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Transglutaminase-mediated setting in bigeye snapper Surimi

Soottawat Benjakul[a,*], Wonnop Visessanguan[b]

[a]*Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand*

[b]*National Center for Genetic Engineering and Biotechnology, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani, 12120, Thailand*

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Abstract

Effect of setting induced by endogenous transglutaminase (TGase) in two species of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*, on gel properties and protein cross-linking was investigated. Setting at either 25 or 40 °C, prior to heating at 90 °C resulted in the increase in both breaking force and deformation of surimi from both species, particularly when setting time increased ($P<0.05$). A decrease in solubility of surimi gels in a mixture of sodium dodecyl-sulfate, urea and β-mercaptoethanol suggested increased formation of non-disulfide covalent bonding which coincided with increased gel strength and the decrease in myosin heavy chain (MHC) polypeptide. The optimum conditions for setting of surimi sol was found to be 40 °C for 2 h for *P. tayenus* and 25 °C for 3 h for *P. macracanthus*. Assayed by monodancylcadaverine (MDC)-incorporation method, TGase from *P. tayenus* and *P. macracanthus* exhibited an optimum temperature at 40 and 25 °C, respectively. In addition, the breaking force and deformation of surimi from both species increased markedly with the addition of calcium chloride, while they decreased considerably in the presence of EDTA, *N*-methylmaleimide and ammonium chloride. The results confirmed that endogenous transglutaminase played an important role in gel enhancement of surimi from both species of bigeye snapper.

Keywords: Setting; Surimi; Suwari; Kamaboko; Transglutaminase; Bigeye snapper

1. Introduction

"Setting" or "suwari" is a well known occurrence in the surimi paste during the incubation at temperatures lower than 40 °C. This phenomenon involves network formation of myosin due to the cross-linking induced by endogenous transglutaminase (TGase) (Seki et al., 1990; Tsukamasa et al., 1993). TGase has been reported to catalyze an acyl transfer reaction between γ-carboxamide groups of glutamyl residues in proteins as the acyl donors and a variety of primary amine and water as the acyl acceptor (Folk & Chung, 1973, Kumazawa, Numazawa, Seguro, & Motoki, 1995). When the ε-amino group of lysine acts as acyl acceptor, cross-linking of proteins is mediated through the formation of ε-(γ-glutamyl) lysine linkages. The increase in gel strength of surimi from Alaska pollack was associated with the increased cross-linking of myosin heavy chain and ε-(γ-glutamyl) lysine content formed (Kumazawa et al, 1995). Conversely, inhibition of endogenous TGase resulted in complete suppression of myosin cross-linking of walleye pollack surimi gel and the cross-linking was also inhibited above 45°C due to the inactivation of TGase (Takeda & Seki, 1996). The ε-(γ-glutamyl) lysine formation in Alaska pollack gel was suppressed by addition of EDTA and ammonium chloride (Kumazawa et al., 1995).

Setting response has been found to vary among fish species (Morales, Ramirez, Vivanco, & Vazquez, 2001; Shimizu, Machida & Takanemi, 1981; Tsukamasa & Shimizu, 1990). Wan, Kimura, Satake & Seki (1995) found that the poorer gel-forming ability of chum salmon surimi was primarily attributable to lower TGase activity as well as the lower contents of myosin and calcium ion, compared with walleye pollack surimi. Moreover, the reactivity of TGase to various fish actomyosin was markedly different (Araki & Seki, 1993) and depended on the conformation of actomyo-

* Corresponding author. Tel.: +66-74-286334; fax: +66-74-212889.

E-mail address: bsoottaw@ratree.psu.ac.th (S. Benjakul).

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sin. Fish TGase has been found to be Ca^{2+}-dependent, however, the requirement varies among fish species (Nozawa, Mamagoshi, & Seki, 1997). The addition of calcium compounds to surimi enhanced TGase-mediated setting, resulting in stronger gels (Lee & Park, 1998).

Bigeye snapper is commonly used for surimi production in Thailand. Two major species, *Priacanthus tayenus* and *Priacanthus macracanthus*, are commonly caught in both the Gulf of Thailand and Indian Ocean. *P. tayenus* render the superior gel-forming ability due to the higher aggregation of myosin heavy chain with the higher hydrophobic interaction and disulfide formation (Benjakul, Visessanguan, Ishisaki, & Tanaka, 2001). Apart from the intrinsic property of myofibrillar proteins previously reported, the setting phenomenon caused by endogenous TGase may be varied between both species and contributes to the different gelation characteristics. Therefore, the objectives of this study were to investigate TGase-mediated setting of surimi from two species of bigeye snapper and to study some characteristics of TGase in both species.

2. Materials and methods

2.1. Chemicals

Ethylenediaminetetraacetic acid (EDTA), ammonium chloride, *N*-ethylmaleimide (NEM), monodansylcadaverine (MDC) and dithiothreitol (DTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) and *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Bigeye snapper preparation

Bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*, were caught from Songkhla-Pattani Coast, stored in ice and off-loaded approximately 24–36 h after catching. Fishes were transported in ice to Department of Food Technology, Prince of Songkla University, Hat Yai with the approximate duration of 2 h.

2.3. Surimi and surimi gel preparation

Bigeye snapper were deboned manually and temperature of fish was controlled to be lower than 4 °C. The mince was then washed with cold water (5 °C) at a mince/water ratio of 1:2 (w/w). The mixture was stirred gently for 3 min and washed mince was filtered with a layer of Nylon screen. The washing process was repeated twice. Finally the washed mince was subjected to centrifugation using a model CE 21 K basket centrifuge (Grandiumpiant, Belluno, Italy) with a speed of 700 $\times g$ for 15 min. Washed mince or fresh surimi was kept in ice until used. For surimi gel preparation, fresh surimi was mixed with 2.5% salt (w/w) and the moisture content was adjusted to 80%. The mixture was chopped for 5 min at 4 °C to obtain the homogenous sol. To study the effect of each compound on surimi gel properties, calcium chloride (10, 20, 50, 80 and 100 mmol/kg), EDTA (0.5, 1, 3 and 5 mmol/kg), ammonium chloride (0.05, 0.1, 0.2 and 0.5 mol/kg) and NEM (1, 3, 5 and 10 mmol/kg) were added and mixed thoroughly prior to heating. Surimi sol was then stuffed into polyvinylidine casing with a diameter of 2.5 cm and tightly sealed. Suwari gels were prepared by incubating surimi sol in a temperature-controlled water bath (Memmert, Schwabach, Germany) either at 25 °C for 1, 2, 3 and 5 h or at 40 °C for 0.5, 1, 1.5 and 2 h. To prepare kamaboko gels, suwari gels were subjected to heating at 90 °C for 20 min in a temperature-controlled water bath. Directly heated gels were prepared by heating the sol at 90 °C for 20 min without prior setting. The gels were then cooled in iced water and stored for 24 h at 4 °C prior to analysis.

