รายงานวิจัยฉบับสมบูรณ์

โครงการ การปรับปรุงคุณภาพของเจลซูริมิจากปลาตาหวานหนังบาง

(*Priacanthus macracanthus*)

โดย รองศาสตราจารย์ ดร. สุทธวัฒน์ เบญจกุล

กุมภาพันธ์ 2547

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

ขอขอบพระคุณสำนักงานกองทุนสนับสนุนการวิจัย สำหรับการสนับสนุนทุนพัฒนานักวิจัยเรื่องการปรับปรุงคุณภาพของเจลซูริมิจากปลาตาหวานหนังบาง(*Priacanthus macracanthus*) และมหาวิทยาลัยสงขลานครินทร์ในการสนับสนุนเงินวิจัยเพิ่มเติม

รองศาสตราจารย์ ดร.สุทธวัฒน์ เบญจกุล

บทคัดย่อ

รหัสโครงการ: RSA/19/2544

ชื่อโครงการ: การปรับปรุงคุณภาพของเจลซูริมิจากปลาตาหวานหนังบาง (*Priacanthus macracanthus*)

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ระยะเวลาโครงการ: 3 ปี

จากการเปรียบเทียบองค์ประกอบกล้ามเนื้อปลาตาหวานหนังหนา (*Priacanthus tayenus*) และปลาตาหวานหนังบาง (*Priacanthus macracanthus*) พบว่าปลาทั้งสองสายพันธุ์มีองค์ประกอบคล้ายกันและมีความคงตัวระหว่างการเก็บรักษาในน้ำแข็งภายหลังการจับแตกต่างกัน ปลาตาหวานหนังหนามีคุณสมบัติการเกิดเจลที่ดีกว่าปลาตาหวานหนังบาง เนื่องจากสามารถจัดเรียงตัวกันโดยอันตรกิริยาไฮโดรโฟบิกและพันธะไดซัลไฟด์ได้สูงกว่า นอกจากนี้พบว่าเอนไซม์ทรานสกลูตามิเนสซึ่งมีบทบาทต่อการเพิ่มความแข็งแรงของเจลมีแอกติวิตีสูงสุดที่อุณหภูมิ 70 และ 25 องศาเซลเซียส สำหรับปลาตาหวานหนังหนาและปลาตาหวานหนังบางตามลำดับ ซึ่งการเซ็ตตัวที่อุณหภูมิที่เหมาะสมก่อนให้ความร้อนมีผลให้ค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุของเจลซูริมิของปลาทั้งสองสายพันธุ์เพิ่มขึ้น กล้ามเนื้อปลาตาหวานหนังบางมีการย่อยสลายตัวเองสูงกว่าปลาตาหวานหนังหนา กล้ามเนื้อปลาทั้งสองสายพันธุ์มีเอนไซม์โปรตีนเอสในส่วนของของเหลวซาร์โคพลาสมิกแตกต่างกันทั้งในด้านชนิดและระดับของแอกติวิตี โดยปลาตาหวานหลังบางมีแอกติวิตีที่สูงกว่า เมื่อทำบริสุทธิ์เอนไซม์โปรตีเอสจากกล้ามเนื้อปลาตาหวานหนังบางพบว่าเอนไซม์หลักเป็นเอนไซม์ชนิดซีรีนที่มีน้ำหนักโมเลกุล 72 กิโลดัลตัน และสามารถย่อยสลายไมโอซินเส้นหนักแต่ไม่ย่อยสลายแอกติน

การเพิ่มความแข็งแรงของเจลซูริมิจากปลาตาหวานหนังบางสามารถกระทำโดยการเติมเอนไซม์ทรานสกลูตามิเนสจากจุลินทรีย์ร่วมกับแคลเซียมคลอไรด์และโปรตีนเติมแต่ง(ไข่ขาวผงหรือโปรตีนพลาสมาเลือดวัว) และความแข็งแรงของเจลเพิ่มขึ้นเมื่อเพิ่มระยะเวลาการเซ็ตตัว

คำหลัก: ปลาตาหวาน ซูริมิ ความแข็งแรงของเจล การเซ็ตตัว การย่อยสลาย การปรับปรุง

Abstract

Project Code: RSA/19/2544

Project Title: Improvement of Gel Quality of Surimi from Bigeye Snapper (*Priacanthus macracanthus*)

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Project Period: 3 years

Compositions and properties of muscle from two species of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus* were compared. Both species had the similar composition but showed slight difference in some properties, especially stability during iced storage. Superior gel-forming ability was observed for *P. tayenus*, compared to *P. macracanthus* due to the higher aggregation of protein, caused by both hydrophobic interaction and disulfide bond. Additionally, Endogenous transglutaminase (TGase) from both species played a role in gel enhancement of surimi. TGase from *P. tayenus* and *P. macracanthus* exhibited an optimum temperature at 40 and 25°C. Therefore, setting at appropriate temperature prior to heating at 90C, resulting in the increase in both breaking force and deformation of surimi from both species. Furthermore, muscle proteins from *P. macracanthus* was more prone to autolysis than those from *P. tayenus.* Two species contained the different sarcoplasmic proteases in term of compositions and activity level. *P. macracanthus* muscle generally had higher sarcoplasmic proteolytic activities than *P. tayenus* muscle.Heat stable alkaline protease from *P. macracanthus* was purified and characterized to be serine protease with the MW of 72 kDa. It mainly degraded myosin heavy chain, not actin.

The gel strength of surimi from bigeye snapper (*P. macracanthus*) increased with the addition of microbial transglutamianse (MTGase) in combination with calcium chloride and protein additives, either egg white or beef plasma protein. The gel strength slightly increased with increasing setting time.

Key words: bigeye snapper, surimi, gel strength, setting, degradation, improvement

Chapter 1

Comparative study on compositions and gelling properties of muscle protein from two species of bigeye snapper, *Priancathus tayenus* and *Priancanthus macracanthus*

CHARACTERISTICS OF MUSCLE FROM TWO SPECIES OF BIGEYE SNAPPER, *PRIACANTHUS TAYENUS* AND *PRIACANTHUS MACRACANTHUS*

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ABSTRACT

Composition and some properties of muscle from two species of bigeye snapper, P. tayenus *and* P. macracanthus, *were investigated. Both species had a similar composition with the same myofibrillar protein content. However, muscle proteins from* P. tayenus *had higher thermal stability than those from* P. macracanthus, *as indicated by the higher enthalpy for transitions as well as the lower inactivation rate constant (K_D). Upon 15 days of iced storage, natural actomyosin Ca^{2+}-ATPase and Mg^{2+}-Ca^{2+}-ATPase activities decreased, whereas Mg^{2+}-EGTA-ATPase activity increased, suggesting the denaturation of myosin, actomyosin and troponin/tropomyosin complexes, respectively. Increased surface hydrophobicity and decreased sulfhydryl groups indicated the denaturation possibly occurred via hydrophobic interaction and disulfide formation. Heading and eviscerating of fish retarded the denaturation and physicochemical changes of proteins during iced storage. The results indicated that a rapid and proper post harvest handling was of importance to maintain the muscle quality of bigeye snapper.*

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INTRODUCTION

Bigeye snapper has become increasingly interesting as a potential raw material for surimi production in Thailand. It is not commonly consumed due to its appearance and thick skin (Morrissey and Tan 2000). In essence, it possesses good gel quality, comparable to fish ordinarily used for surimi production, e.g. threadfin bream, croaker, lizardfish, etc. As a consequence, most of the catch has been used for surimi production. Bigeye snapper caught in Thailand normally includes two species, *Priacanthus tayenus* and *Priacanthus macracanthus*. The former exhibits the superior gel forming ability to the latter (Benjakul *et al.* 2001a). Therefore, *Priacanthus tayenus* is more preferable among surimi processors, while *Priacanthus macracanthus* is sorted out and used for animal feed or for low-grade surimi production.

Due to overexploitation of fishery resources, the fish fleet must travel for a longer distance. As a consequence, the fish may undergo chemical and microbiological deterioration during post-harvest handling, leading to the lower quality of raw materials for surimi production. Benjakul *et al.* (2001b) reported that gel-forming ability of surimi produced from two species of bigeye snapper, *P. tayenus* and *P. macracanthus* continuously decreased throughout 15 days of iced storage. The rate of deterioration, especially degradation, was also found to be faster in *P. macracanthus* compared to *P. tayenus*.

Integrity of myofibrillar proteins is of prime importance for surimi production (An *et al.* 1996; Benjakul *et al.* 1997). Different fish have different protein composition (Hashimoto *et al.* 1979; Kano *et al.* 1986). Furthermore, endogenous proteinase and transglutaminase can influence the gelling properties of surimi by hydrolyzing or cross-linking myosin, respectively (An *et al.* 1996; Benjakul *et al.* 2001a). Properties and stability of muscle proteins, which vary per species, have been reported to be a function of pH (Kamal *et al.* 1990; Tsai *et al.* 1989), salt concentration (Nagai *et al.* 1999), temperature (Tsai *et al.* 1989; Jiang *et al.* 1989), actin/myosin ratio (Jiang *et al.* 1989) and storage time (Nagai *et al.* 1999; Benjakul *et al.* 1997). Differences in properties and composition of fish muscle may contribute to the differences in gel forming ability. The objective of this investigation was to determine the composition and properties as well as the physicochemical changes of muscle protein from two species of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*, during iced storage.

MATERIALS AND METHODS

Chemicals

Adenosine 5'-triphosphate (disodium salt), 1-anilinonapththalene-8-sulfonic

acid (ANS), 5,5'-dithio-bis(2-nitrobenzoic acid), ethylene glycol-bis (β-aminoethyl) N,N.N',N'-tetraacetic acid (EGTA) were purchased from Sigma Chemical Co. (St.Louis, MO). Trichloroacetic acid was obtained from Riedel-deHaen (Seelze, Germany).

Fish Samples

Bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*, caught off Songkhla-Pattani Coast along the Gulf of Thailand, were stored in ice and off-loaded within 10-12 h of capture. Fish were placed in ice and transported to Department of Food Technology, Prince of Songkla University, Hat Yai. The ordinary muscles were excised from the dorsal part for natural actomyosin preparation and composition/property analyses.

Fish were separated into two groups, whole fish and headed/eviscerated fish. The fish were placed uniformly between the layers of ice in a styrofoam box. The box containing fish and ice was kept at room temperature (28-30C). The fish/ice ratio was maintained at 1:2 (w/w) throughout the study by removing the melted ice and replacing the same amount of ice every day. Fish were randomly taken every 3 days for analyses.

Compositional Analyses

Protein, ash, fat and moisture contents of whole muscle were determined according to the methods of AOAC (1999). To determine nonprotein and protein compositions, the ordinary muscle were separated into several fractions using different extracting buffers according to the method of Hashimoto *et al.* (1979). The muscle (20 g) was homogenized in 200 mL of phosphate buffer (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4), pH 7.5 using IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was centrifuged at 5,000 × g for 15 min at 4C using a Sorvall Model RC-B Plus centrifuge (Newtown, CT). The residue was added with 200 mL of the same buffer, homogenized and centrifuged again. These two supernatants were combined and trichloroacetic acid was added to obtain a final concentration of 5%. The resulting precipitate was collected by filtration and referred to as 'sarcoplasmic protein fraction'. The filtrate was used as nonprotein nitrogenous compound fraction. For above residue, 10 volumes of phosphate buffer (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4) containing 0.45 M KCl, pH 7.5 were added, homogenized and centrifuged at 5,000 × g for 15 min at 4C. The process was repeated twice. Both supernatants were combined and used as myofibrillar protein fraction. The pellet obtained was mixed with 5 volumes of 0.1 N NaOH and stirred for 12 h at 4C. The mixture was then centrifuged at 5,000 × g for 15 min at 4C. The supernatant was used as alkali-soluble protein fraction. The final residue was used as stroma protein fraction.

