Chapter 3

Results and discussion

1. Effect of phosphate compounds on setting and gel forming ability of surimi

1.1 Effect of type and levels of phosphate compounds on setting and gel forming ability of surimi

Kamaboko gel and directly heated gel from bigeye snapper and threadfin bream surimi added with different phosphate compounds at various levels had the varying breaking force and deformation ($P<0.05$) (Figures 8 and 9). Generally, both breaking force and deformation decreased as the amount of phosphate compounds added increased up to 0.50% ($P<0.05$). However, the addition of 0.05% PP resulted in the increase in breaking force of kamaboko gel and directly heated gel from bigeye snapper surimi by 17.4% and 11.5%, compared with that of the control, respectively. For kamaboko gel and directly heated gel from threadfin bream surimi, the breaking force increased by 7.70% and 6.45%, respectively. At the same level, TPP addition had no effect on breaking force, while HMP caused the decrease in breaking force of kamaboko gels (Figures 8 and 9). Nevertheless, the increase in breaking force of directly heated gel ($P<0.05$) was observed in surimi from bigeye snapper added with 0.05% TPP. The addition of all phosphate compounds at a level of 0.05% resulted in the increased deformation of kamaboko gels from both surimi ($P<0.05$). For directly heated gel, PP addition at level of 0.05% caused the increase in deformation, while HMP resulted in the reduced deformation of bigeye snapper

surimi gel ($P<0.05$). However, an increase in deformation was obtained in threadfin bream surimi gel added with 0.05% HMP. TPP had no effect on deformation of the gel from bigeye snapper surimi. With the addition of PP at a level of 0.05%, the deformation of kamaboko and directly heated gels from bigeye snapper and threadfin bream surimi increased by 13.5%, 8.21% and 3.5%, 23.70%, compared with that of the control, respectively. The result indicated that PP at 0.05% showed the enhancing effect on gel formation. PP causes the actomyosin complex to be dissociated into actin and myosin (Torigai and Konno, 1996). When phosphate at optimal concentration was added, actomyosin was dissociated and strong gel network was formed (Ellinger, 1975). Xiong and Kupski (1999) found that reduced salt concentration would produce a synergism with phosphate to dissociate actomyosin in chicken fillets. Generally, the ability of TPP on protein extraction was very similar to PP because TPP can be dephosphorylized to PP most likely by a phosphatase, which may be present in myofibril preparations (Torley and Young, 1995). From the result, PP, which has the lowest molecular weight, might distribute uniformly and solubilize or dissociate the actomyosin complex more effectively, compared with other phosphate compounds used. As a result, it exhibited the greater gel strengthening effect than others, particularly at the appropriate concentration. Regardless of phosphate addition, the breaking force of kamaboko gel was higher than that of directly heated gel. Two-step heating used for kamaboko gel preparation possibly allowed the dissociated protein to align gradually during setting (40°C) to form the well-organized network. In addition, setting has been known to induce the formation of non disulfide covalent bond caused by endogenous TGase (Benjakul and Visessanguan, 2003; Kimura *et al*., 1991; Kumazawa *et al*., 1995; Benjakul *et al*., 2004a,b; Benjakul *et al*., 2003). Subsequent

heating resulted in the formation of the large protein aggregate stabilized by various bonding (Benjakul *et al*., 2001).

The decrease in breaking force and deformation in the presence of higher amount of all phosphates used might be due to their chelating property towards calcium ion, which is required for TGase activity (Nozawa *et al*., 1997). Generally, at the same amount of phosphate, HMP showed the greater adverse effect on the gelation. Long-chain polyphosphates are strong complexing agents for the alkaline-earth and heavy-metal ions, while the ring phosphates form weaker complexes. Those phosphates form precipitates with metal ions at higher concentrations (Van Wazer and Callis, 1958; Van Wazer and Campanella, 1950). The polyphosphate anion can bind calcium more firmly than sodium (Ellinger, 1975). Since pyrophosphates as well as the long-chain polyphosphates form soluble complexes of metal ions, the complexing ability of the phosphates apparently does not necessarily depend on chain length (Ellinger, 1975). From the result, phosphate compounds might form the calcium ion complex to a greater extent with increasing amount added. As a consequence, it could impede the activity of endogenous TGase, a Ca^{2+}-dependent enzyme, more effectively as evidenced by the lowered gel strength.

For suwari gel, phosphate addition generally resulted in the decrease in breaking force and deformation, especially with increasing concentration ($P<0.05$) (Figure 8). No changes in breaking force were found in suwari gel from threadfin bream surimi added with 0.05% PP ($P<0.05$). Setting at 40°C possibly caused the changes in protein conformation, resulting in the exposure of reactive groups (glutamine and lysine) for the cross-linking reaction via TGase. Two substrate protein molecules and the enzyme must become associated in a highly oriented and conformation-dependent fashion at some stage of the catalytic process (Folk and

Chung, 1973). The methylene group of glutamine residues is necessary to confer substrate properties and is essential for interaction with a hydrophobic region near the active site of the enzyme (Folk and Chung, 1973). The reactivity of TGase towards various fish actomyosins varied, depending on the conformation of actomyosin (Araki and Seki, 1993). From the result, though PP at 0.05% was shown to increase the breaking force of kamaboko and directly heated gels of both surimi from bigeye snapper and threadfin bream (Figures 8 and 9), it caused the decrease in breaking force of suwari gel. It was suggested that the adverse effect of phosphate addition via chelating of calcium ion required for endogenous TGase was more pronounced in suwari gel, though PP could help the dissociation of actomyosin to form the firm network. The sufficient amount of Ca^{2+}-ion is generally required for TGase activity (Nozawa *et al*., 1997; Lee and Park, 1998). As a result, the less Ca^{2+}-ion available after phosphate addition led to the poorer gel property of suwari gel.

Kamaboko gels had much higher breaking force and deformation than suwari gels and directly heated gel, respectively (Figures 8 and 9). Also, bigeye snapper surimi gels showed the greater breaking force than those from threadfin bream surimi used. The result suggested the superior gel forming ability of proteins in the former surimi to the latter. Generally, gel network development involves two steps. First, interaction of the tail portion of myosin molecules occurs, followed by hydrophobic interaction among the head portions (Sano *et al*., 1990). Because of the instability of the hydrogen bonds during heating, α-helices unfold, exposing hydrophobic amino acids and leading to hydrophobic interaction (Niwa, 1992). Benjakul *et al*. (2001) reported that natural actomyosin from bigeye snapper underwent aggregation at temperatures above 30°C as observed by an increase in

turbidity. Formation of large aggregates is presumably a prerequisite to formation of a good elastic gel (Chan *et al*., 1992). High temperatures during heating led to further oxidation of sulfhydryl groups with a subsequent disulfide bond formation (Benjakul *et al*., 2001).

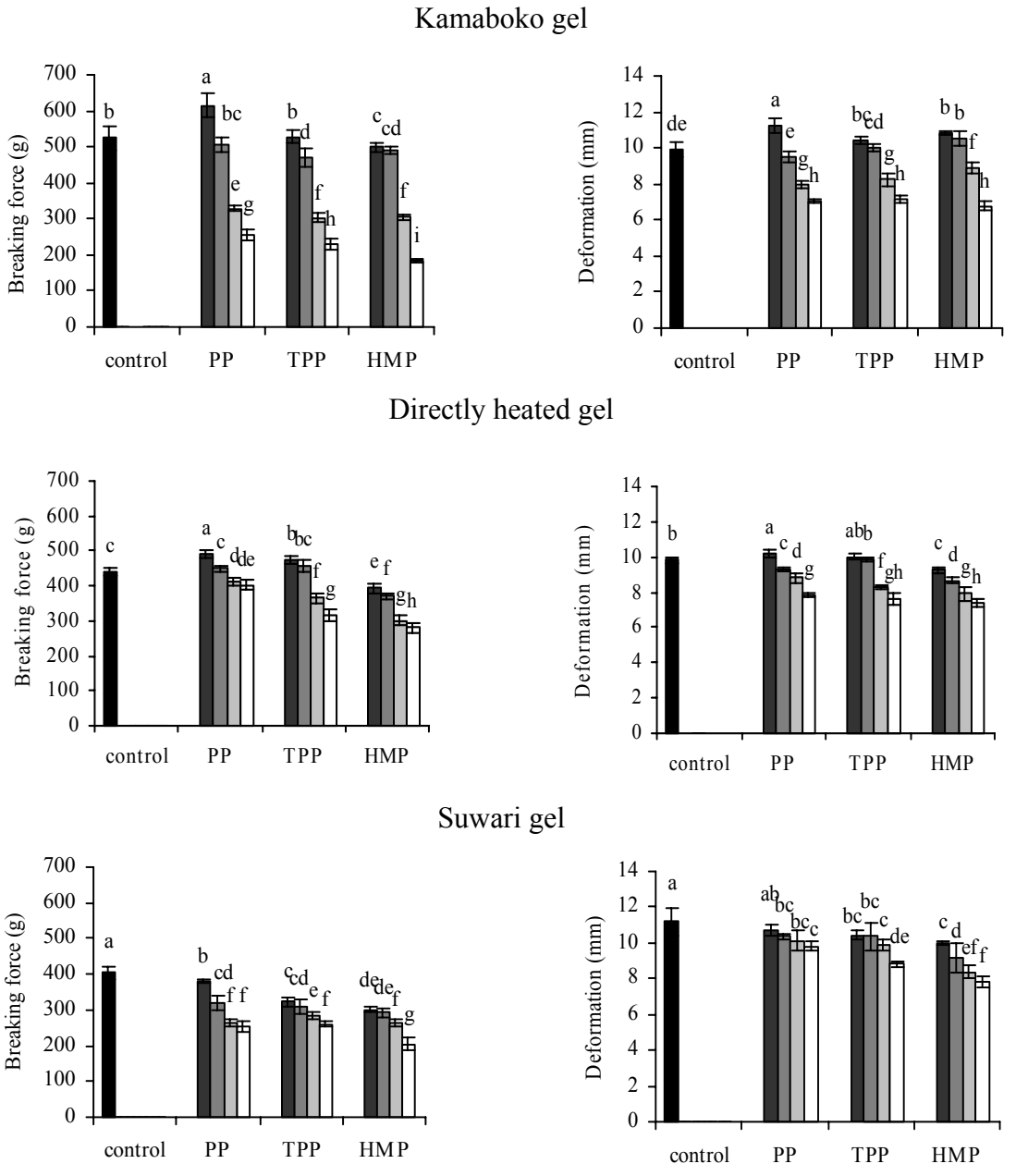

Figure 8. Breaking force and deformation of kamaboko, directly heated and suwari gels from bigeye snapper surimi added with phosphate compounds at different levels. Bars represents the standard deviation from five determinations. Different letters on the bars indicate the significant difference ($P<0.05$).

■ Control ■ 0.05% ■ 0.1% ■ 0.3% □ 0.5%

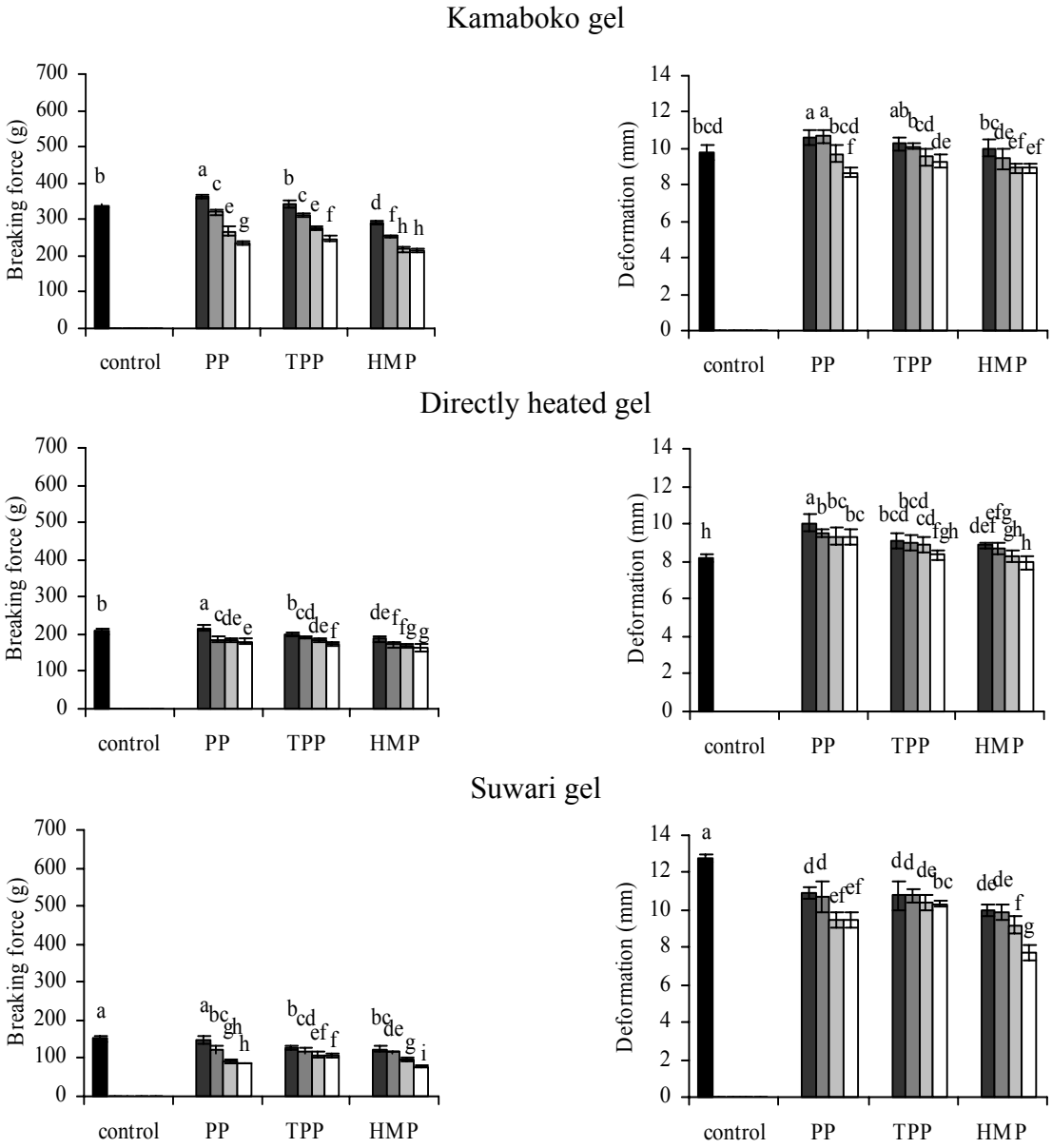

Figure 9. Breaking force and deformation of kamaboko, directly heated and suwari gels from threadfin bream surimi added with phosphate compounds at different levels. Bars represents the standard deviation from five determinations. Different letters on the bars indicate the significant difference ($P<0.05$).

■ Control ■ 0.05% ■ 0.1% ■ 0.3% □ 0.5%

1.2 Effect of phosphate compounds on expressible moisture of surimi gels

Expressible moisture content of kamaboko, directly heated and suwari gels from bigeye snapper and threadfin bream surimi added with different phosphate

compounds at different levels is shown in Tables 3 and 4. Addition of PP in bigeye snapper surimi gel at different levels had no effect on expressible moisture content of all gels ($P>0.05$), except for suwari gel in which higher expressible moisture was obtained with PP addition. Additionally, the lower expressible moisture was noticeable in kamaboko gel added with 0.05% PP ($P<0.05$), suggesting the greater water holding capacity of protein network. For suwari gel from threadfin bream surimi (Table 4), the addition of PP at levels above 0.1% caused the increased expressible moisture content. The result suggested that PP affected the gel network differently in term of water imbibing. For TPP added gels from both surimi, no differences in expressible moisture content were found in kamaboko gels added with different levels of phosphates ($P>0.05$). HMP addition had no effect on the expressible moisture of bigeye snapper surimi gels but resulted in the increased expressible moisture in threadfin bream kamaboko gels. Generally, the increased expressible moisture content was observed with suwari gels added with increasing phosphate content, suggesting the decreased water holding capacity of gel matrix. Phosphate anions act as polyelectrolyte to increase ionic strength, resulting in increased water holding capacity by direct binding of water to the phosphate anions and by the repulsion of protein groups due to the predominance of negative charges on the protein groups. This repulsing effect opens up protein structures, and increases the number of binding sites available for water, which allows for more water to be contained in the meat (Xiong, 2005).

From the result, no changes in expressible moisture content of kamaboko gels from both surimi were observed with increasing phosphate levels, though the decrease in breaking force was found. This reconfirmed the important role of phosphates in retaining the water in the gel matrix. The higher expressible moisture

content was observed in directly heated gels from bigeye snapper surimi added with TPP and HMP, compared with that of kamaboko gel. Therefore, setting was most likely crucial for development of gel network of surimi from bigeye snapper, which can imbibe the water. Without setting, the poorer water holding capacity was noticeable, particularly with increasing phosphate levels. However, no marked differences in expressible moisture content were noticeable between kamaboko gel and directly heated gel of surimi from threadfin bream. It was suggested the differences in TGase activity and susceptibility of proteins to phosphate between two species.

Table 3. Expressible moisture content of surimi gels from bigeye snapper added with various phosphate compounds at different concentrations and heated under various conditions.

Type	Conc (%)	Kamaboko	Directly heated	Suwari
PP	0	5.08±0.45abAB*	5.26± 0.35eA	2.54 ± 0.09cB
	0.05	4.72±0.18bB	5.23±0.12eA	2.79±0.25bcAB
	0.10	5.11±0.23aA	5.27±0.16eA	2.86±0.05bcAB
	0.30	5.29±0.16aA	5.29±0.30eA	2.97±0.20abcdA
	0.50	5.31±0.11aA	5.30±0.18eA	3.06±0.26abA
TPP	0	5.08±0.45abA	5.26±0.35eB	2.54±0.09cB
	0.05	5.22±0.03aA	5.30±0.23eB	2.87±0.20bcAB
	0.10	5.24±0.17aA	6.88±0.69cdA	2.86±0.08bcAB
	0.30	5.37±0.19aA	6.99±0.59cdA	2.88±0.11bcAB
	0.50	5.41±0.18aA	7.30±0.69bcA	3.04±0.38abA
HMP	0	5.08±0.45abA	5.26±0.35eC	2.54±0.09cD
	0.05	5.04±0.32abA	5.42±0.14eC	3.05±0.23abA
	0.10	5.15±0.29abA	6.26±0.53dB	2.87±0.26bcCD
	0.30	5.30±0.38aA	7.96±0.39abA	3.05±0.33abBC
	0.50	5.33±0.19aA	8.61±0.66aA	3.39±0.20aAB

*Values are mean ± standard deviation. Values with the same letter in the same column are not significantly different (P>0.05). Values with the same capital letter in the same column under the same phosphate type are not significantly different (P>0.05).