2.4. Texture analysis

Texture analysis of surimi gels was carried out using a texture analyzer TA-XT2 (Stable Micro System, Surrey, UK). Gels were equilibrated and evaluated at room temperature (28–30 °C). Five cylinder-shaped samples with a length of 2.5 cm were prepared and subjected to determination. Breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyzer equipped with a cylindrical plunger (diameter 5 mm; depression speed 60 mm/min).

2.5. Determination of solubility

Solubility of suwari and kamaboko gel with or without different compounds was determined as described by Benjakul, Visessanguan, and Srivilai (2001). The sample (1 g) was homogenized in 20 ml of 20 mM Tris–HCl, pH 8.0 containing 1% (w/v) SDS, 8 M urea and 2% (v/v) β-ME for 1 min using homogenizer (IKA Labortechnik, Malaysia). The homogenate was heated in boiled water (100 °C) for 2 min and stirred at room temperature for 4 h. The resulting homogenate was centrifuged at 10,000$\times g$ for 30 min using Sorvall Model RC-B plus. Protein in the supernatant (10 ml) was precipitated by the addition of 50% (w/v) cold TCA to a final concentration of 10%. The mixture was kept at 4 °C for 18 h and then centrifuged at 10,000$\times g$ for 30 min. The precipitate was washed with 10% TCA and solubilized in 0.5 M NaOH. The protein content was measured using Biuret test (Robinson & Hodgen, 1940). To obtain the total amount of protein, gels were solu-

bilized in 0.5 M NaOH. The solubility was reported as percentage of total protein.

2.6. SDS–polyacrylamide gel electrophoresis

SDS–polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970) using 10% running gel and 4% stacking gel. Gel samples (3 g) with or without the compounds tested were mixed with 27 ml of 5% (w/v) SDS solution (85 °C). The mixture was then homogenized using IKA Labortechnik homogenizer. The homogenate was incubated in an 85 °C water bath for 1 h to dissolve the proteins, followed by centrifuging to remove undissolved debris. After separation by SDS–PAGE, proteins were fixed and stained with Coomassie Brilliant Blue R-250.

2.7. Preparation of bigeye snapper transglutaminase crude extract

Crude extract was prepared as fish juice as described by Benjakul, Visessanguan, and Srivilai (2001). Bigeye snapper muscle was finely chopped and centrifuged at $5000\times g$ for 30 min at 4 °C. The supernatant obtained was used as TGase crude extract.

2.8. pH and temperature profile of TGase activity of bigeye muscle extract

MDC-incorporating activity of TGase was assayed according to the method of Takagi, Saito, Kikuchi, and Inada (1986) with slight modifications. For pH profile, activity was assayed under different pHs ranging from 2 to 10 at 37 °C for 30 min. The reaction mixture containing 100 μl of 0.2 M DTT, 2.4 ml of McIlvaine's buffer, 100 μl of 0.5 mM MDC and 0.4 ml of 2 mg/ml *N*,*N*′-dimethylated casein was prepared. The reaction was initiated by the addition of 0.2 ml of enzyme extract (0.1 g protein/ml) and the mixture was incubated at 37 °C for 30 min. The reaction was terminated by the addition of 0.2 ml of 1.0 M ammonium sulfate. The fluorescence intensity was measured by using a Hitachi fluorescence spectrophotometer (Hitachi, Japan) at the excitation and emission wavelengths of 350 and 480 nm, respectively. One unit of MDC-incorporating activity was defined as nmol of MDC incorporated per min under assay condition. To study the temperature profile, the assay was performed at various temperatures (25, 30, 35, 40, 45, 55 and 65 °C) under the optimum pH for 30 min. The activity was expressed as mU/ml extract.

2.9. Protein determination

Protein concentration was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as standard.

2.10. Statistical analysis

Analysis of variance (ANOVA) was performed and mean comparisons were run by Duncan's multiple range test (Steel & Torrie, 1980).

3. Results and discussion

3.1. Effect of setting on properties of suwari and kamaboko gel

Setting of surimi paste with or without subsequent heating resulted in the increase in both breaking force and deformation of suwari and kamaboko gels (Figs. 1 and 2, respectively). *P. tayenus* suwari gels had higher breaking force *than P. macracanthus* suwari gels, however the deformation of suwari gels from two species varied, depending upon setting temperature and time used (Fig. 1). *P. tayenus* kamaboko gels generally possessed higher breaking force and deformation than *P. macracanthus* gels under all conditions tested ($P<0.05$; Fig. 2). Breaking force and deformation of suwari and kamaboko gels from both species increased with increasing setting temperature and time.

Suwari gels set at 25 °C exhibited lower breaking force and deformation than those set at 40 °C ($P<0.05$; Fig. 1). Breaking force and deformation of *P. tayenus* suwari gel with a setting time of 5 h increased by 169.9 and 49.2%, respectively, compared to those of suwari gel set for 1 h. At the same setting time (5 h), breaking force and deformation of *P. macracanthus* suwari gel increased by 155.6 and 123.4%, respectively, compared with suwari gel subjected to 1 h setting. Though similar increasing rate of breaking force was obtained between two species, *P. tayenus* suwari gels generally exhibited the higher breaking force than those from *P. macracanthus*.

When setting temperature of 40 °C was used for *P. tayenus* suwari gel preparation, breaking force and deformation increased when the setting time increased ($P<0.05$; Fig. 1). Suwari gel from *P. tayenus* surimi subjected to 2 h setting had 88.8 and 37.3% increase in breaking force and deformation, respectively, compared with gel subjected to 0.5 h setting. Nevertheless, *P. macracanthus* suwari gel had no increase in breaking force when setting time was longer than 1 h. No marked changes in deformation of suwari gel from *P. macracanthus* surimi were found with the increasing setting time ($P>0.05$). With setting time of 1 h, breaking force of *P. macracanthus* suwari gel increased by 53.2%, compared with that of suwari set for 0.5 h. The differences in setting response between two species at 40 °C were presumed to be due to the differences in protein and TGase stability at 40 °C. From the result, *P. macracanthus* surimi did not exhibit a good setting response at 40 °C. Benjakul, Visessanguan, and Leela-