Each fraction was subjected to nitrogen analyses using Kjeldahl method (AOAC 1999).

ATPase Activity Assay

ATPase activity was assayed according to the method of Benjakul *et al.* (1997). Natural actomyosin prepared by the method of Benjakul *et al.* (1997) was diluted to 3-5 mg/mL with 0.6 M KCl, pH 7.0. A 1 mL of natural actomyosin solution was added into 0.6 mL of 0.5 M Tris-maleate buffer, pH 7.0. To the mixture (final volume of 9.5 mL), various chemicals were added for different assays as follows: 10 mM $CaCl_2$ for Ca^{2+}-ATPase, 2 mM $MgCl_2$ for Mg^{2+}-Ca^{2+}-ATPase; 2 mM $MgCl_2$ and 0.5 mM EGTA for Mg^{2+}-EGTA-ATPase. To initiate the reaction, 0.5 mL of 20 mM ATP was added to the assay solution. The reaction was conducted at 25C for 8 min and terminated by adding 5 mL of chilled 15% (w/v) trichloroacetic acid. The reaction mixture was centrifuged at 3,500 *xg* for 5 min at room temperature (28-30C). The inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as μmol inorganic phosphate (Pi) released per mg protein per minute. A blank measurement was performed by adding the chilled trichloroacetic acid prior to the addition of ATP. Ca^{2+} sensitivity was calculated as described by Benjakul *et al.* (1997) as follows:

$$Ca^{2+}\ \text{sensitivity} = 1-\left[\frac{Mg^{2+}\text{-EGTA-ATPase activity}}{Mg^{2+}\text{-}Ca^{2+}\text{-ATPase activity}}\right]\times 100$$

Determination of Hydrophobicity

Protein hydrophobicity was determined according to the method described by Benjakul *et al.* (1997) using 1-anilinonapththalene-8-sulphonic acid (ANS) as a probe. Natural actomyosin solutions with different concentrations were mixed with ANS. The fluorescence intensity of ANS-protein conjugates was measured at an excitation wavelength of 374 nm and an emission wavelength of 485 nm by FP 750 spectrofluorometer (Jasco, Japan). The initial slope of the plot of fluorescence intensity versus actomyosin concentration was referred to as SoANS.

Determination of Sulfhydryl Content

Total sulfhydryl group content was determined using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) according to the method of Ellman (1959) as

described by Benjakul and Bauer (2000). A 1 mL of natural actomyosin solution (0.4%) was mixed with 9 mL of 0.2 M Tris-HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA. To 4 mL of the mixture, 0.4 mL of 0.1% DTNB was added and then incubated at 40C for 25 min. The absorbance at 412 nm was measured using a UV-1601 spectrophotometer (Shimadzu, Japan). A blank was performed by replacing the sample with 0.6 M KCl, pH 7.0. The total sulfhydryl group content was calculated using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was performed using a Model DSC 7 (Perkin Elmer, USA). Temperature calibration was run using the Indium thermogram. The samples (15-20 mg) were accurately weighed into aluminum pans and sealed. The samples were scanned at 10C/min over the range of 30-90C using iced water as the cooling medium. The empty pan was used as the reference. Total denaturation enthalpy (ΔH) was estimated by measuring the area in the DSC thermogram. The maximum transition temperature (T_{max}) was estimated from the thermogram.

Thermal and pH Stability

A 1 mL of natural actomyosin solution (3-5 mg/mL) was incubated at different temperatures (0, 10, 20, 30, 40, 50 and 60C). At definite times (0, 5, 10, 20, 30 and 60 min), a sample solution was immediately cooled in iced water. The sample was then equilibrated at 25C prior to Ca^{2+}-ATPase activity analyses. To study the effect of pH on the stability, natural actomyosin solution (3-5 mg/mL) was mixed thoroughly with McIlvaine buffer (0.2 M Na-phosphate and 0.1 M Na-citrate) at different pHs (4.5, 5.5, 6.5, 7.5, 8.5 and 9.5) at room temperature (28-30C). At definite times (0, 5, 10, 20, 30 and 60 min), a sample was mixed with assay buffer and equilibrated at 25C before assay. The inactivation rate constant (K_D) of actomyosin was calculated according to Arai *et al.* (1973) as follows:

$$K_L = (\ln C_0 - \ln C_t)/t$$

Where C_0 = Ca^{2+}-ATPase activity before treatment, C_t = Ca^{2+}-ATPase activity after treatment for time t, and t = treatment time(s).

Electrophoresis Analyses

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970). Protein fractions were

applied on the gel. Proteins were stained in 0.125% Coomassie brilliant blue R-250 and destained in 25% ethanol and 10% acetic acid.

Protein Determination

Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistical Analyses

The experiments were run in triplicate. The data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Composition of Bigeye Snapper Muscle

Ordinary muscle of two species of bigeye snapper, *P. tayenus* and *P. macracanthus* exhibited a similar proximate composition ($P>0.05$) (Table 1). As a major constituent in fish muscle, water accounted for approximately 79%, followed by protein (18%) and trace amounts of ash and lipids. Depending on the species and the nutritional status of the animal, the muscle of marine fish and invertebrates contain 50-80% water (Sikorski *et al.* 1990). In addition, the composition of muscle can vary depending on the season, size, sex, spawning and feeding condition (Pigott and Tucker 1990). Since fish used in this study were caught and handled under the same time and conditions, the effects of those extrinsic factors could be negligible.

TABLE 1.
PROXIMATE COMPOSITION OF BIGEYE SNAPPER ORDINARY MUSCLES

Composition (%wet wt.)	*P. tayenus*	*P. macracanthus*
Moisture	78.75±0.25*	78.87±0.13
Protein	18.25±0.11	18.19±0.15
Ash	1.30±0.03	1.36±0.04
Fat	1.13±0.02	1.13±0.12

*Average±SD from triplicate determinations

Further fractionation of muscle proteins based on solubility was used to classify muscle proteins into five fractions (Table 2). The content of nonprotein nitrogen in the muscle of both species ranged from 2.89 to 3.14 mg N/g (9.82-10.05% total nitrogen). Nonprotein nitrogen fraction included amino acids, imidazole, dipeptide, nucleotides, trimethylamine oxide, trimethylamine, urea and the products of postmortem changes (Sikorski 1994; Foegeding *et al.* 1996). Generally, nonprotein nitrogenous compounds in white fish muscle make up 9-15% of total nitrogen (Sikorski 1994). Muscle of both species constituted with a similar amount of myofibrillar proteins, accounting for ~44-45% of total muscle protein ($P>0.05$). Electrophoretic studies of myofibrillar protein fractions obtained from both species indicated the presence of protein bands corresponding to myosin heavy chain, actin, troponin, tropomyosin as well as myosin light chains (Fig. 1).

TABLE 2.
PROTEIN AND NONPROTEIN CONSTITUENTS IN BIGEYE SNAPPER MUSCLES

Compositions (mgN/ g Muscle)	*P. tayenus*	*P. macracanthus*
Nonprotein nitrogen	3.14±0.16*	2.89±0.43
Sarcoplasmic protein	10.07±0.24	7.89±0.30
Myofibrillar protein	12.25±0.49	11.89±0.49
Alkali soluble protein	4.42±0.03	5.67±0.05
Stroma protein	1.36±0.03	1.06±0.07

*Average±SD from triplicate determinations

P. tayenus muscle possessed higher contents of sarcoplasmic and stroma proteins but slightly lower content of alkaline soluble fraction than *P. macracanthus* muscle. For sarcoplasmic fraction, proteins with low molecular weight were observed (Fig. 1). Hashimoto *et al.* (1979) found that myoglobin was dominant in dark muscle, while parvalbumin was prevalent in white muscle of sardine and mackerel. Stroma proteins consist not only of collagen and elastin, but also connectin and other proteins (Sikorski and Borderias 1990). Teleost and elasmobranch species generally contain 3 and 10% stroma, respectively. The protein pattern of alkali-soluble fraction was somewhat like that of myofibrillar fraction. The results were in agreement with those of Hashimoto *et al.* (1979) who reported that alkali-soluble fraction of sardine and mackerel muscle exhibited a similar protein pattern to the myofibrillar fraction. However, a

protein band with higher molecular weight than myosin heavy chain was found as a major band.

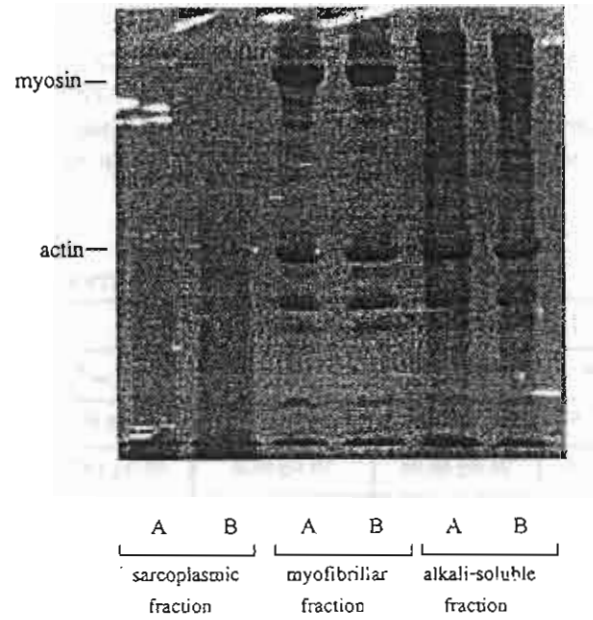

FIG. 1. ELECTROPHORETIC PATTERN OF VARIOUS PROTEIN FRACTIONS FROM BIGEYE SNAPPER MUSCLE
A: *P.tayenus*, B: *P. macracanthus*

Since most of sarcoplasmic proteins and connective tissues are generally removed during surimi processing, myofibrillar proteins in muscle from both species can be increased and thus play an essential role in muscle functional properties, especially gelling property (Foegeding *et al.* 1996). Due to their similar muscle compositions, especially the content of myofibrillar protein fraction, the differences in gelation of muscle protein between two species of bigeye snapper possibly resulted from the differences in the intrinsic properties of proteins, e.g. thermal stability, aggregation rate and the conformational changes, especially during postmortem handling.

Thermal Stability of Muscle Protein

Analysis of DSC thermograms of muscle proteins from both species of bigeye snapper revealed two major endothermic peaks with peak maximum temperatures (T_{max}) at 47 and 70C, respectively (Table 3). The observed T_{max} was within the temperature range observed among various fish species in which the first and second peaks were postulated to be the transitions of myosin and actin, respectively. Cod muscle had two endothermic transitions with T_{max} at 45 and 75C (Hasting *et al.* 1985). Fresh hake muscle also showed two transitions with T_{max} at 46 and 75C (Beas *et al.* 1990). Myosin and actin of Japanese stingfish had T_{max} of 40.9 and 61.1C, respectively (Nagai *et al.* 1999). The different T_{max} of the transitions among fish species seems to be correlated with the habitat temperature of the fish (Akahane *et al.* 1985; Davies *et al.* 1988).

