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Effect of medium temperature setting on gelling characteristics of surimi from some tropical fish

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Abstract

Effects of setting at 25 °C on textural properties and cross-linking of myofibrillar proteins in surimi produced from threadfin bream (*Nemipterus bleekeri*), bigeye snapper (*Priacanthus tayenus*), barracuda (*Sphyraena jello*) and bigeye croaker (*Pennahai macrophthalmus*) were investigated. Increase in setting time (0–8 h) resulted in a higher breaking force and deformation for all surimi gels tested ($P<0.05$). Increased gel strength was associated with increase in non-disulfide bond formation and decreased heavy chain myosin. Proteins underwent degradation during setting; however polymerization occurred to a much higher extent, leading to a strengthened gel matrix. Therefore, setting at 25 °C, for an appropriate time, should be a promising means to improve gelling properties of surimi produced from tropical fish.

Keywords: Setting; Suwari; Surimi; Gelation; Cross-linking

1. Introduction

Surimi, washed minced fish muscle, consisting of salt-soluble myofibrillar proteins, has unique gelling properties that make it useful as a food base in seafood analogues. Gel strength of surimi can be increased by subjecting surimi sol to setting below 40 °C prior to cooking (An, Peters, & Seymour, 1996; Kimura, Sugimoto, Toyoda, Seki, Arai, & Fujita, 1991). Setting or suwari has been widely applied in surimi manufacture. Gelation of fish paste during setting has been reported to have a close relationship to the formation of cross-linking between myosin heavy chain induced by endogenous transglutaminase (TGase) (Kumazawa, Numazawa, Seguro &, Motoki, 1995; Seki et al., 1990) as well as to the thermal formation of non-covalent bonds and disulfide bonds (Hossain, Ito, Kanoh, & Niwa, 1998).

Setting response can be varied, depending on fish species (Shimizu, Machida, & Takanemi, 1981). The setting phenomenon is related to habitat temperature of fish species (Morales, Ramirez, Vivanco, & Vazquez. 2001). The optimum temperature for setting among species may be determined by the heat stability of myosin. Additionally, TGase has been reported to contribute to the polymerization of myosin (Araki & Seki, 1993; Seki et al., 1990). However, the rate of TGase-mediated cross-linking of myosin heavy chain may be primarily dependent on the conformation of substrate myosin at a given temperature rather than on the optimum temperature of TGase (Araki & Seki, 1993; Kamath, Lanier, Foegeding, & Hamann, 1992). TGase-mediated cross-linking reaction of myosin heavy chain varies, depending upon species (Araki & Seki, 1993).

Thailand is one of the largest surimi producers in southeast Asia. At present, twelve surimi factories are located in Thailand, with a total production of about 60,000 metric tons per year (Morrissey & Tan, 2000). Most of the fish used for surimi production includes threadfin bream (*Nemipterus* spp.), bigeye snapper (*Priacanthus* spp.), croaker (*Pennahia* and *Johnius* spp.) and lizardfish (*Saurida* spp.). Though setting has been used in surimi industries in Thailand for many decades, no information about the effect of setting on gelling properties of surimi produced from fish commonly used in Thailand has been reported. Generally, setting can be performed at low (0–4 °C), medium (25 °C) and high

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(40 °C) temperatures (Lanier, 1992). Setting at different temperatures may lead to different gel characteristics, especially with different fish species. Since setting at low temperature takes a longer time, it is not commonly implemented in the industries. So far, high-temperature settings are more widely used in Thailand, due to the shorter time required, but protein degradation, induced by modori-inducing proteinase, generally active at 50–60 °C can occur (Jiang, 2000). Therefore, medium temperature setting can be an alternative for the manufacturer to obtain a better gel quality without severe proteolytic degradation. The objective of this investigation was to study the effect of medium-temperature setting on gel properties and cross-linking of myofibrillar proteins in surimi produced from four tropical fish species, commonly used in Thailand.

2. Materials and methods

2.1. Surimi and surimi gel preparation

Frozen surimi (grade A), produced from threadfin bream (*Nemipterus bleekeri*), bigeye snapper (*Priacanthus tayenus*), baracuda (*Sphyraena jello*) and bigeye croaker (*Pennahai macrophthalmus*), was purchased from Man A Frozen Foods Co., Ltd., Muang, Songkhla. Surimi was kept at −20 °C until used.