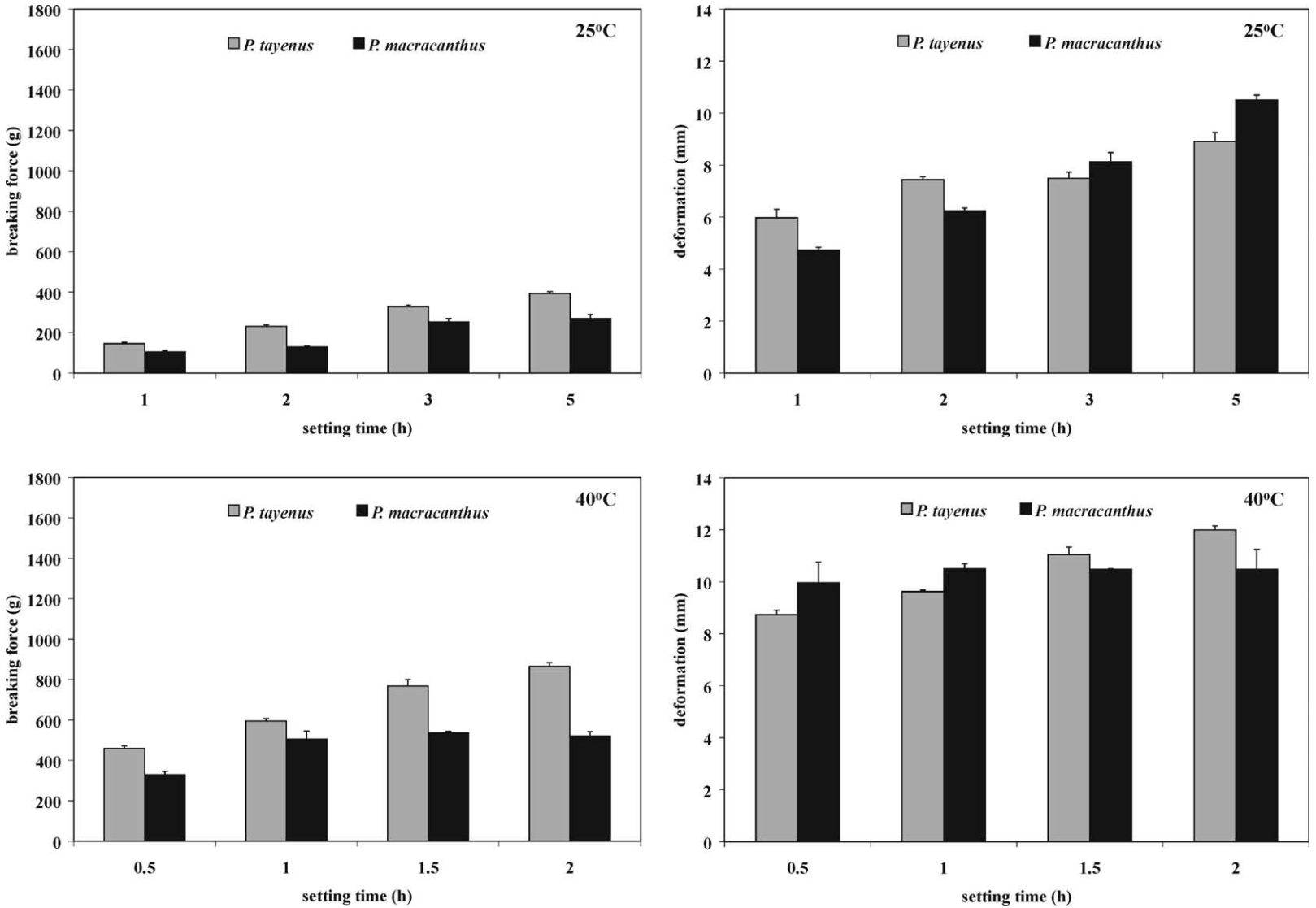

Fig. 1. Breaking force and deformation of suwari gels of surimi from *P. tayenus* and *P. macracanthus* prepared by setting at 25 °C and 40 °C for different times. Error bars indicate the standard deviations from the mean of five determinations.

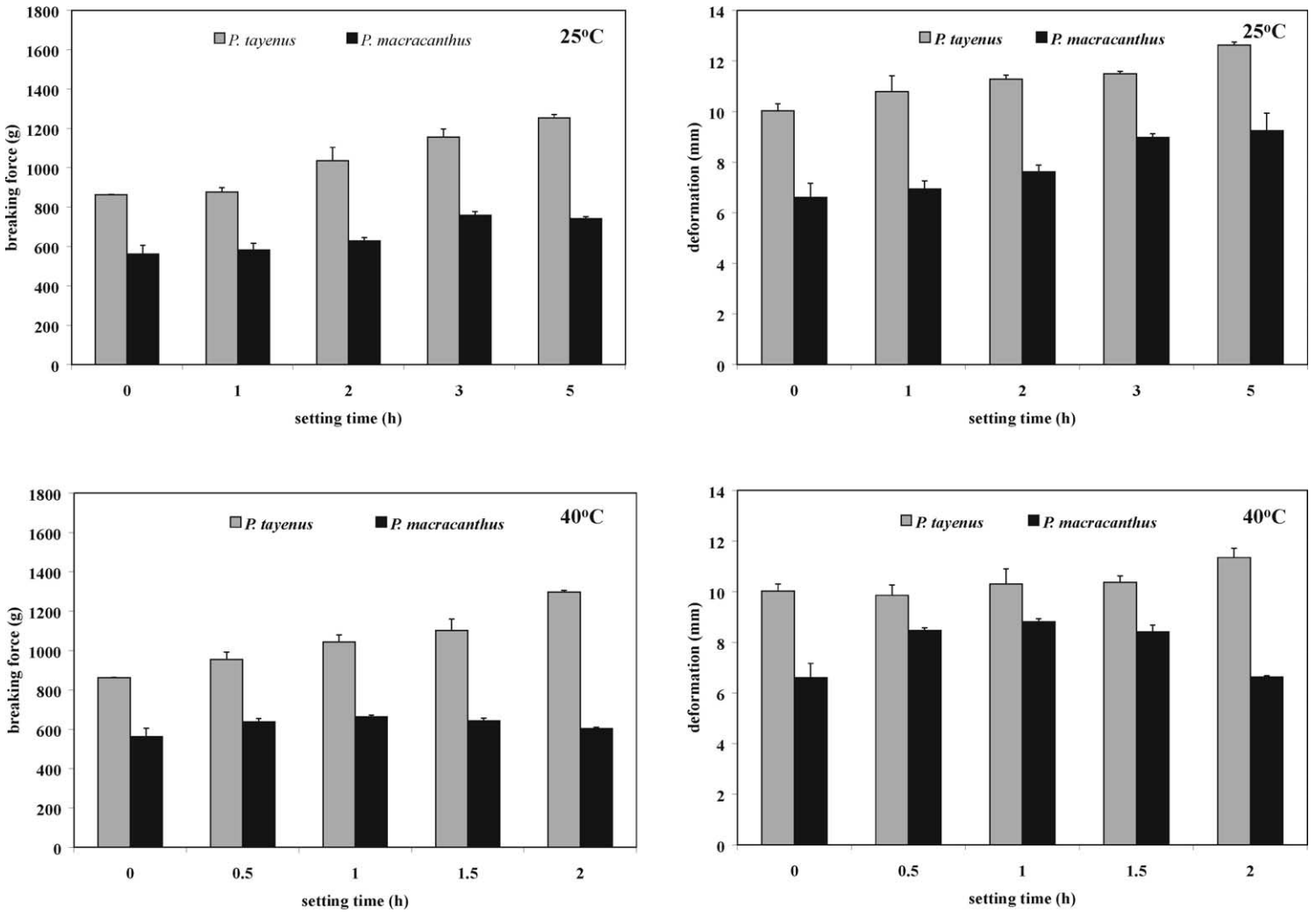

Fig. 2. Breaking force and deformation of kamaboko gels of surimi from *P. tayenus* and *P. macracanthus* prepared by setting at 25 °C and 40 °C for different times. Error bars indicate the standard deviations from the mean of five determinations.