TABLE 3.
T_{max} AND ENTHALPY (ΔH) OF BIGEYE SNAPPER MUSCLES

Samples	Peak 1		Peak 2	
	T_{max} (C)	ΔH (J/g)	T_{max} (C)	ΔH (J/g)
P. tayenus	47.72±0.05*	1.42±0.17	70.64±0.50	0.69±0.11
P. macracanthus	47.61±0.57	0.92±0.07	69.86±0.10	0.144±0.02

*Average±SD from triplicate determinations

Even though muscles of both species of bigeye snapper showed similar T_{max} for both peaks, pronounced differences in enthalpy, energy required for denaturation, were observed. Enthalpies of the first and second peaks of *P. tayenus* muscle were 0.54 and 3.93 folds higher than that of *P. macracanthus* muscle, respectively (Table 3). The results suggested that both myosin and actin of *P. tayenus* muscle were relatively more stable to thermal denaturation, compared to those of *P. macracanthus*. The thermal stability of fish proteins from different species resulted in varied thermal gelation of meat paste. A small quantity of heat was used for the easy-setting meat and the easy-disintegrating meat (Iso *et al.* 1991). Discrepancies in thermal stability may affect how proteins behave or interact with each other during heating, which directly relates to their ability to form a gel under a particular condition.

The inactivation rate constant or K_D value of actomyosin and 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). Compared to heating at tempera-

tures below 20C, a significant increase in K_D value of natural actomyosin from both species of bigeye snapper was observed at 30C (Table 4). K_D values increased substantially at temperature above 40C. The results implied that natural actomyosin from both species were not stable at high temperatures, particularly at temperatures above 40C. K_D values of different fish species determined at definite temperature were varied (Tsai *et al.* 1989), indicating the different stability of muscle protein from different species. At the same temperature, natural actomyosin from *P. macracanthus* had a higher K_D value, compared to that from *P. tayenus*. From the results, it was presumed that muscle proteins of *P. macracanthus* were more susceptible to thermal denaturation than those of *P. tayenus*. The results of K_D were compared to DSC results, in which *P. macracanthus* muscle proteins had a lower enthalpy for transitions. Thus, the stability of muscle proteins from *P. tayenus* was higher than that from *P. macracanthus*. The differences in stability between the 2 species possibly resulted from the different intrinsic properties, amino acid composition as well as actin/myosin ratio. K_D of natural actomyosin was significantly lower than that of myosin. Actin was suggested to play a protective role in the stability of myosin (Jiang *et al.* 1989).

TABLE 4.
INACTIVATION RATE CONSTANT OF NATURAL ACTOMYOSIN Ca^{2+}-ATPase OF BIGEYE SNAPPER AT VARIOUS TEMPERATURES

Temperatures (C)	K_D ($10^{-5}.s^{-1}$)	
	P. tayenus	*P. macracanthus*
0	0.10±0.00*e	0.10±0.00f**
10	0.19±0.01e	0.20±0.00ef
20	0.38±0.17e	0.48±0.00e
30	4.46±0.33d	4.72±0.16d
40	6.53±0.28c	6.51±0.00c
50	28.46±0.20b	30.28±0.12b
60	46.97±1.32a	52.50±0.43a

*Mean±SD from triplicate determinations
**The same letter in the same column indicates nonsignificant differences ($P>0.05$).

pH Stability of Muscle Protein

Natural actomyosin from both species of bigeye snapper showed the highest stability in the pH range of 6.5 to 7.5 at 25C as indicated by the lowest K_D values observed among all pH tested (Table 5). Actomyosins from milkfish, tilapia hybrid, tilapia and carp were stable at pH 6.5-7.9 (Jiang *et al.* 1989). From the results, higher K_D values at both acidic or alkaline pHs possibly indicated the irreversible denaturation of myosin. The denaturation of sardine myofibrils during storage was affected by pHs. Changes in ATPase activity in different pHs might be due to the different degree of modification of actin-myosin interaction by the oxidation of the thiol groups of myosin moiety (Kamal *et al.* 1990).

TABLE 5.
INACTIVATION RATE CONSTANT OF NATURAL ACTOMYOSIN Ca^{2+}-ATPase OF BIGEYE SNAPPER AT VARIOUS pHS

pH	K_D ($10^{-5}.s^{-1}$)	
	P. tayenus	*P. macracanthus*
4.5	27.67±1.03*a	30.11±0.01a**
5.5	18.28±0.27b	16.32±0.48b
6.5	1.12±0.01d	1.15±0.05c
7.5	1.16±0.04d	1.26±0.02c
8.5	15.37±0.11c	15.93±0.12b
9.5	17.63±0.39b	19.08±1.58b

*Mean±SD from triplicate determinations
**The same letter in the same column indicates nonsignificant differences ($P>0.05$).

Changes in ATPase Activities During Iced Storage

A significant decrease in Ca^{2+}-ATPase activity was found in natural actomyosin from *P. macracanthus* stored in ice up to 6 days of storage ($P<0.05$); while no changes in Ca^{2+}-ATPase activity were observed in natural actomyosin from *P. tayenus* ($P>0.05$) (Fig. 2A). For the same species, similar activity was obtained between whole and headed/eviscerated samples. A marked decrease in Ca^{2+}-ATPase activity was noted after 6 days of iced storage. The natural actomyosin from whole samples showed a higher rate of decrease, compared to that from headed/eviscerated samples. A decrease in ATPase

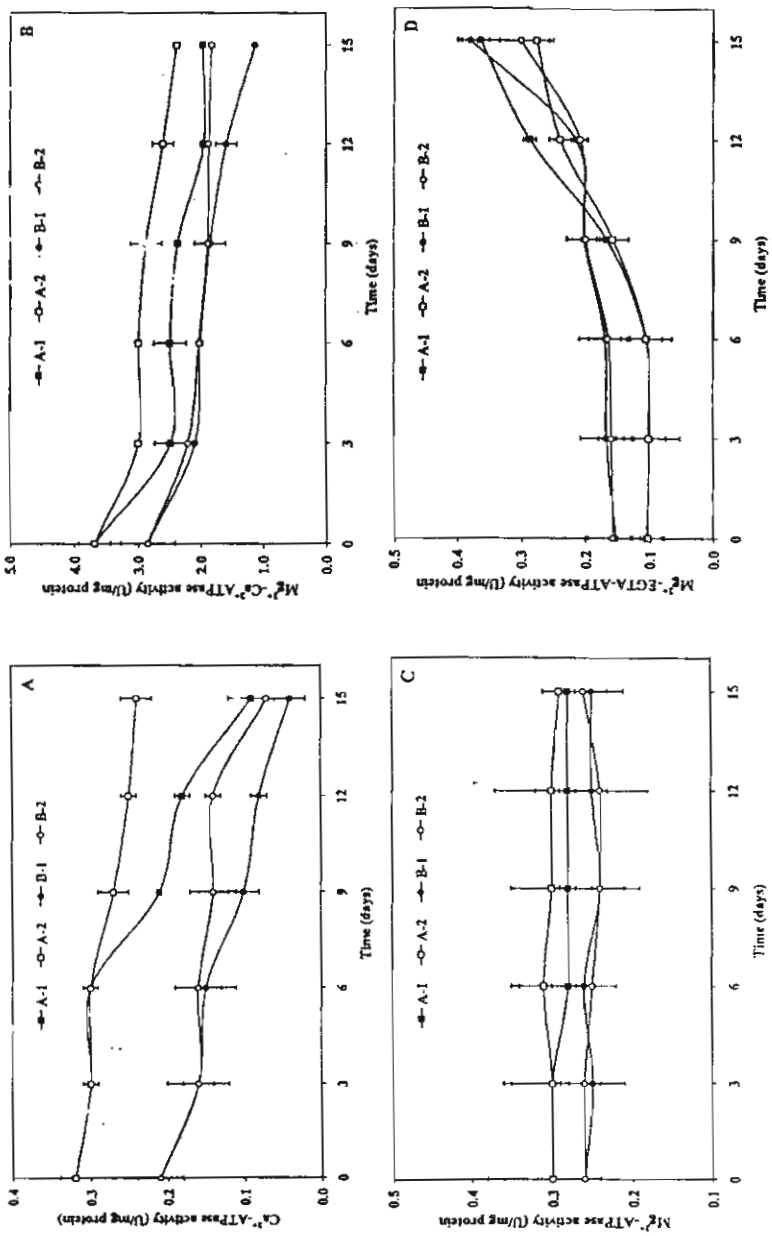

FIG. 2. CHANGES IN Ca^{2+}-ATPase (A), Mg^{2++}-Ca^{2+}-ATPase (B), Mg^{2+}-ATPase (C) and Mg^{2+}-EGTA-ATPase (D) OF BIGEYE SNAPPER NATURAL ACTOMYOSIN DURING ICED STORAGE

A1: *P. tayenus*, whole; A2: *P. tayenus*, headed/eviscerated; B1: *P. macracanthus*, whole; B2: *P. macracanthus*, headed/eviscerated. Bars indicate standard deviation from triplicate determinations.

activity of sardine muscle during 10 days of iced storage was reported by Kamal *et al.* (1991). Ca^{2+}-ATPase activity is used as a good indicator of the integrity of the myosin molecule (Roura and Crupin 1995). Differences in Ca^{2+}-ATPase activity between whole and headed/eviscerated samples were presumed to be due to proteolytic degradation of myofibrillar proteins. From our previous results, it was found that *P. macracanthus* muscle underwent more proteolytic degradation than *P. tayenus* muscle (Benjakul *et al.* 2001a). Seki and Narita (1980) found that Ca^{2+}-ATPase activity in carp myofibrillar proteins decreased by about 50% during 6 days of iced storage with the concomitant degradation of myosin heavy chain due to proteolysis. Deheading and eviscerating could remove the proteinases localized in the viscera as well as the head and prevent myosin from proteolytic degradation.

Changes in Mg^{2+}-Ca^{2+}-ATPase activities during 15 days of iced storage showed a similar pattern to the changes in Ca^{2+}-ATPase previously observed (Fig. 2B). Natural actomyosin from whole *P. tayenus* muscle had lower Mg^{2+}-Ca^{2+}-ATPase activity than headed/eviscerated muscle throughout the storage ($P<0.05$). However, no changes in Mg^{2+}-ATPase activity were observed during iced storage ($P>0.05$) (Fig. 2C). Activities of Mg^{2+}-ATPase and Mg^{2+}-Ca^{2+}-ATPase are the indicators of the integrity of the actin-myosin complexes in the presence of endogenous and exogenous Ca^{2+} ions, respectively (Benjakul *et al.* 1997). No changes in Mg^{2+}-EGTA-ATPase activity of both species were observed during the first 6 days of iced storage ($P>0.05$) (Fig. 2D). A pronounced increase in activity was observed after 6 and 12 days for *P. tayenus* and *P. macracanthus*, respectively. In general, the rate of increase in activity was higher in *P. tayenus*, particularly with the extended storage. Mg^{2+}-EGTA-ATPase indicates the integrity of the troponin-tropomyosin complexes (Ouali and Valin 1981; Benjakul *et al.* 1997). The increase in Mg^{2+}-EGTA-ATPase was concomitant with the loss of Ca^{2+} sensitivity (Fig. 3). Our results confirmed the increase in Mg^{2+}-EGTA-ATPase of carp (Seki and Narita 1980) and Pacific whiting myofibrillar proteins (Benjakul *et al.* 1997) during iced storage. Natural actomyosin from whole samples generally had a higher Mg^{2+}-EGTA-ATPase activity than that from headed/eviscerated samples, especially when the storage time increased, indicating that more changes in troponin-tropomyosin complexes occurred in whole samples, compared to the headed/eviscerated samples.