To prepare the gel, frozen surimi was tempered for 30 min in running water (25 °C). The surimi was then cut into small pieces with an approximate thickness of 1 cm. The surimi was placed in a mixer (National Model MK-K77, Tokyo, Japan). The moisture was adjusted to 80% and 2.5% salt was added. To obtain a homogeneous paste, the mixture was chopped for 5 min at 4 °C. The temperature of surimi sol was kept below 10 °C. The surimi sol was stuffed into polyvinylidine casings (2.5 cm diameter) and both ends were sealed tightly. Suwari gels were prepared by setting sol at 25 °C for different times (1, 2, 3, 5 and 8 h) in a temperature controlled water bath (Memmert, Germany). Suwari gels prepared by setting for 0.5 h were too soft and textural properties could not be measured. After setting for designated times, the gels were immediately cooled using iced water. For kamaboko gel, suwari gels obtained were then subjected to heating at 90 °C for 20 min in a temperature-controlled water bath. The gels were then cooled in iced water and stored at 4 °C for 24 h before analysis.

2.2. Texture analysis

Texture analysis of suwari and kamaboko gels was performed using a texture analyzer Model TA-XT2 (Stable Micro Systems, Surrey, England). Gels were equilibrated and tested at room temperature. Five cylinder-shaped samples of 2.5 cm in length were prepared. The breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyzer equipped with a cylindrical plunger (5 mm diameter; 60 mm/min deformation rate).

2.3. Solubility studies

Solubility of suwari and kamaboko gels in 20 mM Tris-HCl, pH 8.0, containing 1% (w/v) SDS, 8M urea and 2% (v/v) β-ME, were determined as described by Chawla, Venugopol, and Nair (1996). The sample (1 g) was homogenised in 20 ml of solution for 1 min using a homogeniser (IKA Labortechnik, Malaysia). The homogenate was heated in boiling water (100 °C) for 2 min and stirred at room temperature for 4 h. The resulting homogenate was centrifuged at 10,000×*g* for 30 min using a 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×g for 30 min, The precipitate was washed with 10% TCA and solubilised in 0.5 M NaOH. To obtain the total amount of protein, gels were directly solubilised in 0.5 M NaOH. The protein content was measured using the Biuret test (Robinson & Hodgen, 1940). The solubility was expressed as percent of the total protein.

2.4. Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method described by Morrissey, Wu, Lin, and An (1993). Suwari gel (3 g) was homogenised with 27 ml of 5% TCA (w/v). The homogenate was kept in ice for 1 h and centrifuged at 5000×*g* for 5 min. Soluble peptides in the supernatant were measured and expressed as μmol tyrosine/10 g muscle.

2.5. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein pattern of suwari gels was analysed on SDS-PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85 °C were added to the sample (3 g). The mixture was then homogenised using a homogeniser (IKA Labortechnik, Malaysia) for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500×*g* for 20 min to remove undissolved debris. Protein concentration was determined according to the method of Lowry, Rosebrough, Fan, and Randall (1951), using bovine serum albumin as standard. SDS-PAGE gel was 10% running gel and 4% stacking gel. After separation, the proteins were fixed and stained with Coomassie Blue R-250.

2.6. Protein determination

Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.7. 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. Gel properties of surimi gels as affected by medium temperature setting

Setting surimi at the medium temperature (25 °C) resulted in increases of both breaking force and deformation of suwari gels of all fish species tested (Figs. 1–4). Without setting, suwari gels had no measurable force and deformation. However, both force and deformation significantly increased with the increasing setting time ($P<0.05$). In general, bigeye snapper suwari gel had the highest force, followed by barracuda, bigeye croaker, and threadfin bream, respectively. Observed setting responses of both force and deformation of suwari gels were different among all fish species. With increasing setting time, bigeye snapper surimi showed a more rapid response to setting, than other fish species. Setting at 25 °C for 2 h resulted in about a 2 times increase in breaking force of bigeye snapper suwari gel, while only 1–1.5 times increase was achieved for those of other fish species. For all species, setting for 5–8 h rendered suwari gels with higher breaking forces (2.5–3.5 times) and deformations (1.3–1.5 times), than surimi gels set at 25 °C for 1h.

