shown in Figure 35. In general, all protein additives including EW, WPC and BPP showed the inhibitory activity towards the autolysis of Pacific white shrimp mince in a concentration-dependent manner ($P<0.05$). Inhibitory activity of EW on autolysis of Pacific white shrimp mince increased as the concentration increased up to 3% ($P<0.05$). For WPC and BPP, the inhibitory activity increased up to 2% and no difference in inhibitory activity was found at the level above 2% ($P>0.05$). At the same level used (0.5-1%), BPP exhibited the higher inhibitory activity than did WPC and EW. At a level of 1%, inhibition of autolysis by 38.8, 35.4 and 30.5% was observed for Pacific white shrimp mince added with BPP, WPC and EW, respectively. Nevertheless, no differences in inhibition were found when WPC and BPP at levels of 2 and 3% were used ($P>0.05$). From the result, it indicated that BPP WPC and EW contained the inhibitors, which were able to inhibit proteinases in Pacific white shrimp mince. The proteinase inhibitory activity of BPP was reported to be due to alpha-2-macroglobulin (α_2-M) and kininogen (Morrissey *et al*., 1993; Choi *et al*., 1999; Kang and Lanier 1999). α_2-M acts as a nonspecific

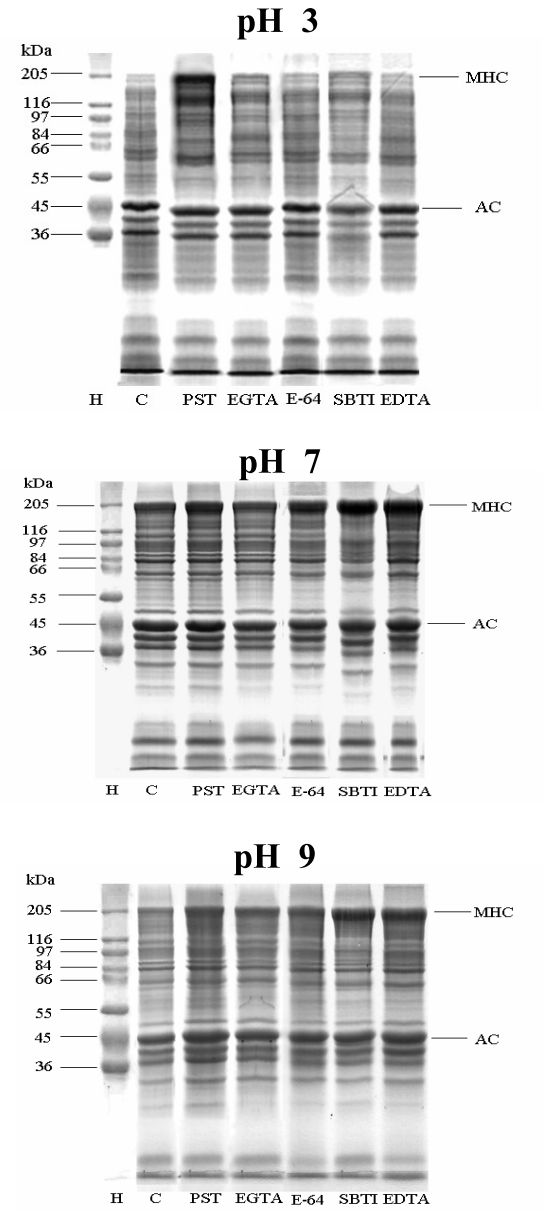

Figure 33 Protein patterns of Pacific shrimp mince incubated at 40°C for 60 min in the presence of 2.5% NaCl without and with proteinase inhibitors. H: high molecular weight marker; C: control (without proteinase inhibitor); PST: pepstatin A; EGTA: ethylene - bis (oxyethylenenitrilo) tetraacetic acid; E-64: trans-epoxysuccinyl-L-leucyl-amido (4-guanidino) butane; SBTI: soybean trypsin inhibitor**;** EDTA**:** ethylenediaminetetraacetic acid.

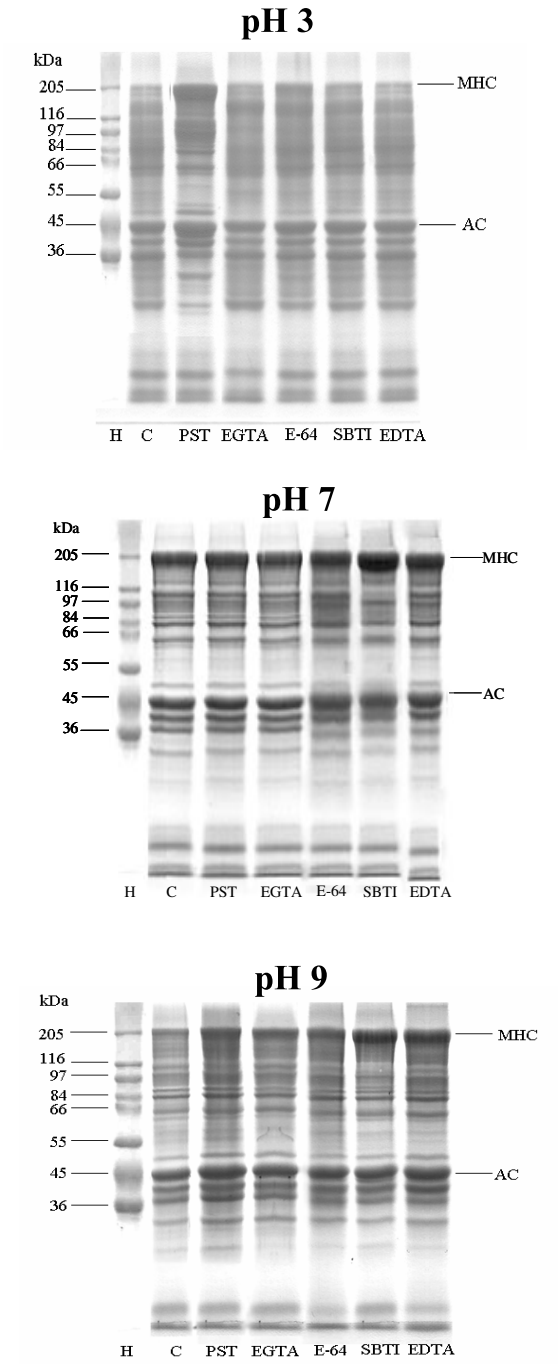

Figure 34 Protein patterns of Pacific shrimp mince incubated at 35°C for 60 min in the absence of 2.5% NaCl without and with proteinase inhibitors. H: high molecular weight marker; C: control (without proteinase inhibitor); PST: pepstatin A; EGTA: ethylene - bis (oxyethylenenitrilo) tetraacetic acid; E-64: trans-epoxysuccinyl-L-leucyl-amido (4-guanidino) butane; SBTI: soybean trypsin inhibitor; EDTA: ethylenediaminetetra acetic acid.

inhibitor for all classes of proteinases (Starkey and Barrett 1977), while kininogen is a specific cysteine proteinase inhibitor (Rawlings and Barrett 1990). EW showed the inhibitory activity due to the presence of some proteinase inhibitors, such as cystatin, ovoinhibitor and ovomacroglobulin, which are specific to cysteine proteinase, serine proteinase and aspartic proteinase, respectively (Garcia-Carreno and Hernandez-Cortes 2000). WPC was more effective in inhibiting cysteine proteinase rather than serine proteinase (Weerasinghe *et al*., 1996).

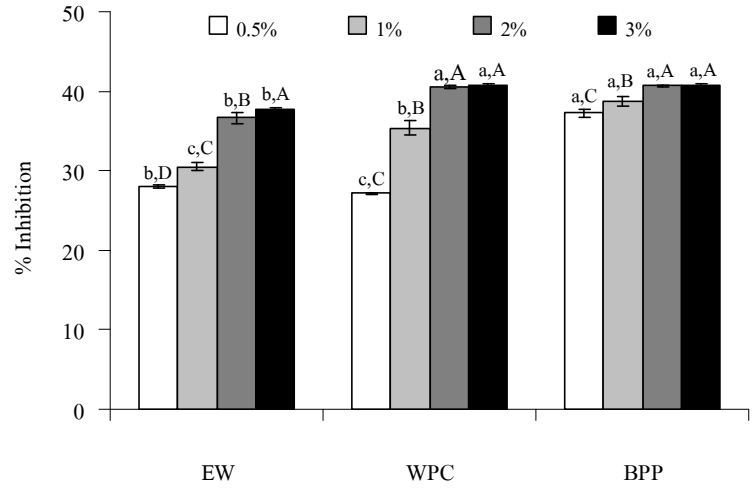

Figure 35 TCA-soluble peptide content of Pacific white shrimp mince incubated at 40°C for 60 min in the presence of 2.5% NaCl and added with various protein additives at different concentrations. Error bars represent the standard deviation from triplicate determinations. Different letters on the bars within the same protein additive and different letter cases within the same level of protein additive indicated the significant differences ($P<0.05$). EW: egg white; WPC: whey protein concentrate; BPP: bovine plasma protein.

Protein patterns of Pacific white shrimp mince added with protein additive at different levels after incubation at 40°C are shown in Figure 36. The extensive degradation of MHC was observed in the sample without the addition of protein additives as indicated by the lowest band intensity retained. However, no changes in actin were noticeable. The result indicated that MHC is the primary target of proteinase. MHC was more susceptible to degradation than was actin (An *et al*., 1994). Disappearance of protein with 65 kDa was found in all samples, regardless of the addition of protein additives. The result indicated that all protein additives had no impact

on inhibition of this protein. Cathepsin B exhibited degradation activity on myosin from mackerel surimi incubated at 40°C (Jiang *et al*., 1997). From the result, the use of protein additives could provide the protection against proteolysis. When comparing the MHC band intensity of Pacific white shrimp mince added without and with protein additives, it was found that MHC was more retained in samples added with protein additives, especially with increasing amounts added. With the addition of protein in the range of 2-3%, marked increases in MHC band intensity were observed, suggesting the sufficient amount of protein additive in inhibiting the proteolytic activity in Pacific white shrimp mince. This result was in accordance with the autolysis study (Figure 36). Morrissey *et al* (1993) found that BPP showed the strongest inhibition of autolysis in fish mince and surimi made from Pacific whiting, followed by egg white and potato extract. BPP also exhibited the higher inhibition towards papain and trypsin than did WPC (Weerasinghe *et al*., 1996). Benjakul *et al* (2004) reported that BPP and EW contained the inhibitors, which are able to inhibit proteinases in lizardfish sarcoplasmic fluid. Autolysis of Pacific white shrimp mince could be minimized when appropriate protein additives were used. Nevertheless, MHC band could not be completely recovered, compared with that found in shrimp meat. Protein additives with high molecular weight possibly could not distribute uniformly throughout the mince. As a result, the lower efficacy in inhibition of proteinases could be found, particularly those associated tightly with muscle. The remaining activity could show the detrimental effect on the properties of resulting gel incubated or set at temperature ranges, where the proteolytic activity was enhanced. WPC could inhibit the proteolysis and increase the breaking force to a lower extent than BPP. However, BPP has not been used due to the mad cow disease awareness. Thus WPC at level of 0.5% was used for the improvement of shrimp gel properties.

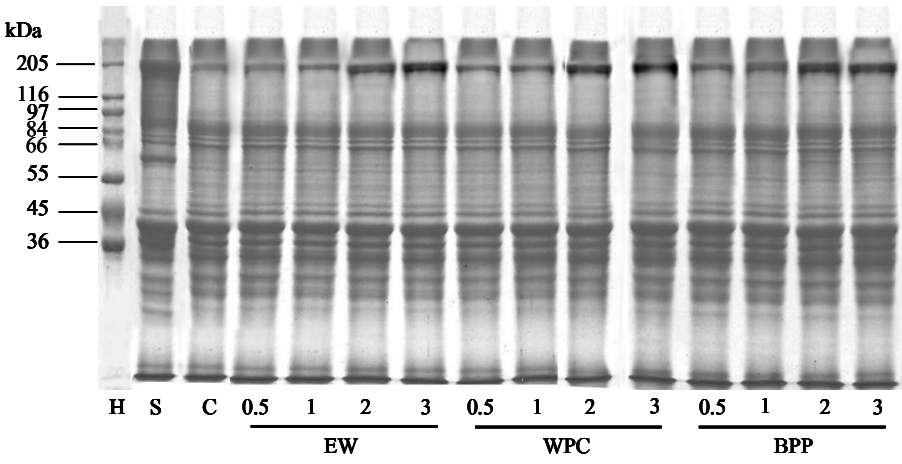

Figure 36 Protein patterns of Pacific white shrimp mince incubated at 40°C for 60 min in the presence of 2.5% NaCl and added with various protein additives at different concentrations. MHC: myosin heavy chain; AC: actin; H: high molecular weight marker; S: shrimp mince; C: without protein additive; EW: egg white; WPC: whey protein concentrate; BPP: bovine plasma protein. Numbers designate the concentration (%w/w).