Ca^{2+} sensitivity of natural actomyosin decreased with an increase in storage time (Fig. 3). Decreasing rate of Ca^{2+} sensitivity in whole samples was higher than that observed in headed/eviscerated samples. Ca^{2+} sensitivity is a good indicator for Ca^{2+} regulation of myofibrillar proteins (Roura and Crupin 1995). It was dependent on the affinity of the troponin molecule for Ca^{2+} ion (Ebashi *et al.* 1968). Benjakul *et al.* (1997) hypothesized that the decreased Ca^{2+} sensitivity of Pacific whiting actomyosin was related to proteinase activity. Removal of troponin resulted in a decrease in Ca^{2+} binding capacity (Ebashi *et*

al. 1968). From the result, the decreased Ca^{2+}-sensitivity was presumed to be due to the denaturation and proteolysis of troponin.

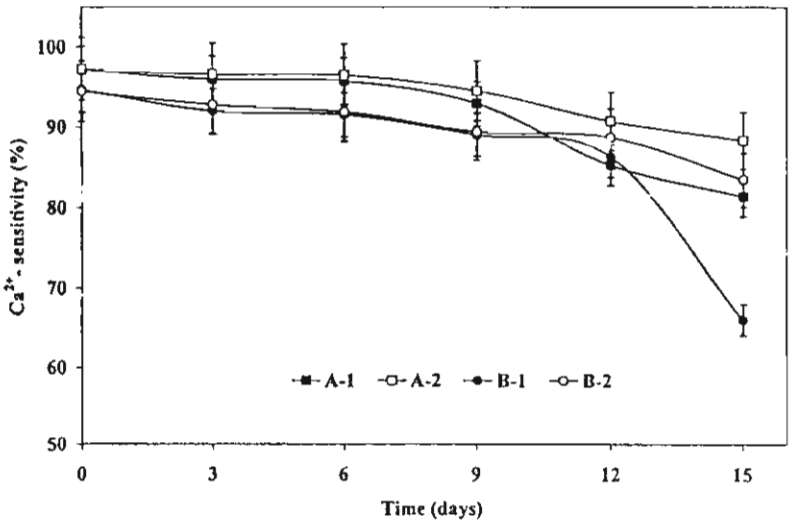

FIG. 3. CHANGES IN Ca^{2+}-SENSITIVITY OF BIGEYE SNAPPER NATURAL ACTOMYOSIN DURING ICED STORAGE

A1: *P. tayenus*, whole; A2: *P. tayenus*, headed/eviscerated; B1: *P. macracanthus*, whole; B2: *P. macracanthus*, headed/eviscerated. Bars indicate standard deviation from triplicate determinations.

Changes in Surface Hydrophobicity of Muscle Protein

Surface hydrophobicity of all samples remained constant in the first 3 days ($P>0.05$) and slightly increased up to 12 days during iced storage (Fig. 4). Surface hydrophobicity increased substantially at days 12-15. Natural actomyosin from *P. macracanthus* showed a higher surface hydrophobicity, compared to that from *P. tayenus* ($P<0.05$). For the same species, natural actomyosin from whole samples exhibited a slightly higher surface hydrophobicity than that from headed/eviscerated samples. The increased surface hydrophobicity indicated the structural and conformational changes of myofibrillar proteins. The results confirmed the increase in surface hydrophobicity of hake and Pacific whiting

actomyosin during iced storage (Roura *et al.* 1992; Benjakul *et al.* 1997). Nevertheless, the sharp increase in surface hydrophobicity obtained in hake and Pacific whiting was found in the first 3 days of iced storage, while no changes were found in this study. The differences in changing pattern between bigeye snapper and those species were possibly caused by the differences in amino acid compositions as well as the different stability of protein during iced storage. From the result, the denaturation or degradation of proteins presumably resulted in an exposure of the interior of molecule, where the hydrophobic portion was located. Exposed hydrophobic amino acids containing an aromatic ring, e.g. phenylalanine and tryptophan, were able to bind to ANS (Kato and Nakai 1980). Hydrophobic interaction between amino acids and oxidation of sulfhydryl groups affected surface hydrophobicity (Hill *et al.* 1982). Therefore, natural actomyosins from both species were susceptible to conformational changes during prolonged iced storage, as indicated by the increased surface hydrophobicity. *P. macracanthus* muscle was more slightly prone to this change than *P. tayenus* muscle.

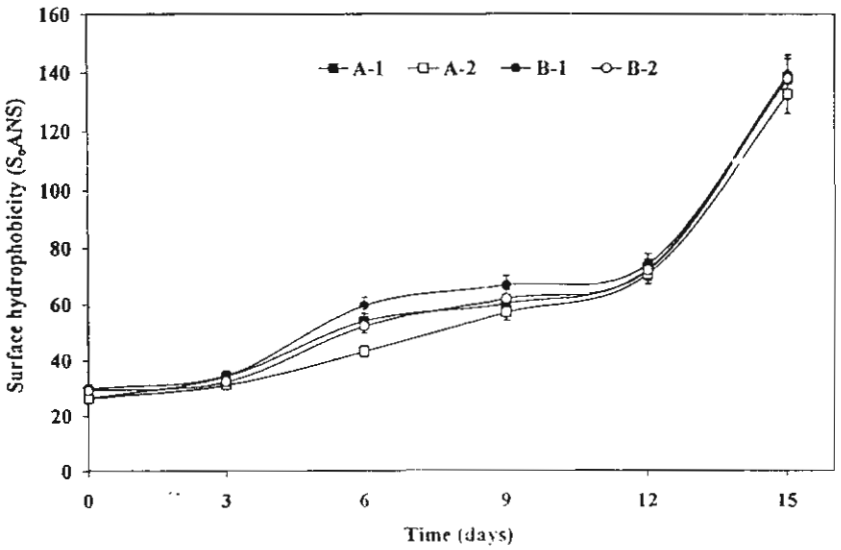

FIG. 4. CHANGES IN SURFACE HYDROPHOBICITY OF BIGEYE SNAPPER NATURAL ACTOMYOSIN DURING ICED STORAGE

A1: *P. tayenus*, whole; A2: *P. tayenus*, headed/eviscerated; B1: *P. macracanthus*, whole; B2: *P. macracanthus*, headed/eviscerated. Bars indicate standard deviation from triplicate determinations.

Changes in Sulfhydryl Content of Muscle Protein

Total sulfhydryl content of all samples decreased gradually throughout 15 days of iced storage (Fig. 5). Higher decrease in total sulfhydryl content was observed in the whole sample, compared to the headed/eviscerated samples, especially in *P. tayenus* natural actomyosin. The decrease in sulfhydryl group generally resulted from the formation of disulfide bonds through oxidation of sulfhydryl groups or disulfide interchanges (Hayakawa and Nakai 1985). Sulfhydryl groups on the myosin head portion, named SH_1 and SH_2, were reported to involve in ATPase activity of myosin (Kielley and Bradley 1956; Seikine *et al.* 1962). SH_3, another sulfhydryl group on the light meromyosin region of myosin molecule, was susceptible to oxidation during iced storage of carp actomyosin (Sompongse *et al.* 1996). The dimer formation via the oxidation of SH_3 resulted in an increase in Mg^{2+}-EGTA-ATPase (Sompongse *et al.* 1996). From our results, the decrease in sulfhydryl groups was coincidental with the increase in Mg^{2+}-EGTA-ATPase (Fig. 2D). Additionally, the decrease in Ca^{2+}-ATPase (Fig. 2A) and Mg^{2+}-Ca^{2+}-ATPase (Fig. 2B) was concomitant with the

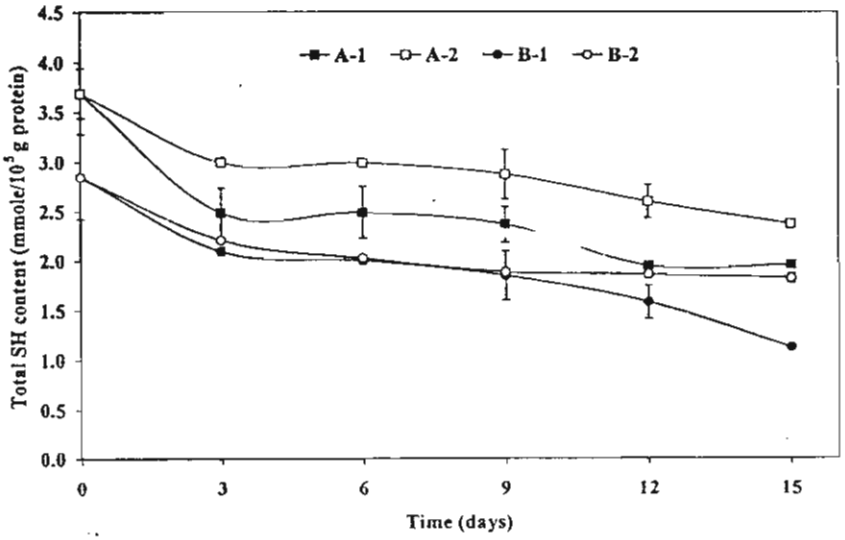

FIG. 5. CHANGES IN TOTAL SULFHYDRYL GROUP CONTENT OF BIGEYE SNAPPER NATURAL ACTOMYOSIN DURING ICED STORAGE

A1: *P. tayenus*, whole; A2: *P. tayenus*, headed/eviscerated; B1: *P. macracanthus*, whole; B2: *P. macracanthus*, headed/eviscerated. Bars indicate standard deviation from triplicate determinations.

decrease in sulfhydryl group content. Therefore, it was postulated that both sulfhydryl groups localized on either head or tail portions of myosin molecules underwent oxidation, leading to less available free sulfhydryl groups. The oxidation of sulfhydryl groups on the head portion presumably resulted in the decreased Ca^{2+}-ATPase and Mg^{2+}-Ca^{2+}-ATPase activity, whereas oxidation of sulfhydryl groups on the tail portion possibly caused the increase in Mg^{2+}-EGTA-ATPase activity. Thus, oxidation of sulfhydryl groups possibly involved in denaturation of myofibrillar proteins in the 2 species during iced storage. Jiang *et al.* (1989) suggested that sulfhydryl groups on F-actin might compete with those on myosin molecules for the oxidation into disulfides. As a result, the denaturation of myosin may also be dependent on actin/myosin ratio, which may be different between species.

CONCLUSION

Muscles of bigeye snapper, *P. tayenus* and *P. macracanthus*, possessed similar composition. However, muscle protein of *P. tayenus* had higher thermal stability than that of *P. macracanthus*. Denaturation of proteins such as loss of Ca^{2+}-ATPase activity during iced storage was presumed to occur via either hydrophobic interaction or disulfide formation. Troponin-tropomyosin complexes were possibly altered by either proteolytic degradation or the oxidation of sulfhydryl groups. Pretreatment of both species by heading and eviscerating retarded the denaturation and physicochemical changes of proteins during iced storage.