pongwatana (in press) reported that muscle proteins from *P. tayenus* had a higher thermal stability than those from *P. macracanthus* as indicated by higher enthalpy for transitions and lower inactivation rate constant. Therefore, protein molecules *of P. macracanthus* may undergo excess unfolding at 40 °C, leading to the aggregation via hydrophobic interaction or some disulfide bonding. As a result, glutamine or lysine residues were possibly imbedded and not available for cross-linking mediated by TGase. Conversely, unfolding of *P. tayenus* protein might occur to a proper extent, which resulted in the exposure of those reactive residues for TGase mediated reaction. Tsukamasa and Shimizu (1991) found that heat susceptibility of myosin heavy chain (MHC) was a factor affecting TGase associated setting phenomenon in fish muscle. Thus, the setting phenomenon was more dominant at 40 °C for *P. tayenus* and 25 °C seemed to be a appropriate temperature for *P. macracanthus*.

From the result, suwari gels of both species prepared by setting at 40 °C rendered higher breaking force and deformation, compared with those prepared at 25 °C. Apart from non-disulfide covalent bond formation, the increases in breaking force and deformation of suwari gels at higher temperature were presumed to be due to higher thermal aggregation of myofibrillar proteins stabilized by both covalent and non-covalent bonding. Benjakul et al., (2001) reported that natural actomyosin (NAM) from two species of bigeye snapper underwent aggregation at temperature higher than 30 °C. The aggregation was associated with the increase in disulfide formation and hydrophobic interaction.

For kamaboko gels of surimi from both species, it was noted that the setting time of 1 h at 25 °C did not increase the breaking force of kamaboko gels (Fig. 2). However, the marked increase in breaking force and deformation of kamaboko gel was found as the setting time increased. *P. tayenus* kamaboko gel prepared by setting at 25 °C for 5 h, prior to heating had the increase in breaking force and deformation by 45.2 and 23.2%, respectively. A similar result was observed with *P. macracanthus* kamaboko gel. With the prior setting time of 5 h, breaking force and deformation of kamaboko gel of this species increased by 31.9 and 39.8%, respectively, compared with directly heated gel.

Breaking force and deformation of kamaboko gels with prior setting at 40 °C for different time are shown in Fig. 2. It was noted that much more increase in breaking force was found, compared to that of surwari gel. For *P. tayenus*, the longer the setting time, the higher breaking force was observed. Breaking force of kamaboko gel from *P. tayenus* surimi prepared by setting at 40 °C for 2 h increased by 50.3%, compared with directly heated gel. However, breaking force of kamaboko gel from *P. macracanthus* tended to decrease when setting time was longer than 1 h and only 18% increase in breaking force was found with 1 h setting. Significant decreases in both breaking force and deformation were noted when *P. macracanthus* sol was subjected to setting at 40 °C for 2 h. Therefore, setting temperature was another factor determining the final kamaboko gel quality. Different TGase properties, especially the thermal stability and optimum temperature, between two species were presumed to cause such a difference. Additionally, the setting of *P. macracanthus* surimi sol at high temperature (40 °C) for a longer time may cause more degradation caused by heat activated proteinase. The proteolysis was detected at optimum setting temperature for surimi paste of threadfin bream, hoki, walleye pollack and Atlantic croaker (Kamath, Lanier, Foegeding, & Hamann, 1992; Lee, Seki, Kato, Nakagawa, Terui, & Arai, 1990a, 1990b; Takeda & Seki, 1996). Therefore, setting at temperatures, which maximize TGase activity but minimize proteinase activity may be used to achieve good quality kamaboko gels.

After setting, the elevated temperature during heating resulted in further oxidation of sulfhydryl groups with the accompanied disulfide bond formation. The α-helix unfolds due to the instability of hydrogen bonds, exposing greater numbers of hydrophobic amino acids, resulting higher hydrophobic interaction (Niwa, 1992). As a consequence, kamaboko gels had higher breaking force and deformation than suwari gels. When comparing the properties of kamaboko gels between two species, it was found that *P. tayenus* surimi had much higher breaking and deformation than *P. macracanthus* surimi. The present result is in agreement with Benjakul et al. (2001) who found that *P. tayenus* NAM exhibited the superior gel-forming ability to *P. macracanthus* NAM.

3.2. Effect of setting on solubility of suwari and kamaboko

Solubility of suwari and kamaboko gels with the setting at 25 °C is shown in Table 1. In general, suwari gels from *P. tayenus* had a lower solubility, when the setting time increased ($P<0.05$). However, no decrease in solubility was found when setting time was longer than 2 h. For kamaboko gels, the decrease in solubility was observed when setting time increased up to 5 h. Different phenomenon was found in suwari gel from *P. macracanthus*. No marked changes in solubility of suwari gels with different setting times at 25°C were noted, but the gradual decrease in solubility was observed in kamaboko with increasing setting time. From the result, suwari and kamaboko gels from *P. tayenus* surimi showed the lower solubility than those from *P. macracanthus* surimi. The decrease in solubility indicated the formation of non-disulfide crosslinks induced by endogenous TGase.

Table 1
Solubility of suwari and kamaboko gels of surimi from *P. tayenus* and *P. macracanthus* prepared by setting at 25 °C for different times[a]

Setting time (h)	Solubility (%)			
	Suwari gel		Kamaboko gel	
	P. tayenus	*P.macracanthus*	*P. tayenus*	*P.macracanthus*
Surimi	100d	100b	100d	100f
Sol	97.37±1.54c	98.46±1.62ab	97.37±1.54d	98.46±1.62ef
0	–	–	88.28±0.91c	96.59±1.12de
1	88.64±2.54b	95.23±3.21a	88.26±0.92c	95.23±0.34cd
2	85.36±1.68a	95.23±3.20a	84.49±2.48b	94.14±1.26c
3	84.98±1.67a	94.17±1.85a	84.47±2.24b	87.85±1.68a
5	83.89±0.64a	94.17±1.82a	81.25±1.86a	90.84±1.27b

Mean±S.D. from triplicate determinations
[a] Different letters in same column indicate significant differences ($P<0.05$).