For kamaboko gels, which were prepared by setting under the same conditions and subsequently heated at 90 °C for 20 min, both force and deformation increased as the setting time increased ($P<0.05$). Depending on species, setting at 25 °C for 8 h led to an increase in breaking force, about 1.9–2.4 times more than gels without prior setting. Kamaboko gels from bigeye croaker and bigeye snapper generally had higher breaking forces than those from threadfin bream and barracuda. Numakura et al. (1985) found that gel strengths of suwari and kamaboko gels from Alaska pollack surimi increased as the setting times at 20 and 30 °C increased. Setting has been applied in the surimi industry for a long time to increase the gel strength of surimi. The improved gel property via setting was found to result from polymerization of heavy chain myosin induced by

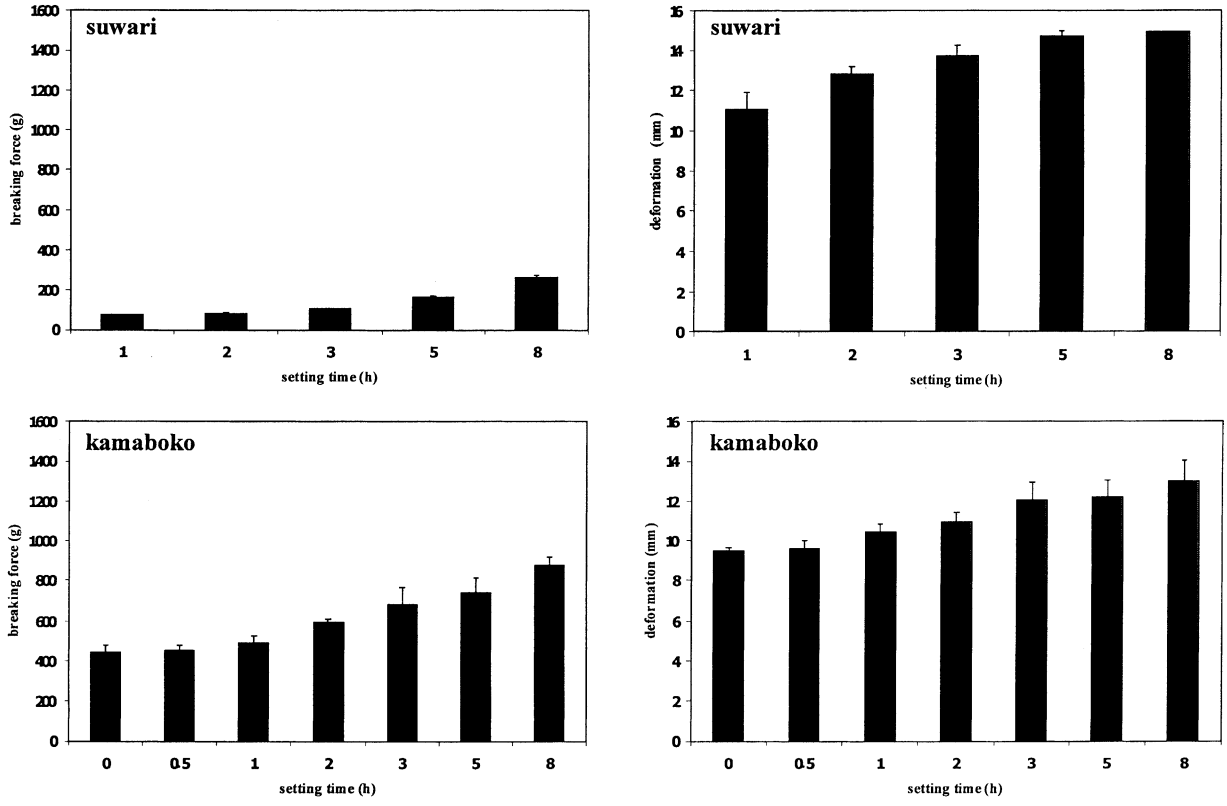

Fig. 1. Force and deformation of suwari and kamaboko gels from threadfin bream surimi prepared by setting at 25 °C for different times. Bars indicate the standard deviations from five determinations.