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Differences in Gelation Characteristics of Natural Actomyosin from Two Species of Bigeye Snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*

S. BENJAKUL, W. VISESSANGUAN, S. ISHIZAKI, AND M. TANAKA

ABSTRACT: Natural actomyosin (NAM) of *P. tayenus* exhibited higher turbidity and storage modulus (G') upon heating, compared to that of *P. macracanthus*, suggesting the higher protein aggregation and rigidity. At temperature above 35 °C, *P. tayenus* NAM had higher surface hydrophobicity and disulfide bond formation than *P. macracanthus* NAM. The α-helix content of NAM from both fish species decreased as the temperature increased, indicating changes in structural conformation during heating. NAM gel from *P. tayenus* rendered more three-dimensional network than that from *P. macracanthus*. These results indicated that *P. tayenus* NAM possessed superior gelling characteristic to *P. macracanthus* NAM due to the higher aggregation of protein caused by both hydrophobic interaction and disulfide bond.

Keywords: natural actomyosin, bigeye snapper, gelation, surimi

Introduction

GEL-FORMING ABILITY IS ONE OF THE MOST IMPORTANT AT tributes of surimi, which can be affected by both intrinsic and extrinsic factors including species, endogenous enzymes, additives as well as cooking procedure (Shimizu and others 1981; Niwa 1992; Lee 1986; Araki and Seki 1993; Morrissey and others 1993). Myosin has been reported to be an important protein mainly responsible for fish gel formation (Niwa 1992). Differences in cross-linking of myosin heavy chain contribute to the differences in gel-forming ability among the muscles of various fish (Nishimoto and others 1987). Endogenous proteinases and transglutaminases can influence the gelling properties of surimi by hydrolyzing myosin or cross-linking myosin, respectively (An and others 1996). Thermal gelation of fish muscle has been reported to occur in a three-step process including (1) dissociation of myofibril structures by protein solubilization in the presence of salt; (2) partial unfolding of protein structure caused by heat treatment; and (3) aggregation of unfolded protein via both covalent and noncovalent bonds to form a three-dimensional network (Stone and Stanley 1992). It is essential to allow heat-denatured fish muscle proteins to align, allowing aggregation, before further heating at high temperature, which strengthen the interaction formed during setting (Chan and others 1992b). Setting (suwari) was associated with transglutaminase (Kimura and others 1991). Araki and Seki (1993) suggested that transglutaminase-mediated cross-linking reaction was mainly regulated by conformation of actomyosin, which varied among fish species. To improve surimi gel quality, many approaches have been implemented. Microbial transglutaminase has shown potential to increase the gel strength of surimi by inducing non-disulfide covalent bond formation (Seguro and others 1995), whereas protein additives have been widely used to alleviate the softening (modori) induced by endogenous thermostable proteinases (Morrissey and others 1993).

Thailand is one of the most important surimi producing countries in the Southeast Asia with a total production of about 60,000 metric ton per y. An approximate 90% of products are exported to Japan and South Korea (Morrissey and Tan 2000). The surimi production in Thailand is primarily based on threadfin bream (*Nemipterus* spp.) due to its high gel quality and white color. However, with the overexploitation of resources in the Gulf of Thailand as well as Indian Ocean, harvest of threadfin bream has been decreasing. As a consequence, other fish species, for example bigeye snapper (*Priacanthus* spp.), lizardfish (*Saurida* spp.) as well as croaker (*Pennahia* spp.), have become more economically important as a raw material for surimi production. Among those species, bigeye snapper is more common for surimi manufacture due to the high gel quality and availability. Additionally, it is not consumed directly due to its appearance and thick skin. Bigeye snapper caught in Thailand normally includes two species, *Priacanthus tayenus*, which is more abundant and *Priacanthus macracanthus*. *P. macracanthus* has a slightly whiter mincemeat, however, it possesses a much poorer gel quality, compared to *P. tayenus*. Due to higher quality gels obtained, *P. tayenus* is more preferable among processors, while *P. macracanthus* is either manually sorted out or used for low-grade surimi production. In order to understand the differences in gel forming ability of these two fish species, the physicochemical characteristics of fish muscle and gelation mechanism are needed to elucidate. The objective of this study was to compare the inherent gel-forming ability of NAM during thermal gelation between two species of bigeye snapper, *P. tayenus* and *P. macracanthus*.

Materials and Methods

Chemicals

8-Anilino-1-naphthalenesulfonic acid was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Other chemicals used were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Fish samples

Two species of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*, were caught off the Songkhla–Pattani Coast along the Gulf of Thailand, stored in ice and off-loaded within 10 to 12 h capture. Fish were transported in ice to Dept. of Food Technology, Prince of Songkla Univ., then washed and filleted. The muscle was excised and kept on ice prior to NAM extraction.

Preparation of NAM

NAM from fish muscle was prepared according to the method of MacDonald and Lanier (1994) with a slight modification. Bigeye snapper muscle was homogenized in chilled 0.6 M KCl, pH 7.0 at a ratio of 1:10 (w/v) using a homogenizer (IKA Labortechnik, Selangor, Malaysia). To avoid overheating, the sample was placed in ice and homogenized for 20 sec, followed by a 20 sec rest interval for a total extraction time of 4 min. The extract was centrifuged at 5000 × *g* for 30 min at 4 °C. Three volumes of chilled deionized water were added to precipitate NAM. The NAM was collected by centrifuging at 5000 × *g* for 20 min at 4 °C. NAM pellet was mixed thoroughly with glycerol to the final concentration of 30% (v/v) and stored at –20 °C. Prior to analysis, frozen NAM was partially thawed with running water (20 °C). The glycerol was removed by addition of 10 volumes of 20 mM Tris-HCl buffer, pH 7.0. The mixture was stirred gently at 4 °C for 15 min. To obtain the NAM, the mixture was centrifuged at 7800 × *g* using a refrigerated centrifuge (Tomy CX250, Tokyo, Japan) at 4 °C for 15 min. The pellet was kept on ice until used.

Dynamic rheological measurements

Dynamic rheological properties of NAM samples during heating were measured using a Haake RheoStress RS-50 (EKO Instruments Trading Co., Ltd., Tokyo, Japan) operated in the oscillatory mode. The rheometer was equipped with a concentric cylinder measurement system with a bob dia of 10 mm and a cup dia of 48 mm. Dynamic viscoelastic measurements were taken with a gap of 2.1-mm at a fixed frequency of 2.1 Hz and the maximum strain amplitude of 0.05 was used. This condition had been previously determined to give a linear response in the viscoelastic region. NAM samples (45 mg/mL) were prepared in 20 mM Tris-HCl buffer, pH 7.0 and added with NaCl to a final concentration of 0.45 M, prior to the determination. Samples (1 g) were loaded into a cup and heated over the range of 20 to 75 °C at 1 °C/min using a Haake DC 5 temperature control unit. To avoid evaporation of the sample during heating, 0.5 mL of liquid paraffin was added. Data were collected every 30 sec and measurements were carried out in duplicate.

Turbidity measurement

NAM (1 mg/mL) dissolved in chilled 50 mM potassium phosphate buffer, pH 7.0 containing 0.6 M KCl was placed in a cuvette (light path length of 1 cm) and heated at a heating rate of 1 °C/min from 20 to 75 °C. Heating was conducted with a temperature-controlled unit (Hitachi, Tokyo, Japan) connected with spectrophotometer U-3200 (Hitachi). Protein aggregation was monitored continuously by measuring the absorbance at 660 nm (Sano and others 1994).

Total sulfhydryl and disulfide bonds determination

Total sulfhydryl groups were measured according to the method of Ellman (1959) with a slight modification. NAM solution was prepared at 1 mg/mL in chilled 50 mM potassium phosphate buffer, pH 7.0 containing 0.6 M KCl and a 5 mL aliquot was transferred to screw-capped test tube. The solution was linearly heated from 20 to 70 °C using a circulating bath (Haake K20) equipped with a temperature-controlled unit (Haake DC5, Haake Mess-Technik GmbH, Co., Karlsruhe, Germany). The heated aliquot was cooled immediately with iced water (0 to 2 °C). To 0.25 mL aliquot of protein solution (1 mg/mL), 3 mL of 0.2 M Tris-HCl buffer, pH 6.8 containing 8 M urea, 2% SDS and 10-mM EDTA were added. After incubation with 0.25 mL of 10-mM DTNB in 0.2-M Tris-HCl buffer, pH 6.8 at 40 °C for 40 min, absorbance at 412 nm was measured using spectrophotometer (Hitachi U-1100). Reagent blank was prepared by replacing the sample with 50 mM potassium phosphate buffer, pH 7.0 containing 0.6 M KCl. For sample blank, the reaction was run without DTNB solution. Sulfhydryl content was calculated using a molar extinction coefficient of 13600 M-1cm-1. Disulfide bonds in proteins were determined by using 2-nitro-5-thiosulfobenzoate (NTSB) assay as described by Thannhauser and others (1987). To 0.5 mL of protein sample (1 mg/mL), 3.0 mL of freshly prepared NTSB assay solution were added. The mixture was incubated in dark at room temperature (25 °C) for 25 min. Absorbance was then measured at 412 nm. Disulfide bond was calculated from absorbance using the extinction coefficient of 13900 M-1cm-1.

Surface hydrophobicity

Protein surface hydrophobicity was measured according to the method of Li-Chan and others (1985). NAM solution (1 mg/mL in 50 mM potassium phosphate buffer, pH 7.0) was linearly heated from 20 to 70 °C at a rate of 1 °C/min. The heated solution was diluted to 0.05, 0.1, 0.2 and 0.3 mg/mL using the same buffer. A 2 mL aliquot of NAM solution was mixed with 10 mL of 100 mM ANS in 50 mM potassium phosphate buffer, pH 7.0. The fluorescence intensity of ANS-protein conjugates was measured using a spectrofluorometer RF-1500 (Shimadzu, Tokyo, Japan) at the excitation and emission wavelengths at 374 nm and 485 nm, respectively. Protein hydrophobicity was calculated from initial slope of plot of fluorescence intensity against protein concentration using linear regression analysis. The initial slope was referred to as S_oANS.

Circular dichroism

Circular dichroism (CD) analysis was performed at 20 °C using a JASCO spectropolarimeter (J-720, Japan Spectroscopic Co., Ltd., Tokyo, Japan). NAM solution (0.2 mg/mL in chilled 50 mM potassium phosphate buffer, pH 7.0 containing 0.6 M KCl) was linearly heated from 20 to 70 °C at a rate of 1 °C /min. After heated to different temperatures (25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 °C) and cooled with iced water, NAM solution was subjected to CD analysis using scanning wavelengths from 200 to 260 nm. Molar ellipticities at 222 nm, $[\theta]_{222}$, were measured in 1.0-cm path length cell which was thermostated at 20 °C. α-helicity was estimated using molar ellipticities at 222 nm according to the method of Ogawa and others (1993).

Electrophoretic analysis

Protein compositions of NAM preparation were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Samples were loaded on the PAGEL®-Compact precast gel 5-20% gradient and subjected to electrophoresis at a constant voltage of 100 V using a Compact-PAGE apparatus (Atto Corp., Tokyo, Japan). After electrophoresis, gel was

stained with 0.125% (w/v) Coomassie brilliant blue R-250 in 25% (v/v) ethanol and 10 % (v/v) acetic acid and destained with 25% (v/v) ethanol and 10 % (v/v) acetic acid. High and low molecular weight markers (Sigma Chemical Co., St. Louis, Mo., U.S.A.) were used to estimate the molecular weight of proteins.