Table 2
Solubility of suwari and kamaboko gels of surimi from *P. tayenus* and *P. macracanthus* prepared by setting at 40 °C for different times[a]

Setting time (h)	Solubility (%)			
	Suwari gel		Kamaboko gel	
	P. tayenus	*P.macracanthus*	*P. tayenus*	*P.macracanthus*
Surimi	100d	100d	100f	100b
Sol	97.37±1.54c	98.46±1.62dc	97.37±1.54e	98.46±1.62b
0	–	–	88.26±0.92d	96.59±1.12b
1	82.42±1.10b	96.30±1.84bc	85.03±1.88c	88.28±3.86a
2	80.22±1.11a	95.16±0.14b	82.33±1.61b	84.98±6.72a
3	79.85±1.27a	91.48±0.94a	80.18±0.94b	85.35±0.63a
5	79.82±0.54a	90.40±1.62a	77.49±1.62a	89.01±3.30a

Mean±S.D. from triplicate determinations
[a] Different letters in same column indicate significant differences ($P<0.05$).

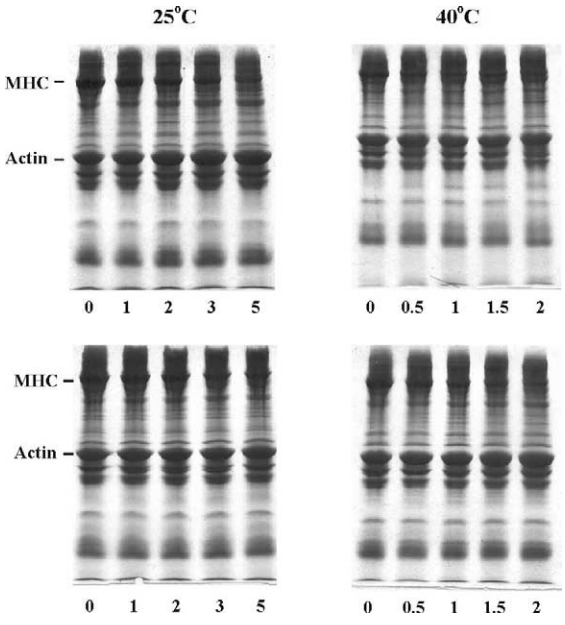

Fig. 3. SDS–PAGE pattern of suwari gels of surimi from *P. tayenus* (upper) and *P. macracanthus* (lower) prepared by setting at 25 °C and 40 °C for different times. Numbers designate setting time (h).

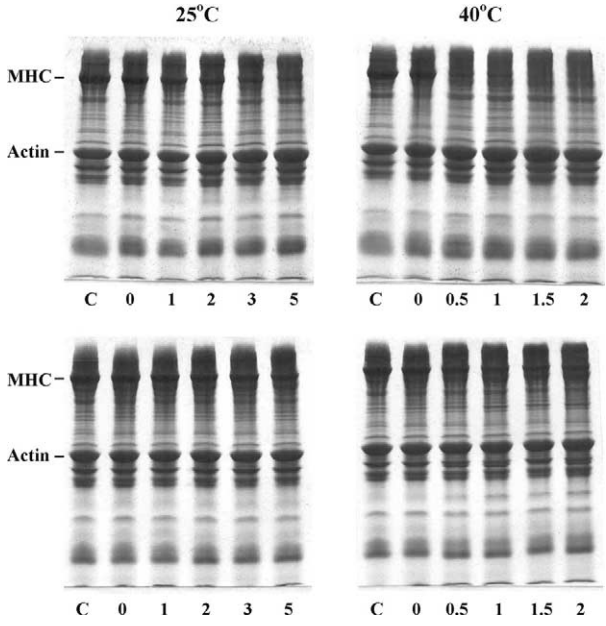

Fig. 4. SDS–PAGE pattern of komaboko gels of surimi from *P. tayenus* and *P. macracanthus* prepared by setting at 25 °C (a) and 40 °C (b) for different times. Numbers designate setting time (h).

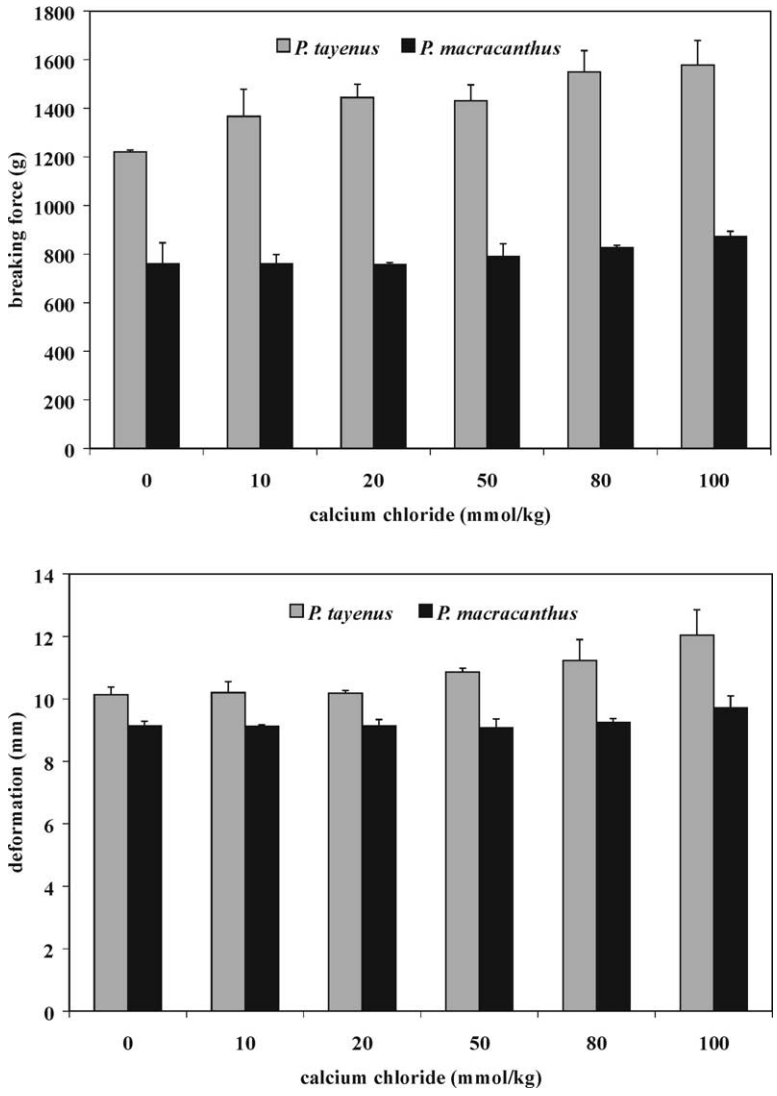

Fig. 5. Breaking force and deformation of kamaboko gels of surimi from *P. tayenus* and *P. macracanthus* as affected by different calcium chloride concentrations. Error bars indicate the standard deviations from the mean of five determinations.