From the result, suwari and kamaboko gels tended to have lower expressible moisture content than directly heated gel, indicating the higher water

holding capacity of the gels. During setting at 40°C, proteins underwent some denaturation and aligned themselves gradually to form the network, which can imbibe water (Benjakul and Visessanguan, 2003). Alverez and Tejada (1997) found that suwari gel had lower water holding capacity than kamaboko gels. However, the heating process might ruin some hydrogen bonding, which is involved in water holding in gel matrix, particularly of those without prior setting.

Table 4. Expressible moisture content of surimi gels from threadfin bream added with various phosphate compounds at different concentrations and heated under various conditions.

Type	Conc.(%)	Kamaboko	Directly heated	Suwari
PP	0	5.03±0.16bB*	5.17±0.10abAB	3.71 ±0.31dC
	0.05	4.94±0.20bB	4.96±0.30bB	3.93±0.16cdBC
	0.10	5.23±0.13bB	5.12±0.13abAB	4.23±0.13abcAB
	0.30	5.62±0.17aA	5.33±0.15abA	4.48±0.13aA
	0.50	5.79±0.15aA	5.34±9.15abA	4.49±0.20aA
TPP	0	5.03±0.16bA	5.17±0.10abA	3.71 ±0.31dB
	0.05	5.04±0.36bA	5.17±0.17abA	3.99±0.15 bcdAB
	0.10	5.17±0.14bA	5.21±0.21abA	4.26±0.36 abcA
	0.30	5.19±0.09bA	5.33±0.14abA	4.34±0.14bcA
	0.50	5.23±0.17bA	5.44±0.17aA	4.35±0.18bcA
HMP	0	5.03±0.16bB	5.17±0.10abA	3.71 ±0.31dB
	0.05	5.15±0.20bB	5.31±0.33abA	4.26±0.11abcA
	0.10	5.76±0.25aA	5.34±0.29abA	4.28±0.18abcA
	0.30	5.83±0.22aA	5.37±0.24abA	4.44±0.28aA
	0.50	5.88±0.15aA	5.41±0.22aA	4.53±0.27aA

*Values are mean ± standard deviation. Values with the same letter in the same column are not significantly different (P>0.05). Values with the same capital letter in the same column under the same phosphate type are not significantly different (P>0.05).

1.3 Effect of phosphate compounds on whiteness of surimi gels

Whiteness of all surimi gels increased as the phosphate concentrations increased (P<0.05) (Tables 5 and 6). This might be associated with the increase in expressible moisture content of gel, especially when the phosphate amount increased.

Table 5. Whiteness of surimi gels from bigeye snapper added with various phosphate compounds at different concentrations and heated under various conditions.

Type	Conc (%)	Kamaboko	Directly heated	Suwari
PP	0	71.37±0.13iD*	71.93±0.13fD	59.56±0.18iD
	0.05	72.43±0.25eC	72.14±0.09fD	60.11±0.29fC
	0.10	73.06±0.21cB	72.45±0.18eC	63.14±0.10dB
	0.30	73.87±0.10aA	73.37±0.12dB	64.15±0.19abA
	0.50	73.92±0.02aA	73.97±0.08bA	64.34±0.21abA
TPP	0	71.37±0.13iD	71.93±0.13fC	59.56±0.18iD
	0.05	72.14±0.24fC	71.93±0.20fC	61.69±0.21eC
	0.10	72.70±0.06dB	73.12±0.35dB	62.90±0.24dB
	0.30	73.18±0.18bcA	73.67±0.11cA	63.66±0.15cA
	0.50	73.33±0.01bA	73.81±0.11bcA	63.77±0.11cA
HMP	0	71.37±0.13iD	71.93±0.13fD	59.56±0.18iE
	0.05	72.14±0.10fC	73.29±0.14dC	61.83±0.11eD
	0.10	72.23±0.04efC	74.08±0.08bB	62.84±0.08dC
	0.30	73.34±0.12bB	74.35±0.16aA	64.15±0.10bB
	0.50	73.73±0.18aA	74.55±0.16aA	64.46±0.05aA

*Values are mean ± standard deviation. Values with the same letter in the same column are not significantly different (P>0.05). Values with the same capital letter in the same column under the same phosphate type are not significantly different (P>0.05).

Table 6. Whiteness of surimi gels from threadfin bream added with various phosphate compounds at different concentrations and heated under various conditions.

Type	Conc.(%)	Kamaboko	Direct heated	Suwari
PP	0	77.27 ± 0.13eD*	77.46 ± 0.20gD	65.40 ± 0.21gD
	0.05	78.10 ± 0.35dC	77.44 ± 0.22gD	70.87 ± 0.17eC
	0.10	78.43 ± 0.14bcBC	78.61 ± 0.23eC	71.83 ± 0.19dB
	0.30	78.57 ± 0.04bB	79.00 ± 0.24cdB	72.94 ± 0.22cA
	0.50	78.94 ± 0.17aA	79.40 ± 0.13bA	73.01 ± 0.17cA
TPP	0	77.27 ± 0.13eC	77.46 ± 0.20gD	65.40 ± 0.21gE
	0.05	77.85 ± 0.17dB	78.06 ± 0.27fC	69.65 ± 0.27fD
	0.10	77.88 ± 0.09dB	78.51 ± 0.16eB	71.77 ± 0.16dC
	0.30	78.13 ± 0.25cdB	78.78 ± 0.10deB	72.70 ± 0.26cB
	0.50	78.60 ± 0.20bA	79.86 ± 0.26aA	73.95 ± 0.10aA
HMP	0	77.27 ± 0.13eB	77.46 ± 0.20gD	65.40 ± 0.21gD
	0.05	76.86 ± 0.21fC	78.96 ± 0.22cdC	71.10 ± 0.17eC
	0.10	77.42 ± 0.22eB	79.31 ± 0.14bcB	72.78 ± 0.20cB
	0.30	77.79 ± 0.19dA	79.88 ± 0.12aA	73.42 ± 0.18bA
	0.50	78.09 ± 0.12dA	80.17 ± 0.15aA	73.61 ± 0.17bA

*Values are mean ± standard deviation. Values with the same letter in the same column are not significantly different (P>0.05). Values with the same capital letter in the same column under the same phosphate type are not significantly different (P>0.05).

The free water released to the gel surface contributed to the light scattering, resulting in the increased whiteness of gel. Among all gels tested, suwari gels had the lowest whiteness when compared with other gels (kamaboko and directly heated gels). This was probably due to the fact that higher temperature caused the denaturation of protein, especially pigments remaining in the muscle, leading to more turbidity as shown by higher whiteness. Directly heated gel had similar whiteness to kamaboko gel. Polyphosphates can exhibit whiting effect via the dispersion of a myosin sol (Grantham, 1981).

1.4 Effect of phosphate compounds on solubility of surimi gel

Solubility of kamaboko, directly heated and suwari gels from bigeye snapper and threadfin bream surimi added with phosphate at different levels is shown in Tables 7 and 8, respectively. From the results, solubility of all gels increased when the levels of phosphates increased ($P<0.05$). The solubility of gels added with PP was lowest, especially when 0.05%PP was used. The decrease in solubility indicated the presence of non-disulfide cross-links formed during setting at high temperature (40°C). From the result, the increase in solubility coincided with decreased breaking force and deformation, especially when the levels of phosphates increased. Thus, the decrease in breaking force with the concomitant increase in solubility might be caused by the lowered cross-links induced by endogenous TGase. A solution containing SDS, urea and β-mercaptoethanol was used to solubilize protein by destroying all bonds, except ε-(γ-glutamyl) lysine linkage (Benjakul *et al*., 2001a). Thus, the lowered solubility indicated non-disulfide covalent bond formation induced by endogenous TGase. TGase has been known to play an essential role in ε-(γ-glutamyl) lysine

linkage formation in surimi gel (Kumazawa *et al*., 1995). From the result, kamaboko gels of bigeye snapper surimi added with all phosphates at levels lower than 0.1% showed the lower solubility than those of threadfin bream surimi. Additionally, suwari gels from bigeye snapper surimi had the lower solubility than those from threadfin bream at all levels of phosphates used. The result suggested that non-disulfide cross-linking occurred in surimi from bigeye snapper to a greater extent than surimi from threadfin bream, possibly due to either higher TGase activity or the more reactive myofibrillar proteins in the former toward TGase-induced reaction.

Table 7. Solubility of bigeye snapper surimi gels added with various phosphate compounds at different concentrations and heated under various conditions.

Type	Conc.(%)	Kamaboko	Direct heated	Suwari
PP	Control	100f*	100d	100e
	0	51.60 ± 0.98b	62.69 ± 1.24a	23.99 ± 3.58a
	0.05	48.34 ± 1.11a	59.39 ± 1.49a	27.88 ± 2.11a
	0.10	53.76 ± 0.88c	62.22 ± 1.69a	35.19 ± 3.28b
	0.30	69.51 ± 1.24d	67.21 ± 2.16b	45.15 ± 1.90c
	0.50	76.74 ± 1.10e	70.59 ± 2.83c	49.80 ± 1.91d
TPP	Control	100e	100d	100e
	0	51.60 ± 0.98a	62.69 ± 1.24a	23.99 ± 3.58a
	0.05	52.50 ± 1.86a	61.33 ± 2.02a	32.63 ± 0.78b
	0.10	66.04 ± 1.35b	62.72 ± 2.00a	36.36 ± 1.55b
	0.30	70.87 ± 2.70c	75.17 ± 2.20b	41.03 ± 1.78c
	0.50	77.31 ± 2.18d	79.65 ± 1.73c	51.16 ± 3.31d
HMP	Control	100f	100d	100e
	0	51.60 ± 0.98a	62.69 ± 1.24a	23.99 ± 3.58a
	0.05	55.82 ± 2.44b	70.96 ± 1.69b	35.32 ± 3.70b
	0.10	67.48 ± 1.60c	72.48 ± 1.92b	37.35 ± 2.40b
	0.30	75.21 ± 2.07d	82.44 ± 1.86c	50.07 ± 2.85c
	0.50	80.73 ± 2.69e	84.34 ± 3.71c	59.24 ± 1.71d

*Solubility in 20 mM Tris-HCl containing 1%SDS, 8 M urea and 2% β-MW, pH 8.0. Control: solubility in 0.5 N NaOH, PP = Sodium pyrophosphate, TPP = Sodium tripolyphosphate, HMP = Sodium hexametaphosphate. The same letter in the same column under the same phosphate type indicate non-significant difference ($P>0.05$).

Table 8. Solubility of threadfin bream surimi gels added with various phosphate compounds at different concentrations and heated under various conditions.

Type	Conc.(%)	Kamaboko	Direct heated	Suwari
PP	Control	100d*	100d	100d
	0	67.03±0.61b	61.09±0.88b	54.45±0.71a
	0.05	63.53±1.05a	58.48±1.03a	56.52±1.32b
	0.10	68.40±1.75b	61.36±1.27b	58.23±1.29b
	0.30	72.32±1.02c	62.14±1.16b	61.77±1.17c
	0.50	73.59±1.66c	66.49±1.91c	62.85±0.95c
TPP	Control	100d	100d	100e
	0	67.03±0.61a	61.09±0.88a	54.45±0.71a
	0.05	65.33±1.04a	61.84±2.01ab	56.75±1.00b
	0.10	70.52±1.27b	63.88±1.95ab	58.90±0.90c
	0.30	71.66±1.56b	64.25±2.16ab	59.10±1.01c
	0.50	75.63±1.49c	64.52±2.00c	61.61±1.01d
HMP	Control	100d	100d	100d
	0	67.03±0.61a	61.09±0.88a	54.45±0.71a
	0.05	69.73±1.65b	62.52±1.44a	56.71±1.21b
	0.10	75.71±2.13c	65.38±1.26b	58.41±1.22b
	0.30	76.11±2.13c	66.84±0.94bc	62.20±0.78c
	0.50	77.17±1.48c	67.68±1.26c	62.76±1.53c

*Solubility in 20 mM Tris-HCl containing 1%SDS, 8 M urea and 2% β-MW, pH 8.0. Control: solubility in 0.5 N NaOH, PP = Sodium pyrophosphate, TPP = Sodium tripolyphosphate, HMP = Sodium hexametaphosphate. The same letter in the same column under the same phosphate type indicate non-significant difference (P>0.05).

1.5 Effect of phosphate compounds on protein patterns of surimi gels

The protein patterns of gels from bigeye snapper and threadfin bream surimi without and with addition of different phosphate compounds at various levels are depicted in Figures 10 and 11, respectively. MHC band intensity increased as the phosphate levels increased. However, no marked differences in MHC band intensity were noticeable when the phosphate compounds at level of 0.05% were used, compared with those of the control (without phosphate addition). Generally, no changes in actin band intensity were observed, even phosphate compounds were added at a higher level. MHC was most susceptible to cross-linking during setting (Benjakul *et al*., 2003a; Benjakul *et al*., 2004a). The increase in MHC band intensity

with a higher level of phosphate added revealed that non-disulfide cross-link was reduced. Phosphates might chelate the calcium ions required for endogenous TGase, resulting in the insufficient amount of calcium ion. As a consequence, polymerization of MHC was reduced as shown by the more retained MHC. Benjakul *et al*. (2003) reported the decrease in MHC of surimi gel from bigeye snapper, particularly when the setting was implemented. However, the cross-linking of surimi proteins from tropical fish was inhibited in the presence of EDTA, calcium ion chelator (Benjakul *et al*., 2004c). From the result, the increase in MHC band intensity was coincidental with the decrease in breaking force (Figures 8 and 9). It suggested that phosphate compounds showed the detrimental effect on gelling properties of surimi, most likely due to their chelating property towards calcium ion needed for cross-linking induced by endogenous TGase. Additionally, the excessive charge modification of proteins by phosphates might induce the protein repulsion, leading to the less aggregation with poorer gel properties. When comparing the protein patterns of surimi gel from both surimi, it was formed that bigeye snapper surimi gels, particularly the control gel or gel added with 0.05% phosphate, showed the lower MHC band intensity than gels from threadfin bream surimi, indicating the superior setting of the former. This was coincidental with the higher gel strength of the former. Due to the higher gel strengthening effect of PP, it was chosen for further use in combination with $CaCl_2$ to improve the gel property.

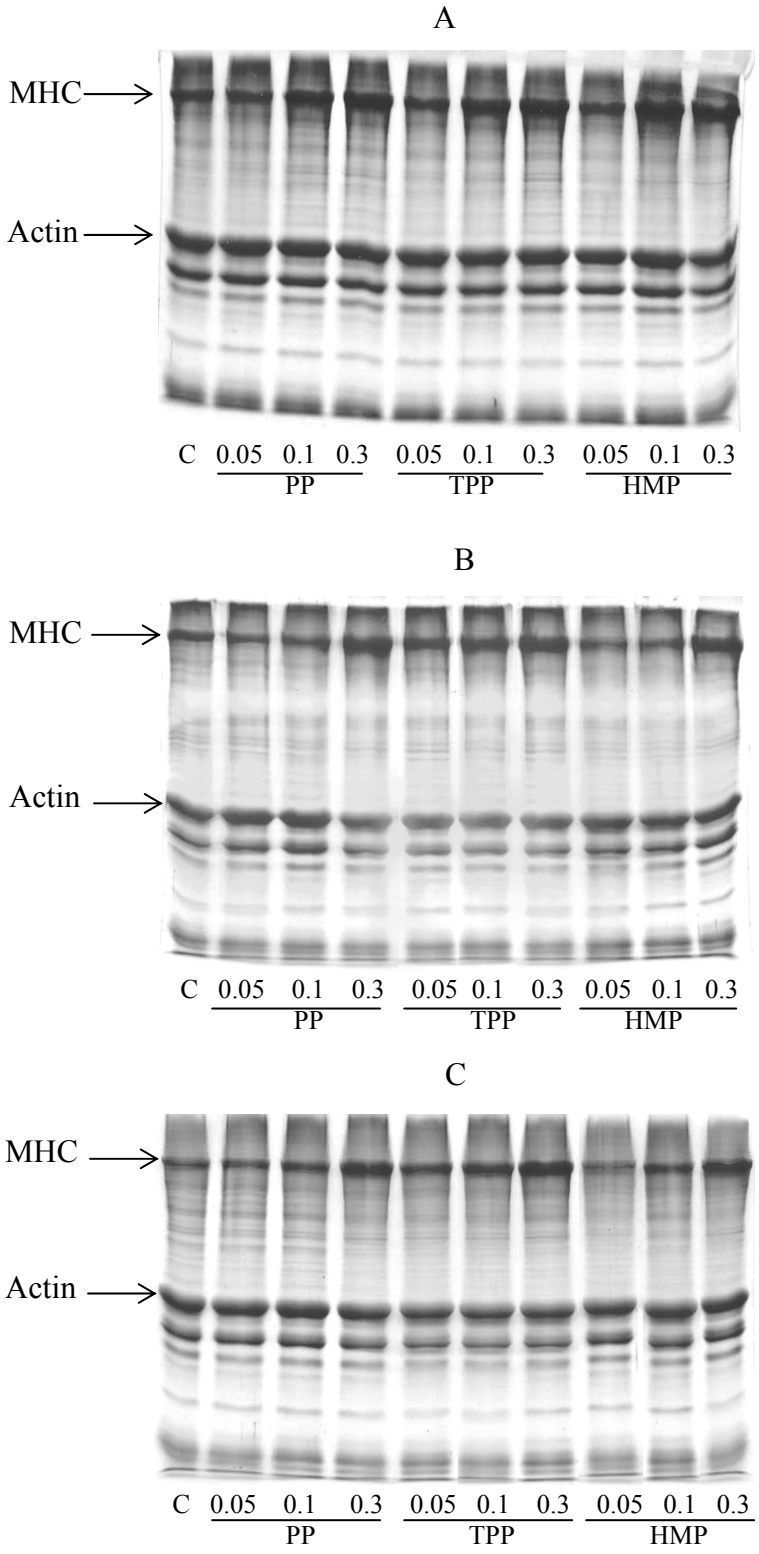

Figure 10. SDS-PAGE patterns of protein in kamaboko (A), directly heated (B) and suwari gels (C) from bigeye snapper surimi added with various phosphate compounds at different levels (%). MHC; myosin heavy chain, C; control gel (without phosphate addition), PP; pyrophosphate, TPP; tripolyphosphate, HMP; hexametaphosphate. Numbers designate the amount of phosphate added.