Scanning electron microscopy (SEM)

NAM gels from *P. tayenus* and *P. macracanthus* (45 mg/g) obtained after heating at 1 °C/min from 20 to 45 and 75 °C were immersed in 2.5% glutaraldehyde in 50 mM potassium phosphate buffer containing 0.45 M NaCl, pH 7.0 for 2 h. Fixed specimens were dehydrated in graded ethanol solutions with serial concentrations of 50, 70, 80, 90 and 100%, followed by 100% acetone. The samples were air-dried, coated with platinum and observed with a Hitachi scanning electron microscope (Hitachi).

Protein determination

Protein content was measured using Biuret method (Itzhaki and Gill 1964). Bovine plasma protein was used as a standard.

Statistical analysis

The experimental design was a completely random design with three replications. Data were presented as mean values with standard deviations. Statistical analyses were performed with the statistical program SPSS (SYSTAT 7.0, SPSS, Inc., Chicago, Ill., U.S.A.). One-way analysis of variance (ANOVA) was carried out and mean comparisons were run by Duncan's multiple range test (Steel and Torrie 1980).

Results and Discussion

Protein compositions of NAM

Similar electrophoretic patterns of NAM preparation were observed between two species of bigeye snapper (Figure 1). NAM preparations from *P. tayenus* and *P. macracanthus* mainly contained myosin and actin. Other proteins naturally associated with myosin and actin, for example troponin, tropomyosin, and C-proteins were less than 10% as determined by the densitometric analysis of SDS-PAGE gel. Due to no marked differences in protein composition, the different gelling properties between two species were postulated to be the result from the differences in physicochemical characteristics, protein conformation as well as reactive groups, which directly involve in gelation.

Dynamic rheological measurement

Typical gelation profiles of NAM from *P. tayenus* and *P. macracanthus* during thermal scanning from 10 to 75 °C are shown in Figure 2. Even though a similar pattern in gelation was observed, changes in storage modulus (G') indicated three major differences in gelation between *P. tayenus* and *P. macracanthus*, including 1) a maximum peak temperature, 2) the rate of G' development during heating, and 3) the magnitude of G' developed during and the end of heating process. Gelation patterns of NAM from both *P. tayenus* and *P. macracanthus* could be characterized into three main transitions (Figure 2a). The first transition, shown by a sharp increase in G', indicated that NAM of *P. tayenus* and *P. macracanthus* formed gel networks at 35 °C with showing a maximum G' at 40 °C and at 38 °C, respectively. Heating of NAM at temperature less than 40 °C generally resulted in dissociation of some myofibrillar components, for example tropomyosin from the F-actin backbone and F-actin from its

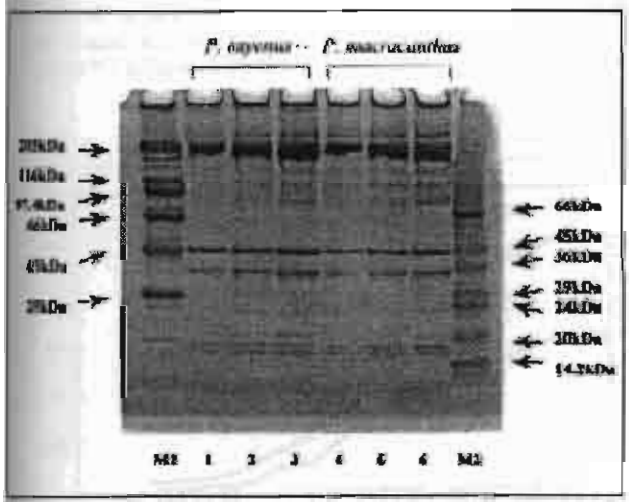

Figure 1—Electrophoretic pattern of NAM from two species of bigeye snapper, *P. tayenus* and *P. macracanthus*. M1 and M2 denote high and low molecular weight standards; Lane 1,4: 2 mg protein; Lane 2,5: 4 mg protein; Lane 3,6: 10 mg protein

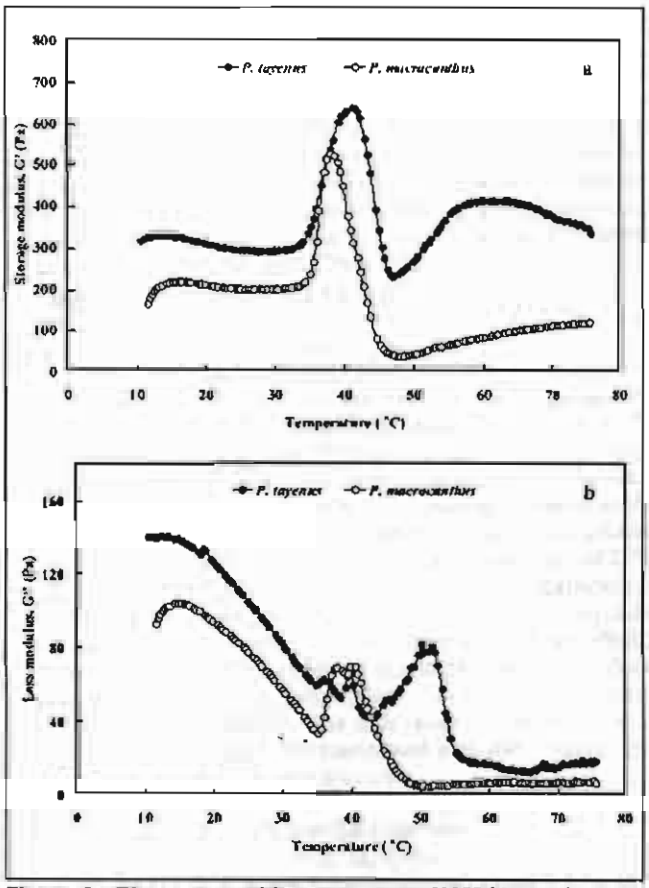

Figure 2—Rheogram of bigeye snapper NAM (45 mg/g, 0.45 M NaCl) heated linearly from 20 to 75 °C at 1 °C/min

helix structure (Ziegler and Acton 1984). However, conformational changes of these proteins had no significant contributions on the changes of gel modulus (Xiong 1997). Although it is present in a complex form with actin and other proteins, myosin is responsible for the gel elasticity development of NAM during the thermal gelation (Sano and others 1988; Sano and others 1989). It is postulated that partial unfolding of the protein structure initiated by the dissociation of myosin light chain subunits from the heavy chains may lead to an interfilamental association of myosin and form a three-dimensional structure. In the second transition, G′ decreased drastically as heating proceeded from 41 to 46 °C for *P. tayenus* NAM and from 39 to 46 °C for *P. macracanthus* NAM. The decrease in G′ up to 48 °C was possibly caused by dissociation of actin-myosin complex (Egelandsdal and others 1986) and the denaturation of myosin tail. It is assumed that helix-coil transformation can lead to a large increase in fluidity of the semi gel and may disrupt some of protein network already formed (Xiong 1997). Since NAM from *P. macracanthus* reached the peak at the temperature lower than that from *P. tayenus*, it suggested that actomyosin complex in *P. macracanthus* underwent dissociation at the lower temperature, compared to that in *P. tayenus*. The third transition, characterized by the second increase in G′ with heating at 47 to 75 °C, indicated that *P. tayenus* NAM gel networks were formed with a higher rate than *P. macracanthus* NAM, especially within the temperature range of 47 to 58 °C. As heating proceeded, it was evident that magnitude of G′ developed of NAM from *P. tayenus* was higher than that of *P. macracanthus*, suggesting more rigidity of protein matrix formed. The increase in G′ after 47 °C probably attributed to the formation of irreversible gel networks (Egelandsdal and others 1986; Lou and others 2000). At final temperature, NAM from *P. tayenus* exhibited three times higher G′, compared to that from *P. macracanthus*. This indicated that NAM from *P. tayenus* exhibited the superior gelling property, compared to that from *P. macracanthus*. This might involve the higher cross-links between protein aggregates or strands and deposition of denatured proteins in the existing protein networks, leading to the strengthening of gel matrix.

For the loss modulus, G″, continuous decrease was observed between 10 to 35 °C for NAM from both species (Figure 2b). G″ of NAM from *P. macracanthus* started to increase at 35 °C and reached the maximum at temperature range of 37 to 40 °C, followed by the gradual decrease up to 50 °C. At temperature higher than 50 °C, no changes in G″ were obtained. For NAM from *P. tayenus*, a small peak was found at 40 °C, and G″ began to increase and reached a maximum at temperature of 50 to 52 °C, followed by a sharp decrease up to 55 °C. However, no marked changes in G″ was obtained at temperature higher than 60 °C. In general, the changes in G″ were in accordance with the changes in G′. The result suggested that elastic gel network of NAM from *P. tayenus* was formed at higher temperature, resulting from the gradual unfolding of the myosin, followed by the formation of gel network.

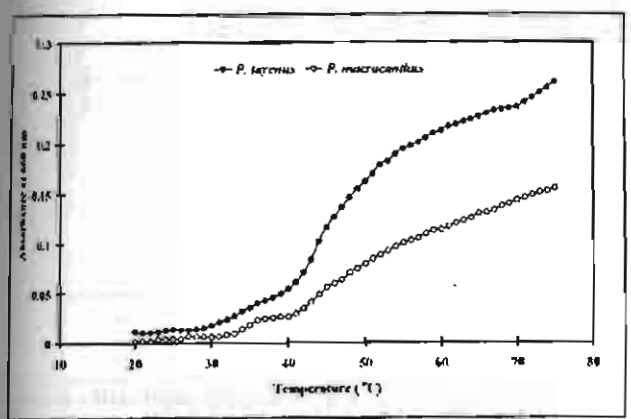

Figure 3—Turbidity of bigeye snapper NAM solution (1 mg/mL) during linear heating from 20 to 75 °C at 1 °C/min

Turbidity measurement

Changes in turbidity of NAM solution indicated the formation of protein aggregate during heating process (Figure 3). Absorbance reading is commonly used to monitor the extent of protein aggregates (Chan and Gill 1994; Sano and others 1994). NAM solution became more turbid as temperature increased, suggesting an increased extent of protein aggregate. An increase in turbidity of *P. tayenus* and *P. macracanthus* NAM solution was observed at 30 °C. NAM consists of long filaments, in which approximately 1 mm long thin filaments of actin, tropomyosin, and troponin are conjugated with a great number of myosins all along the filament. Each myosin molecule is bound to the actin filament at its head portion with its tail portion sticking out (Sano and others 1988). Thus, it seems reasonable that the NAM molecules tend to interact with each other and form protein aggregates upon heating. However, at the temperature above 40 °C, *P. tayenus* NAM exhibited higher rate in turbidity development than that of *P. macracanthus*. The results suggested that NAM from *P. tayenus* could undergo aggregation at a higher extent, compared to NAM from *P. macracanthus*. The differences in turbidity of heat-treated myofibril and myosin solutions among fish species were most probably due to the size and/or rate of aggregation (Chan and Gill 1994). Muscle proteins can associate with one another through hydrophobic, electrostatic and hydrogen bonds, Van der Waals interactions, and disulfide bonds. The relative contributions of each type of bond are different in aggregated proteins than in the native proteins (Xiong 1997). From the result, the difference in aggregation of NAM from two species was possibly due to the difference in hydrophobic domain exposed during heating process between two species. Extent of aggregation for fish myosin seemed to depend on the amount of hydrophobic (Chan and others 1992a). Moreover, the less aggregation of NAM from *P. macracanthus* was presumably due to the lower intermolecular disulfide bond formation during heating, compared to NAM from *P. tayenus*. Smyth and others (1998) found that turbidity of myosin solution heated in the absence of dithiothreitol (DTT), reducing agent, was greater than those of myosin solution heated in the presence of DTT, indicating the role of disulfide bond in thermal aggregation. Formation of large aggregates is presumably a prerequisite to formation of a good elastic gel (Chan and others 1992b). The poorer aggregating ability of herring actomyosin reflected the inferior gelling properties of surimi from herring (Chan and others 1992b). The differences in aggregation of NAM were postulated to be associated with the different gelling properties between two species.