4. Effect of setting condition on gel property of Pacific white shrimp meat

4.1 Characterization of endogenous TGase

The temperature profile of endogenous TGase in crude extract of Pacific white shrimp muscle is shown in Figure 37. The optimal temperature of endogenous TGase was 55°C (0.07 units/mg protein). A decrease in TGase activity observed at temperature above 55°C was presumably as a result of thermal inactivation. At high temperature, partial unfolding of the enzyme molecule could take place and led to the loss in activity. Purified TGase from red sea bream had a temperature optimum of 55°C (Yusada *et al.*, 1994), while optimum TGase activity for walleye pollack liver was observed at 50°C (Kumazawa *et al.*, 1996). Tsukamasa *et al* (2002) reported that TGase activity of carp showed the highest value at 50°C, while TGase from threadfin bream and white croaker had the optimal temperature of 40 and 30°C, respectively. Yongsawatdigul *et al* (2002) reported that TGase activity from threadfin bream was highest at 55°C. This demonstrated that TGase activity and optimum temperatures vary with species. The

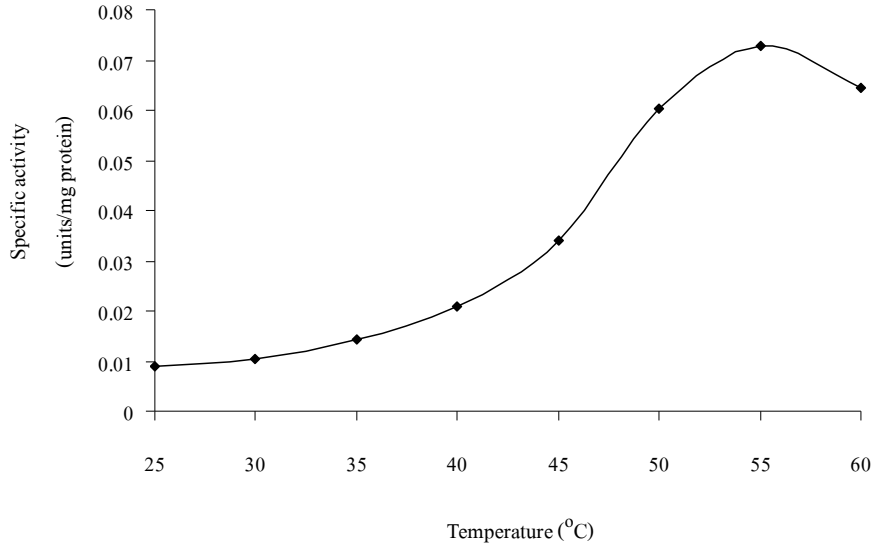

Figure 37 Effect of temperature on TGase activity of crude extract from Pacific white shrimp muscle. Error bars represent the standard deviation for triplicate determinations.

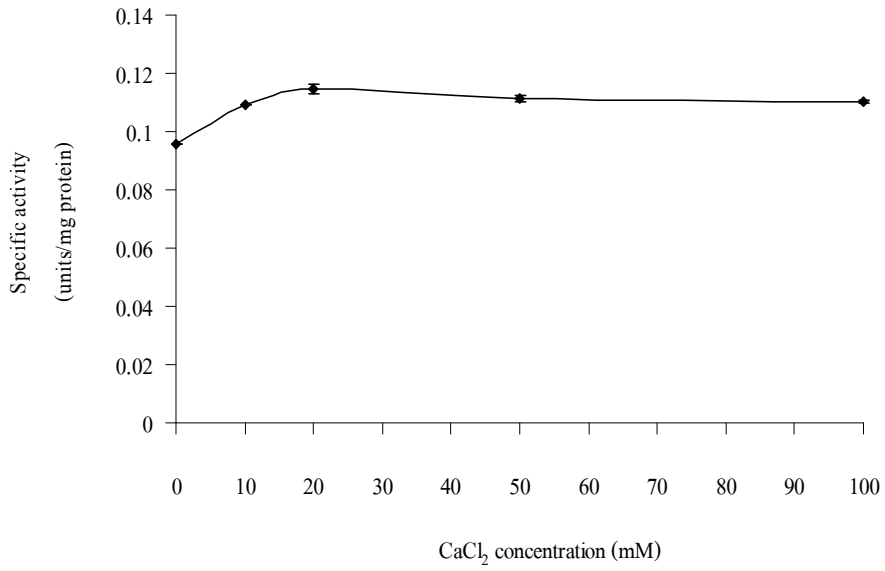

Figure 38 Effect of $CaCl_2$ concentrations on TGase activity of crude extract from Pacific white shrimp muscle. Error bars represent the standard deviation for triplicate determinations.

difference in optimal temperature of TGase from different species is probably affected by the habitat temperature (Nozawa *et al*., 2001). The activity of endogenous TGase of Pacific white shrimp muscle increased with increasing $CaCl_2$ up to 20 mM (Figure 38). Thereafter, no changes in TGase activity were found with $CaCl_2$ range of 20-100 mM. Calcium possibly induced the conformational changes of enzyme, which consequently exposed the cysteine located at the active site to substrate (Jiang and Lee., 1992). Noguchi *et al.* (2001) reported that the calcium ion bound to a binding site of red sea bream TGase molecule, resulting in conformational changes. Subsequently, Tyr covering the catalytic Cys was removed. Then, the acyl donor bind with the Cys at the active site, forming an acyl-enzyme intermediate. Optimal $CaCl_2$ concentration for TGase from red sea bream liver, Japanese oyster, scallop and pollock liver were 0.5, 25, 10 and 3 mM, respectively (Kumazawa *et al*., 1996, 1997; Nozawa *et al*., 2001; Yasueda *et al*., 1994). Nozawa *et al* (1999) reported that carp TGase showed its maximum activity at 0.8 mM $CaCl_2$. On the other hand, scallop TGase required over 50 mM of $CaCl_2$ for its full activation. Yongsawatdigul *et al* (2006) also reported that TGase in small scale mud carp muscle showed Ca^{2+}- dependent characteristic with the minimum activity in the absence of Ca^{2+} and highest activity at 1.25 mmol/kg. From the result, 20 mM $CaCl_2$ was required to fully activate TGase activity of Pacific white shrimp muscle. Endogenous TGase was most likely involved in setting and contributed to gel strength.

4.2 Effect of setting condition on gel properties of Pacific white shrimp meat

4.2.1 Breaking force and deformation

Breaking force and deformation of gel from Pacific white shrimp meat without and with 5mmolePP/kg / 5mmole$MgCl_2$/kg in the presence of 150 mmole$CaCl_2$/kg after setting at 55°C for 0-3 h are shown in Figure 39. At the same time of setting, greater breaking force and deformation were obtained with gels added with PP/$MgCl_2$ compared with gel without PP/$MgCl_2$ ($P<0.05$). The result indicated that PP and $MgCl_2$ had the gel enhancing effect for Pacific white shrimp meat. PP and $MgCl_2$ might induce the dissociation of actomyosin complex (Chang *et al*., 2001). The free MHC or actin could undergo aggregation with the ordered structure.

Regardless of PP/$MgCl_2$ addition, setting at 55°C for 0.5 h increased the breaking force of the gel ($P<0.05$). No differences in breaking force were found with setting time

of 0.5-2 h ($P>0.05$). However, the decrease in breaking force was noticeable with setting time of 3 h ($P<0.05$). The result was in agreement with Luo *et al* (2001) who reported that the breaking force of silver carp surimi incubation at 50°C decreased as the incubation period increased. The maximum breaking force of Pacific white shrimp gels without and with PP/$MgCl_2$ addition increased by 22.51 and 50.18 %, respectively, compared with control gel. Setting for a long time might cause the degradation of muscle protein induced by proteinases, which were active at high temperature (50-60°C) (Figure 29). It has been generally accepted that the formation of a heat-induced gel requires the denaturation of the proteins. This process is accompanied by a conformational change and exposure of the reactive groups and is followed by a second stage in which the denatured proteins establish protein-protein interactions, which lead to aggregation (Ferry, 1948). Alvarez *et al* (1999) reported that the final structure of the kamaboko gel network was the outcome of denaturation-aggregation during setting of the surimi sol. From the result, lowered breaking force was observed in gel without PP/$MgCl_2$ addition for all setting times used, compared with gel with PP/$MgCl_2$ addition. The similar result was found for deformation, in which setting for 0.5 h yielding the highest deformation for the gel with PP/$MgCl_2$ addition. For gel without PP/$MgCl_2$ addition, the highest deformation was found when setting was conducted for 1 h.

Surimi, when solubilized with salt and held at low temperatures, forms an elastic gel, and subsequent heating forms a cooked gel of greater strength than one formed without a setting step (Niwa 1992; Joseph *et al*., 1994; Park *et al*., 1994; An *et al*., 1996; Yongsawatdigul and Park 1996; Shie and Park 1999). Setting for different times possibly caused the different changes in protein conformation, resulting in the difference in the exposure of reactive group (glutamine and lysine) involved in the cross-linking induced by reaction via TGase (Benjakul *et al*., 2004). A hydrophobic methylene group of peptide-bound glutamine residue is necessary to confer substrate properties and is essential for interaction with a hydrophobic region of the active site of the enzyme (Folk, 1970).

Apart from cross-linking induced by endogenous TGase, the appropriate setting time presumably cause optimal exposure and interaction of the hydrophobic portion of protein molecules (Benjakul *et al.*, 2004). The different setting response observed among gel from fish and invertebrate muscle were possibly due to the difference in activity and thermal stability of

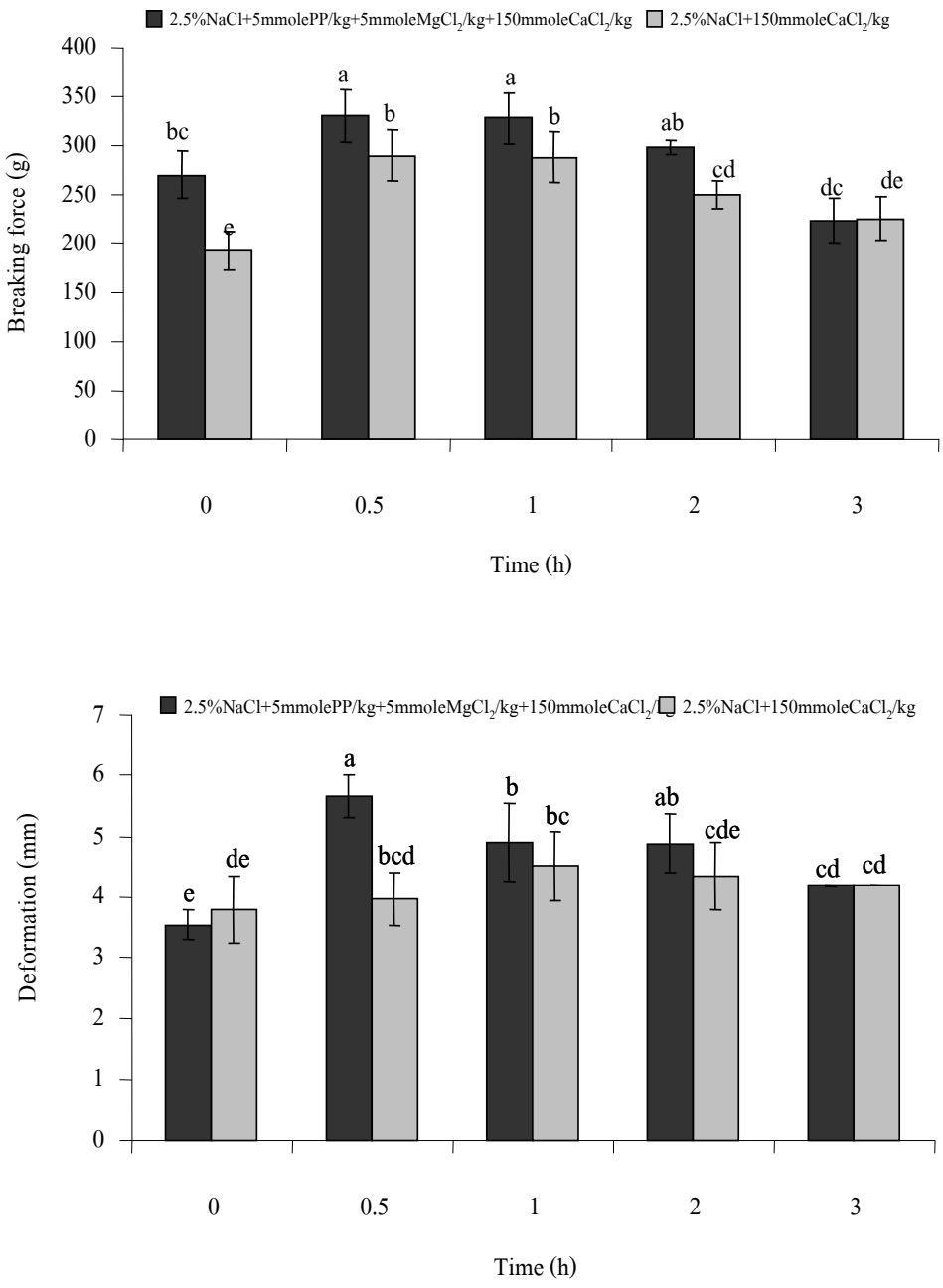

Figure 39 Breaking force and deformation of gels from Pacific white shrimp meat added with $CaCl_2$ with and without PP/$MgCl_2$ during setting at 55°C for different times. Error bars represent the standard deviation from five determinations. Different letters on the bars indicate the significant differences ($P<0.05$).