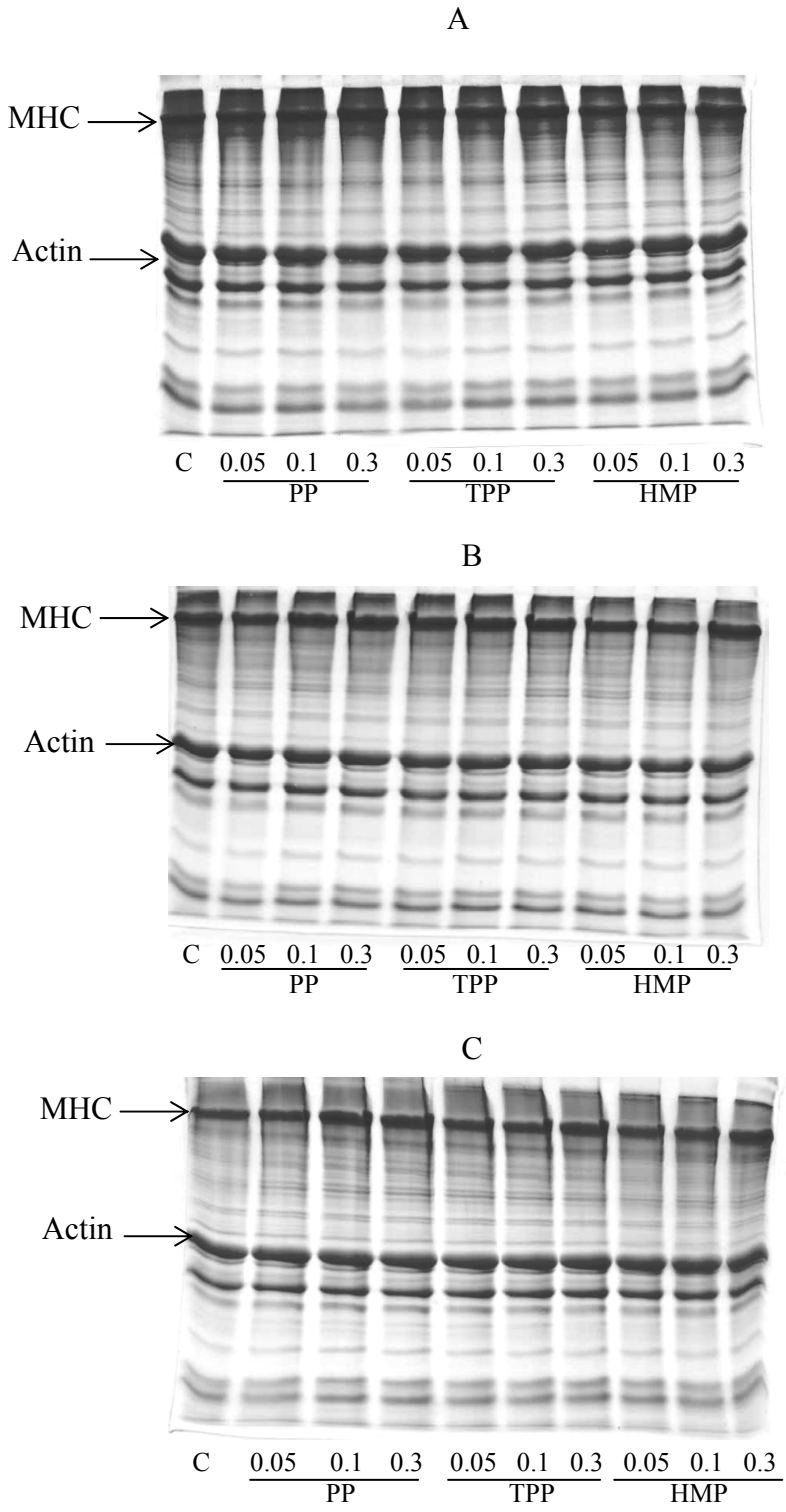

Figure 11. SDS-PAGE patterns of protein in kamaboko (A), directly heated (B) and suwari gels (C) from threadfin bream surimi added with various phosphate compounds at different levels (%). MHC; myosin heavy chain, C; control gel (without phosphate addition), PP; pyrophosphate, TPP; tripolyphosphate, HMP; hexametaphosphate. Numbers designate the amount of phosphate added.

2. Effect of sodium pyrophosphate in combination with calcium chloride on surimi gel properties

2.1 Effect breaking force and deformation

Breaking force and deformation of kamaboko gels of bigeye snapper and threadfin bream surimi added with PP (0, 0.025 and 0.05%) in combination with $CaCl_2$ (0, 25 and 50 mmole/kg) are depicted in Figures 12 and 13, respectively. In the absence of PP, breaking force and deformation of gels increased with increasing $CaCl_2$ concentrations ($P<0.05$). The result suggested that calcium ion might activate endogenous TGase, leading to the enhanced gel strength. Calcium compounds are commonly added as a gel enhancer for surimi (Yamamoto *et al.*, 1991). Benjakul and Visessanguan (2003) found that breaking force and deformation of bigeye snapper surimi increased markedly with the addition of calcium chloride, revealing that endogenous TGase played an important role in gel strengthening of surimi from bigeye snapper. Fish TGase has been found to be Ca^{2+}-dependent, however the requirement varies among fish species (Nozawa *et al.*, 1997). Bigeye snapper and threadfin bream surimi gel containing 0.025%PP had the increase in breaking force and deformation when $CaCl_2$ levels added increased ($P<0.05$). With the addition of 50 mmole $CaCl_2$/kg, breaking force and deformation of kamaboko gels from bigeye snapper surimi increased by 38.68% and 17.95%, respectively, compared with the control gel (without additives). With the same condition, breaking force and deformation of kamaboko gel from threadfin bream increased by 33.66% and 13.98%, respectively. However, the gel enhancing effect of $CaCl_2$ was markedly decreased in the presence of 0.05% PP. From the results, it suggested that the level of PP added determined the gel strength of surimi. Higher amount of PP might cause the repulsion

of protein molecules, leading to the lowered aggregation. Furthermore, phosphate anion can bind calcium more firmly than sodium, resulting in the formation of the complexes of calcium ions. Thus, PP at level of 0.025% would be sufficient to dissociate the actomyosin and the sufficient amount of $CaCl_2$ was also required for TGase activity. Calcium ion remaining after complexing with PP most likely involved in the setting induced by TGase. TGase catalyzes an acyl transfer reaction between γ–carboxamide groups of glutamyl residues in proteins as acyl donors and a variety of primary amine and water as the acyl acceptor to form ε–(γ-glutamyl) lysine linkages (Folk and Chung, 1973; Kishi *et al.*, 1991).

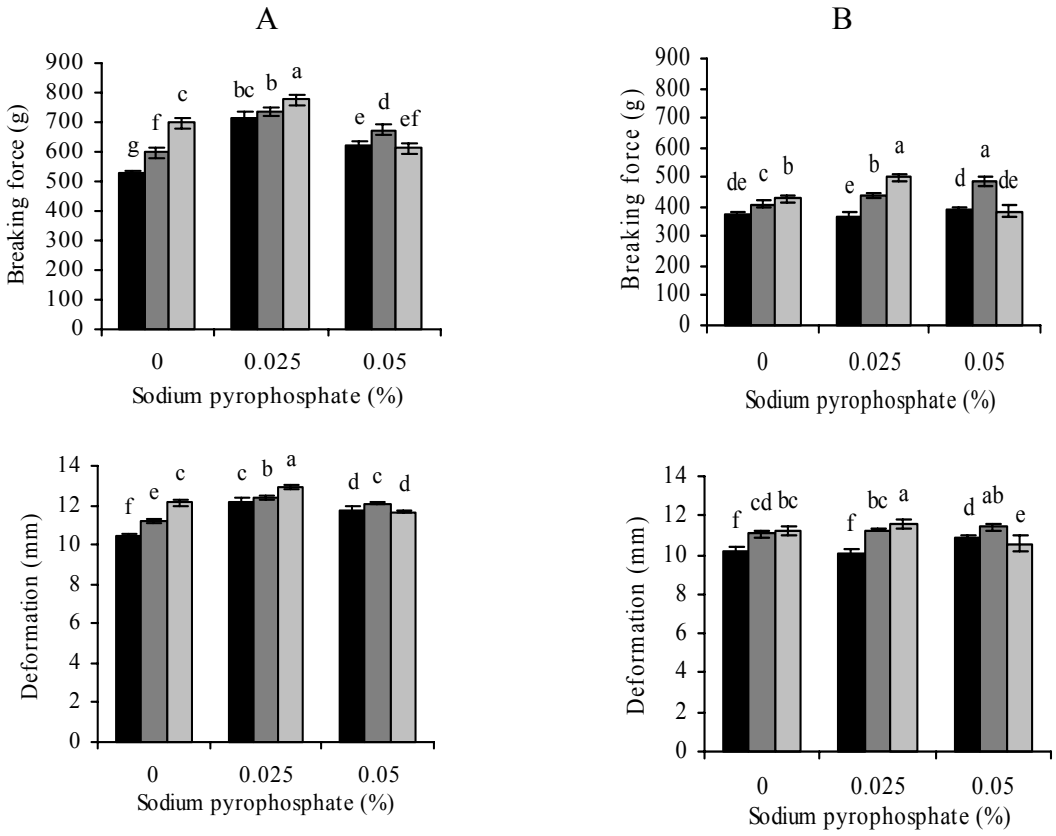

Figure 12. Breaking force and deformation of kamaboko gels from bigeye snapper (A) and threadfin bream (B) surimi added with sodium pyrophosphate and calcium chloride at different levels. Bars represent the standard deviation from five determinations. Different letters on the bars indicate significant difference ($P<0.05$)
■ 0 mole $CaCl_2$/kg ■ 25 mmole $CaCl_2$/kg □ 50 mmole $CaCl_2$/kg

2.2 Effect on expressible moisture

Expressible moisture content of kamaboko gels from bigeye snapper and threadfin bream surimi added with $CaCl_2$ decreased when the concentrations of $CaCl_2$ increased ($P<0.05$) (Figure 13). The gels without and with 0.025% PP had the decrease in expressible moisture content as the concentration of $CaCl_2$ increased ($P<0.05$). With the addition of 0.05% PP, $CaCl_2$ addition had no influence on expressible moisture content of resulting gels of bigeye snapper surimi. Also, the greater expressible moisture was generally found with samples containing 0.05% PP regardless of $CaCl_2$ concentrations, compared with that of gel consisting of 0.025% PP. The increase in breaking force of surimi gel generally correlated with the lowered expressible moisture content. From the result, bigeye snapper and threadfin bream kamaboko gels added with 0.025% PP in combination with 50 mmole $CaCl_2$/kg had the highest gel strength (Figure 12) and the lowest expressible moisture. Therefore, in the presence of PP and $CaCl_2$ at the optimal concentrations, three-dimensional organized matrix of gel with the capability of water holding was formed. Phosphate has negative surface charge, thereby affecting the surface charges of proteins, which enhance the water binding capacity of proteins.

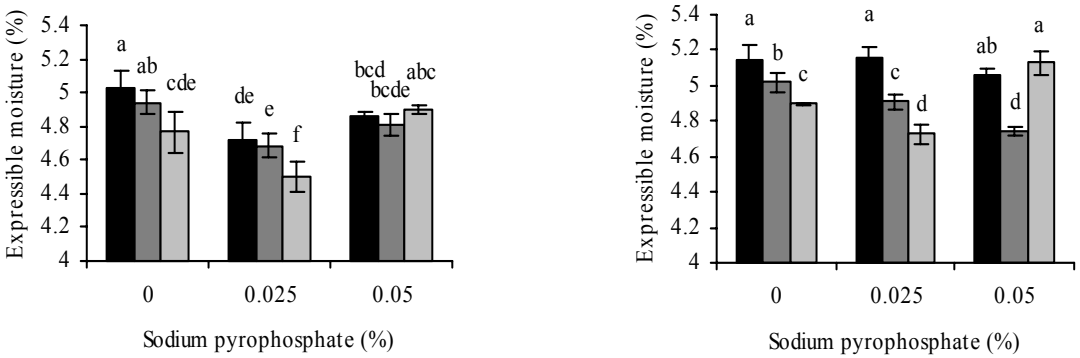

Figure 13. Expressible moisture content of kamaboko gel from bigeye snapper (A) and threadfin bream (B) surimi added with sodium pyrophosphate and calcium chloride at different levels. Bars represent the standard deviation from five determinations. Different letters on the bars indicate significant difference ($P<0.05$)

■ 0 mole $CaCl_2$/kg ■ 25 mmole $CaCl_2$/kg □ 50 mmole $CaCl_2$/kg

2.3 Effect on whiteness

Whiteness of kamaboko gels from bigeye snapper and threadfin bream surimi added with out and with PP at levels of 0.025 and 0.05% in the presence or the absence of $CaCl_2$ is shown in Table 9. Regardless of PP concentration, whiteness of surimi gel increased with increasing $CaCl_2$ concentrations ($P<0.05$). $CaCl_2$ might form the complex with some anions in the muscle, leading to the formation of insoluble particles. At 50 mmole $CaCl_2$/kg, it was noticeable that the greater whiteness was observed in the sample added with greater amount of PP ($P<0.05$). Calcium ion might form the insoluble complex with phosphate, leading to the light scattering in surimi gels. An increase in whiteness was associated with the light scattering effect of insoluble calcium carbonate in surimi gel (Benjakul *et al*., 2004b).

Table 9. Whiteness of kamaboko gels from bigeye snapper and threadfin bream surimi added with sodium pyrophosphate and calcium chloride at various concentrations

PP (%)	$CaCl_2$ (mmole/kg)	Bigeye snapper	Threadfin bream
0	0	72.98±0.15d*	78.51±0.08f
	25	74.28±0.10c	80.51±0.12d
	50	74.62±0.07b	81.99±0.11b
0.025	0	73.13±0.16d	78.20±0.07g
	25	74.49±0.07bc	81.15±0.07c
	50	74.90±0.13a	82.10±0.11b
0.05	0	73.22±0.11d	78.37±0.10e
	25	74.60±0.21b	81.19±0.07c
	50	74.98±0.15a	82.28±0.09a

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different ($P>0.05$).

2.4 Effect on solubility

Solubility of bigeye snapper and threadfin bream kamaboko gels added with PP (0, 0.025 and 0.05%) in combination with $CaCl_2$ (0, 25 and 50 mmole/kg) is

shown in Table 10. From the result, solubility of gels without and with 0.025% PP decreased when levels of $CaCl_2$ added increased ($P<0.05$). Solubility of gels added with 0.025% PP in combination with 50 mmole $CaCl_2$/kg was lowest. This might be due to the cross-linking of protein induced by TGase, which requires Ca^{2+} for full activation (Kishi *et al.*, 1991; Seki *et al.*, 1990). The decrease in gel-forming ability was associated with the decrease in non-disulfide covalent cross-linking, as indicated by an increase in solubility and more MHC retained (Benjakul *et al.*, 2004a). When PP at a level of 0.05% was added, $CaCl_2$ addition showed no pronounced effect on the solubility of kamaboko gels from both surimi. Therefore, amount of PP used in surimi might affect TGase activity.

Table 10. Solubility of kamaboko gels from bigeye snapper and threadfin bream surimi added with sodium pyrophosphate and calcium chloride at different concentrations.

PP (%)	$CaCl_2$ (mmole/kg)	Bigeye snapper	Threadfin bream
Control		100h*	100f
0	0	71.05±0.23g	71.85±0.48d
	25	68.30±0.64ef	70.07±0.33c
	50	64.37±0.26c	68.17±0.91b
0.025	0	63.51±0.54c	72.01±0.51d
	25	62.23±0.81b	67.43±1.34ab
	50	60.04±0.49a	66.75±0.40a
0.050	0	67.18±1.32de	70.59±0.33c
	25	66.72±0.77d	66.91±0.64ab
	50	67.69±1.27def	71.11±0.88cd

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different ($P>0.05$).

2.5 Effect on protein patterns

The protein patterns of kamaboko gels from bigeye snapper and threadfin bream surimi added with PP and $CaCl_2$ at different levels are shown in

Figure 13. Regardless of PP addition, MHC decreased continuously as the concentrations of calcium chloride used increased. The lowest MHC band intensity was observed with surimi gel added with 0.025% PP and 50 mmole $CaCl_2$/kg (Figure 14). No marked changes in actin were observed in all samples. The results suggested that MHC underwent cross-linking to a much higher extent when $CaCl_2$ concentration increased. The decrease in MHC band intensity was coincidental with the increase in breaking force and deformation (Figure 12). Therefore, calcium ion might activate endogenous TGase, which effectively induce cross-linking, especially ε–(γ-glutamyl) lysine (Benjakul *et al*., 2004b). It was noted that the increase in MHC band intensity was found in surimi gel added with 0.05% PP, especially for bigeye snapper surimi. The result reconfirmed that an excessive amount of PP might cause the detrimental effect on cross-linking, even in the presence of higher amount of calcium ion. The repulsion of proteins with charges modified by phosphate anion was postulated to result in the decreased cross-linking. Wan *et al*. (1994) reported that amount of cross-linked MHC in grade A surimi paste was observed with addition of calcium chloride up to 5mM. The cross-linking of MHC in the surimi appeared to be dependent on the Ca^{2+} concentration (Wan *et al.*, 1994). The addition of 5 mmole $CaCl_2$/kg sharply increased the formation of cross-linked MHC in walleye pollack surimi gel (Wan *et al.*, 1994). From the result, the protein cross-linking in bigeye snapper surimi was higher than threadfin bream surimi as evidenced by the more retained MHC band intensity. It was suggested that setting phenomenon in threadfin bream surimi was lower than bigeye snapper surimi.