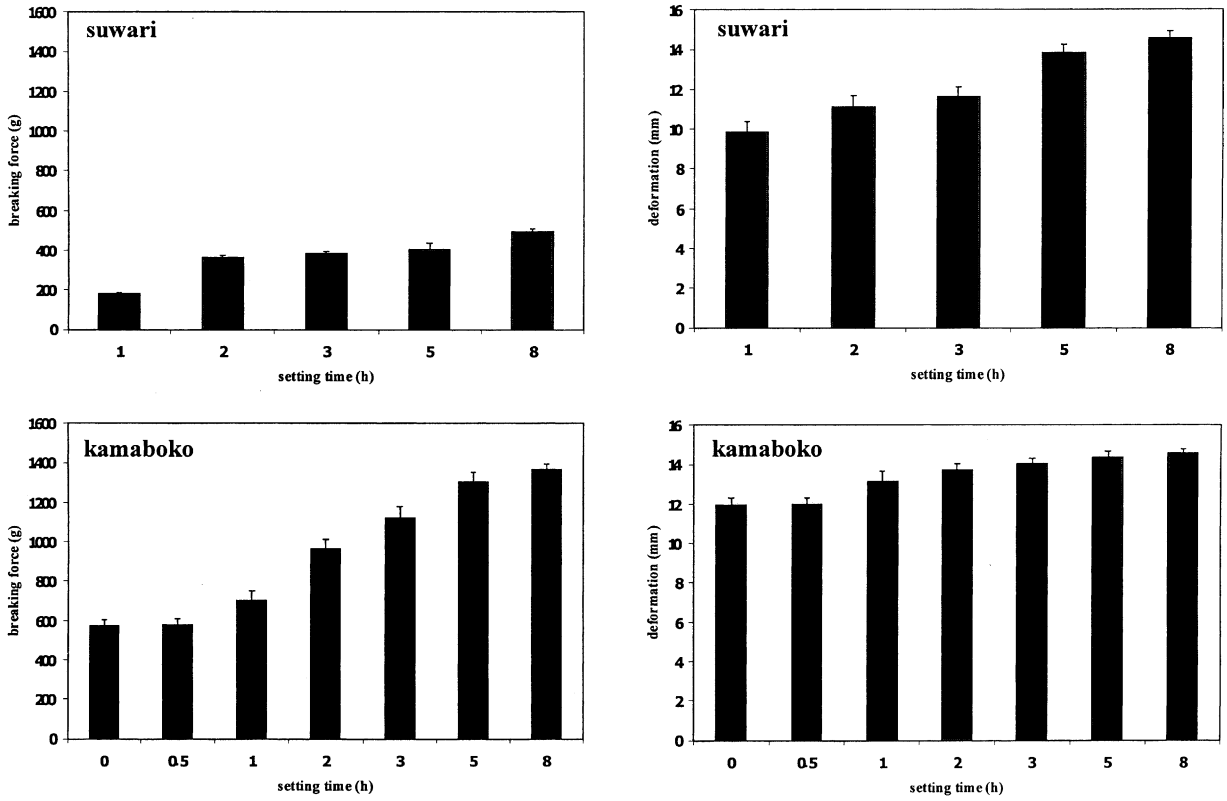

Fig. 2. Force and deformation of suwari and kamaboko gels from bigeye snapper surimi prepared by setting at 25 °C for different times. Bars indicate the standard deviations from five determinations.