Table 11 Expressible moisture content and color of gels from Pacific white shrimp meat added with $CaCl_2$ with and without PP/$MgCl_2$ during setting at 55°C for different times

Treatments	Time (h)	Expressible moisture content (%)	Color		
			*L**	*a**	*b**
2.5%NaCl+5mmole PP/kg+5mmole $MgCl_2$/kg+150mmole $CaCl_2$/kg	0	35.40±0.74a	74.84±0.50a	18.26±0.15c	16.94±0.11c
	0.5	29.96±1.98c	75.19±0.35a	18.77±0.23bc	17.28±0.36b
	1	25.20±1.51d	75.15±0.91a	19.59±0.46ab	17.84±0.65a
	2	31.82±0.61b	73.19±0.78b	19.56±0.48a	18.14±0.46a
	3	34.26±1.04a	74.71±0.60a	19.03±0.15bc	17.42±0.27b
2.5%NaCl+150mmole $CaCl_2$/kg	0	35.68±0.95a	73.87±0.68a	18.81±0.50b	17.97±0.30b
	0.5	33.32±1.70c	73.32±0.52ab	19.10±0.28b	17.83±0.38b
	1	33.48±1.39bc	72.33±0.69b	19.40±0.55ab	18.10±0.40b
	2	35.28±1.60ab	73.36±0.40ab	19.03±0.61b	17.76±0.47b
	3	35.08±1.05abc	73.11±0.67b	20.23±0.33a	18.58±0.24a

Different letters in the same column within the same treatment indicate significant difference ($P<0.05$).

Mean±SD from five determinations.

endogenous TGase. Benjakul *et al* (2004) reported that setting surimi sol at 40°C generally increased the breaking force and deformation of suwari gel from threadfin bream, bigeye snapper, barracuda and bigeye croaker surimi. Setting still occurs in the fish meat in which TGase is inhibited. Nature of myofibril as substrate of TGase make a larger contribution to the setting than activity of TGase (Tsukamasa *et al*, 2002).

4.2.2 Expressible moisture content

Expressible moisture content of Pacific white shrimp gel with and without PP/$MgCl_2$ addition in the presence of 150 mmole$CaCl_2$/kg and set for different times is shown in Table 11. With setting up to 1 h, the expressible moisture content decreased continuously. For both treatments, expressible moisture content of gel increased as setting times were greater than 1 h ($P<0.05$). The decrease in expressible moisture content suggested the high water holding capacity of gel. The increase in expressible moisture content of gel set for a longer time was in agreement with the lowered breaking force and deformation (Figure 39). With the appropriate setting, the aggregation of protein could build up the network which can imbibe the water in the gel matrix. As the setting time increased, the degradation possibly occurred, mainly caused by endogenous proteinase. As a consequence, the network formed could be destroyed to some extent, leading to the lower ability to imbibe water.

4.2.3 Color

Color of gel from Pacific white shrimp gel added with and without PP/$MgCl_2$ in the presence of 150mmole$CaCl_2$/kg at different setting times is shown in Table 11. Setting time slightly affected the change in color of Pacific white shrimp gels. L^*-value slightly decreased, but a^* and b^*-values slightly increased as the setting time increased. The denaturation of proteins might be enhanced with the sufficient setting time. As a result, the pigments, including astaxanthin, could be observed more evidently. In general, pigments complexed with proteins are more stable than free form. Dissociation of the protein moiety brings about changes in color (Sikorski and Pan, 1994). Tammatinna *et al* (2007) reported that setting temperature directly affected lightness of shrimp gel. Higher L^* - value was observed in gel with prior setting (25°C for 2 h or 40°C for 30 min).

4.2.4 TCA-soluble peptide content

During setting at 55°C for up to 3 h, the increases in TCA-soluble peptide content were observed, indicating that the proteolytic degradation took place during the extended setting (Figure 40). The degradation probably occurred during setting at 55°C, which was the optimal temperature for endogenous TGase (Figure 38), though the autolysis was maximized at 40°C (Figure 29). Additionally, the denatured protein generated during extended setting would be a preferable substrate for proteolysis (Benjakul *et al*., 2004). As a result, a gradual decrease in breaking force and deformation was found with Pacific white shrimp gel subjected to setting for extended times (Figure 38). There was no differences in TCA-soluble peptide content in the presence and absence of PP/$MgCl_2$ during setting for 0-0.5 h. Thereafter, TCA - soluble peptide content of the gel added with PP/$MgCl_2$ was lower than that without PP/$MgCl_2$ during setting for 1-3 h. This was possibly due to the fact that PP might chelate the ions required for endogenous proteinases, leading to lower autolysis (Figure 40). From, the result, it was noted that the degradation occurred during setting. Therefore, setting time should be enough for enhancing the cross-linking induced by endogenous TGase, but should not be too long to avoid proteolysis associated with the lowered gel strength.

4.2.5 Solubility

Solubility of Pacific white shrimp gels set for different times at 55°C is shown in Figure 41. In general, a setting time of 3 h rendered the lowest solubility for both Pacific white shrimp gel added with and without PP/$MgCl_2$. This suggested that the cross-linking induced by TGase occurred to a greater extent as the setting time increased. Solution containing SDS, urea and βME has been used to solubilize protein by destroying all bonds, except non-disulfide covalent bonds, particularly the ε- (γ-glutamyl) lysine linkage (Benjakul *et al*., 2001). Therefore, the decrease in solubility indicated that non-disulfide bond formation occurred to a greater extent with increasing setting time. It has been known that endogenous TGase plays a crucial role in ε-(γ-glutamyl) lysine linkage formation (Kumazawa *et al*., 1995). From the result, non disulfide covalent bond was presumed to be a major contributor to the decrease in solubility. At 0.5 h of setting, Pacific white shrimp gel added with PP/$MgCl_2$ had the lower solubility than that without PP/$MgCl_2$ addition, suggesting that slightly greater non-disulfide bonds were formed in the

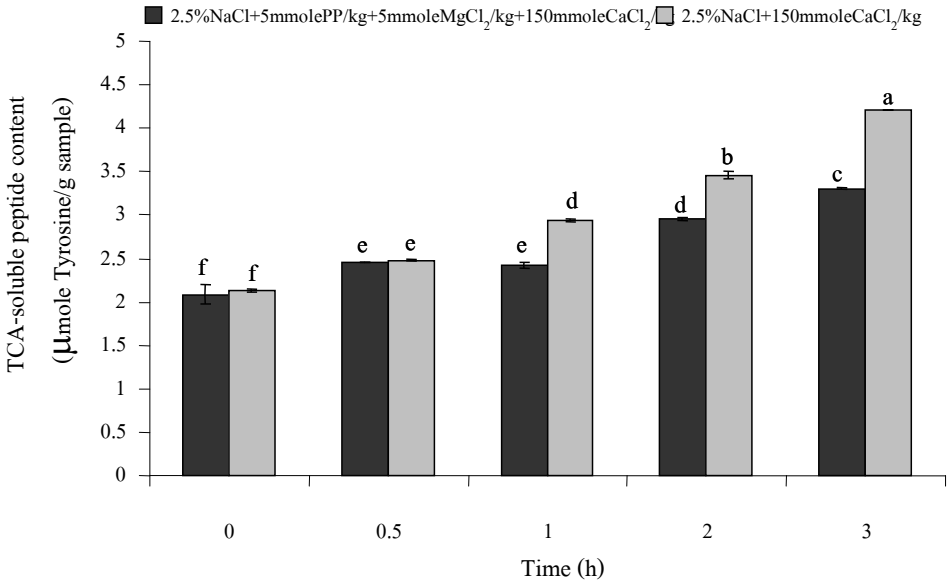

Figure 40 TCA-soluble peptide content of gels from Pacific white shrimp meat added with $CaCl_2$ with and without PP/$MgCl_2$ during setting at 55°C for different times. Error bars represent the standard deviation from five determinations. Different letters on the bars indicate the significant differences ($P<0.05$).

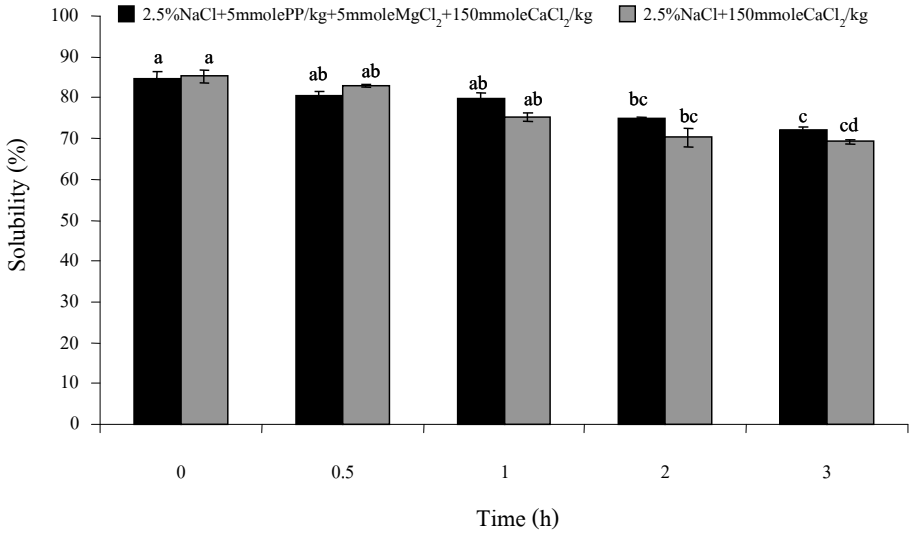

Figure 41 Solubility of Pacific white shrimp gels added with $CaCl_2$ with and without PP/$MgCl_2$ during setting at 55°C for different times. Error bars represent the standard deviation from five determinations. Different letters on the bars indicated the significant differences ($P<0.05$).

former. However, the higher solubility was found in the former with the setting greater than 0.5 h. Though the increase in non-disulfide bond formation was found, it did not reflect the gel strength directly (Figure 39). Therefore, not only the degree of cross-linking, but also degradation could determine the gel properties of Pacific white shrimp meat.

4.2.6 Protein pattern

MHC in both Pacific white shrimp gel with and without PP/$MgCl_2$ addition in the presence of 150 mmole$CaCl_2$/kg gradually decreased as the setting time increased (Figure 42). High molecular weight cross-linkes (>205 kDa) were clearly observed on the top of polyacrylamide gels. However, the degradation products with molecular weight of approximately 120-170 were generated with increasing setting time. Thus polymerization and degradation of MHC occurred simultaneously during the setting process. MHC content of cooked gel of pollock and croaker surimi decreased during pre-incubation ("setting") at temperatures ranging from 4-50°C (Kamath *et al*., 1992). Decreases in MHC content were attributed to either nondisulfide covalent cross-linking or proteolysis. Depending upon which process dominated at a given temperature, the formation of stronger or weaker gel occurred, respectively (Kurth and Roger, 1984). The structure of substrate was more important for TGase reaction than their absolute lysine and glutamine content (Kurth and Roger, 1984). Yongsawatdigul *et al* (2006) found an increase in higher molecular weight polymer on the SDS-PAGE of the small scale mud carp gel preincubated at 55°C. From the result, the continuous decrease in MHC was in accordance with the decrease in solubility (Figure 41).