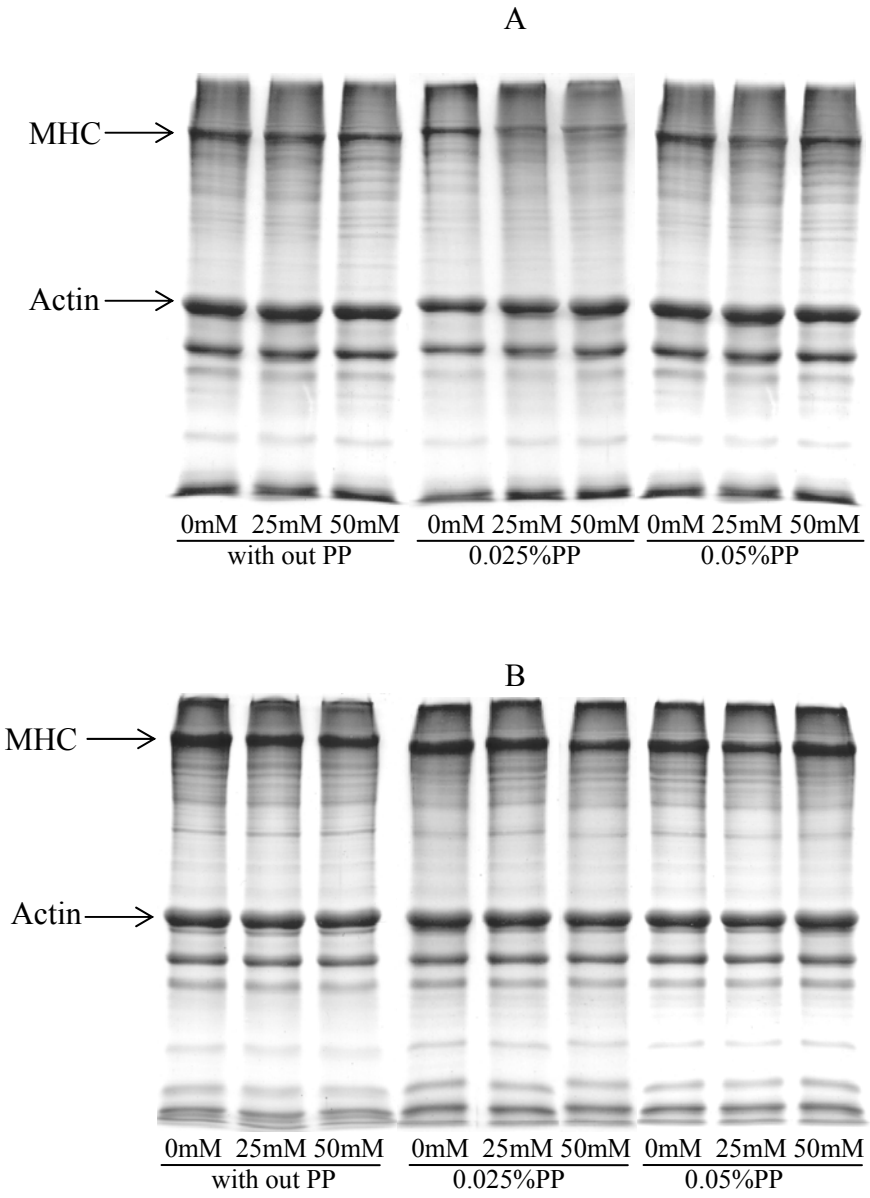

Figure 14. SDS-PAGE pattern of protein in kamaboko gel from bigeye snapper (A) and threadfin bream (B) surimi added with sodium pyrophosphate (PP) and calcium chloride at different levels. MHC; myosin heavy chain. Numbers designate the concentration of calcium chloride.

3. Effect of setting time on gel forming ability of surimi gel added with sodium pyrophosphate in combination with calcium chloride

3.1 Effect on breaking force and deformation

Kamaboko gels from both bigeye snapper and threadfin bream surimi added with 0.025% PP and 50 mmole $CaCl_2$/kg had the increase in breaking force when the setting time increased up to 90 min ($P<0.05$). Thereafter, a slight decrease in breaking force was observed ($P<0.05$). The result indicated that setting at 40°C for 90 min, followed by heating at 90°C for 20 min was the optimal condition to strengthen the gel from both bigeye snapper and threadfin bream surimi (Figure 15). Benjakul and Visessanguan (2003) found that the setting at 25 or 40°C for appropriate time prior to heating can induce endogenous TGase in two species of bigeye snapper (*Priacanthus tayenus* and *P. macracanthus*), resulting in the increase in both breaking force and deformation of surimi from both species. Benjakul *et al*. (2004a) studied the effect of setting at 40°C on the textural properties of surimi gels from threadfin bream, bigeye snapper, barracuda and bigeye croaker. It was found that an increase in setting time generally resulted in higher breaking force and deformation of kamaboko gels. The sufficient setting time might allow the unfolding of proteins at 40°C, resulting in differences in the exposure of reactive groups (glutamine and lysine) involved in the cross-linking reaction induced by TGase. The methylene group of the 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, 1983). The appropriate setting time presumably caused the cross-linking reaction proceed effectively. The similar results were obtained with deformation. Therefore, setting time is another important factor determining the gel strength of surimi.

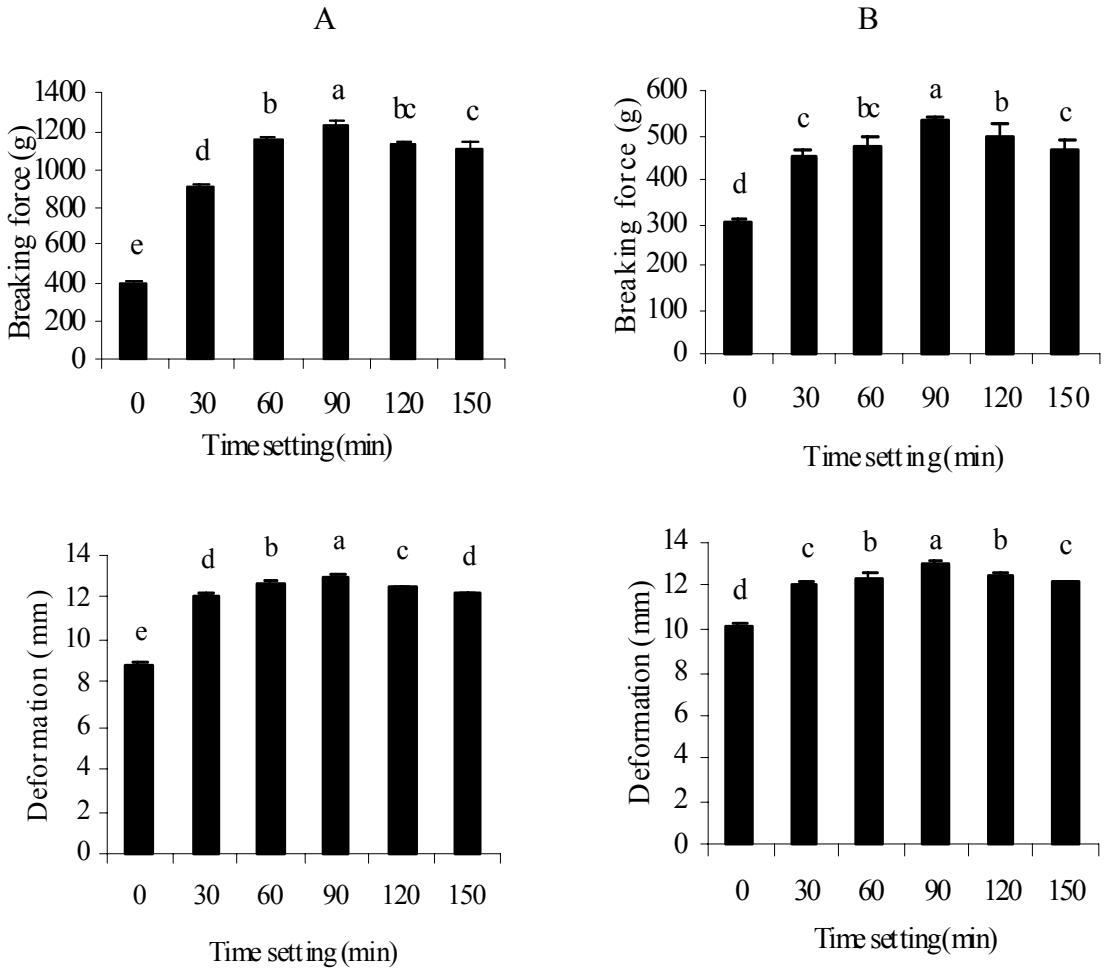

Figure 15. Breaking force and deformation of gels from bigeye snapper (A) and threadfin bream (B) surimi added with 0.025% PP and 50 mmole $CaCl_2$/kg and set at 40°C for different times, followed by heating at 90°C 20 min. Bars represent the standard deviation from five determinations. Different letters on the bars indicate significant difference ($P<0.05$).

3.2 Effect on expressible moisture

Expressible moisture of bigeye snapper and threadfin bream surimi kamaboko gel added with 0.025% PP and 50 mmole $CaCl_2$/kg and set at 40°C for different times, followed by heating at 90°C for 20 min is shown in Table 11. From the result, directly heated surimi gel (setting 40°C for 0 min) had the higher expressible moisture than other gels with prior setting at 40°C. Nevertheless, setting time showed no effect on expressible moisture of kamaboko gels of both surimi

(P>0.05). The result suggested that water could be retained in gel matrix potentially after setting for at least 30 min. The use of PP in combination of $CaCl_2$ might render gel matrix with well-organized structure; the polypeptide could form the strong three-dimensional matrix, which can imbibe water effectively, particularly with sufficient setting time.

Table 11. Expressible moisture content of bigeye snapper and threadfin bream kamaboko gels added with 0.025% PP and 50 mmole $CaCl_2$/kg and set for various times at 40°C.

Time setting (min)	Bigeye snapper	Threadfin bream
0	4.92±0.03a*	4.97±0.04a
30	4.53±0.10b	4.79±0.17b
60	4.45±0.36b	4.76±0.22b
90	4.40±0.20b	4.71±0.29b
120	4.46±0.10b	4.74±0.20b
150	4.48±0.16b	4.77±0.24b

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

3.3 Effect on whiteness

Whiteness of bigeye snapper and threadfin bream kamaboko gels added with 0.025% PP and 50 mmole $CaCl_2$/kg with different setting times at 40°C is present in Table 12. With increasing setting time, the lowered whiteness was observed for both surimi gels. It was postulated that Maillard reaction could take place to a higher extent when the setting time increased. Kamaboko gel also showed the lower whiteness, compared with directly heated gel (P<0.05), possibly due to the lower expressible moisture of the former. As a result, light scattering was lowered with the corresponding lower whiteness.

Table 12. Whiteness of kamaboko gels from bigeye snapper and threadfin bream surimi added with 0.025% PP and 50 mmole $CaCl_2$/kg and set at 40°C for different times.

Time setting (min)	Bigeye snapper	Threadfin bream
0	77.53±0.05a*	82.24±0.04a
30	77.08±0.09b	81.49±0.04b
60	76.28±0.07c	81.08±0.11c
90	76.16±0.03d	81.05±0.03c
120	76.07±0.09de	81.03±0.06c
150	75.99±0.03e	80.99±0.08c

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

3.4 Effect on solubility

Solubility of bigeye snapper and threadfin bream kamaboko gels containing 0.025% PP and 50 mmole $CaCl_2$/kg with setting at 40°C for various times is shown in Table 18. Directly heated surimi gel (setting at 40°C for 0 min) showed the highest solubility. Decrease in solubility was observed as the setting time increased ($P<0.05$). In the presence of 50 mmole $CaCl_2$/kg, TGase could be activated and induced the cross-linking more effectively with increasing setting time. Thus, sufficient setting time could allow the polymerization of surimi protein take place to a greater extent. The result was in agreement with the increase in breaking force and deformation when setting time increased (Figure 15). The lowest solubility was found in kamaboko gel set at 40°C for 90 min, followed by heating at 90°C for 20 min. The surimi gels from threadfin bream, bigeye snapper and barracuda had the decreased solubility with extended setting time at 40°C (Benjakul *et al*., 2004a). A decrease in solubility of surimi gels in the mixture of SDS, urea and βME suggested the increased formation of non-disulfide covalent bonding (Benjakul and Visessanguan, 2003).

Table 13. Solubility of kamaboko gels from bigeye snapper and threadfin bream surimi added with 0.025%PP and 50 mmole $CaCl_2$/kg and set at 40°C for different times.

Time setting (min)	Bigeye snapper	Threadfin bream
control	100g*	100g
0	76.74±0.26f	75.56±0.30f
30	64.33±0.35e	68.72±0.60d
60	55.55±0.17b	65.21±0.34c
90	50.35±0.60a	62.10±0.43a
120	58.52±0.54c	63.68±0.17b
150	61.76±0.84d	67.47±0.26e

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

3.5 Effect on protein pattern

The protein patterns of bigeye snapper and threadfin bream kamaboko gels containing 0.025% PP and 50 mmole $CaCl_2$/kg with various setting times at 40°C are depicted in Figure 16. MHC band intensity decreased continuously as the setting time increased. No MHC band was retained after setting more than 60 min, suggesting the cross-linking of MHC with increasing setting time. Actin band intensity increased markedly when setting time increased. This was possibly because the cross-linked MHC could be removed during solubilization. As a consequence, the ratio of actin to total proteins could be increased as indicated by the greater band intensity.

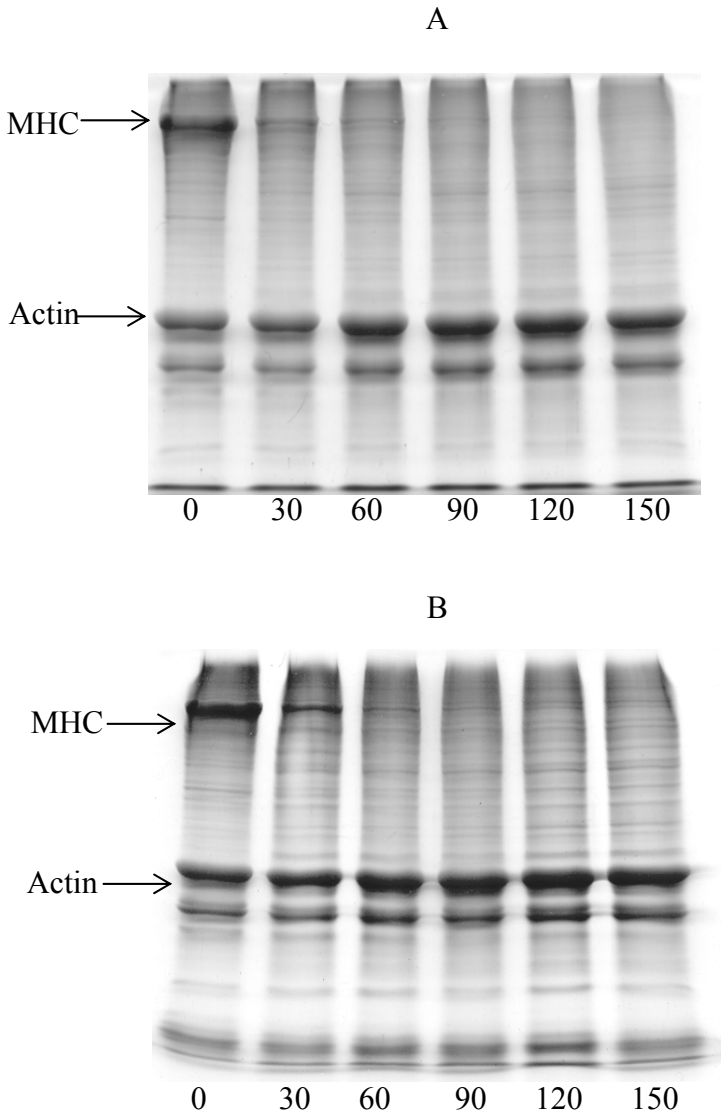

Figure 16. SDS-PAGE pattern of protein in kamaboko gels from bigeye snapper (A) and threadfin bream (B) surimi added with 0.025% sodium pyrophosphate and 50 mmole/kg calcium chloride and set at 40°C for different times. MHC; myosin heavy chain. Numbers designate the setting time (min).

4. Effect of sodium pyrophosphate in combination with microbial transglutaminase on surimi gel properties

4.1 Effect on breaking and deformation

Breaking force and deformation of bigeye snapper kamaboko gel added with PP (0, 0.025, 0.05 and 0.1%) without and with 0.05% MTGase and set at 40°C for 30 min, followed by heating at 90°C for 20 min are depicted in Figure 17A. In the presence of 0.05% MTGase, all gel samples with different PP levels had the higher breaking force and deformation ($P<0.05$). Gel with 0.025% PP and 0.05% MTGase had the increase in breaking force and deformation by 31.51% and 25.54%, respectively, compared with the control gel (without additives). For kamaboko gels from threadfin bream surimi, gel added with 0.05% PP in combination with 0.05% MTGase had the increase in breaking force and deformation by 32.96% and 17.83%, respectively, compared with the control gel (without additives) (Figure 17B). With PP addition at levels higher than 0.025%, the decreases in breaking force and deformation were found regardless of MTGase addition. PP at higher levels might cause the repulsion of protein molecule after charge modification. Although, PP might chelate some endogenous calcium ion in surimi but MTGase, Ca^{2+}-independent enzyme, could catalyze the MHC cross-linking and increased gel forming ability of bigeye snapper and threadfin bream surimi. PP at an appropriate level could dissociate proteins, thereby endogenous TGase and MTGase could cross-link those proteins easily. The increase in breaking force and deformation of gels added with MTGase was presumably caused by the increased cross-linking induced by MTGase. Jiang *et al.* (2000a) found that MTGase catalyzed the MHC cross-linking of both pollack and golden threadfin bream surimi and increased the gel forming ability of surimi.

However, the addition of MTGase at a high level (higher than 0.03%) resulted in the decrease in breaking force of surimi made from some fish species (Asagami *et al.*, 1995). Tsai *et al.* (1996a) found that the gel strength of minced mackerel product increased with the addition of purified TGase and reached the highest level, which was about 3.9 fold over that of the control, when the amount of TGase was 0.34 units/g of meat. From the result, the addition of MTGase in combination with PP could strengthen the gel matrix as evidenced by the increase in both breaking force and deformation.