For suwari and kamaboko gels subjected to setting at 40 °C, the solubility is shown in Table 2. Suwari gels from *P. tayenus* surimi set for up to 1 h rendered the lowest solubility and no changes in solubility were found with the increasing time. For suwari gel from *P. macracanthus* surimi, the setting time of 1.5 rendered the lowest solubility. Generally, lower solubility was observed in suwari gels from *P. tayenus*. When considering the solubility of kamaboko gels, continuous decrease in solubility was found in *P. tayenus* gels as the setting time increased ($P<0.05$), while no changes in solubility was found in kamaboko gels from *P. macracanthus* with setting time longer than 0.5 h ($P>0.05$). From the result, a lower solubility was obtained in both suwari and kamaboko gels from *P. tayenus*, compared to those from *P. macracanthus*. This further confirms that *P. tayenus* had superior setting phenomenon to *P. macracanthus*. This was presumed to be due to the higher TGase activity. Moreover, the myosin heavy chain of *P. tayenus* was presumably more preferable for cross-linking induced by TGase. Numakura et al. (1985) found that solublity of surimi gel in SDS-urea-β-ME medium decreased with the prolonged setting time at 20 or 30 °C.

3.3. Effect of setting on protein pattern of suwari and kamaboko gel

Myosin heavy chain (MHC) in both suwari and kamaboko gels from both species gradually decreased as the setting time increased (Figs. 3 and 4, respectively). Generally, slightly higher decrease in MHC was found in the gels from *P. tayenus*, compared to those from *P. macracanthus*. This indicated that *P. tayenus* MHC underwent cross-linking to a higher extent, compared with that from *P. macracanthus*. Carp endogenous

TGase preferentially polymerized MHC rather than other myofibrillar proteins (Nakahara, Nozawa, & Seki, 1999). Seki, Nakahara, Takeda, Maruyama, and Nozawa (1998) found that rod portion of MHC was polymerized preferentially than subfragment-1. Nevertheless, no marked changes in actin were found in both species. The result was in accordance with Nakahara et al. (1999) who reported that endogenous TGase from carp could not cross-link actin molecules due to the lack of reactive lysyl residue as the acyl acceptor. Decrease in MHC as appeared on SDS–PAGE under reducing condition was coincidental with the decreased solubility in the mixture of denaturants, including SDS, urea and β-ME (Tables 1 and 2). Such a decrease was due to the non-disulfide covalent cross-linking presumably induced by endogenous TGase during setting. Polymerization proceeded effectively as setting time increased, leading to the decrease in MHC and solubility.

Overall, the increase in gel strength of suwari or kamaboko gels was evident as the setting time at either 25 or 40 °C increased. Increased gel strength was associated with the decrease in MHC with a concomitant increase in non-disulfide covalent cross-linking. From the result, optimum condition for setting of surimi sol was found to be 40 °C for 2 h for *P. tayenus* and 25 °C for 3 h for *P. macracanthus*. Those conditions were used for further study.

3.4. Effect of calcium chloride on gel properties and protein cross-linking

With addition of calcium chloride at various concentrations ranging from 10 to 100 mmol/kg, breaking force and deformation of kamaboko increased as calcium chloride concentration increased ($P < 0.05$; Fig. 5). Since kamaboko gel, a completely heated gel, is a final gelling product, the strengthening effect of calcium chloride on gel quality was investigated in only kamaboko gel to maximize final gel quality. Breaking force of kamaboko gel from *P. tayenus* and *P. macracanthus* added with 100 mmol calcium chloride/kg increased by 29.3 and 14.9%, while deformation increased by 18.8 and 6.5%, respectively, compared with those of directly heated gel (Fig. 2). From the results, calcium chloride showed an enhancing effect on gel strength. Calcium ion showed more influence in gel improvement of surimi from *P. tayenus* than that from *P. macracanthus*. This was possibly due to the differences in reactivity toward calcium ion, different amount of TGase between two species as well as the differences in calcium ion concentration. Fish TGase show differences in their sensitivity to calcium ion (Ashie & Lanier, 2000). Walleye pollack TGase required 3 mM calcium ion, whereas carp muscle TGase required 5 mM calcium ions for full activation (Kishi, Nozawa, & Seki, 1991; Yasueda, Kumazawa, & Motoki, 1994).

The continuous decrease in solubility was observed in kamaboko gels from both species as the calcium concentration increased (data not shown). This indicated that calcium ion at a sufficient concentration played an important role in full activation of TGase activity, leading to the more cross-linking of MHC via non-disulfide covalent bond. The decrease in MHC was found as the calcium chloride concentration increased as shown on SDS–PAGE (Fig. 6). This result indicated that MHC underwent more polymerization with the addition of calcium chloride. The decreased MHC was coincidental with the lower solubility (data not shown). However, no changes in actin were noted even with the higher concentration of calcium chloride. This result reconfirmed that actin was not a good substrate for endogenous TGase.

3.5. Effect of TGase inhibitors on kamaboko gel properties

Breaking force and deformation of kamaboko gels from both species markedly decreased with the increasing concentration of EDTA, ammonium chloride and NEM (Fig. 7). At a level of 5 mmol EDTA/kg, the breaking force of kamaboko from *P. tayenus* and *P. macracanthus* decreased by 51.2 and 38.2% and deformation decreased by 17.8 and 33.29%, respectively (Fig. 7a). In the presence of EDTA, a chelator, less calcium ion was available for TGase activation. As a consequence, a lower TGase activity was assumed, leading to a lower cross-linking of protein, especially MHC. Gel formation of Alaska pollack suirmi was totally inhibited in the presence of 5 mmol EDTA/kg (Kumazawa et al., 1995). This result indicated the importance of calcium ion on TGase activity. The gel strength of walleye pollack surimi did not increase without calcium ion (Wan, Kimura, Satake, & Seki, 1994). The decrease in breaking force and deformation of kamaboko gels that contained EDTA was associated with the higher solubility (data not shown). The markedly increased solubility was obtained as EDTA concentration increased. This result indicated the inhibitory effect of EDTA on cross-link formation in kamaboko gels.

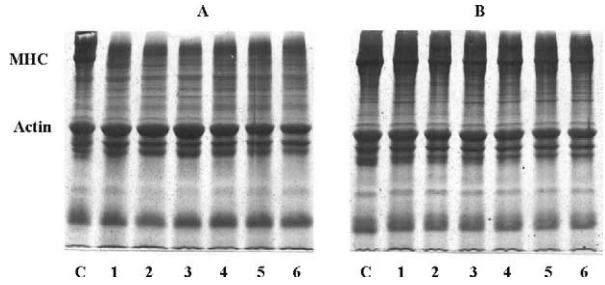

Fig. 6. SDS–PAGE pattern of kamaboko gels of surimi from *P. tayenus* (A) and *P. macracanthus* (B) as affected by different calcium chloride concentrations. C: surimi sol; 1, 2, 3, 4, 5 and 6 designate 0, 10, 20, 50, 80 and 100 mmol $CaCl_2$/kg.