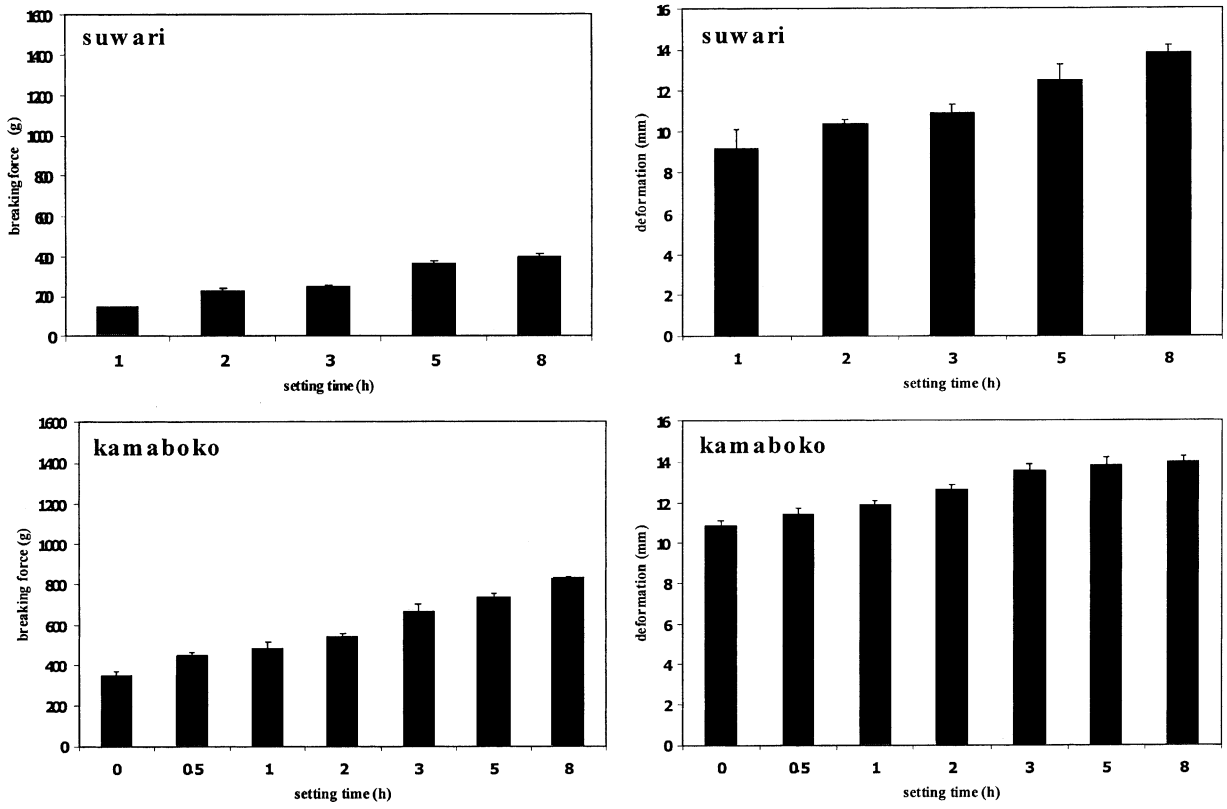

Fig. 3. Force and deformation of suwari and kamaboko gels from barracuda surimi prepared by setting at 25 °C for different times. Bars indicate the standard deviations from five determinations.