When comparing the protein pattern between gel without and with PP/$MgCl_2$ addition, similar protein patterns were generally found. Addition of PP/$MgCl_2$ did not show much impact on protein patterns of the resulting gel. Apart from non-disulfide covalent bond formed, other interactions such as ionic interaction as well as hydrophobic interaction were most likely involved in gel formation. This might result in the differences in gel properties.

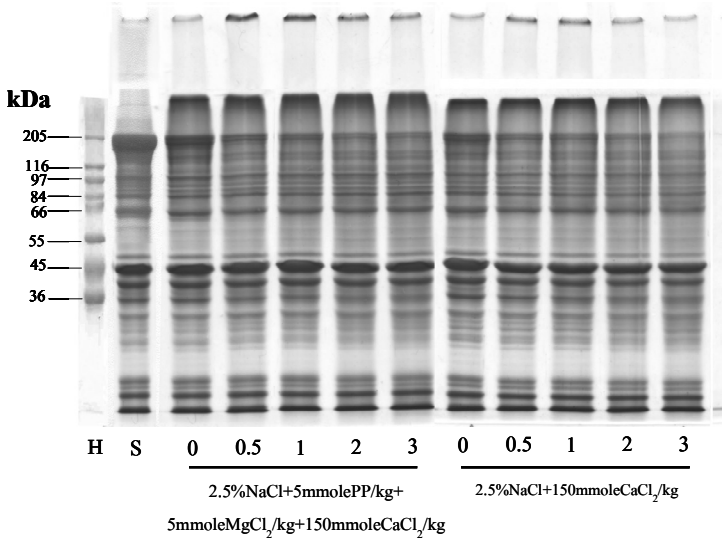

Figure 42 Protein patterns of gels from Pacific white shrimp meat added with $CaCl_2$ with and without PP/$MgCl_2$ during setting at 55°C for different times. H: high molecular weight marker; S: shrimp mince. Number designate incubated times (h).

4.3Effect of $CaCl_2$ concentrations on gel property

4.3.1 Breaking force and deformation

Breaking force and deformation of Pacific white shrimp gels containing 5 mmolePP/kg and 5 mmole$MgCl_2$/kg in the presence of $CaCl_2$ at different concentrations (0, 10, 20, 50, 100 and 150 mmole$CaCl_2$/kg) are depicted in Figure 43. After sols were set at 55°C for 30 min, followed by heating at 90°C for 20 min, gels with different breaking force were obtained depending on $CaCl_2$ concentration (Figure 43). There was no difference in breaking force between gel without and with 10 mmole$CaCl_2$/kg ($P>0.05$). Breaking force of Pacific white shrimp gel increased as the $CaCl_2$ concentration increased up to 100 mmol/kg ($P<0.05$). Breaking force of gel added with $CaCl_2$ at the levels of 100 and 150 mmol/kg was not different ($P<0.05$). Two combined effects would subsequently promote the cross-linking reaction in the Pacific white shrimp gel. Firstly, Ca^{2+} and high temperature setting activated endogenous TGase activity. Secondly, these conditions protmoted the unfolding of TGase substrate myofibrillar protein and more available reactive groups of glutamine and lysine could be exposed (Hemung and

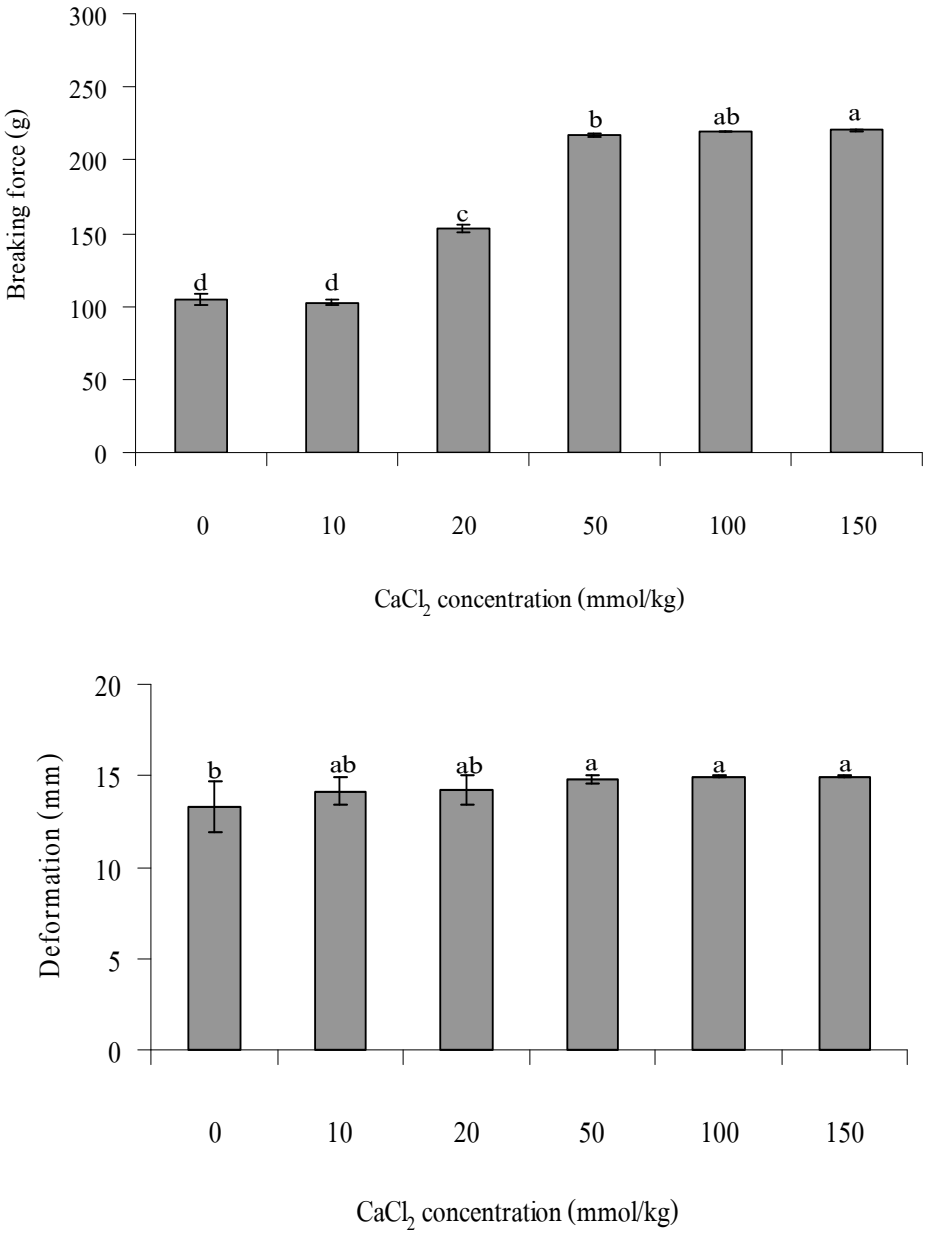

Figure 43 Breaking force and deformation of Pacific white shrimp gels (2.5%NaCl +5 mmole PP/kg + 5 mmole$MgCl_2$/kg) in the presence of different $CaCl_2$ concentrations. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min. Error bars indicate the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

Youngsawatdigul, 2003). Benjakul *et al* (2004) reported the difference in TGase activity and its reactivity toward the protein substrates.

At high levels of calcium chloride, calcium or chloride ion might cause changes in protein conformation. Ions interact with oppositely charged groups on protein molecules to form a double layer of ionic groups, which decreases electrostatic interactions between protein molecules (Vojdani, 1996). However, an excessive amount of calcium could result in "salting out" effect, in which salt could compete with protein in water binding. Deformation was found to vary with $CaCl_2$ concentration. Addition of $CaCl_2$ above 20 mmol/kg resulted in the increases in deformation ($P<0.05$), compared with the control (without $CaCl_2$). From the result, $CaCl_2$ showed an enhancing effect on the gel strength in a concentration dependent maner. Fish TGase showed the difference in their sensitivity to calcium ion (Ashie and Lanier, 2000). Walleye pollack TGase required 3 mM calcium ion, whereas carp muscle TGase required 5 mM calcium ions for full activation (Kishi *et al.*, 1991; Yasueda *et al*., 1994).

Since NaCl or PP in combination with $MgCl_2$ were added to dissociate the myofibrillar proteins, the addition of excessive amount of calcium ion may cause salting-out, leading to loss in solubility of protein. Thus, an appropriate amount of calcium chloride is needed to maximize the setting of Pacific white shrimp gel. Xiong and Breakke (1991) reported that $CaCl_2$ displayed a peculiar effect on the breast myofibrils. Protein extractability of breast myofibrils was initially increased by $CaCl_2$ (<5mM), then declined rapidly with addition $CaCl_2$ to 40 mM, followed by increase with further addition of $CaCl_2$ to 100 mM. Calcium ions function by changing charge density of the myofibrillar protein and influence the structure of myofibrils and alter the protein solubility. At intermediate concentration (e.g. 30-50 mM), calcium may act as a neutralizer or electrostatic depressor to reduce protein interaction. Further elevation in calcium concentration presumably brings about a net positive charge to the proteins. Hence, more protein became extracted and solubilized. Benjakul *et al* (2004) reported that the addition of calcium chloride at a level of 20 mmol/kg increased breaking forces of suwari from bigeye snapper, threadfin bream, barracuda and bigeye croaker by 256.8, 24.7, 67.3 and 29.9%, compared with the control (without calcium chloride), respectively. Lee and Park (1998) found that the addition of 0.2% calcium compounds improved shear stress of Pacific whiting surimi whereas the lower concentrations (0.05 to 0.1%) effectively increased gel texture of Alaska pollack surimi.

4.3.2 Expressible moisture content

Expressible moisture content of Pacific white shrimp gel added with $CaCl_2$ at different concentrations (0, 10, 20, 50, 100 and 150 mmole$CaCl_2$/kg) is shown in Table 12. Expressible moisture content decreased continuously as the concentration of $CaCl_2$ increased ($P<0.05$). The result suggested that the higher amount of $CaCl_2$ could enhance the gelation of protein either by the activation of endogenous TGase or formation of Ca^{2+}- bridge between the protein molecules. This might result in the greater ability of gel to hold more water. Calcium ion showed more influence on gel improvement of surimi from *P. tayenus* than that from *P. macracanthus* (Benjakul and Visessanguan, 2003). This was possibly due to the differences in reactivity toward calcium ion, different amount of TGase between species as well as the differences in calcium ion concentration (Ashie and Lanier, 2000).

Table 12 Expressible moisture content and color of Pacific white shrimp gels (2.5%NaCl+5mmolePP/kg+5mmole$MgCl_2$/kg) in the presence of different $CaCl_2$ concentrations. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min.

$CaCl_2$ concentrations (mmol/kg)	Expressible moisture content (%)	Color		
		*L**	*a**	*b**
0	33.80±1.98a	75.50±0.30d	16.47±0.26a	17.05±0.10c
10	32.84±1.51ab	75.58±0.46d	16.39±0.30a	17.41±0.35b
20	31.88±1.70ab	75.65±0.12d	16.30±0.27a	17.38±0.08b
50	31.18±0.61bc	77.23±0.10c	16.21±0.24ab	17.46±0.09b
100	31.10±1.41bc	77.99±0.38b	15.92±0.20bc	18.36±0.21a
150	29.57±0.86c	78.55±0.30a	15.84±0.17c	18.64±0.32a

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

Mean ± SD from five determinations.

4.3.3 Color

Color of Pacific white shrimp gel added with $CaCl_2$ at different concentrations (0-150 mmole$CaCl_2$/kg) is shown in Table 12**.** *L** and *b**- values increased as $CaCl_2$ concentration increased ($P<0.05$). $CaCl_2$ might form a complex with some anions in the muscle, resulting in the formation of insoluble particles. Calcium ion might form an insoluble complex with phosphate, leading to the light scattering in surimi gels (Benjakul, *et al*., 2004). An increase in whiteness was associated with the light scattering effect of insoluble calcium carbonate in surimi gel (Benjakul, *et al*., 2004). Benjakul *et al* (2000) reported that no differences in whiteness of both suwari and kamaboko gels added with different concentrations of $CaCl_2$ (0, 50, 100 and 150 mM) in the presence of 0.2% fraction I-S and 100 NIH thrombin fraction, were observed. From the result, *a**-value decreased slightly as $CaCl_2$ concentration increased. Calcium particulates formed might contribute to the lower redness observed.