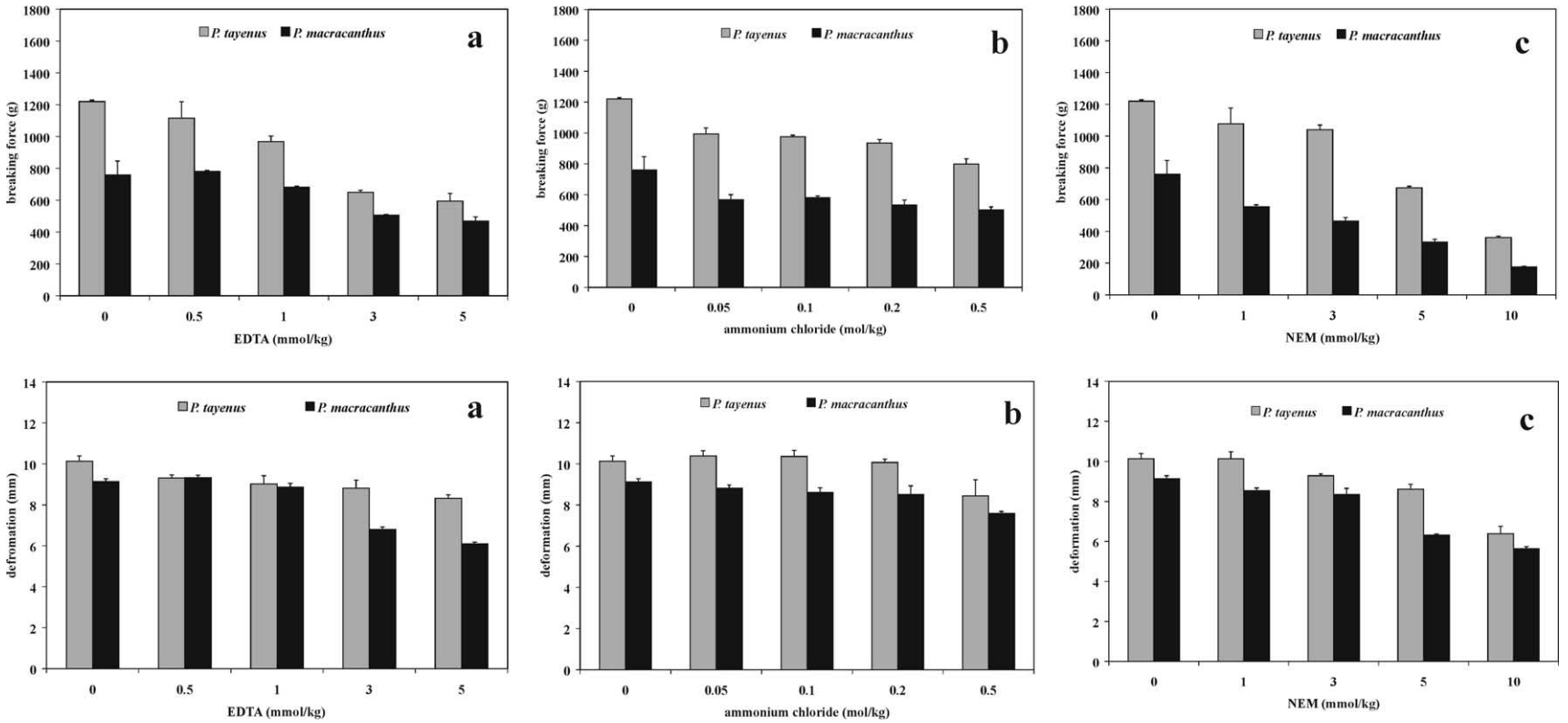

Fig. 7. Breaking force and deformation of kamaboko gels of surimi from *P. tayenus* and *P. macracanthus* as affected by (a) EDTA, (b) ammonium chloride and (c) NEM at different concentrations. Error bars indicate the standard deviations from the mean of five determinations.

When ammonium chloride was added, the decrease in both breaking force and deformation was also observed (Fig. 7b). With the addition of 0.5 mol ammonium chloride/kg, breaking force of kamaboko from *P. tayenus* and *P. macracanthus* decreased by 34.5 and 33.8% and deformation decreased by 16.6 and 16.7%, respectively. Ammonium chloride was used as TGase inhibitor, which acted as gel suppressor (Kumazawa et al., 1995). During acyl transfer reaction between γ-carboxyamide groups of glutamine residues and primary amines, ammonia is generated and an excess amount of ammonium ion should prevent further progress of the reaction (Ashie & Lanier, 2000; Takagi et al., 1986). The decrease in breaking force and deformation was concomitant with the increased solubility (data not shown).

When NEM was added, kamaboko gels had the decreased breaking force and deforamtion, especially as the concentration increased (Fig. 7c). With the addition of 10 mmol NEM/kg, breaking force of kamaboko gel from *P. tayenus and P. macracanthus* decreased by 70.4 and 76.9% and deformation decreased by 36.9 and 38.3%, respectively. NEM has been known as the sulfhydryl blocking agent. From the result, NEM was presumed to block reactive sulfhydryl group in the active site of TGase. As a result, lower activity was observed. TGase is a sulfhydryl enzyme and is readily inactivated by sulfhydryl reagent, which alkylate free sulfhydryl groups (Ashie & Lanier, 2000). However, a considerable decrease in breaking force in kamaboko was observed with NEM addition, compared to other TGase inhibitors. This is because NEM is capable of blocking sulfhydryl groups in myofibrillar proteins. As a consequence, disulfide bond between myosin molecules was suppressed. Increased solubility was observed with higher concentration of NEM (data not shown). Under conditions that activated TGase, suwari gel from NEM treated actomyosin had a lower breaking force than that from untreated actomyosin (Niwa, Inuzuka, Nowsad, Liu, & Kanoh, 1995). Therefore, apart from non-disulfide covalent bond, disulfide bonds directly contribute to the elasticity of the gel.

From SDS–PAGE, it was found that MHC was more retained as the higher concentration of EDTA was added into the gels (Fig. 8). Thus EDTA was shown to impede the non-disulfide covalent cross-linking in surimi from both species. The present results are comparable to those reported by Kumazawa et al. (1995) who showed that no decrease in MHC was found and ε-(γ-glutamyl) lysine formation was completely suppressed by the addition of EDTA. Polymerization of MHC was also suppressed as observed by more retained MHC when ammonium chloride was added, particularly with a higher concentration (Fig. 8). The reduction in non-disulfide cross-linking was presumed to be due to the inactivation of TGase. The ε-(γ-glutamyl) lysine formation was reduced with addition of ammonium chloride in Alaska pollack surimi (Kumazawa et al., 1995). MHC was more retained as the higher concentration of NEM was added (Fig. 8). This result further confirmed