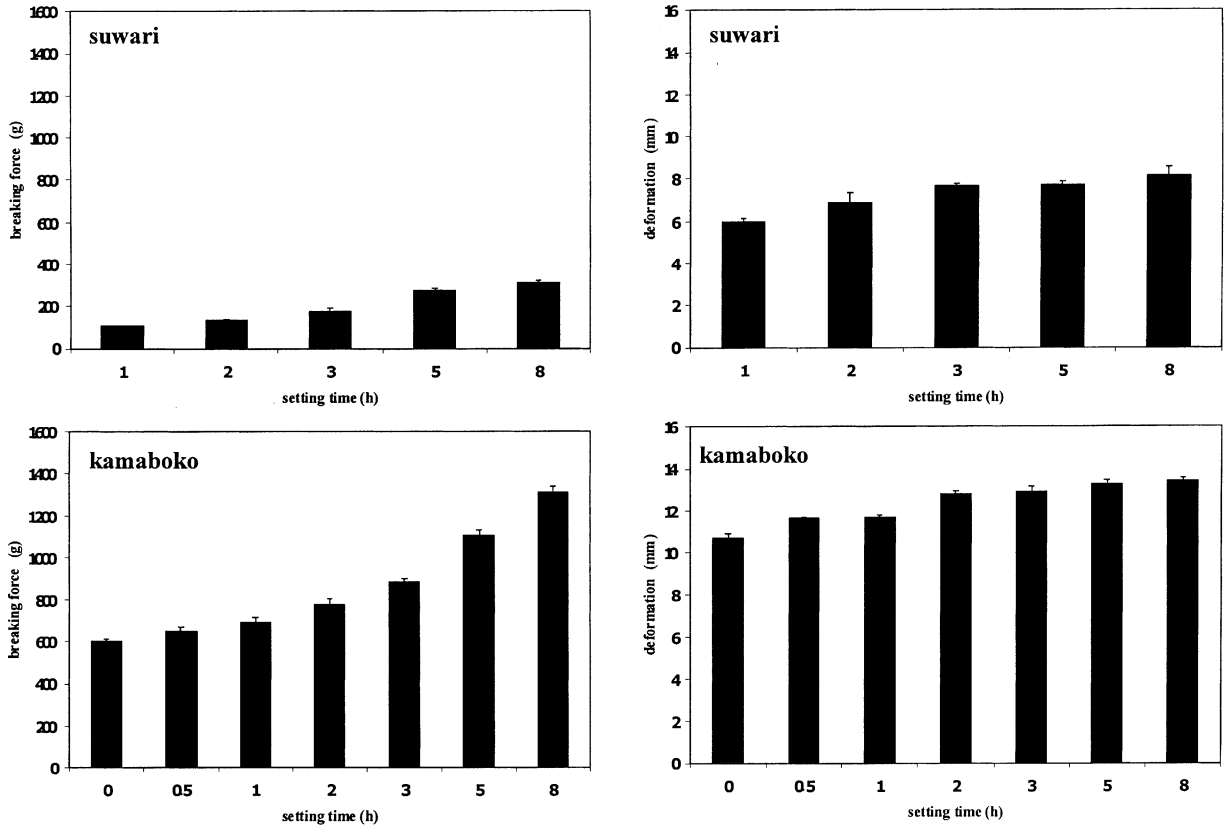

Fig. 4. Force and deformation of suwari and kamaboko gels from bigeye croaker surimi prepared by setting at 25 °C for different times. Bars indicate the standard deviations from five determinations.

Table 1
Solubility of suwari and karnaboko gels of surimi from some tropical fish prepared by setting at 25 °C for different times

Gels	Setting time (h)	Solubility (%)[a]			
		Threadfin bream	Bigeye snapper	Barracuda	Bigeye croaker
Suwari	Control	100f[b]	100f	100e	100h
	0	98.28±0.55e	98.48±0.85ef	99.78±0.89e	97.47±0.83e
	0.5	95.06±0.71d	97.53±1.06e	97.86±1.13e	93.95±0.59d
	1	93.05±0.47c	94.61±0.85d	94.57±1.35d	92.63±1.26d
	2	92.22±0.48bc	90.69±0.84c	87.98±0.67c	90.39±1.94c
	3	91.95±0.47bc	89.22±0.84c	86.44±0.67c	88.08±1.36c
	5	91.11±0.49b	85.78±0.85b	81.78±1.78b	84.72±0.78b
	8	85.55±2.30a	78.43±1.70a	74.33±2.19a	82.10±0.67a
Kamaboko	Control	100f	100f	100e	100h
	0	90.83±0.84e	89.71±1.47e	87.21±1.16e	89.80±0.68g
	0.5	89.72±0.48e	86.27±1.70d	86.28±0.67e	81.57±0.68f
	1	87.22±1.27d	83.33±1.70c	85.66±1.78e	74.51±0.67e
	2	85.55±0.48c	78.43±1.69b	81.78±1.78d	72.55±0.68d
	3	84.45±0.48c	77.45±1.69b	75.97±1.34c	71.37±0.67c
	5	80.28±0.96b	72.55±1.70a	73.74±1.44b	69.02±0.22b
	8	78.33±0.84a	71.57±1.69a	71.43±0.60a	67.15±0.38a

[a] Mean±SD from triplicate determinations.
[b] Different letters in the same column under the same gel denote significant differences ($P<0.05$).

TGase (Kimura et al., 1991). The content of ε-(γ-Glu) Lys in Alaska pollack surimi gel increased as the setting time at 30 °C increased, indicating the participation of endogenous TGase in the setting process (Kumazawa et al., 1995).