4.3.4 TCA-soluble peptide content

With the addition of $CaCl_2$, TCA-soluble peptide content derived mainly from the degradation of myofibrillar proteins in the gel decreased with the increasing concentration of $CaCl_2$ (Figure 44). The lower TCA-soluble peptide content was observed with increasing $CaCl_2$ content. Cross-linked proteins at higher levels of $CaCl_2$ might be more resistant to proteolysis. As a consequence, the lower degradation products were produced. Ca^{2+} at higher amount might bind with protein substrates and caused the conformational changes in which proteinases could not hydrolyze easily. Additionally, cross-links via salt bridges might be more resistant to hydrolysis. Although 10 mM Ca^{2+} increased cross-linking of MHC by activating the endogenous TGase in salted squid muscle paste, simultaneously activated calpain was observed (Park *et al*., 2003).

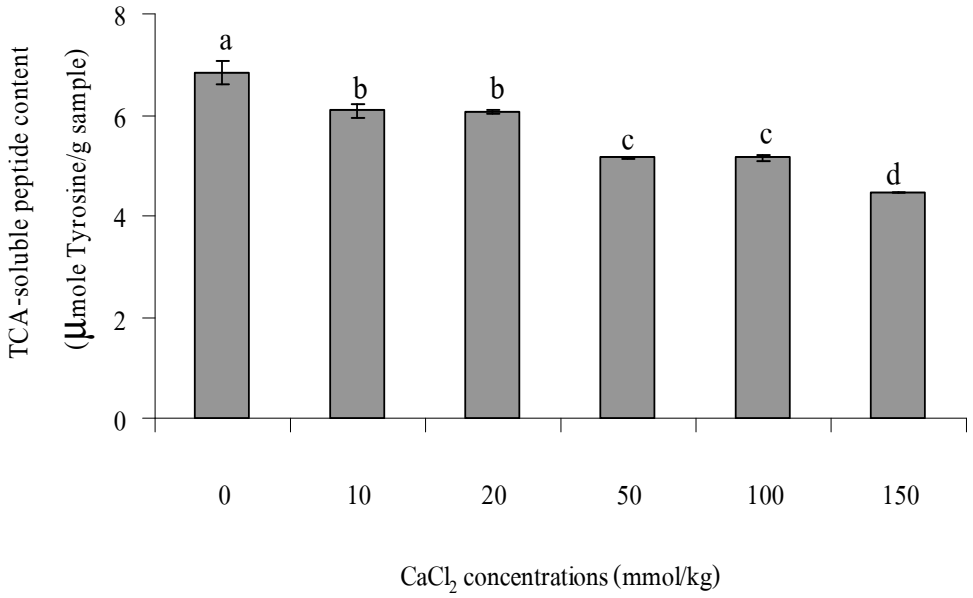

Figure 44 TCA soluble peptide content of Pacific white shrimp gels (2.5%NaCl + 5mmolePP/ kg + 5mmole$MgCl_2$/kg) in the presence of different $CaCl_2$ concentrations. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min. Error bars represent the standard deviation from triplicate determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

3.3.5 Solubility

A gradual decrease in solubility of Pacific white shrimp gel was observed as the calcium chloride concentration increased up to 100 mmol/kg (Figure 45). These indicated that calcium ion at sufficient concentration played an important role in full activation of TGase, leading to the more cross-linked of MHC via nondislufide covalent bond (Figure 46). Decreases in solubility coincided with the increased breaking force and deformation. These result indicated that non-disulfide covalent bond formation induced by endogenous TGase was favored at the $CaCl_2$ concentration of 100 mmol/kg. TGase has been known to play an essential role in ε - (γ-glutamyl) lysine linkage formation in surimi gel (Kumazawa *et al*., 1995). The decreases in solubility of gels from bigeye snapper, threadfin bream and barracuda were observed as the concentration of calcium chloride increased (Benjakul *et al*., 2004).

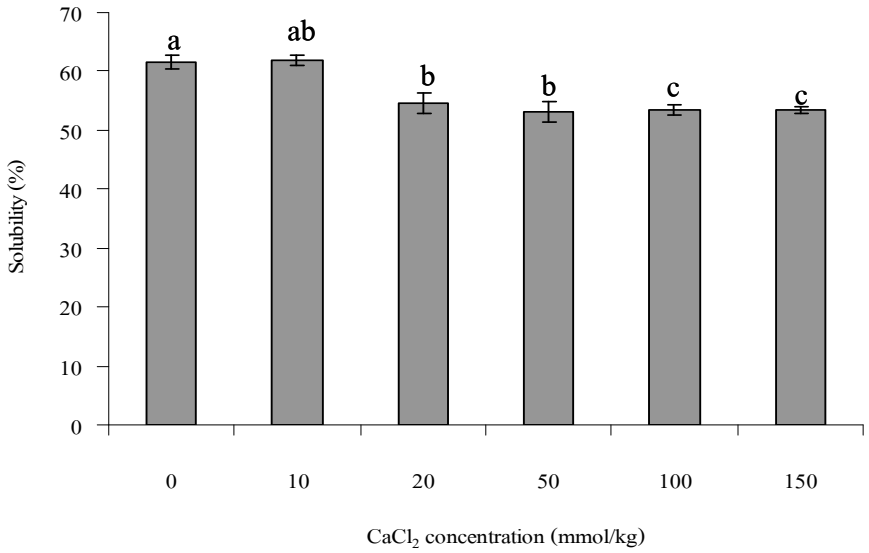

Figure 45 Solubility of Pacific white shrimp gels (2.5%NaCl+5mmolePP/kg+ 5mmole$MgCl_2$/ kg) in the presence of different $CaCl_2$ concentrations. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min. Error bars represent the standard deviation from triplicate determinations. The different letters on the bars indicate the significant differences ($P<0.05$).

4.3.6 Protein pattern

Protein patterns of Pacific white shrimp gel without and with addition of calcium chloride at different levels are depicted in Figure 46. Even without calcium chloride addition, MHC almost underwent cross-linking. This indicated that the setting at 55°C, for a proper time, effectively resulted in the cross-linking of MHC, especially via non-disulfide covalent bonds. This result was in agreement with the highest TGase activity at 55°C (Figure 37). Decreases in MHC band intensity were found as the calcium chloride concentration increased. The decrease in MHC band was presumed to be due to the cross-linking of proteins in the presence of Ca^{2+}-ion (Benjakul *et al*., 2004). This result indicated that MHC underwent more polymerization with the addition of calcium chloride. The decreased MHC band intensity was coincidental with the lower solubility (Figure 45).

Higher molecular weight cross-links that could not pass through 4% acrylamide were observed in concominant with the reduced MHC intensity in the Pacific white shrimp gel

added with $CaCl_2$. Among muscle proteins, TGase preferably catalyzed the cross-linking of MHC (Takeda and Seki, 1996). Therefore, higher molecular weight cross-links of Pacific white shrimp gel added with different $CaCl_2$ and pre-incubated at 55°C resulted from the action of endogenous TGase. From the result, no marked changes in actin band intensity were found when $CaCl_2$ at 0-50 mmol/kg was added. At level greater than 50 mmol/kg, actin band intensity was also decreased. This indicated that actin was also served as the substrate for endogenous TGase, especially when MHC was not available.

The decrease in band intensity of protein MW 116 to 97 was also found as the calcium chloride concentration increased**.** The results reconfirmed that MHC was a preferred protein substrate for polymerization induced by TGase, compared with actin and other proteins. Yongsawatdigul *et al* (2002) reported that MHC intensity of threadfin bream surimi gel decreased with an increased concentration of Ca^{2+} (0-0.5%).

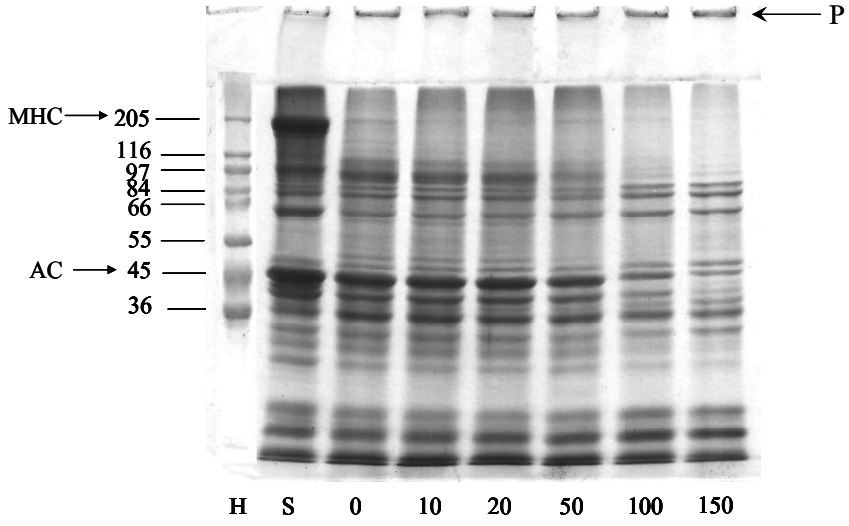

Figure 46 Protein patterns of Pacific white shrimp gels (2.5%NaCl + 5mmolePP/kg + 5mmole $MgCl_2$/kg) in the presence of different $CaCl_2$ concentrations. Sols were incubated at 55°C for 30 min, followed by heating 90°C for 20 min. MHC: myosin heavy chain; AC: actin; H: high molecular weight marker; S: shrimp mince; P: polymerized protein; Number designate $CaCl_2$ concentrations (mmol/kg).

5. Effect of MTGase on gel properties of Pacific white shrimp meat

5.1 Breaking force and deformation

Breaking force and deformation of Pacific white shrimp gel added with MTGase at different levels are shown in Figure 47. The lowest breaking force and deformation were observed in the control gel (without $CaCl_2$ addition). In the presence of 5 mmolePP/kg, 5 mmole$MgCl_2$/kg and 50 mmole$CaCl_2$/kg, breaking force of Pacific white shrimp gel increased by 81%, compared with that of control gel. The increase in breaking force of the gel most likely resulted from the cross-linking in the presence of Ca ion and during pre-incubation at 55°C. The unfolding of myosin and actin induced by $CaCl_2$ resulted in an exposure of free SH groups, which subsequently underwent disulfide interchanges (Hemung and Yongsawatdigul, 2005). However, no differences in deformation between both gels were observed ($P>0.05$). Breaking force and deformation increased as the levels of MTGase increased up to 0.3% ($P<0.05$). The breaking force and deformation of Pacific white shrimp gel added with 0.3% MTGase were 302.82 g and 5.30 mm, respectively, which were increased by 59.03% and 14.06%, compared with the control (without MTGase addition). When the amount of MTGase was higher than 0.3%, significant decreases in breaking force and deformation were obtained ($P<0.05$). Therefore, 0.3% MTGase appeared to be an optimum amount for Pacific white shrimp gel. Several previous studies also indicated that an excess of MTGase might decrease the gel-forming ability of surimi-based products (Asagami *et al*., 1995; Sakamoto *et al*., 1995; Seguro *et al*., 1995). This phenomenon might be due to the excessive formation of the ε- γ- (glutamyl) lysine bond which consequently make the protein gels fragile (Hsieh *et al*, 2002). However, Jiang *et al*. (2000) reported that breaking force and deformation of hairtail surimi gel increased with the addition of MTGase up to 0.6 unit MTGase/g. This was possibly due to the gel-forming ability of hairtail surimi was much lower than other surimi even though 0.6 unit MTGase/g was added. It was obvious that MTGase alone was not sufficient to produce a high quality of hairtail surimi (Jiang *et al*., 2000). In this study, Pacific white shrimp gel was set at 55°C, which was the optimum temperature for endogenous TGase in Pacific white shrimp meat. Coincidentally, MTGase has been known to have the optimal temperature around 45-50°C (Motoki and Seguro, 1998). During the setting, myosin is denatured and polymerized by a calcium dependent endogenous TGase. Thus, unfolding of