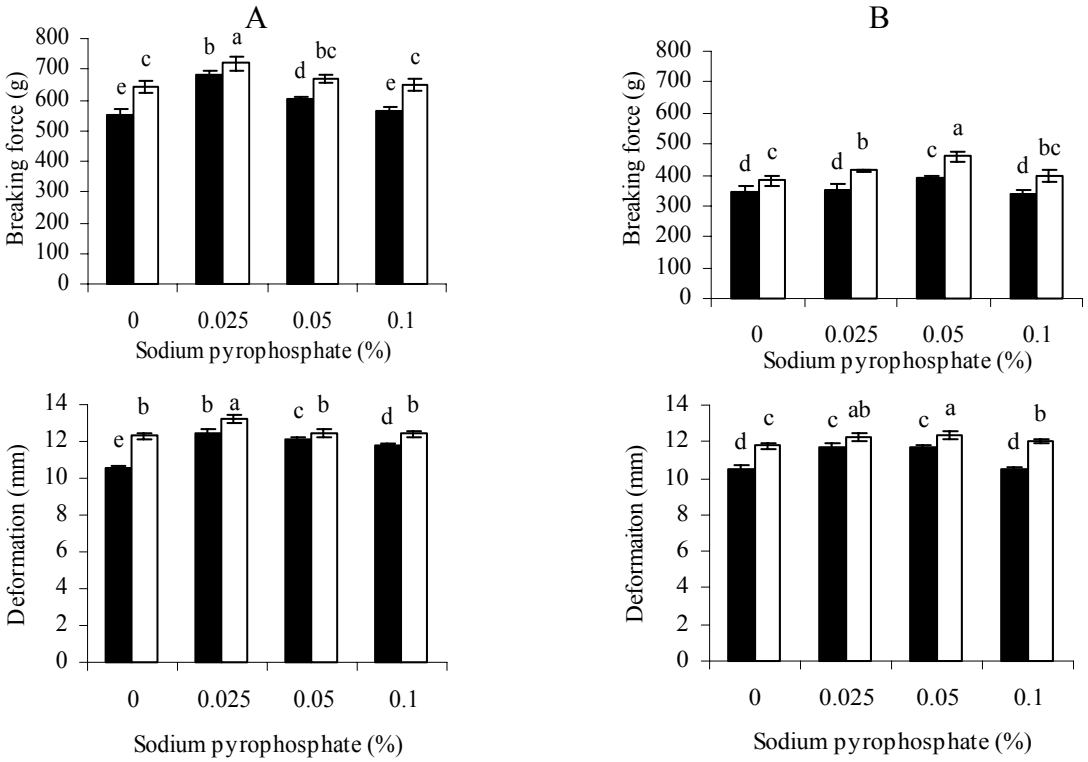

Figure 17. Breaking force and deformation of kamaboko gels from bigeye snapper (A) and threadfin bream (B) surimi added with sodium pyrophosphate and microbial transglutaminase at different level. Bars represent the standard deviation from five determinations. Different letters on the bars indicate the significant difference ($P<0.05$).
■ 0%MTGase □ 0.05%MTGase

4.2 Effect on expressible moisture

The addition PP in combination with MTGase had no marked effect on the expressible moisture of kamaboko gel from bigeye snapper surimi. Conversely,

the addition of 0.05% MTGase decreased the expressible moisture of kamaboko gel from threadfin bream surimi ($P<0.05$). Three dimensional network from surimi gel added with PP and MTGase had water holding capacity more than surimi gel added with only PP (without MTGase), which was coincidental with the increased breaking force, especially with addition of 0.025% PP in combination with 0.05% MTGase. From the result, it was suggested that the matrix of gel of threadfin bream surimi was much improved in fashion which could imbibe more water in the matrix. For bigeye snapper surimi, MTGase might cause the non-significant decrease in expressible moisture content. Ramirez *et al.* (2002) found that the addition of 0.6% MTGase had a negative effect on WHC of restructured product. This behavior suggested that an excess of MTGase caused an increase in protein-protein interactions with a decrease on protein-water interactions, which induced a decrease in WHC. Therefore, the use of high concentration of MTGase (0.6%) might produce an excessive protein aggregation which does not increase the textural properties of restructured products, but rather decreases the WHC value.

Table 14. Expressible moisture content of kamaboko gels from bigeye snapper and threadfin bream surimi added with PP and MTGase at various levels.

PP (%)	MTGase (%)	Bigeye snapper	Threadfin bream
0	0	4.92±0.06a*	4.96±0.16a
	0.05	4.74±0.12ab	4.74±0.03b
0.025	0	4.71±0.07ab	4.94±0.07a
	0.05	4.67±0.03c	4.54±0.15c
0.05	0	4.79±0.07abc	4.73±0.12b
	0.05	4.72±0.06ab	4.21±0.01d
0.1	0	4.86±0.15ab	4.99±0.06a
	0.05	4.73±0.05ab	4.68±0.06bc

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different ($P>0.05$).

4.3 Effect on whiteness

Whiteness of bigeye snapper and threadfin bream kamaboko gels added with PP at different levels with or without MTGase is present in Table 15. The addition of 0.05% MTGase in surimi gel increased the whiteness of all surimi gels regardless of PP concentrations. Polyphosphates can exhibit whiting effect via the dispersion of a myosin sol (Grantham, 1981). In general, MTGase commercially available contains calcium lactate, which can exhibit the light scattering effect in surimi gel. Thus, gels added with MTGase had the increase in whiteness.

Table 15. Whiteness of kamaboko gel from bigeye snapper and threadfin bream surimi added with PP and MTGase at various levels.

PP (%)	MTGase (%)	Bigeye snapper	Threadfin bream
0	0	74.11±0.09a*	77.48±0.09a
	0.05	74.95±0.12c	78.26±0.09b
0.025	0	74.33±0.07b	77.61±0.01a
	0.05	75.24±0.10d	78.70±0.05c
0.05	0	74.80±0.14c	78.75±0.10c
	0.05	75.41±0.08e	78.82±0.09c
0.1	0	75.18±0.08d	79.00±0.06d
	0.05	77.21±0.07f	79.59±0.15e

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

4.4 Effect on solubility

Solubility of bigeye snapper and threadfin bream kamaboko gels added with PP (0, 0.025 and 0.05%) in combination with MTGase (0.05%) is shown in Table 16. From the result, solubility of both surimi gels decreased when MTGase was added, regardless of PP concentrations. Among all samples, the solubility of gels from bigeye snapper added with 0.025% PP and threadfin bream gels added with 0.05% PP in combination with 0.05% MTGase was lowest. These results suggested that MTGase could catalyze the MHC cross-linking in concert with endogenous TGase to

increase gel strength. However, a slight increase in solubility was found with gel containing a higher amount of PP, which was able to chelate Ca^{2+} ion required for endogenous TGase. Ramirez *et al*. (2002) found that the use of 0.3% or 0.6% MTGase decreased protein solubility of restructured fish products resembling hams, compared with the control (without MTGase).

Table 16. Solubility of kamaboko gel from bigeye snapper and threadfin bream surimi added with PP and MTGase at various levels.

PP (%)	MTGase (%)	Bigeye snapper	Threadfin bream
Control		100h*	100f
0	0	70.84±0.25g	73.85±0.43e
	0.05	66.87±0.61d	72.06±0.43d
0.025	0	65.31±0.43b	73.80±0.09e
	0.05	63.75±0.49a	70.12±0.49b
0.050	0	67.94±0.34e	71.63±0.34cd
	0.05	66.06±0.19c	68.00±0.16a
0.100	0	69.45±0.34f	73.91±0.50e
	0.05	66.97±0.41d	71.31±0.25c

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

4.5 Effect on protein patterns

Electrophoretic study revealed that MHC underwent cross-linking when MTGase was added, regardless of PP concentration as evidenced by the lowered MHC band intensity. The lowest MHC band intensity was formed with gel added with 0.05% MTGase and 0.025% PP for bigeye snapper surimi and 0.05% MTGase in combination with 0.05% PP for threadfin bream surimi (Figure 18). This indicated that MTGase was powerful for induction of MHC cross-linking (Figure 18). MTGase catalyze the formation of ε-(γ-glutamyl) lysine cross links which is covalent (Sakamoto *et al.*, 1995a,b; Seguro *et al.*, 1995). The decrease in MHC was concomitant with the increased breaking force and deformation. Nevertheless, no

marked changes in actin were obtained. MHC is a preferable substrate for cross-linking reaction induced by both endogenous and microbial TGase. Nakahar *et al.* (1999) reported that MTGase and carp TGase could not cross-link actin molecules. Thus, MTGase effectively induced the polymerization of MHC, which is a major protein contributing to the gel network formation. As a consequence, the stronger gel network was formed. The result was in accordance with Jiang *et al.* (1998b) who reported that cross-linking of MHC occurred rapidly in mackerel surimi added with MTGase.

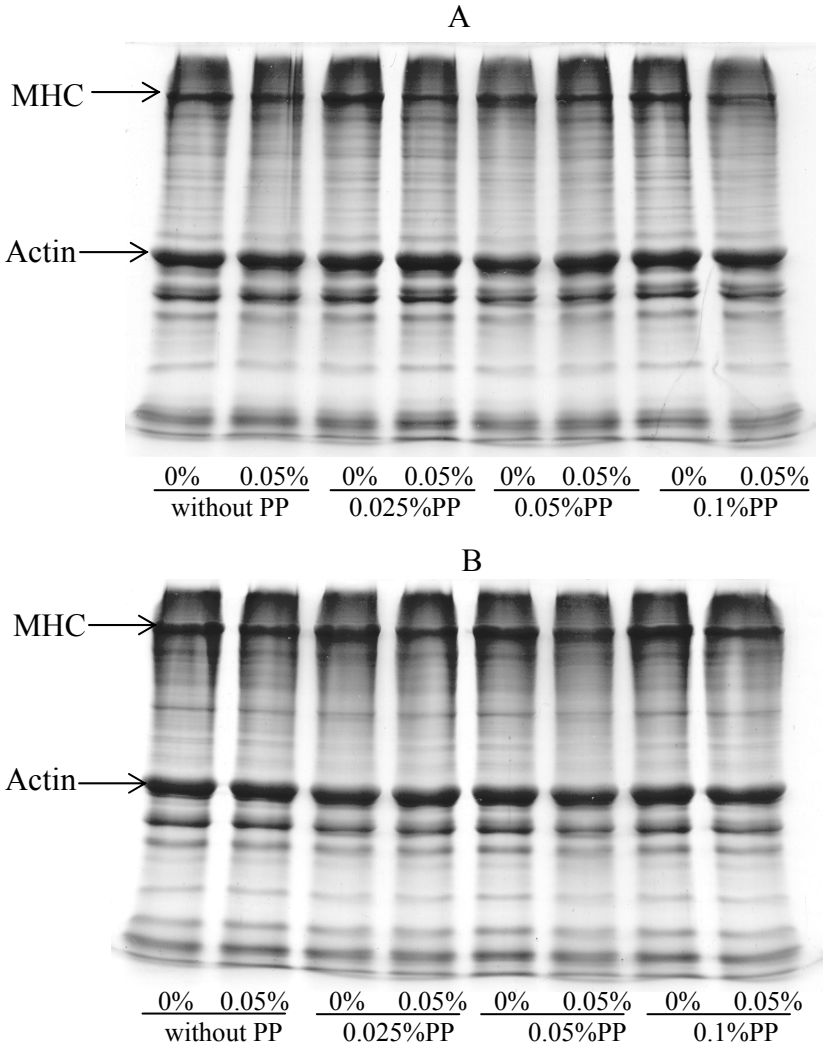

Figure 18. SDS-PAGE patterns of protein in kamaboko gels from bigeye snapper (A) and threadfin bream (B) surimi added with sodium pyrophosphate (PP) and microbial transglutaminase at different levels. MHC; myosin heavy chain. Numbers designate MTGase level added (%)

5. Effect of setting time on gel forming ability of surimi gel added with sodium pyrophosphate in combination with MTGase

5.1 Effect on breaking force and deformation

Addition of 0.025% PP in combination with 0.05% MTGase caused the greatest increase in gel strength for bigeye snapper surimi, while the addition of 0.05% PP in combination with 0.05% MTGase showed the highest gel strength for threadfin bream surimi. These conditions were used to study the effect of setting time on gel properties. From the result, setting at 40°C for 120 min, followed by heating at 90°C for 20 min was the optimal setting condition for bigeye snapper surimi. For threadfin bream surimi, setting time of 60-90 min was found to be optimal (Figure 19 and 20). With the sufficient setting time, the protein molecules could unfold properly and cross-linking could take place effectively with those exposed reactive groups. From the result, both endogenous TGase and MTGase might work spontaneously in polymerization of proteins, especially MHC. The result indicated that both TGase might function more efficiently with increasing setting time. As a consequence, the increased breaking force and deformation were observed. Furthermore, an appropriate setting time presumably caused optimal exposure and interaction of the hydrophobic area of the active site and substrate glutamine residues.

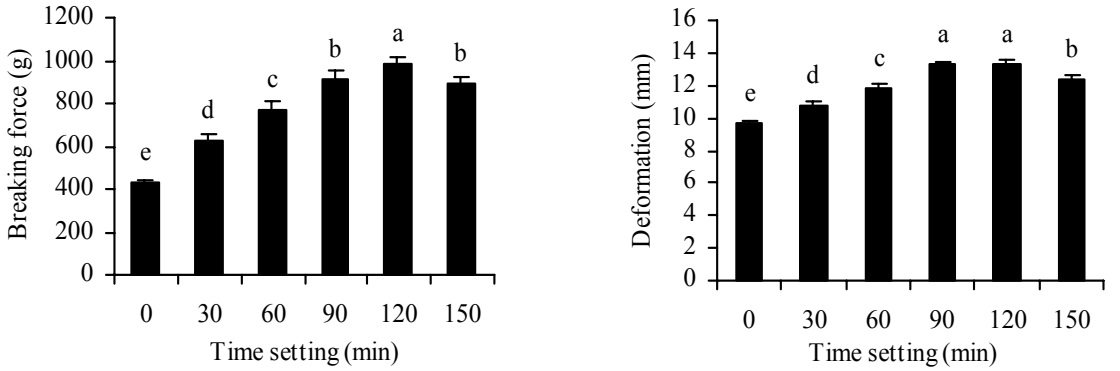

Figure 19. Breaking force and deformation of kamaboko gels from bigeye snapper surimi added with 0.025% sodium pyrophosphate and 0.05% microbial transglutaminase and set at 40°C for different times. Bars represent the standard deviation from five determinations. Different letters on the bars indicate the significant difference (P<0.05).

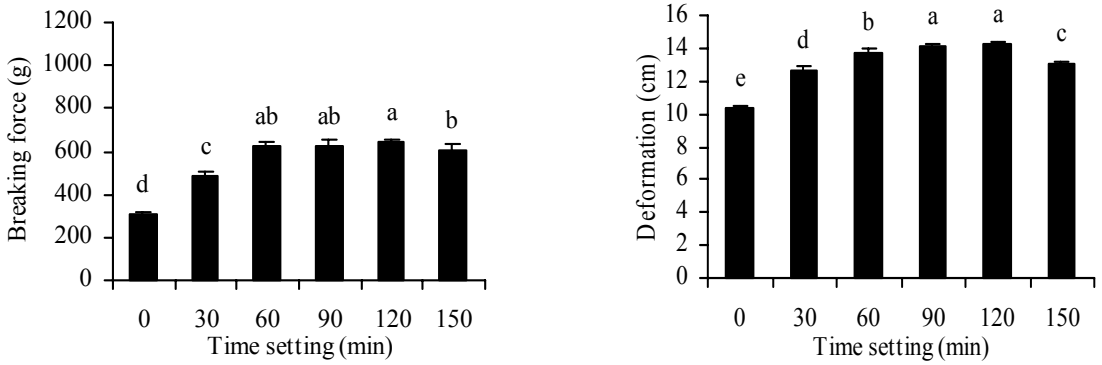

Figure 20. Breaking force and deformation of kamaboko gels from threadfin bream surimi added with 0.05% PP and 0.05% MTGase and set at 40°C for different times. Bars represent the standard deviation from five determinations. Different letters on the bars indicate the significant difference (P<0.05).

5.2 Effect on expressible moisture

Expressible moisture of bigeye snapper and threadfin bream kamaboko gels added with PP and MTGase at the optimal levels with different setting times is shown in Table 17. Without prior setting, the gel had the highest expressible moisture (P<0.05). When setting time at 40°C increased, no marked increase in expressible moisture of gels was observed (P<0.05). The result suggested that water could be retained in gel matrix potentially after setting at least for 30 min.

Table 17. Expressible moisture content of kamaboko gels from bigeye snapper surimi (added with 0.025% PP and 0.05% MTGase) and from threadfin bream surimi (added with 0.05% PP and 0.05% MTGase) and set at 40°C for different times.

Time setting (min)	Bigeye snapper	Threadfin bream
0	4.88±0.16c*	4.44±0.06b
30	4.62±0.02b	4.25±0.03ab
60	4.56±0.10ab	4.15±0.09a
90	4.45±0.12ab	4.14±0.15a
120	4.37±0.11a	4.13±0.17a
150	4.48±0.04ab	4.17±0.10a

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

5.3 Effect on whiteness

Whiteness of bigeye snapper and threadfin bream kamaboko gels added with PP and MTGase with the optimal levels decreased as the setting time increased (P<0.05). Without prior setting, gels had the highest whiteness. The Maillard reaction might occur to a greater extent with increasing setting time (P<0.05). Generally, kamaboko gels from threadfin bream surimi had a higher whiteness than those of bigeye snapper at all setting times studied.

Table 18. Whiteness of kamaboko gels from bigeye snapper (added with 0.025% PP and 0.05% MTGase) and for threadfin bream surimi (added with 0.05% PP and 0.05% MTGase) and set at 40°C for different times.

Time setting (min)	Bigeye snapper	Threadfin bream
0	75.38±0.10e*	79.44±0.09f
30	74.21±0.06d	78.76±0.07e
60	73.53±0.09c	78.28±0.10d
90	73.25±0.03b	78.10±0.07c
120	72.93±0.10a	77.89±0.08b
150	72.79±0.08a	77.72±0.08a

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

5.4 Effect on solubility

Solubility of bigeye snapper and threadfin bream kamaboko gels containing PP and MTGase at optimal levels had the decreased solubility in solutions consisting of 8 M urea, 2% SDS and 2% βME when the setting time increased ($P<0.05$). For both surimi gels, the setting time of 120 min showed the lowest solubility, suggesting the highest non-disulfide band formation. In general, the rate of decrease in solubility was greater in surimi from bigeye snapper, which was coincidental with the higher breaking force and deformation. However, a slight increase in solubility was noticeable with setting for 150 min. With extended setting time at 40°C, proteolysis caused by endogenous proteinase could take place, leading to the formation of degradation peptides. Degradation was observed in surimi gel during setting, especially at the high temperature (Benjakul *et al*., 2004a).