From the result, much higher forces and deformations were obtained with kamaboko gels, than with suwari gels. This was possibly because the heating process induced protein aggregation stabilised by various bonds, including hydrophobic interaction and, disulfide

bonds, (Samejima, Ishioroshi, & Yasui, 1981; Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001). Benjakul, Visessanguan et al. (200l) 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 for formation of a good elastic gel (Chan, Gill, & Paulson, 1992). Elevated temperature during heating resulted in further oxidation of sulfhydryl groups with a subsequent disulfide bond formation (Benjakul, et al., 2001). Due to instability of hydrogen bonds during the heating process, the alpha helix unfolds, exposing hydrophobic amino acids, leading to hydrophobic interaction (Niwa, 1992). Alvarez and Tejada (1997) reported that sardine kamaboko gels had much higher gel strength than the corresponding suwari gels, indicating that protein–protein bonds were established at cooking temperature, which strengthened the network previously formed by setting.

3.2. Solubility of surimi gels

For all surimi tested, solubility of both suwari and kamaboko gels prepared by setting at 25 °C decreased as setting time increased ($P<0.05$). In general, a setting time of 8 h rendered the lowest solubility for both suwari and kamaboko gels. A lower decrease in solubility was observed in gels from threadfin bream, than in surimi gels from other species (Table 1). This result was in agreement with the lowest gel strength observed in surimi from threadfin bream. Solutions containing SDS, urea and β-mercaptoethanol were used to solubilise protein by destroying all bonds, except non-disulfide covalent bonds, particularly the ε-(γ-glutamyl) lysine linkage (Benjakul, Visessanguan, & Srivilai, 2001). Therefore, the decrease in solubility indicated that non-disulfide bond formation occurred to a greater extent as the setting time increased. It has been known that endogenous TGase plays a crucial role in ε-(γ-glutamyl) lysine linkage formation (Kumazawa et al., 1995). From the result, non-disulfide covalent bond was presumed to be a major contributor to strengthening of the gel matrix, which was associated with the decrease in solubility.

Table 2
TCA-soluble peptides in suwari gels from some tropical fish

Fish species	TCA-soluble peptides (μmol tyrosine/10 g)[a]
Threadfin bream	1.74±0.01a[b]
Bigeye snapper	1.15±0.01b
Barracuda	0.81±0.03c
Bigeye croaker	0.52±0.05d

[a] Means±S.D. from triplicate determinations.
[b] Different letters in the same column under the same gel denote significant differences ($P<0.05$).

At the same setting time, kamaboko gels from all species showed the lower solubilities, than suwari gels. This suggested that non-disulfide covalent bonds in kamaboko gels were subsequently formed after setting. During heating up to 40–50 °C, endogenous TGase still worked as the cross-linker by inducing non-disulfide bond formation. At higher temperatures, proteins likely underwent unfolding, allowing the reactive lysine or glutamine residues exposed for ε-(γ-glutamyl) lysine linkage formation. As a result, a lower solubility was found in kamaboko gels, than in suwari gel. However, the solubility was different among surimi from different species. Solubility of bigeye croaker kamaboko gel, was markedly less than that obtained in suwari gel. This suggested that non-disulfide bonds in this species were preferentially formed during heating, probably due to the higher activity as well as more suitable orientation of protein molecules for cross-linking reactions at higher temperatures. Thus, differences in endogenous TGase activity, protein compositions and conformations, which favour the cross-linking reaction induced by TGase, are postulated among fish species. Such differences possibly resulted in the differences in gel characteristics among surimi from different species.