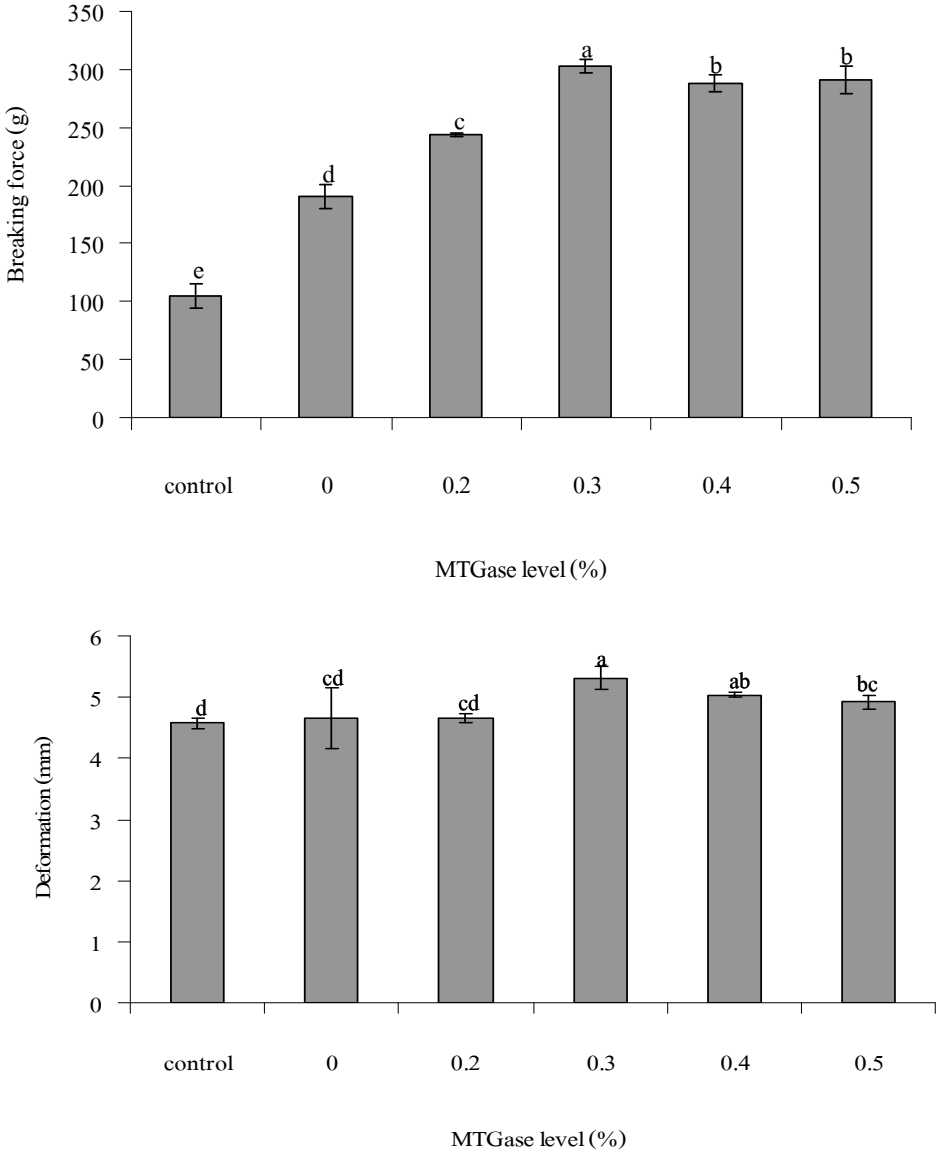

Figure 47 Breaking force and deformation of gels from Pacific white shrimp meat (5mmole PP/kg + 5 mmole$MgCl_2$/kg + 50 mmole$CaCl_2$/kg) in the presence of MTGase at different levels. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 30 min. Error bars represent the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$). Control: gel added with 5mmolePP/kg + 5 mmole$MgCl_2$/kg (without 50 mmole$CaCl_2$/kg).

muscle protein molecules at higher temperature might expose the reactive group for cross-linking by MTGase and endogenous TGase in Pacific white shrimp meat. This led to the increased breaking force and deformation of resulting gel.

5.2 Expressible moisture content

Expressible moisture content of Pacific white shrimp gel added with different level of MTGase is shown in Table 13. From the result, lowered expressible moisture content was noticeable with the addition of MTGase up to 0.3% ($P<0.05$). This result was in agreement with the highest breaking force and deformation at the same MTGase level (Figure 47). Nevertheless, the greater expressible moisture content was obtained in gels added with MTGase greater than 0.3%**.** The excessive cross-linking might lead to the lowered reactive groups available for water binding. As a result, lower water could be retained in the gel matrix.

Table 13 Expressible moisture content of gels from Pacific white shrimp meat (5mmolePP/kg + 5 mmole$MgCl_2$/kg + 50 mmole$CaCl_2$/kg) in the presence of MTGase at different levels

Treatment	Expressible moisture content	color		
	(%)	*L**	*a**	*b**
control	24.63±2.05a	77.83±0.19d	14.67±0.15d	14.7±0.18d
0%MTGase	22.17±2.78a	78.97±0.19c	15.15±0.34bc	14.62±0.45d
0.2%MTGase	19.03±0.68b	79.53±0.29b	15.71±0.14b	14.81±0.22cd
0.3%MTGase	14.02±0.93c	79.66±0.25b	16.42±0.27a	16.25±0.34a
0.4%MTGase	18.44±2.41b	79.61±0.57b	15.04±0.48c	15.22±0.18b
0.5%MTGase	17.96±1.59b	80.41±0.3a	14.99±0.37d	15.15±0.20bc

Control: gel added with 5 mmolePP/kg + 5 mmole$MgCl_2$/kg (without 50 mmole$CaCl_2$/kg). Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 30 min.

Different letters in the same column indicated significant difference ($P<0.05$)

Mean ± SD from five determinations.

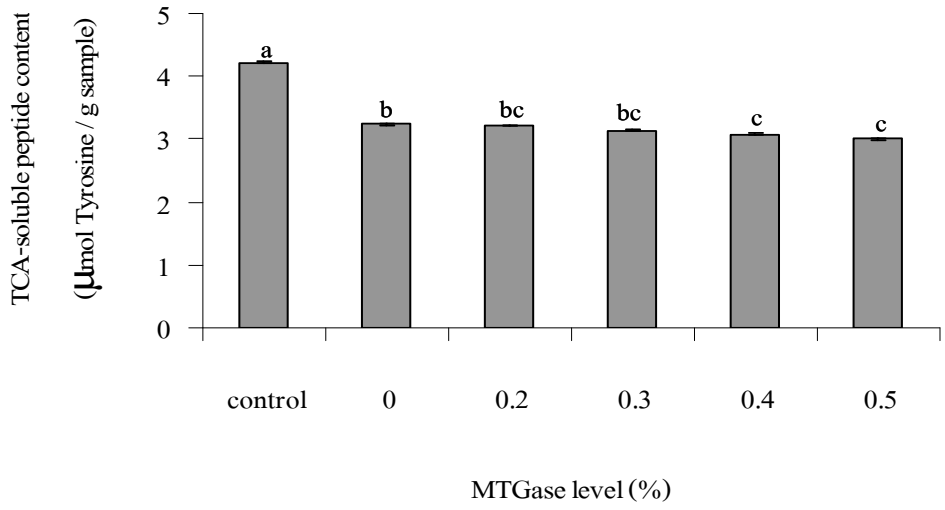

Figure 48 TCA-soluble peptide content of gels from Pacific white shrimp meat (5mmolePP/kg + 5 mmole$MgCl_2$/kg + 50 mmole$CaCl_2$/kg) in the presence of MTGase at different levels. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 30 min. Error bars represent the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$). Control: gel added with 5mmolePP/kg + 5 mmole$MgCl_2$/kg (without 50 mmole$CaCl_2$/kg).

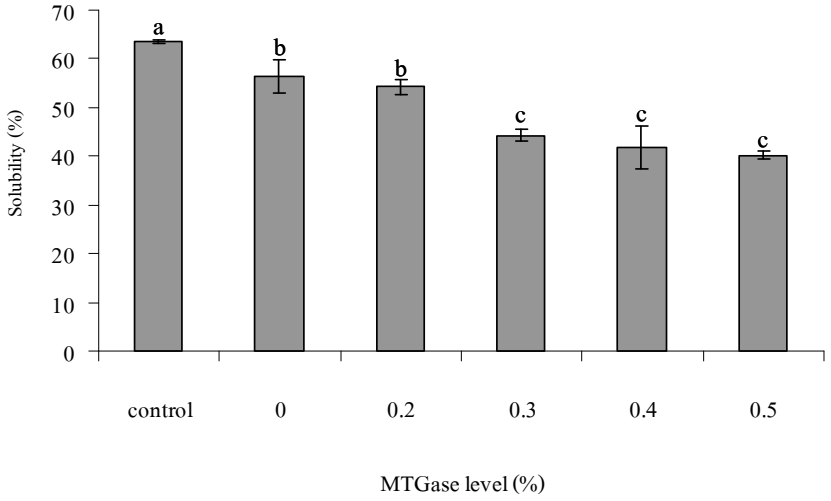

Figure 49 Solubility of gels from Pacific white shrimp meat (5mmolePP/kg + 5 mmole$MgCl_2$ /kg+ 50 mmole$CaCl_2$/kg) in the presence of MTGase at different levels. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 30 min. Error bars represent the standard deviation from five determinations. The different letters on the bars indicate the significant differences ($P<0.05$). Control: gel added with 5mmole PP/kg + 5mmole$MgCl_2$/kg (without 50 mmole$CaCl_2$/kg).

5.3 Color

L, a*, b**-values of Pacific white shrimp gel added with different levels of MTGase are shown in Table 13. *L**-value increased as the concentration of MTGase increased ($P<0.05$). *a** and *b**-value increased when the level of MTGase increased up to 0.3%, followed by the decrease at higher amount of MTGase added. From the result, *L**-value increased with the addition of 0.5% MTGase. As discussed previously, maltodextrin used as MTGase stabilizer possibly exhibited the scattering effect, resulting in the greater of *L**-value of the Pacific white shrimp gel. Additionally, the lower water holding capacity in the gel matrix could be related with the greater released water, particularly on the surface. This could partially result in the increase in whiteness.

5.4 TCA-soluble peptide content

TCA-soluble peptide content of Pacific white shrimp gel added with different levels of MTGase is depicted in Figure 48. The results revealed that protein underwent degradation to a higher extent in the control gel (without MTGase addition). At high temperature, some endogenous protease, such as cathepsin, was active and hydrolyzed muscle proteins. A breakdown of the myofibrillar protein inhibits the development of a three-dimensional gel network of surimi based products (Morrissey *et al*., 1993). Decreases in TCA-soluble peptide content were observed when MTGase was added into the Pacific white shrimp gel. The lowest TCA-soluble peptide content was found when MTGase at levels of 0.4-0.5% was used ($P<0.05$). The cross-linked proteins induced by MTGase as well as endogenous TGase might be less susceptible to hydrolysis by endogenous proteinases, particularly during setting.

5.5 Solubility

Solubility of Pacific white shrimp gel added with higher levels of MTGase had the decreased solubility when the solution consisting of 8 M urea, 2% SDS and 2% βME was used for solubilization (Figure 49). However, no differences in solubility was noticeable in the gels with MTGase addition at levels of 0.3-0.5%. In general, the decrease in solubility was consistent with the increases in breaking force and deformation (Figure 47). The results indicated the increase in non-disulfide bond formation was more pronounced as MTGase level increased.

The result was in accordance with Gomez-Guillen *et al* (2004) who reported that the addition of MTGase resulted in the decrease in solubility of gel from horse mackerel (*Trachurus spp.*) muscle.

5.6 Protein pattern

Changes in protein patterns of Pacific white shrimp gel added with different levels of MTGase are shown in Figure 50. Disappearance of MHC was observed in the control sample (without MTGase addition and set at 55°C for 30 min). It was found that MHC was much more decreased with the concomitant increase of cross-linked proteins on the stacking gel as MTGase levels increased. However, no marked changes in actin were observed in the Pacific white shrimp gel with all MTGase levels used. The decrease in MHC was due to polymerization induced by MTGase (Seguro *et al*., 1995, Jiang *et al*., 1998)**.** The polymerization was greater in the presence of MTGase as evidenced by lowered myosin band intensities (Ashi and Lanier, 1999). Cross-linking rate can be monitored by the disappearance of MHC and the occurrence of high molecular weight protein (MW > 205 kDa) (Chantarasuwan, 2001). The decrease in MHC band intensity was concomitant with the decreases in TCA-soluble peptide content (Figure 48) and solubility (Figure 49). Therefore, MTGase addition and the optimal setting temperature should be applied to retard the degradation and to maximize the cross-linking of proteins.