Sulfhydryls and total disulfide bonds

Changes in disulfide bonds formation and sulfhydryl content of NAM during heating are shown in Figure 4. Disulfide bonds of NAM from both species increased drastically after

treatment at temperature of 35 to 60 °C and remained constant at temperature ranging from 60 to 70 °C (Figure 4a). Kishi and others (1997) found the polymerization of myosin heavy chain via disulfide bond during heating at 80 °C. Corresponding to the formation of disulfide bond formation, sulfhydryl content simultaneously decreased at a temperature higher than 35 °C (Figure 4b). The result indicated that thermally exposed sulfhydryl groups were oxidized to disulfide bonds. Lui and others (1982) observed the decrease in total sulfhydryl groups of Atlantic croaker actomyosin when it was heated from 30 to 60 °C, while Sano and others (1994) found the decrease in total sulfhydryl content in carp NAM from 30 to 80 °C. Formation of disulfide bonds in *P. tayenus* NAM was higher than *P. macracanthus* NAM, particularly at temperature higher than 40 °C. This result indicated that sulfhydryl groups of NAM from *P. tayenus* were more prone to disulfide formation. This was possibly facilitated by the structural changes of NAM from *P. tayenus* during heating, which made the SH groups more susceptible to oxidation. The results suggested that disulfide bonds would play an essential role in actomyosin aggregation. Sulfhydryl group on the surface of F-actin might compete with sulfhydryl group on the myosin molecules for the oxidation into disulfides (Chang and others 1989). Therefore, the different ratio of actin to myosin between two species might lead to the differences in oxidation pattern of sulfhydryl groups. The differences in disulfide bonds formed between NAM from two species were in accordance with the differences in thermal aggregation as indicated by turbidity (Figure 3) as well as rheological changes as monitored by storage modulus (Figure 2). It was postulated that the structural changes of myosin, particularly at S-1 region, of *P. tayenus* during heating possibly occurred in the way which facilitated the oxidation of sulfhydryl groups, leading to more intermolecular disulfide bond formation, compared to those of *P. macracanthus*. As a result, myosin from *P. tayenus* was more susceptible to heat and formed aggregates, possibly via disulfide formation. Polymerization of myosin heavy chain was due to the oxidation of sulfhydryl groups in S-1, while dimerization was caused by the oxidation of sulfhydryl groups in rod (Kishi and others 1997; Runglerdkriangkrai and others 1999).

Surface hydrophobicity

Changes in surface hydrophobicity of *P. tayenus* and *P. macracanthus* NAM during heating from 20 to 70 °C are shown in Figure 5. *P. tayenus* NAM exhibited lower surface hydrophobicity than that of *P. macracanthus* at the temperature below 30 °C. However, during heating process, surface hydrophobicity of NAM from *P. tayenus* was generally higher than that of *P. macracanthus*, particularly at temperature higher than 45 °C. Surface hydrophobicity generally increased with an increase in temperature. Surface hydrophobicity of *P. tayenus* NAM increased in two steps, starting with a rapid increase with heating from 30 to 55 °C, followed by a plateau from 55 to 65 °C. Another increase in surface hydrophobicity occurred between 65 and 70 °C. For NAM from *P. macracanthus*, an increase in surface hydrophobicity was observed from 35 to 45 °C, followed by a constant hydrophobicity up to 55 °C. Another increase was found between 55 and 70 °C. The increased surface hydrophobicity indicated the structural changes of actomyosin during heating, causing the hydrophobic groups emerged at the surface of molecule and subsequently formed hydrophobic interaction to reduce free energy (Sano and others 1994). The increased hydrophobicity also indicated the involvement of hydrophobic interactions in gel formation (Chan and others 1993). This result revealed that hydrophobic groups of *P. tayenus* NAM were more exposed to the surface and more reactive to form hydrophobic interaction. The higher surface hydrophobicity was in good agreement with the higher aggregation (Figure 3) and storage modulus (Figure 2a). Chan and others (1992b)

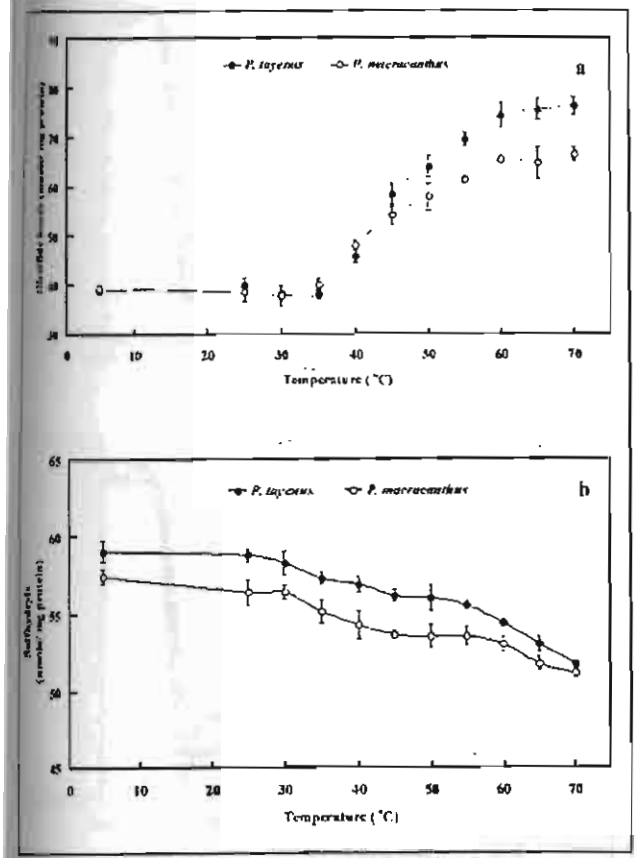

Figure 4—Disulfide bond and total sulfhydryl content in bigeye snapper NAM (1 mg/mL) as affected by heat treatment. Vertical bars represent standard deviation from triplicate determinations.

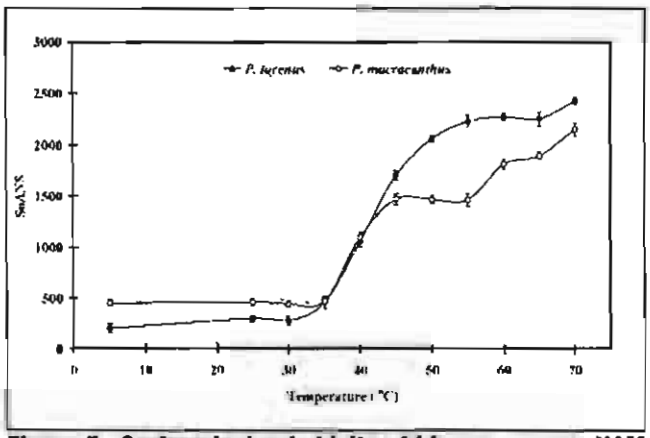

Figure 5—Surface hydrophobicity of bigeye snapper NAM (1 mg/mL) as affected by heat treatment. Vertical bars represent standard deviation from triplicate determinations.

suggested that fish myosin lost its helical content with simultaneous exposure of interior hydrophobic surface while the proteins were thermally denatured. Therefore, higher hydrophobic interactions and disulfide bonds were presumed to occur in NAM from *P. tayenus* during the heating process, compared to NAM from *P. macracanthus*. As a consequence, NAM from *P. tayenus* rendered the higher gelling property than that from *P. macracanthus*. The formation of intermolecular hydrophobic interactions among protein molecules, in addition to the disulfide bonding occurred during heating is thought to be a primary mechanism for surimi gel formation (Lanier 2000).

Circular dichroism and α-helix content

The magnitude of CD spectra of NAM from two species after heating at different temperatures, followed by cooling is present in Figure 6. The magnitude of spectra decreased with a gradual decrease in α-helix content as the temperature increased (Figure 7). This result revealed that α-helical portion of NAM underwent unfolding gradually when the temperature increased. The marked decrease in α-helix content was observed at around 40 and 35 °C for NAM from *P. tayenus* and *P. macracanthus*, respectively. The result was in accordance with the sharp decrease in storage modulus at the same temperature (Figure 2a). Unfolding or denaturation of light meromyosin possibly contributed to increase in fluidity of myofibrillar filaments (Xiong and Blanchard 1994).

The changes in magnitude of spectra and α-helix content were found at temperature of 30 to 40 °C, which is commonly setting temperature. This result was in agreement with Ogawa and others (1995) who reported the decrease in α-helix during setting at temperature of 30 and 40 °C. The α-helix structures are stabilized by hydrogen bonds between –CO and NH– of a polypeptide chain (Sano and others 1994). Hydrogen bonds can be destroyed at high temperature and are stable with decreasing temperature. From the result, no substantial changes in magnitude of spectra were observed in the samples heated at high temperature. This was due to cooling induced refolding of unfolded α-helix via restabilization of hydrogen bonds. This result was coincidental with Sano and others (1994) who found that unfolded α-helix of carp NAM refolded during cooling process. When comparing the changes in CD spectra and α-helix content between NAM from two species, it was noted that magnitude of spectra for NAM from *P. tayenus* decreased to a higher extent, compared to those of *P. macracanthus* at the same temperature tested. This suggested that NAM complexes from *P. tayenus* unfolded to a higher extent than those from *P. macracanthus*. As a result, the buried reactive sulfhydryl groups or hydrophobic residues were more exposed and associated each other through disulfide bonding or hydrophobic interaction, respectively. Although uncoiling is necessary to expose reactive groups, chemical and/or physical properties of the unfolded domains seem to be more important for aggregation (Chan and others 1992a). Sano and others (1990) suggested that the interaction of myosin tail portion with high α-helix content mainly involved in aggregation at temperature of 30 to 44 °C. From the result, it can be presumed that NAM from *P. tayenus* exhibited the superior properties for aggregation, as observed by the higher disulfide formation and α-helix unfolding with higher surface hydrophobicity. As a consequence, higher aggregation with more rigidity of NAM from *P. tayenus* was obtained, compared to that of *P. macracanthus*.