Table 19. Solubility of kamaboko gels from bigeye snapper surimi (added with 0.025% PP and 0.05% MTGase) and from threadfin bream surimi (added with 0.05% PP and 0.05% MTGase) and set at 40°C for different times.

Time setting (min)	Bigeye snapper	Threadfin bream
control	100f*	100f
0	72.01±0.53e	74.64±0.55e
30	63.71±0.33d	69.66±0.31d
60	60.02±0.38c	63.69±0.47b
90	55.51±0.67b	63.06±0.16ab
120	52.93±0.25a	62.54±0.51a
150	55.74±0.42b	65.94±0.64c

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different ($P>0.05$).

5.5 Effect on protein patterns

The protein patterns of bigeye snapper kamaboko gels added with PP and MTGase with different setting times are depicted in Figure 21. MHC band intensity decreased continuously as the time setting increased for both surimi.

However, MHC in bigeye snapper surimi decreased to a higher rate, compared with that found in threadfin bream surimi gel. This result suggested that MTGase together with endogenous TGase could cross-link MHC of bigeye snapper surimi more effectively than that of threadfin bream. Moreover, the higher endogenous TGase activity was presumed in the bigeye snapper surimi. Bigeye snapper surimi was reported to exhibit the excellent setting phenomenon associated with the good quality (Benjakul and Visessanguan, 2003).

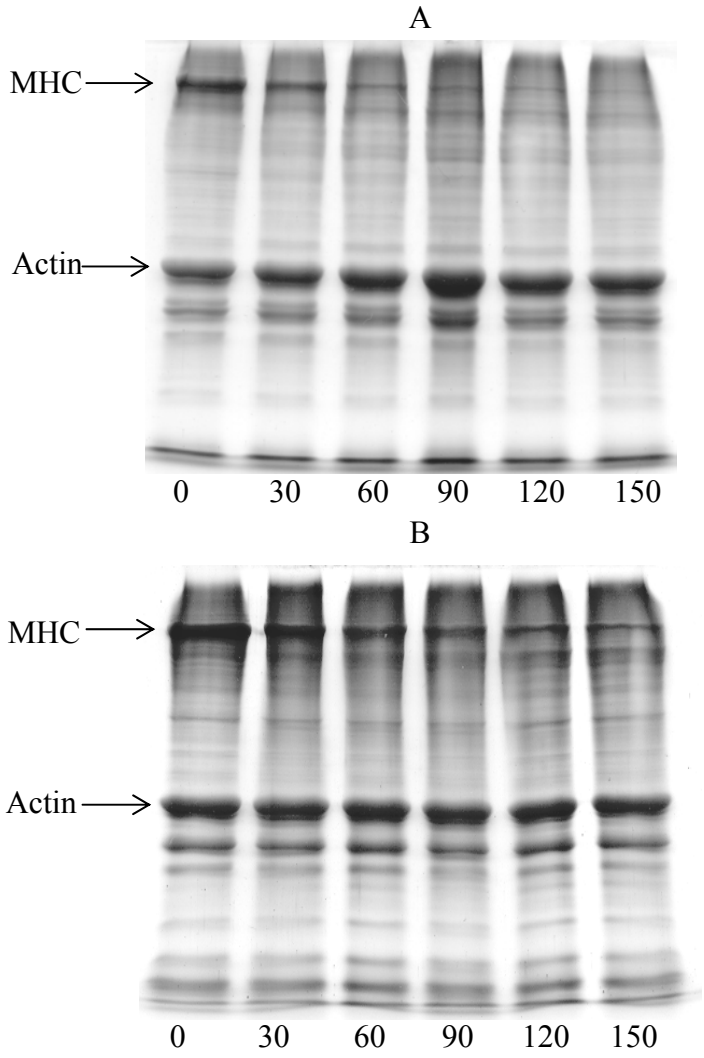

Figure 21. SDS-PAGE patterns of proteins in kamaboko gel from bigeye snapper surimi (added with 0.025% PP and 0.05% MTGase) and from threadfin bream surimi (added with 0.025% PP and 0.05% MTGase) and set at 40°C for different times. MHC; myosin heavy chain. Numbers designate the setting time (min).

6. Effect of sodium pyrophosphate and/or calcium chloride and/or microbial transglutaminase on microstructure of surimi gel

Microstructures of kamaboko gels from bigeye snapper and threadfin bream surimi prepared with different conditions were visualized by scanning electron microscopy as shown in Figures 22 and 23, respectively. All gels had a three-dimensional network with different microstructures. It was suggested that this network structure was formed through intermolecular ε-(γ-glutamyl) lysine cross-links induced by endogenous TGase or MTGase in co-operation with protein aggregation via hydrophobic interaction, disulfide bond and/or other interactions during heating process. In general, fibrous structure was observed for bigeye snapper and threadfin bream surimi. However, the surimi gel without additive had the larger voids, and denser structure compared with those containing PP or PP in combination with $CaCl_2$ or PP in combination with MTGase. These observations suggested that the addition of PP resulted in the formation of an ordered structure with the finer strands and more fibrous structure. However, surimi gel added with 0.025% PP and 50 mmole $CaCl_2$/kg exhibited the finer and more compact structure with smaller voids in the matrix than that containing only 0.025% PP or 0.025% PP / 0.05% MTGase for bigeye snapper surimi or 0.05%PP / 0.05% MTGase for threadfin bream surimi. Calcium ion therefore exhibited the important role in induction of protein cross-linking, possibly by activating endogenous TGase. It was also reported that calcium might cause the cross-linking of protein via calcium bridges (Lanier, 2000). From the result, gel matrix of bigeye snapper surimi was finer than that of threadfin bream surimi, regardless of additives used. This was in accordance with the higher gel strength of the former.

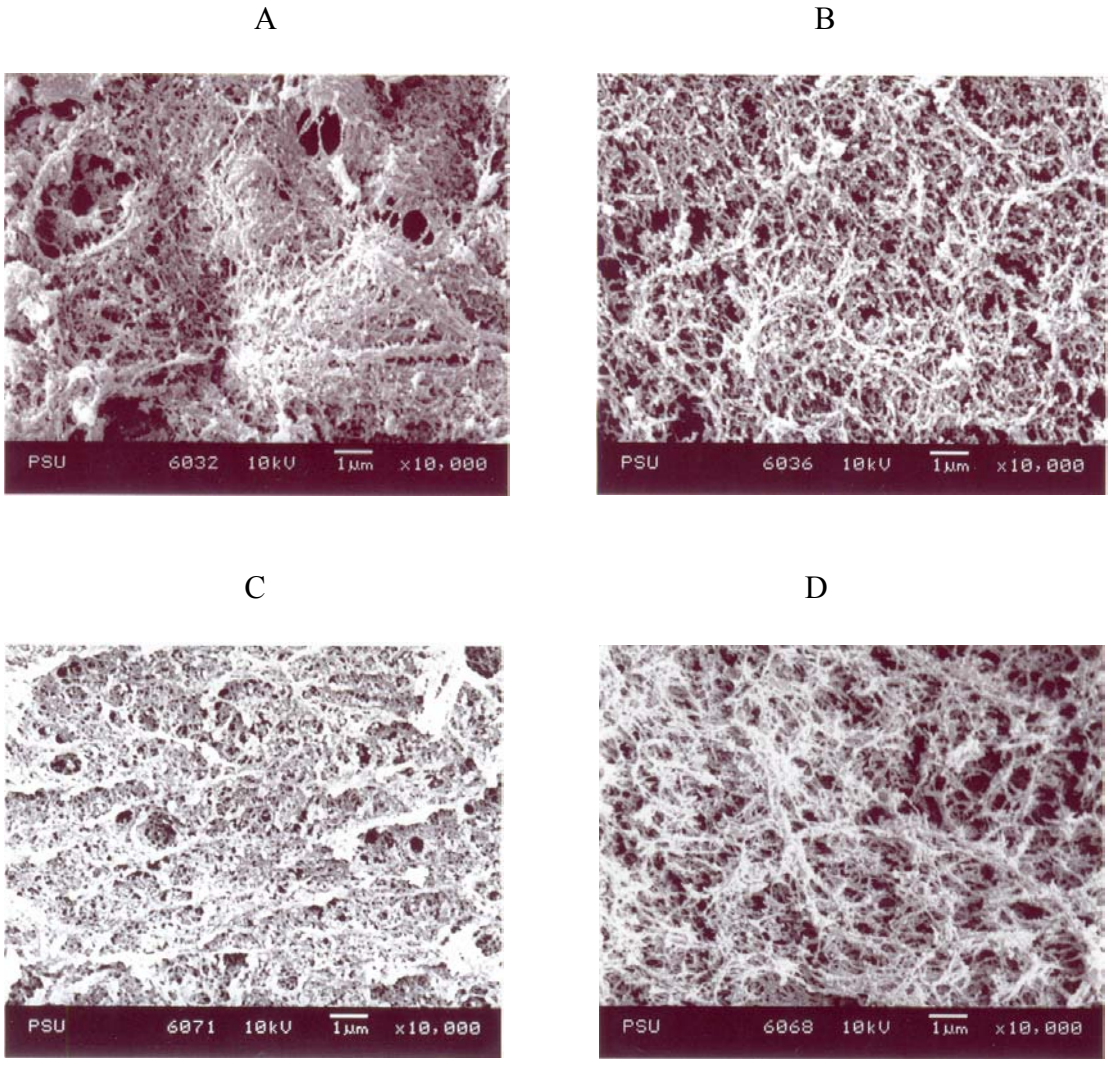

Figure 22. Microstructure of kamaboko gels from bigeye snapper surimi without additive (A), with 0.025% PP (B), with 0.025% PP and 50 mmole $CaCl_2$/kg (C), and with 0.025% PP and 0.05% MTGase (D).

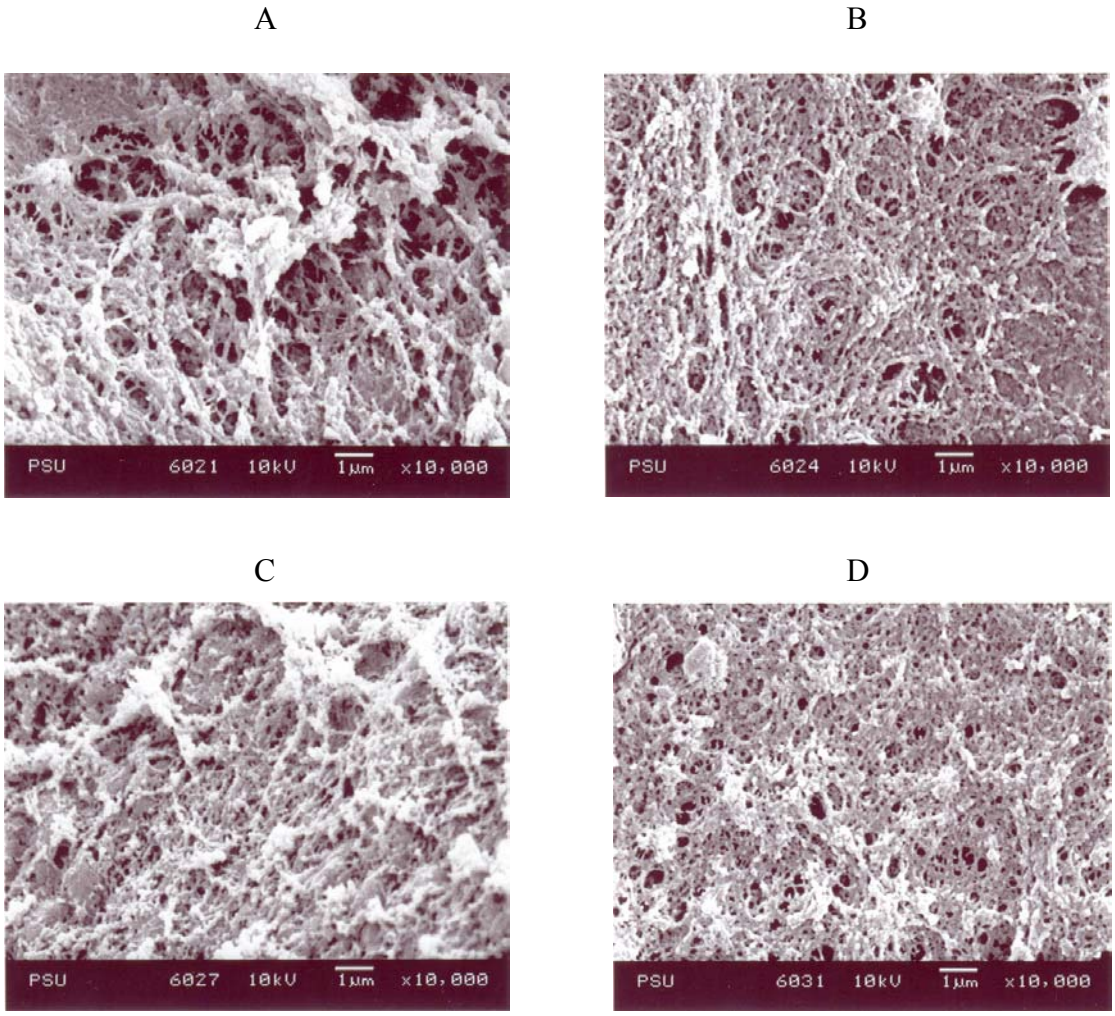

Figure 23. Microstructure of kamaboko gels from threadfin bream surimi without additive (A), with 0.025%PP (B), with 0.025%PP and 50 mmole $CaCl_2$/kg (C), and with 0.05% PP and 0.05% MTGase (D).

7. Effect of phosphate compounds on gel forming ability of surimi

7.1 Effect on breaking force and deformation

The lowered breaking force and deformation of bigeye snapper and threadfin bream surimi gels were observed in kamaboko gel or directly heated gel with and without PP or TPP when 20 mmole EGTA/kg was added (Figures 24 and 25). With the addition of 20 mmole EGTA/kg, breaking force of kamaboko gels of bigeye snapper and threadfin bream decreased by 65.01% and 44.56%, respectively, and deformation decreased by 51.71% and 47.69%, respectively, when compared with the control kamaboko gel. For directly heated gel of bigeye snapper and threadfin bream surimi, breaking force decreased by 58.03% and 65.86%, respectively, and deformation decreased by 53.34% and 39.40%, respectively, in the presence of 20 mmole EGTA/kg, when compared with the control directly heated gel. EGTA generally showed an inhibitory effect on gel formation in a concentration-dependent manner (Ashie and Lanier, 2000). EGTA inhibits endogenous TGase activity through chelating Ca^{2+}, a divalent cation required for the activation of tissue TGase (Folk, 1980). These results suggested that endogenous TGase played an essential role in setting of surimi gel, regardless of PP or TPP addition.

The higher breaking force and deformation of kamaboko gels from both surimi were obtained when PP at a level of 0.025% was added, when compared with gel without PP addition (Figures 24 and 25) ($P<0.05$). These results revealed that PP at 0.025% showed an enhancing effect on kamaboko gels from bigeye snapper and threadfin bream surimi. PP at low levels should induce the dissociation of actomyosin and sufficient amount of Ca^{2+} ion was still remained. As a consequence, the cross-linking induced by endogenous TGase still occurred. However, addition of 0.025% PP had no enhancing effect on gel strength of directly heated gel from threadfin bream

surimi (Figure 24). This reconfirmed the important role of setting on gel strengthening.

Addition of 0.025% PP in combination with 20 mmole EGTA/kg resulted in the increased breaking force and deformation, however the increased concentration of PP decreased breaking force and deformation of resulting gels. For surimi gel added with TPP, lowered breaking force and deformation were noticeable. When the concentration of TPP increased, more adverse effect on breaking force and deformation was found. TPP at higher levels might chelate Ca^{2+} ion together with EGTA added. This led to the less availability of calcium ion required for endogenous TGase. As a result, the lowered gel strength was observed.