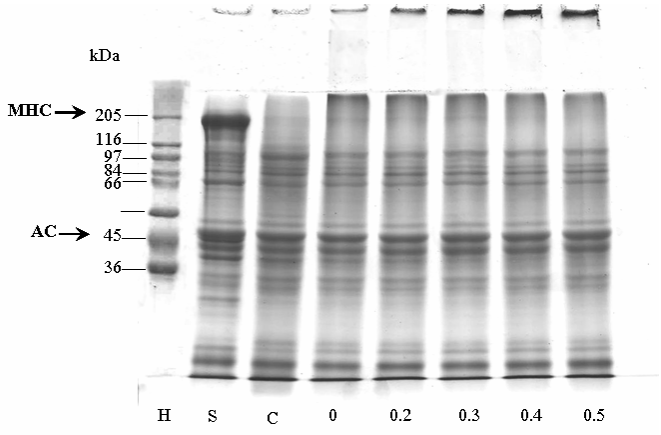

Figure 50 Protein patterns of gels from Pacific white shrimp meat (5mmolePP/kg + 5 mmole $MgCl_2$/kg+ 50 mmole$CaCl_2$/kg) in the presence of MTGase at different levels. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 30 min. H: high molecular marker; S: shrimp mince; C: control (gel added with 5mmolePP/kg + 5 mmole$MgCl_2$/kg); MHC: myosin heavy chain; AC: actin; Numbers designate MTGase level (%).

6. Effect of hydrocolloids on freeze-thaw stability of Pacific white shrimp gel

6.1 Breaking force and deformation

Breaking force and deformation of Pacific white shrimp gel added with modified starch and carrageenan at different levels (0, 2 and 4% (w/w)) and subjected to freeze-thawing for 0, 1 and 3 cycles are shown in Figures 51 and 52, respectively. Breaking force and deformation were affected by the amount of hydrocolloids added. Generally, breaking force of the Pacific white shrimp gel increased by 7.15% when 2% modified starch was added ($P<0.05$). Breaking force of gel added with 4% was not different from that of the control at 0 freeze-thaw cycle ($P<0.05$). Yang and Park (1998) reported that the highest gel strength of Alaska pollack surimi was obtained when 30 g/kg potato starch were added. The textural characteristics of protein gels containing fillers depend on the molecular structure of the fillers that can either depress or reinforce the primary gel structure (Aguilera and Kessler, 1989). As starch granules absorb water from the surroundings during heating, the expanded starch granules exert pressure to the gel matrix, resulting in increased gel strength (Lee *et al*., 1992). There were probably three reasons for such decreasing gel strength when excessive amount of starch was added. Obviously the first reason was a greatly decreased concentration of myofibrillar proteins (Yang and Park, 1998). The second was probably due to a greatly increased concentration of starch, which is also a gelling agent but much weaker than myofibrillar proteins. When the effect of the structure formed by an additive (e.g., starch) is more than that of another one (e.g., myofibrillar proteins) in the system, the textural properties are more dependent on the gel network of the former (starch) (Lanier, 1986). Even though the reinforcement of the starch granules on the gel network might increase at high starch concentration, it would probably not compensate for the effect of decreased myofibrillar proteins. The third reason was probably related to the gelatinizing properties of the starch. However, in the surimi-starch system, there are not only starch and water but also fish proteins, salt, and sugar. Proteins can bind water during salt solubilization and thermal denaturation, which occur prior to starch gelatinization. Those proteins could bind most of the water, leaving less water available for starch than in the starch–water system (Wu *et al.*, 1985). From the result, deformation of gel added with modified starch decreased as the level of modified starch increased ($P<0.05$).

Breaking force and deformation of control gel (without modified starch) increased gradually as freeze-thaw cycles increased up to 3 cycles ($P<0.05$). On the other hand, breaking force of the gel added with 2 and 4% modified starch decreased with increasing freeze thaw cycles. This suggested that the aggregation was more pronounced in the control gel, particularly with repeated freeze-thawing. During freezing or frozen storage, water migrated to form ice crystals. As a result, the gel matrix was collapsed and became more rubbery. For deformation, the marked increases were observed in all samples as freeze thaw cycles increased up to 3 cycles. The highest increase in deformation was found in the control. It indicated that the gel became more rubbery in texture, particularly for the control gel. These indicated that the addition of modified starch could retard the changes in textural properties to some extent during freeze-thawing process. Colmenero *et al* (1996) also reported that the addition of modified waxy maize starch (5 and 10%) caused an increase in penetration force and a decrease in elasticity of bologna sausages and also resulted in the increased freeze-thaw stability.

The Pacific white shrimp gel containing ***ι***-carrageenan had the increases in breaking force and deformation as the amount of ***ι***-carrageenan increased ($P<0.05$) (Figure 52). Breaking force and deformation of the control gel increased gradually as freeze-thaw cycle increased (Figures 51 and 52). For, gels samples added with 4% carrageenan, breaking force and deformation decreased with increasing freeze-thaw cycles. The decrease in breaking force was found in the gel added with 2% carrageenan after 3 freeze-thaw cycles ($P<0.05$). From the result, modified starch and carrageenan might act differently in the gel, particularly during freeze thawing. Starch acts as a "simple filler" or "passive filler" (Ziegler and Foegeding, 1990). ***ι***-carrageenan form fine mesh structure irrespective of temperature. These networks provide connection between adjacent structures within the gel and may serve a supporting function (Gomez-Guillen *et al.*, 1996). Increased gel forming ability of surimi gel when carrageenan is added, is due to the interaction of the carrageenan's sulfate groups with myofibrillar proteins (Bullens *et al.*, 1990). *K*-carrageenan solubilizes at 60-70°C, when the myosin has already begun to gel, whereas ***ι***-carrageenan solubilized at 50°C before the

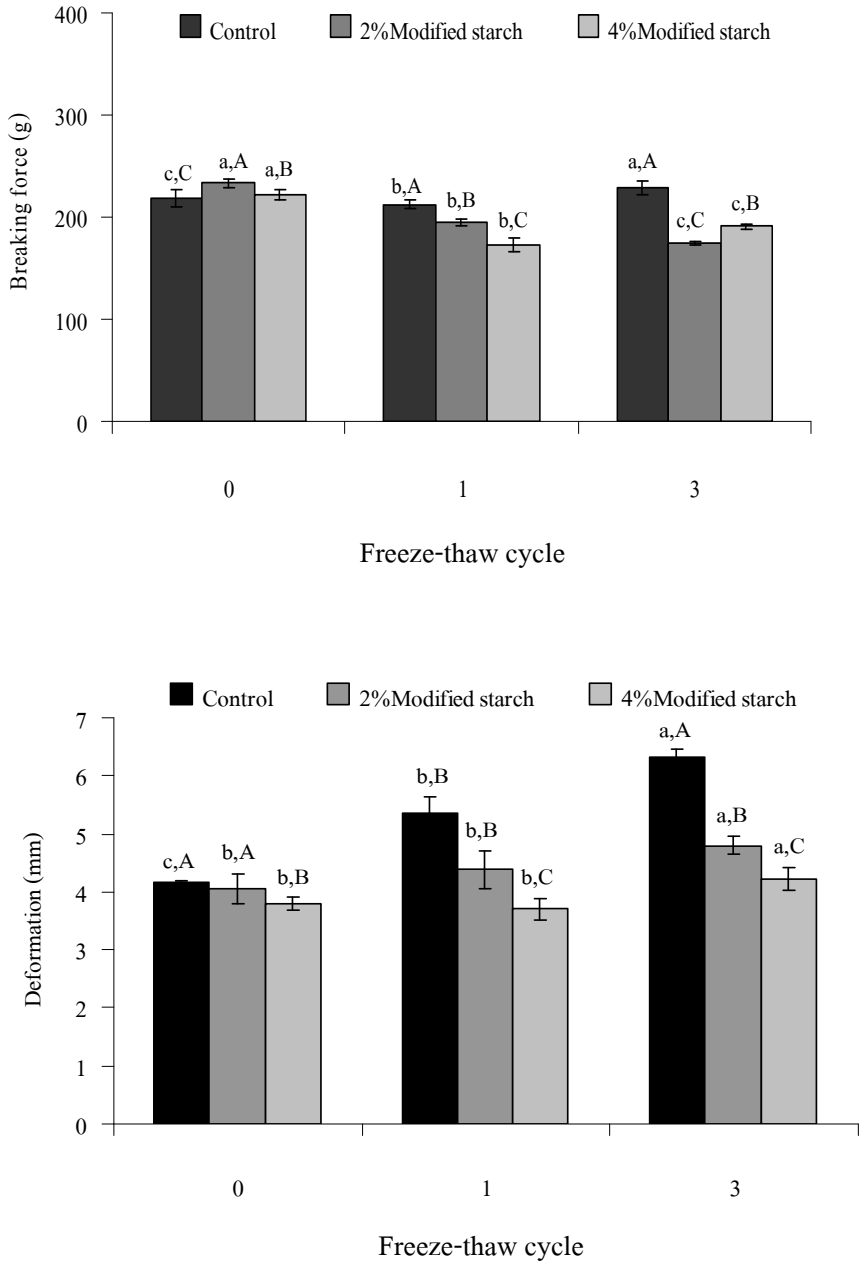

Figure 51 Breaking force and deformation of gels from Pacific white shrimp meat added with modified starch at different levels and subjected to different freeze-thaw cycles. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min. Gels were subjected to freeze-thawing up to 3 cycles. Error bars represent the standard deviation for five determinations. Different letters on the bars within the same freeze-thaw cycle and different letter cases within the same level of modified starch indicate significant differences ($P<0.05$).

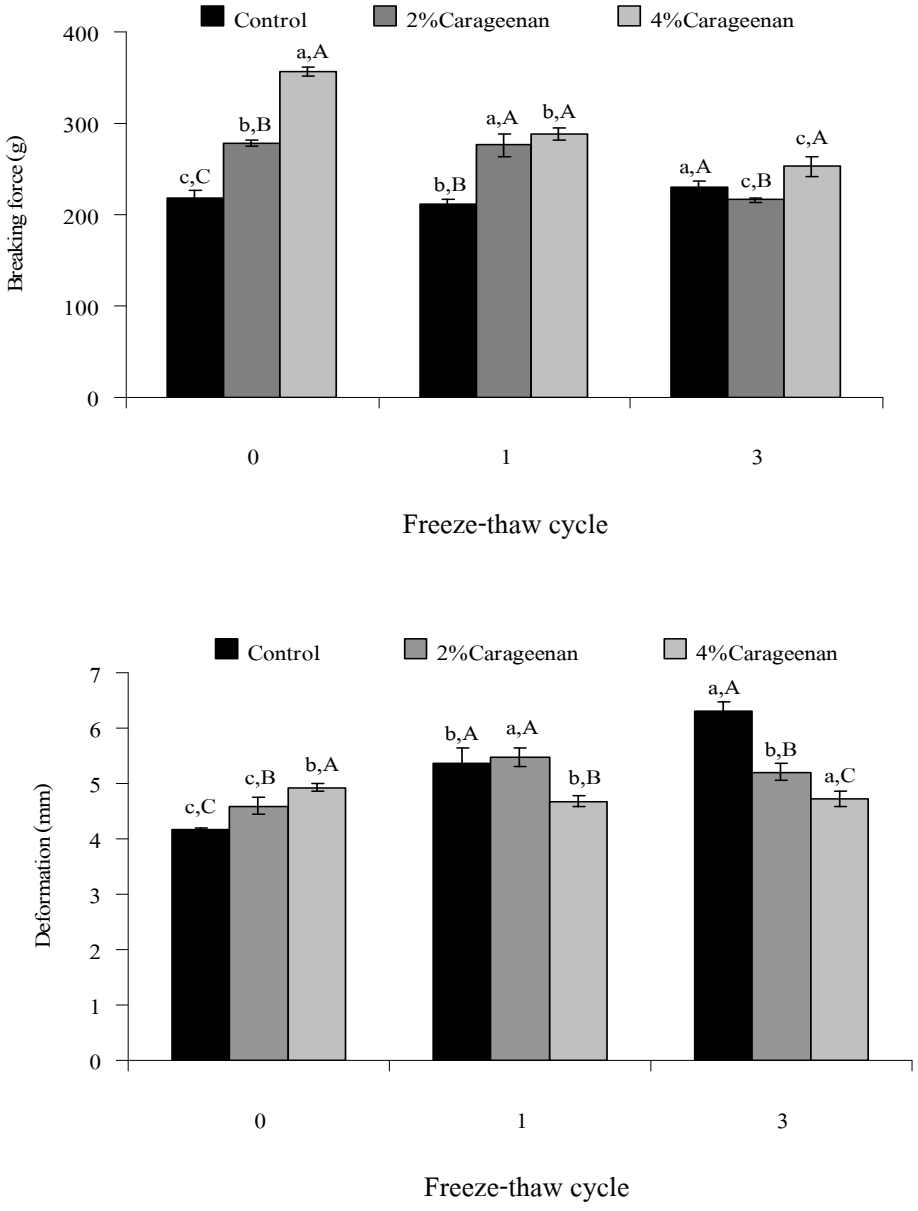

Figure 52 Breaking force and deformation of gels from Pacific white shrimp meat added with ***ι***-carrageenan at different levels and subjected to different freeze-thaw cycles. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min. Gels were subjected to freeze-thawing up to 3 cycles. Error bars represent the standard deviation for five determinations. Different letters on the bars within the same freeze-thaw cycle and different letter cases within the same level of ***ι***-carrageenan indicate significant differences ($P<0.05$).

myofibrillar protein has gelled and thus the latter can penetrate into the protein matrix more regularly. From the result, the addition of 2% modified starch or *ι*-carrageenan in Pacific white shrimp gel could maintain the mechanical properties of resulting gel effectively, irrespective of freeze-thaw cycles.