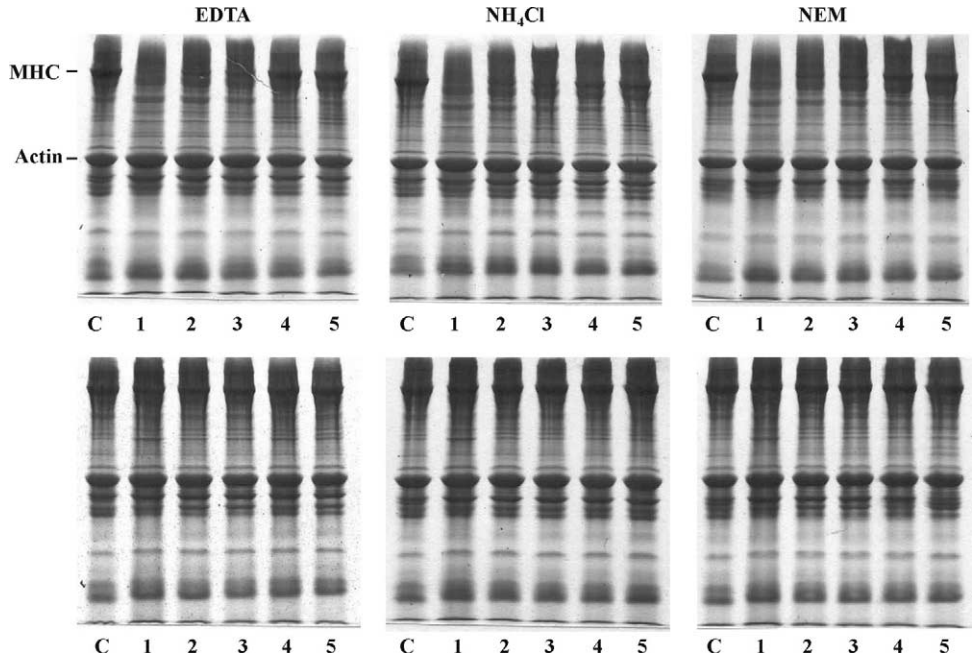

Fig. 8. SDS-PAGE pattern of kamaboko gel of surimi from *P. tayenus* (upper) and *P. macracanthus* (lower) added with EDTA, ammonium chloride and NEM at different concentrations. C: surimi sol; 1, 2, 3, 4 and 5 designate 0, 0.5, 1, 3 and 5 mmol/kg for EDTA and NEM and 0, 0.05, 0.1, 0.2 and 0.5 mmol/kg for NH_4Cl.

that NEM possibly reduced TGase activity and the formation of disulfide bond, leading to the lower polymerization.

From the results, we inferred that endogenous TGase was involved in gel setting, especially via the induction of non-disulfide covalent cross-linking formation. This phenomenon directly contributed to gel strengthening. Therefore, gel enhancement can be carried out by optimizing the TGase activity as well as maximizing protein reactivity toward TGase.

3.7. pH and temperature profiles of endogenous TGase in bigeye muscle

TGases from *P. tayenus* and *P. macracanthus* muscle had optimum pHs of 6 and 6.5, respectively (Fig. 9). TGase activity decreased substantially at acidic or alkaline pHs. This was probably due to the denaturation of enzymes under the acidic and alkaline conditions. Optimum pHs of TGase was found in pH ranges commonly found in surimi. Thus, TGase could exhibit maximum activity in surimi, particularly during setting. For temperature profile, it was noted that TGase from both species had the different optimum temperatures. TGase from *P. tayenus* muscle showed the highest activity at 40 °C, whereas that from *P. macracanthus* muscle had the optimum temperature at 25 °C. The results indicated marked differences in enzymatic properties of TGases between the two species of bigeye snapper. Red sea bream liver TGase had an optimum temperature of 55 °C (Yasueda et al., 1994), while walleye pollack liver TGase showed the maximum activity at 50 °C (Kumazawa, Nakanishi, Yasueda, & Motoki, 1996). Corresponding to the optimal setting temperatures previously observed for*P. tayenus* and *P. macracanthus* surimi, setting is presumably mediated by endogenous muscle TGases found in two species of bigeye snapper. To maximize TGase activity in surimi from both species, setting should be conducted at an appropriate temperature.

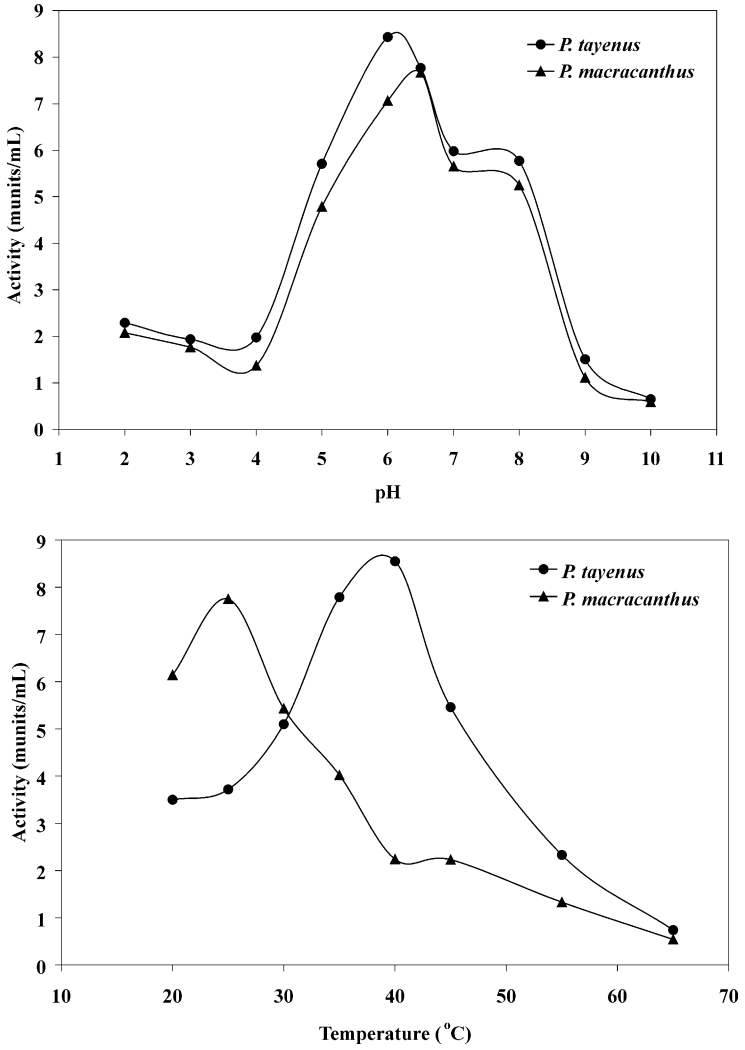

Fig. 9. pH and temperature profile of crude TGase from *P. tayenus* and *P. macracanthus* muscle.

4. Conclusion

Setting mediated by endogenous TGase at the appropriate temperature and time contributed to an enhanced gel quality of surimi from both species of bigeye snapper. Highest setting response in *P. tayenus* and *P. macracanthus* surimi could be maximized at 40 and 25 °C, respectively, corresponding to the optimum temperature of TGase activity from each species.

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