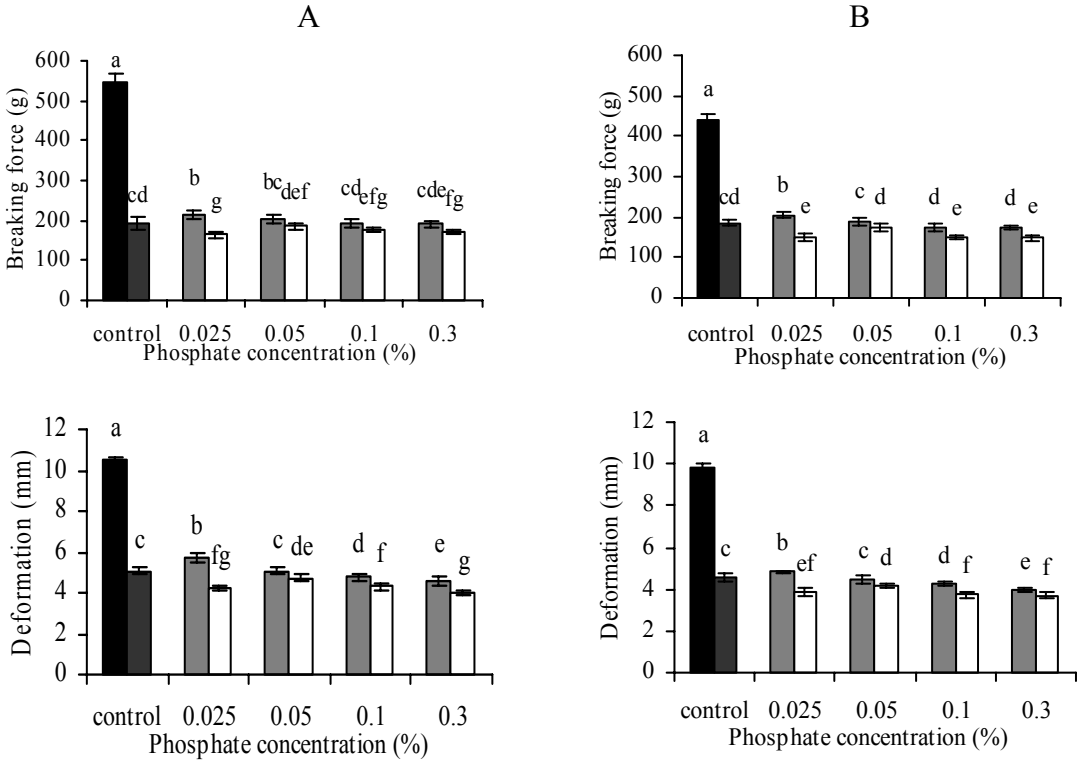

Figure 24. Breaking force and deformation of kamaboko gels (A) and directly heated gel (B) from bigeye snapper surimi added with PP or TPP at different levels in the presence of 20 mmole EGTA/kg. Bars represent the standard deviation from five determinations. Different letters on the bar indicate the significant difference ($P<0.05$).
■control; no phosphate and EGTA■ control+EGTA▒ PP□ TPP

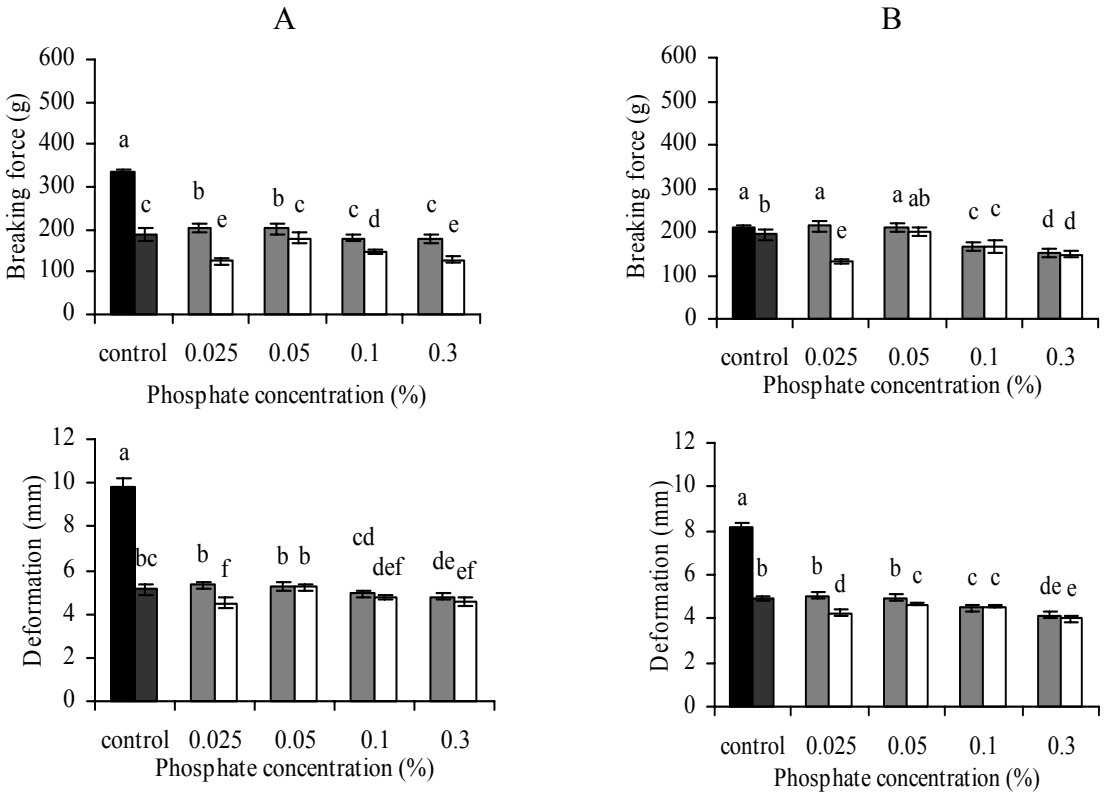

Figure 25. Breaking force and deformation of kamaboko gels (A) and directly heated gel (B) from threadfin bream surimi added with PP or TPP at different levels in the presence of 20 mmole EGTA/kg. Bars represent the standard deviation from five determinations. Different letters on the bar indicate the significant difference ($P<0.05$).
■ control; no phosphate and EGTA ■ control+EGTA □ PP □ TPP

7.2 Effect on whiteness

Whiteness of kamaboko and directly heated gels of bigeye snapper and threadfin bream surimi added with PP or TPP in the presence of EGTA is present in Tables 20 and 21, respectively. Slight increase in whiteness was found in both kamaboko and directly heated gels from both surimi when the level of phosphate increased ($P<0.05$). This was in agreement with the increase in expressible moisture when the greater levels of phosphates were added. The unbound water on the surface of gels was possibly associated with light scattering effect, causing the increased whiteness of gels.

Table 20. Whiteness of kamaboko gels from bigeye snapper and threadfin bream surimi added with PP or TPP at various levels in the presence of 20 mmole EGTA/kg

Type	Conc. (%)	Bigeye snapper	Threadfin bream
Control		73.98 ± 0.10f*	78.53 ± 0.06f
Control+EGTA		85.15 ± 0.07e	87.12 ± 0.04e
PP	0.025	85.24 ± 0.08de	87.25 ± 0.11de
	0.05	85.40 ± 0.09bc	87.34 ± 0.08cd
	0.1	85.54 ± 0.10ab	87.50 ± 0.13bc
	0.3	85.69 ± 0.10a	87.71 ± 0.06a
TPP	0.025	85.22 ± 0.06de	87.18 ± 0.08de
	0.05	85.36 ± 0.07cd	87.31 ± 0.15de
	0.1	85.53 ± 0.09ab	87.50 ± 0.12bc
	0.3	85.65 ± 0.14a	87.62 ± 0.05ab

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

Table 21. Whiteness of directly heated gels from bigeye snapper and threadfin bream surimi added with PP or TPP at various levels in the presence of 20 mmole EGTA/kg

Type	Conc. (%)	Bigeye snapper	Threadfin bream
Control		76.67 ± 0.11e*	78.51 ± 0.15d
Control+EGTA		85.37 ± 0.13d	87.31 ± 0.03c
PP	0.025	85.44 ± 0.04d	87.38 ± 0.09c
	0.05	85.49 ± 0.07cd	87.44 ± 0.05c
	0.1	85.63 ± 0.08abc	87.60 ± 0.09b
	0.3	85.73 ± 0.10a	87.81 ± 0.04a
TPP	0.025	85.41 ± 0.13d	87.34 ± 0.05c
	0.05	85.47 ± 0.08cd	87.41 ± 0.11c
	0.1	85.54 ± 0.08bcd	87.59 ± 0.06b
	0.3	85.70 ± 0.08ab	87.73 ± 0.13ab

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

7.3 Effect on solubility

Solubility of kamaboko and directly heated gels from bigeye snapper and threadfin bream surimi added with PP or TPP in the presence of EGTA is present

in Tables 22 and 23, respectively. When EGTA was added, solubility of kamaboko and directly heated gels from both surimi increased, suggesting the inhibitory activity of EGTA on cross-linking activity induced by endogenous TGase. For kamaboko gel, the addition of PP at 0.025% resulted in the lowered solubility. The results suggested that 0.025% PP could assist in the conformational changes, in which the polymerization could take place effectively. However, PP addition exhibited no marked effect on solubility of directly heated gel. This somehow indicated the synergistic effect between PP and setting. For TPP, it caused the greater solubility, compared with that observed in gels added with 0.025% PP. Therefore, it can be inferred that PP at an appropriate amount was able to improve the gel strength via facilitating the setting phenomenon.

Table 22. Solubility of kamaboko gels from bigeye snapper and threadfin bream surimi added with PP or TPP at various levels in the presence of 20 mmole EGTA/kg

Type	Conc. (%)	Bigeye snapper	Threadfin bream
NaOH		100a*	100a
Control		51.60±0.98g	67.03±0.61f
Control+EGTA		68.27±1.30de	68.66±0.90d
PP	0.025	66.18±1.21f	66.87±0.95e
	0.05	67.71±0.61ef	66.99±0.71e
	0.1	68.22±1.16de	68.91±0.90d
	0.3	68.99±1.42de	68.99±0.76d
TPP	0.025	71.74±0.62b	73.19±1.40b
	0.05	69.65±1.00cd	68.82±0.72d
	0.1	71.03±0.79bc	71.49±0.31c
	0.3	71.69±1.07b	72.94±0.73b

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

Table 23. Solubility of directly heated gels from bigeye snapper and threadfin bream surimi added with PP or TPP at various levels in the presence of 20 mmole EGTA/kg

Type	Conc. (%)	Bigeye snapper	Threadfin bream
NaOH		100a*	100a
Control		62.69±1.24g	61.09±0.88e
Control+EGTA		68.54±0.79def	68.61±0.47d
PP	0.025	67.79±0.69f	67.16±1.52de
	0.05	68.09±0.61ef	67.24±1.49de
	0.1	69.25±0.63cde	70.82±1.42c
	0.3	70.06±0.30c	71.53±0.87c
TPP	0.025	71.88±0.79b	73.94±0.62b
	0.05	69.60±0.95cd	67.87±0.62d
	0.1	71.72±0.66b	70.99±0.95c
	0.3	71.77±0.61b	71.94±0.69bc

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

7.4 Effect on protein patterns

Electrophoretic study revealed that cross-linking of MHC was markedly impeded when EGTA was added regardless of phosphate addition as shown in Figures 26 and 27. In general, MHC band intensity increased as the higher amounts of both PP and TPP were added. PP or TPP at higher levels might chelate the Ca^{2+} ion in concert with EGTA. This led to the lowered Ca^{2+} ion available for TGase. As a result, cross-linking of MHC was decreased. Rawdkuen *et al*. (2005) found that the cross-linking of MHC in surimi proteins were markedly suppressed by the addition of EGTA, TGase inhibitors. Nevertheless, no marked changes in actin were obtained. This results reconfirmed the negative effect of PP and TPP on cross-linking of protein via their chelating properties. It was presumed that EGTA amount used (20 mmole/kg) was not enough to totally chelate all endogenous Ca^{2+} ion. Thus, the effect of phosphate on the gel-forming ability cannot of thoroughly discussed from the study

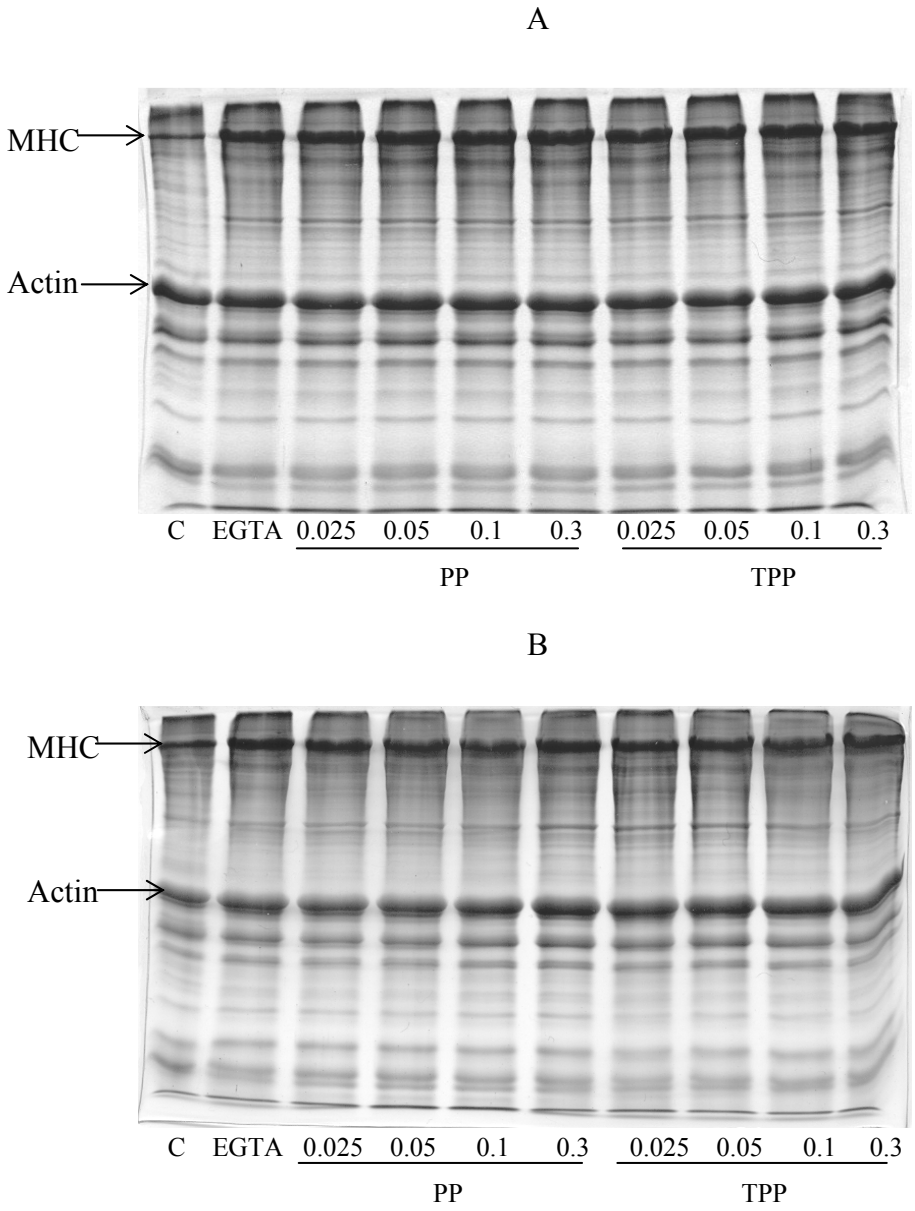

Figure 26. SDS-PAGE patterns of proteins in kamaboko gels from bigeye snapper (A) and threadfin bream (B) surimi added with PP or TPP at different levels in the presence of 20 mmole EGTA/kg. MHC; myosin heavy chain. Numbers designate the concentration of phosphate added (%).

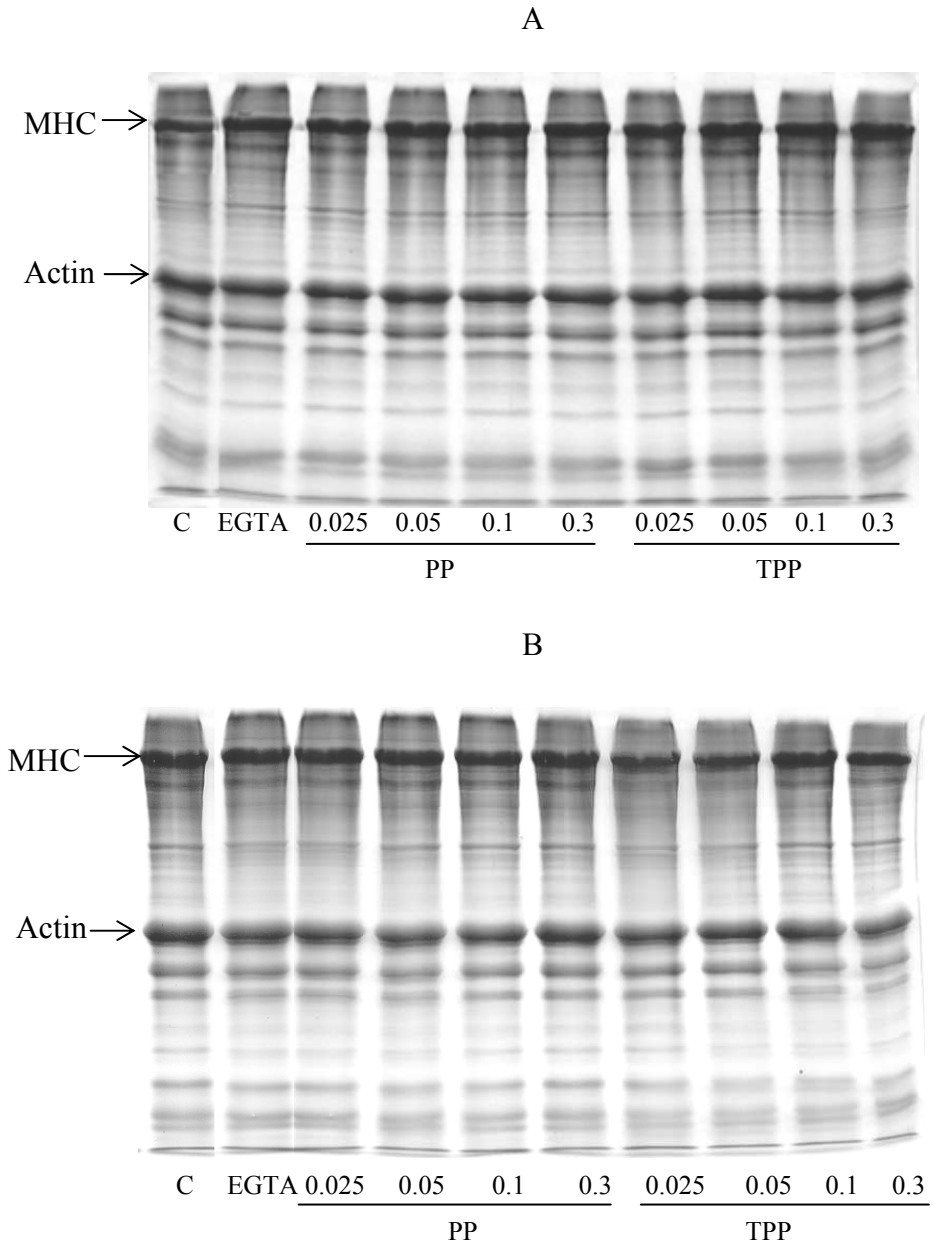

Figure 27. SDS-PAGE patterns of proteins in directly heated gels from bigeye snapper (A) and threadfin bream (B) surimi added with PP or TPP at different levels in the presence of 20 mmole EGTA/kg. MHC; myosin heavy chain. Numbers designate the concentration of phosphate added (%).