6.2 Expressible moisture content

Expressible moisture contents of Pacific white shrimp gels added with modified starch or carrageenan at different levels are shown in Figure 53. Generally, expressible moisture content of the Pacific white shrimp gel decreased as the concentration of modified starch increased ($P<0.05$). The increase in expressible moisture content by 37.04% was found in the control gel after 3 freeze-thaw cycles. The gels added with 2% modified starch had the increase in expressible moisture content when the freeze-thawing increased ($P<0.05$). For gel added with 4% modified starch, no marked changes in expressible moisture content were observed with increasing freeze-thaw cycle ($P<0.05$). The fluctuations in temperature during storage causes re-distribution of ice-crystals, resulting in the growth of ice crystals. After being thawed, the water from these large crystals can not diffuse back into the cells (Giddings and Hill, 1978). Kim *et al* (1986) reported that increasing the number of freeze-thaw cycles reduced the strength and deformation of fish surimi gel and suggested that certain protein may be particularly labile to freeze-thawing. The results suggested that water holding capacity of mince gel was increased when the appropriate level of modified starch was added. During gelatinization, several successive changes occur. Granule swelling, disruption of crystalline regions, loss of birefringence, increase of viscosity and fragmentation of the granules take place (Suzuki, 1981). Starch favors the formation of stronger heat-induced structure through swelling of the starch granules embedded in the protein gel matrix. This increased pressure and water binding in the gel matrix caused a more compact and firm structure in the casing (Chen *et al.*, 1993). Furthermore, the hydrophilic nature of the hydroxypropyl group of modified starch could keep the water in the starch paste from separating or syneresis when subjected to freeze-thaw cycling (Pal *et al*., 2002). Pal *et al* (2002) reported that the poor freeze-thaw stability exhibited by unmodified starch indicates extensive retrogradation of starch during frozen storage. However, the substitution

improved the water holding capacity of starch gel by decreasing the extent of retrogradation. The ability of hydroxypropyl group reducing retrogradation could be the bulky hydroxyl groups which prevented proper alignment of starch chains for maximum retrogradation (Pal *et al*., 2002).

At the same freeze thaw cycle, the addition of 4% modified starch could reduce the expressible moisture content effectively. However, modified starch at 4% might cause the dryness of gel since water could be imbibed much more in starch molecules. Pacific white shrimp gel added with 4% ***ι***-carrageenan had the lowest expressible moisture content, compared to the control and gel containing 2% ***ι***-carrageenan ($P<0.05$). After freeze-thawing, the control gels had the marked increases in expressible moisture content. For the gel containing 2% ***ι***-carrageenan, expressible moisture content increased up to 1 cycle of freeze thawing. However, the increase in expressible moisture content was found in gel containing 4% carrageenan with 3 cycles of freeze-thawing ($P<0.05$). ***ι***-carrageenan has greater water holding capacity than ***K***-carageenan and further prevents syneresis during thawing of frozen fish gel (de Ponte *et al.,* 1985). Charged groups of carrageenan might involve in water binding as well as interaction between carrageenan and protein. This might lead to the formation of well-arranged network, while can imbibe more water. Once the molecules are brought closed together by the ionic bonding, hydrogen bonds also form, building up immense aggregation containing large amount of trapped immobilized water (Sand, 1985)**.**

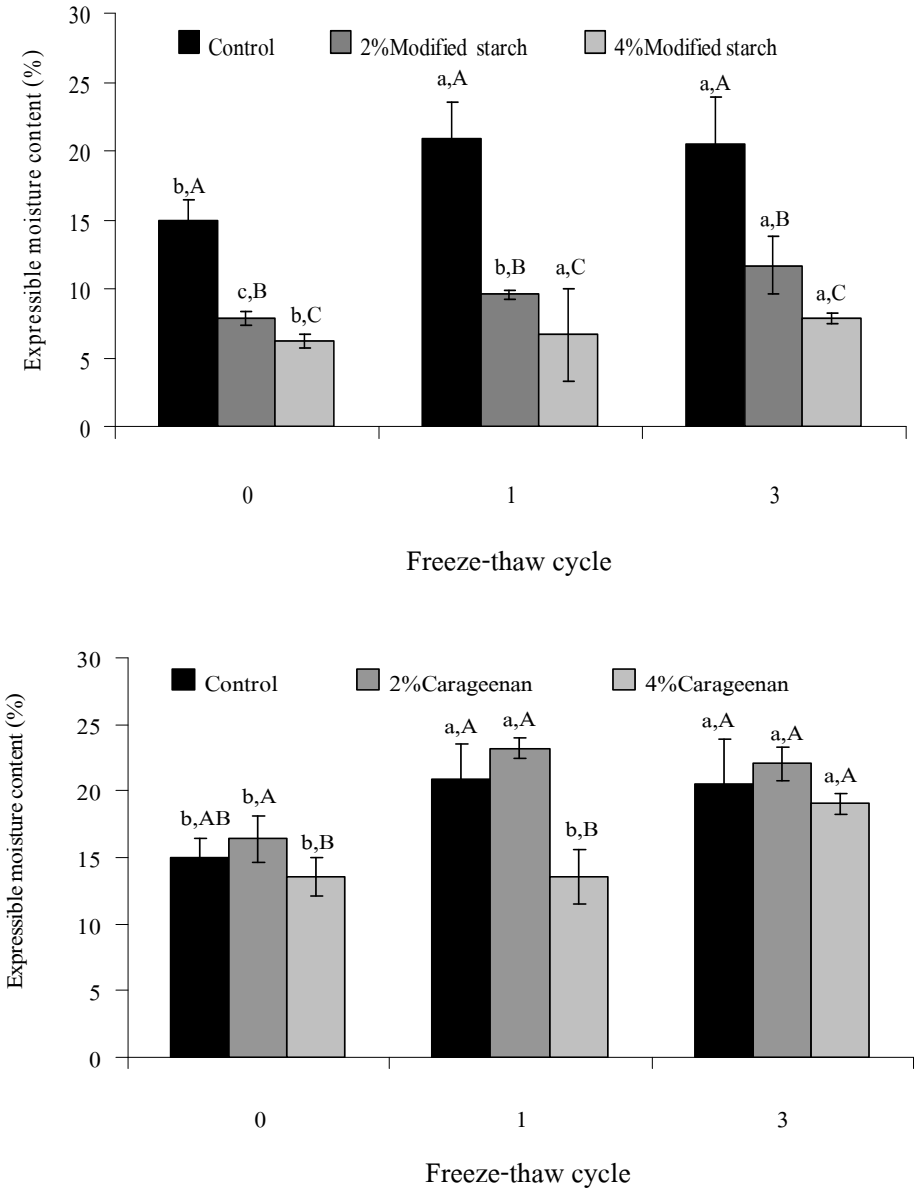

Figure 53 Expressible moisture content of gels form Pacific white shrimp meat added with modified starch or *ι*-carrageenan at different levels and subjected to different freeze-thaw cycles. Sols were incubated at 55°C for 30 min, followed by heating at 90°C for 20 min. Gels were subjected to freeze-thawing up to 3 cycles. Error bars represent the standard deviation for five determinations. Different letters on the bars within the same freeze-thaw cycle and different letter cases within the same level of modified starch or *ι*-carrageenan indicate significant differences ($P<0.05$).

6.3 Microstructure

The selected micrographs of Pacific white shrimp gels (Figure 54A to 53D) revealed that the additive-free control gel made by setting at 55°C for 30 min before heating at 90°C for 20 min had an irregular, porous matrix with some cavities (Figure 54A). The gels added with modified starch had the finer and denser structure with smaller void (Figure 54C). After subjected to multiple freeze-thaw cycles, the control Pacific white shrimp gel become more spongy with the large voids (Figure 54B). Open pockets were present in the gel structure that had poorer gel properties as found in gel after subjected to freeze-thaw cycle (B and D)). Wang and Smith (1992) reported that gels with the lowest expressible moisture content had the most uniform and continuous gel matrices, which was confirmed by the present results. In the gel added with 2% modified starch and subjected to 3 cycles of freeze thawing, more fibrous gel network was observed in comparison with the gel without freeze-thawing (Figure 54D). However, more ordered structure was noticeable, compared with the control gel after freeze-thawing. Modified starch might help in retarding the migration of water to form the ice crystals. Freezing temperature influences the retrogradation of starch pastes. Retrogradation is responsible for the shrinkage and syneresis of starch pastes and gel when held for a long period of time and the effect is highly magnified when gel is frozen and thawed in many cycles (Pomeranz, 1991). Nevertheless, starch which had undergone hydroxypropylation could overcome the problem of syneresis (Kim and Eliasson, 1993; Wu and Seib, 1990; Yeh, 1993). The hydrophobic nature of the hydroxylpropyl starch might prevent the water in the gel network from separation during freezing and thawing process. These result suggested that modified starch was an excellent additive to improve the gel stability of Pacific with shrimp meat during frozen storage.

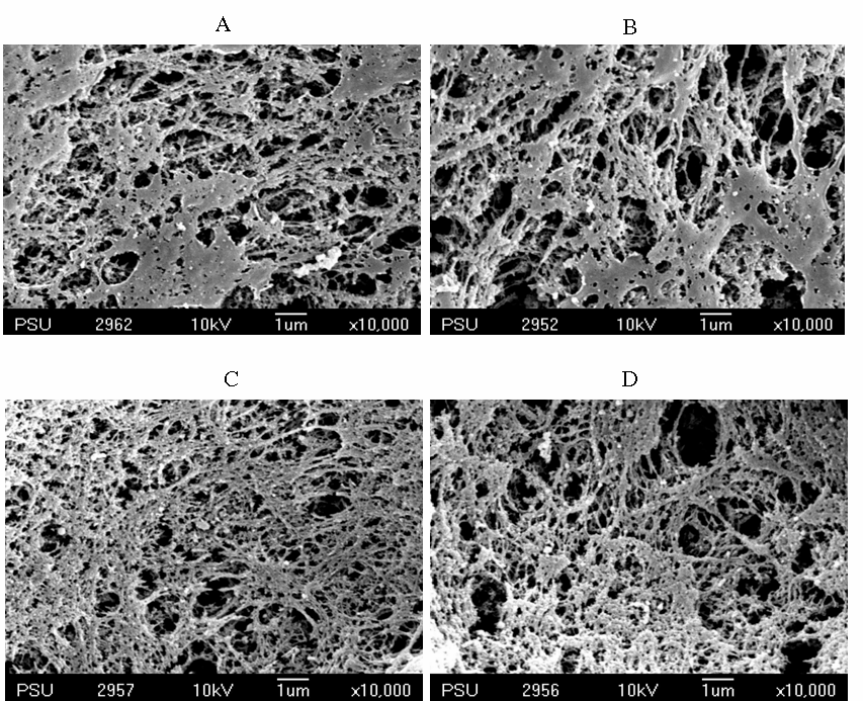

Figure 54 Microstructures of Pacific white shrimp gels added without and with 2% modified starch and subjected to different freeze-thaw cycles. A: control / 0 freeze-thaw cycle; B: control / 3 freeze-thaw cycles; C: 2% modified starch / 0 freeze-thaw cycle; D: 2% modified starch / 3 freeze-thaw cycles.