3.3. Protein pattern in suwari gels

Protein patterns of suwari gels prepared by incubating the sol at 25 °C for 8 h are depicted in Fig. 5. Heavy chain myosin decreased in suwari gels, compared to that observed in surimi sol (without setting). Among all suwari gels tested, heavy myosin chain in suwari from bigeye snapper and barracuda decreased to a greater extent, than that from bigeye croaker and threadfin bream. The decrease in heavy chain myosin was presumed to be due to the cross-linking of protein during setting, leading to the lower band intensity appearing on the SDS-PAGE. From the result, the decrease in myosin heavy chain intensity was coincidental with the increase in breaking force and deformation (Figs. 1–4). The

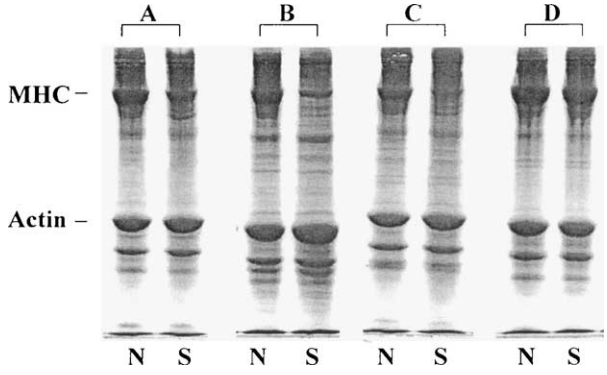

Fig. 5. . SDS-PAGE pattern of suwari gels of surimi from threadfin bream (A), bigeye snapper (B), barracuada (C) and bigeye croaker (D). N: surimi sol (no setting), S: setting for 8 h at 25 °C.

more heavy chain myosin retained, the less were the breaking force and deformation. However, it was found that heavy chain myosin in suwari gels from bigeye croaker and threadfin bream decreased markedly after setting at 40 °C for a proper time (Benjakul, Visessanguan & Chatarasuwan, 2003). This indicated that maximum polymerization occurred at different temperatures, depending on species. Kamath et al. (1992) reported that optimum setting temperatures for Alaska pollock and Atlantic croaker suirmi were 25 and 40 °C, respectively. The cross-linking reaction induced by TGase occurs when protein molecules and the enzyme become associated in a highly oriented and conformation-dependent fashion at some stage of the catalytic process (Gorman & Folk, 1981). Furthermore, optimum temperature for setting among species may be determined by the heat-stability of myosin and the rate of cross-linking may be dependent on the conformation of the substrate myosin at a given temperature rather than on the optimum temperature of TGase (Kamath et al., 1992). Therefore, an appropriate temperature of setting for individual surimi should be maximized to obtain the highest gel quality.

3.4. Protein degradation in suwari gels

During setting at 25 °C for 8 h, TCA soluble peptides were found, indicating that proteolytic degradation occurred during setting (Table 2). From the result, TCA-soluble peptides were different, depending on species. Threadfin bream suwari gel contained the highest amount of TCA-soluble peptides, suggesting the highest proteolytic activity during setting at 25 °C, while bigeye croaker showed the lowest degradation. The differences in degradation among species were postulated to be due to the differences in proteolytic activity, optimum temperature and types of proteolytic enzymes in surimi. Moreover, the differences in susceptibility of myofibrillar proteins to hydrolysis may vary among species tested. Proteolytic activity in fish muscle is high at 50–60 °C and causes rapid and severe degradation of myofibrillar proteins, particularly myosin (An et al., 1996). This phenomenon, named "modori", lowers the gel strength (An et al., 1996; Morrissey et al., 1993). From the result, though the setting was conducted at 25 °C, which is generally lower than the temperature ranges suitable for heat-activated proteinases, degradation products were found. Suwari gel, prepared by setting at 40 °C, showed the most degradation products, as shown by the increased TCA-soluble peptides and formation of degradation protein bands appearing on the SDS-PAGE (Benjakul et al., 2003). The result was in accordance with Takeda and Seki (1996) and Ando, Tsukamasa, and Makinodan (1998) who found that some proteolysis occurred in walleye pollack paste during setting at 25 °C. Since increased breaking force and deformation were obtained with extended setting at 25 °C, it suggested that cross-linking occurs to a greater extent, than proteolysis. As a consequence, negligible proteolysis at 25 °C had no markedly detrimental effect on gel-forming ability of any surimi produced from the four tropical fish species.

4. Conclusion

Setting of surimi from some tropical fish at 25 °C rendered improved gel quality, especially when the setting time was increased. This resulted from polymerization of heavy chain myosin via non-disulfide bond formation, induced by endogenous TGase. Therefore, setting at 25 °C is a promising means to improve gel quality of surimi from tropical fish without severe protein degradation.

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