8. Effect of phosphate compounds on fish muscle protein

8.1 Changes in ATPase activities

ATPase activities of NAM were monitored in bigeye snapper and threadfin bream NAM added with PP, TPP or HMP (0, 0.025, 0.05, 0.1 and 0.3%) followed by dialysis against 0.6 M KCl (Figure 28). No changes in Ca^{2+}-ATPase were observed with all treatments, but Mg^{2+}-ATPase activity slightly increased when TPP and HMP amounts increased. Conversely, Mg^{2+}-ATPase activity decreased when PP amounts increased. For Mg^{2+}-EGTA-ATPase, no changes were observed in NAM added with TPP and HMP. Slight decrease in Mg^{2+}-EGTA ATPase activity was noticeable as PP amount increased. Ca^{2+}-ATPase activity has been used as an indicator of myosin integrity (Benjakul *et al*., 1997). Mg^{2+}-ATPase activity has been used to indicate of actin integrity (Azuma and Konno, 1998) while Mg^{2+}-EGTA ATPase activity has been used as an indicator of the tropomyosin-troponin complex integrity (Ouali and Valin, 1981; Ebasi *et al*., 1968; Watabe *et al*., 1989). The result suggested that actin denaturation occurred in NAM treated with PP as indicated by the decrease in Mg^{2+}-ATPase activity. No changes in myosin and tropomyosin-troponin complex in the presence of endogenous calcium ions in bigeye snapper and threadfin bream NAM added with PP, TPP and HMP. Increased myofibrillar Mg^{2+}-ATPase activity can enhance ATP consumption of muscle (Hwang *et al*., 1991; Sikorski *et al.*, 1990; Watabe *et al*., 1989). From the result, it can be concluded that PP at concentration up to 0.05% was able to dissociate the actomyosin. Free actin released was easily denatured in the presence of high salt (Torigai and Konno, 1996). Mg^{2+}-ATPase activity of myosin was activated by F-actin (Collins and Korn, 1986). Generally, myosin plays a role in protection of actin from salt (Torigai and Konno, 1996). Therefore, PP most likely induced the dissociation of actomyosin complex,

leading to the more pronounced network formation of myosin, which is a major contributor for gelation. This was evidenced by the increase in gel strength of surimi gel added with PP (Figure 8 and 9).

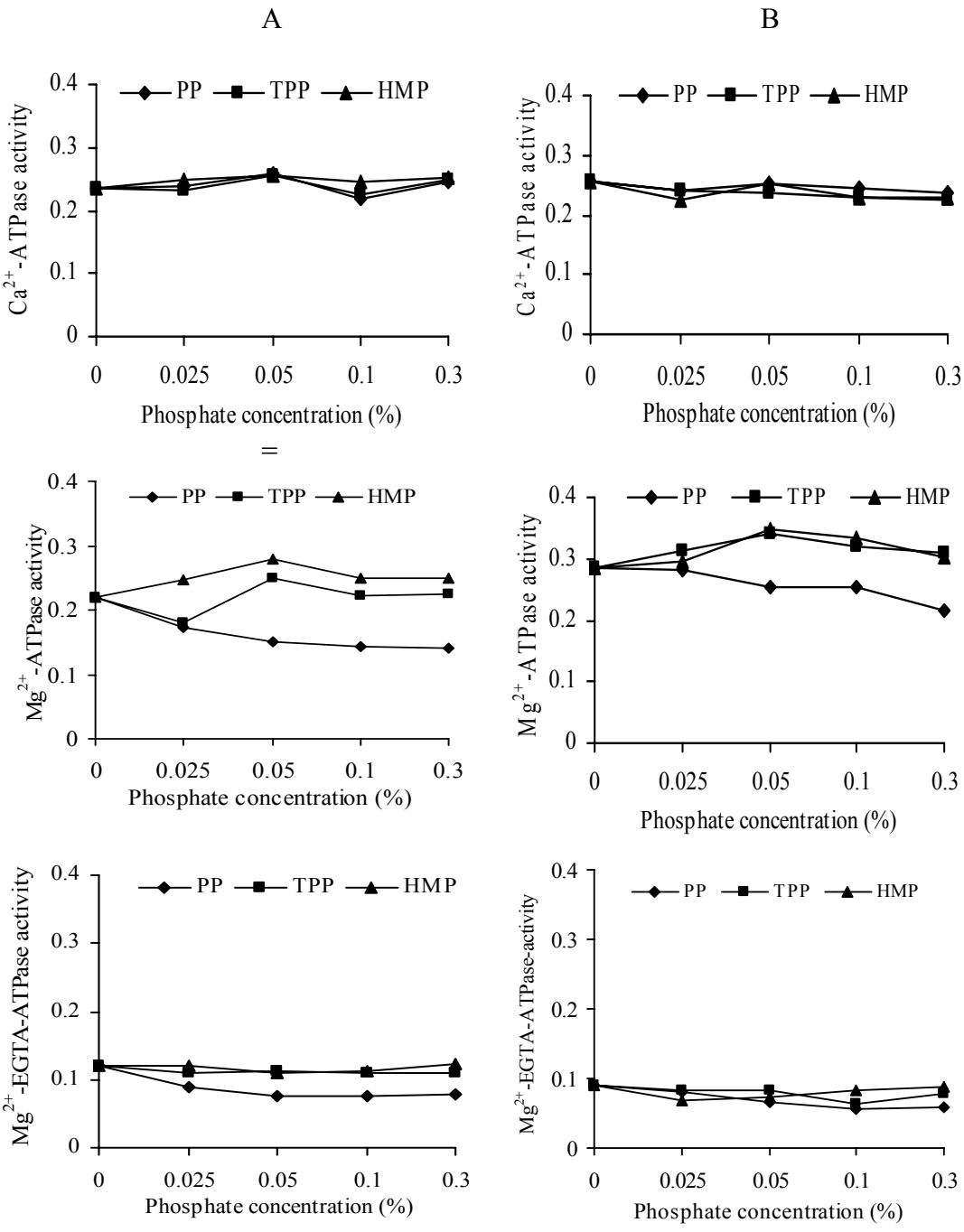

Figure 28. ATPase activity of NAM from bigeye snapper (A) and threadfin bream (B) treated with various phosphates at different concentrations. Activities were determined after dialysis of treated NAM against 0.6 M KCl.

8.2 Thermal stability of muscle protein

The inactivation rate constant or K_D value of actomyosin or myosin Ca^{2+}-ATPase activity has been generally used to evaluate the thermal stability of fish proteins (Tsai *et al*., 1989; Jiang *et al.*, 1989). A significant increase in K_D value of natural actomyosin from bigeye snapper and threadfin bream was observed at 20°C (Table 24). K_D value increased substantially at temperature above 30°C.

At the same temperature, NAM added with 0.1% PP had a higher K_D value, compared with NAM with no phosphate addition. NAM of threadfin bream had a higher K_D value than NAM of bigeye snapper at all conditions tested. From the results, it was presumed that muscle proteins of threadfin bream were more susceptible to thermal denaturation than those of bigeye snapper. The addition of 0.1% PP in NAM from both bigeye snapper and threadfin bream caused NAM more susceptible to thermal denaturation. The differences in stability between the kind of fish possibly resulted from the different intrinsic properties, amino acid composition as well as actin/myosin. Actin was suggested to play a protective role in the stability of myosin (Jiang *et al*., 1989).

Mg^{2+}-ATPase activity has been used as an indicator of the actin integrity. K_D value of Mg^{2+}-ATPase of NAM increased continuously with temperatures up to 30ºC (Table25). At temperature above 30ºC, no Mg^{2+}-ATPase activity was remained. In the presence of 0.1% PP, K_D of NAM was higher at all temperatures tested. The results suggested that the actin underwent denaturation in the presence of salt and when exposed to increasing temperature easily as PP was present. This result reconfirmed that PP could induce the dissociation of actomyosin complex. As a consequence, the stronger gel matrix could be formed (Figure 8 and 9).

Table 24. Inactivation rate constant of natural actomyosin Ca^{2+}-ATPase of bigeye snapper and threadfin bream at various temperatures

Temp (°C)	Kd x 10^5			
	Bigeye snapper		Treadfin bream	
	0%PP	0.1%PP	0%PP	0.1%PP
0	0.10±0.09d	0.15±0.23d	0.11±0.10d	0.16±0.00d
10	0.25±0.18d	0.30±0.23d	0.28±0.10d	0.34±0.00d
20	2.01±0.10c	2.29±0.09c	2.15±0.18c	2.32±0.11c
30	6.03±0.11b	12.95±0.14b	7.92±0.13b	10.72±0.15b
40	33.63±0.521a	40.64±0.65a	35.99±0.35a	41.74±0.80a
50	-	-	-	-
60	-	-	-	-
70	-	-	-	-

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

Table 25. Inactivation rate constant of natural actomyosin Mg^{2+}-ATPase of bigeye snapper and threadfin bream at various temperatures

Temp (°C)	Kd x 10^5			
	Bigeye snapper		Treadfin bream	
	0%PP	0.1%PP	0%PP	0.1%PP
0	0.36±0.11d	0.46±0.14d	0.52±0.07d	0.79±0.11d
10	8.32±0.14c	9.44±0.19c	9.17±0.10c	12.10±0.16c
20	11.79±0.16b	14.15±0.22b	11.81±0.11b	14.22±0.17b
30	12.83±0.17a	16.14±0.24a	12.81±0.19a	15.14±0.18a
40	-	-	-	-
50	-	-	-	-
60	-	-	-	-
70	-	-	-	-

*Values are mean ± standard deviation from triplicate determinations. Values with the same letter in the same column are not significantly different (P>0.05).

8.3 Effect of phosphate compounds on transglutaminase activity in fish muscle

The residual TGase activities of bigeye snapper and threadfin bream muscle treated with 2.5% NaCl, 2.5% NaCl and PP or TPP or HMP at different levels (0.025, 0.05, 0.1 and 0.3%) are shown in Figure 29. Salt reduced TGase activity from bigeye snapper and threadfin bream muscles by 8.62% and 3.71%, respectively, compared with the control (no additive). Phosphate compounds also reduced TGase activity to a greater extent when the level of phosphate compound increased. Among

all phosphates tested, HMP showed a higher inhibitory activity against TGase activity. High-molecular-weight phosphates exhibit the higher efficacy in metal chelation than those with lower-molecular-weight. These result suggested that NaCl, PP, TPP and HMP were able to reduce TGase activity, possibly via the chelation of Ca^{2+} ion required for TGase activity. When $CaCl_2$ at levels of 25 and 50 mmole/kg was added, TGase activity regained slightly. Greater increase in activity was observed with the higher $CaCl_2$ concentration (50 mmole/kg) (Figure 30). When PP at higher levels was added, slight decrease in TGase activity was found. The lower decrease was obtained in the sample containing 50 mmole $CaCl_2$/kg. It was suggested that sufficient Ca^{2+} ion could compensate for the loss of Ca^{2+} ion caused by phosphate addition. Residual TGase activity was greater in samples with added calcium, which can function as TGase activator (Montero *et al.,* 2005). This result indicated that phosphate compounds could chelate calcium ions, leading the insufficient amount of calcium ion required for endogenous transglutaminase. As a result, the lowered gel strength was observed in the presence of phosphate, particularly at a higher concentration (Figure 12).

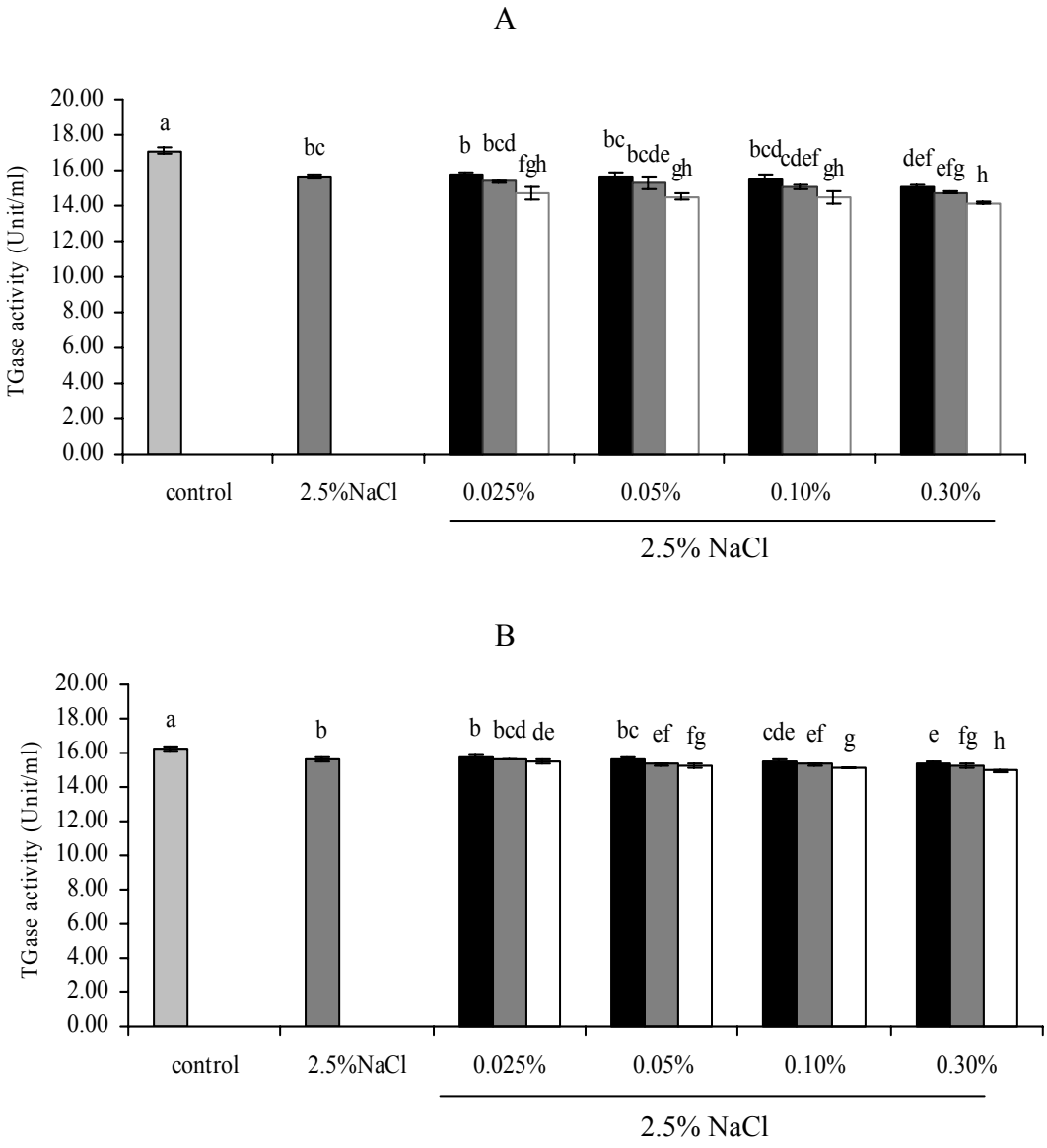

Figure 29. TGase activity of fish muscle from bigeye snapper (A) and threadfin bream (B) in the presence of salt or salt in combination with phosphates at different levels. Different letters on the bars indicate the significant difference ($P<0.05$).

■ PP ▒ TPP □ HMP

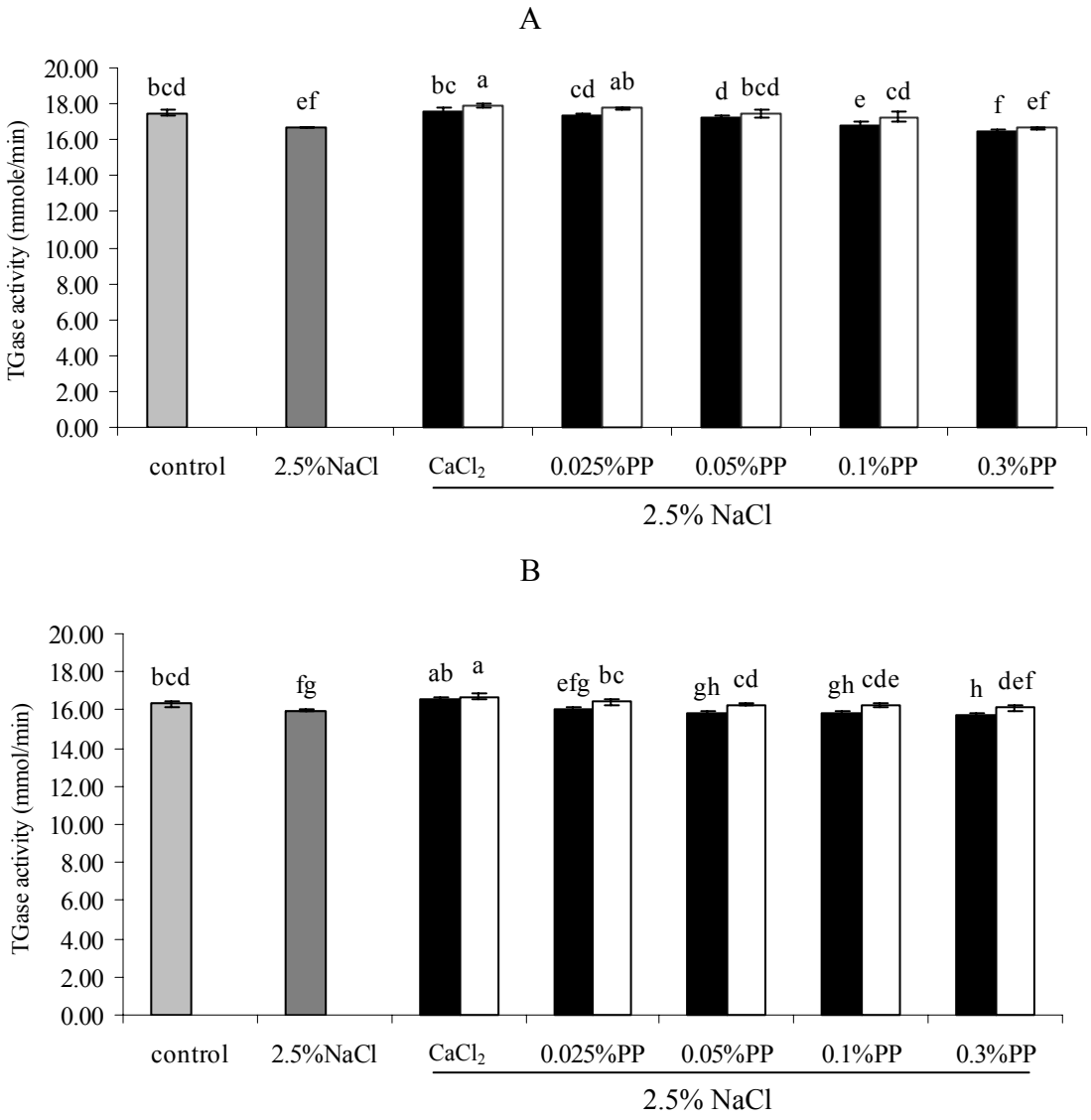

Figure 30. TGase activity of fish muscle from bigeye snapper (A) and threadfin bream (B) in the presence of salt or salt in combination with $CaCl_2$ or PP at different levels. Different letters on the bars indicate the significant difference ($P<0.05$).

■ 25 mmole/kg $CaCl_2$ □ 50 mmole/kg $CaCl_2$