Ultrastructure of NAM gels

The ultrastructure of NAM gels from two species of bigeye snapper in the presence of 0.45 M NaCl is shown in Fig-

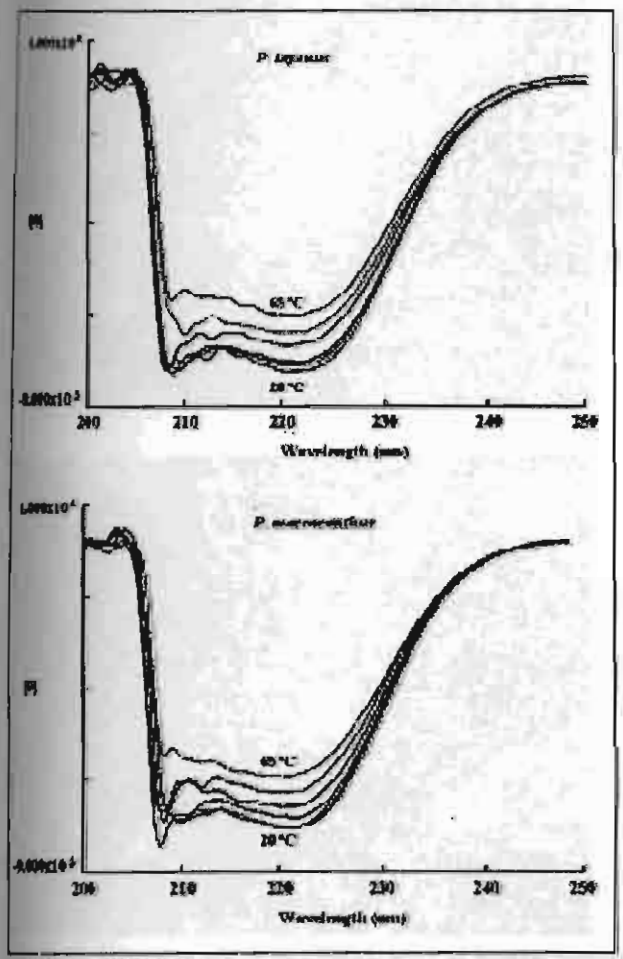

Figure 6—CD spectra of bigeye snapper NAM after heat treatment to different temperatures, followed by cooling

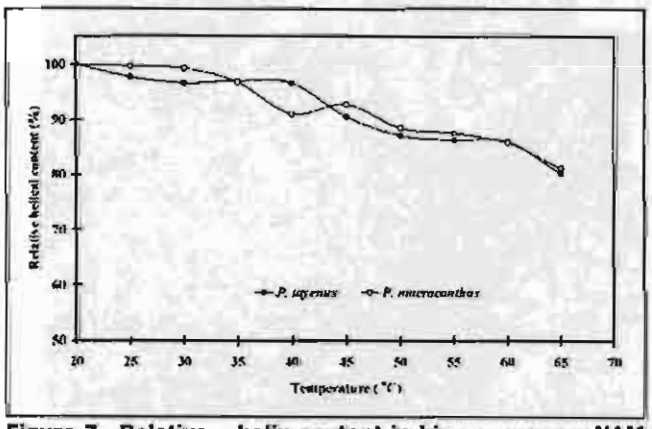

Figure 7—Relative α-helix content in bigeye snapper NAM after heat treatment to different temperatures, followed by cooling

...l. NAM gels from *P. tayenus* and *P. macracanthus* prepared by heating from 5 to 45 °C at the constant heating rate of 1 °C/min exhibited the granular structure. Gel structure of NAM from *P. tayenus* has a larger pore size with the coarser structure (Figure 8a), compared to that from *P. macracanthus* (Figure 8c). The result suggested that *P. tayenus* NAM was postulated to undergo more aggregation when heated up to 45 °C, at which the storage modulus began to increase after the sharp declining. The result corresponded with the higher aggregation (Figure 3) and higher storage modulus (Figure 2a). For the gels formed by heating up to 75 °C, more three-dimensional structure with a lacy-like network was observed in both NAMs. However, NAM from *P. tayenus* exhibited more three-dimensional network with a larger granular structure (Figure 8b) than that from *P. macracanthus* (Figure 8d). This was possibly due to the higher aggregation of NAM from *P. tayenus*, particularly at the tail region, leading to the lacy-like structure. Ishioroshi and others (1982) reported that both LMM and HMM contributed to heat induced network of the gel. Sponge-like and lacy-like network structures were produced by HMM and LMM, respectively. Therefore, the α-helix tail possibly played an important role in heat-induced gelation of myosin at the higher temperature, while the globular head underwent the aggregation at the lower temperature. From the result, α-helix content in NAM from both fish species decreased at the higher content at higher temperature. Therefore, it might contribute to the formation of aggregate, resulting in more lacy-like network. Chan and others (1993) proposed that thermal aggregation of cod and herring myosins initiated by the unfolding and interaction of HMM S-2, and further aggregation was mediated through the interaction of LMM to form clusters of aggregates at higher temperature.

From the results, the differences in gelation between two species were caused by different intrinsic properties of muscle proteins, mainly myosin and actin. However, the presence of other proteins possibly contributed to the differences in gelling characteristics. Actin/myosin ratio was found to affect the stability of milkfish actomyosin (Jiang and others 1989) and thermal gelation of carp actomyosin (Sano and others 1989). Thus, different actin/myosin ratio as well as free myosin/bound myosin ratio probably caused the differences in gelation of NAM between two species of bigeye snapper.

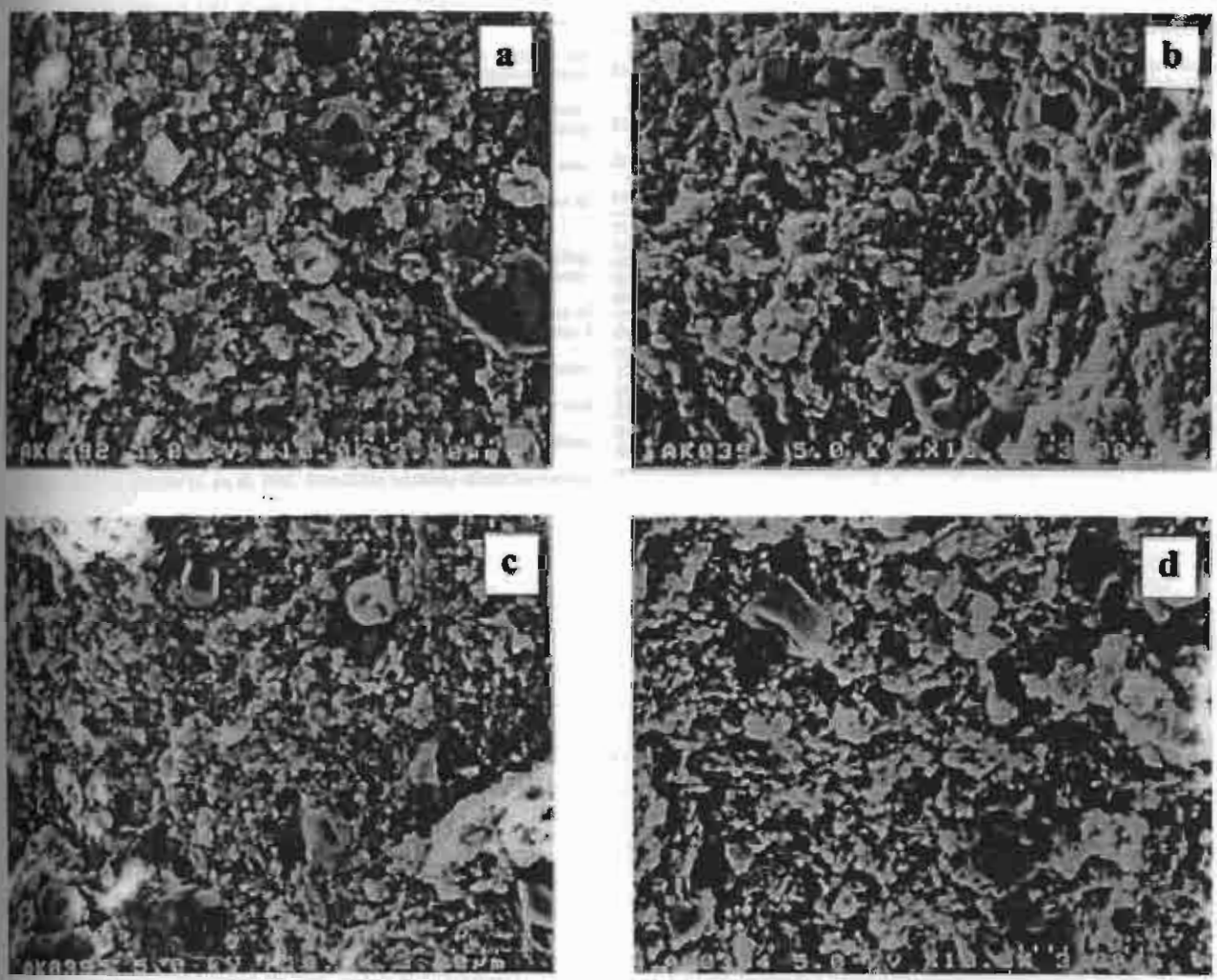

Figure 8—Scanning electron micrographs of bigeye snapper NAM gels (45 mg/g, 0.45M NaCl) heated at 1 °C/min from 20 to either 45 or 75 °C. a: *P. tayenus*, 45 °C; b: *P. tayenus*, 75 °C; c: *P. macracanthus*, 45 °C; d: *P. macracanthus*, 75 °C.

Conclusion

DIFFERENCES IN GEL-FORMING ABILITY OF TWO SPECIES OF bigeye snapper were attributed to the inherent properties of NAM. NAM from *P. tayenus* underwent aggregation via hydrophobic interaction and/or disulfide bonds to a higher extent, compared to that from *P. macracanthus*. As a consequence, NAM from *P. tayenus* rendered a superior rheological property with higher gel-forming ability.

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Gel-forming properties of surimi produced from bigeye snapper, *Priacanthus tayenus* and *P macracanthus*, stored in ice

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Abstract: The influence of iced storage of two species of bigeye snapper, *Priacanthus tayenus* and *P macracanthus*, on the gel-forming ability of the resulting surimi was investigated. Upon iced storage, whole fish underwent deterioration faster than beheaded/eviscerated fish. Total volatile base and trimethylamine contents of whole fish were higher than those of beheaded/eviscerated fish, particularly after 9 days of storage ($P < 0.05$). *P macracanthus* muscle was more susceptible to proteolytic degradation than *P tayenus* muscle. Ca^{2+}-ATPase activity decreased as the storage time increased ($P < 0.05$), indicating the denaturation of myosin. A marked decrease in Ca^{2+}-ATPase activity was found in whole fish kept for more than 6 days in ice ($P < 0.05$). Breaking force and deformation of surimi gels from both species decreased, with a concomitant decrease in whiteness, as the storage time increased ($P < 0.05$). Beheading and evisceration of fish retarded the deterioration. However, the gel-forming ability of surimi produced from both species decreased continuously throughout iced storage ($P < 0.05$), probably owing to the denaturation and degradation of myofibrillar proteins.

Keywords: bigeye snapper; gelation; surimi; iced storage; degradation; denaturation

INTRODUCTION

Freshness of fish is generally considered as the most crucial requirement for the raw material to be processed into surimi. Time and temperature of the fish between capture and processing can affect the final surimi quality.[1] Lower gel quality can be found with gel made from fish stored over time in ice. However, the rate of loss of gel strength appears to vary among species. The rate of decline in gel strength is dependent on the denaturation and extent of proteolysis of myofibrillar proteins. The gel strength of kamaboko made from lizard fish kept in ice for 3 days was 50% of that made from fresh fish.[2] Northern squawfish surimi could be made from fish stored for up to 9 days.[3] MacDonald *et al*[4] revealed that good-quality surimi could be produced from hoki stored in ice for up to 10 days. Surimi gel quality can be influenced by many factors affecting protein structure.[5] Prolonged holding times and elevated temperatures can cause severe proteolysis of myofibrillar proteins, which is directly associated with inferior gel quality.[6] However, degradation of myosin heavy chain also occurred during iced storage of Pacific whiting.[7] During handling, leakage of digestive enzymes into the muscle also results in subsequent hydrolysis of muscle proteins. Therefore pretreatment of fish, including beheading and evisceration prior to handling, can be another means to retard the deterioration caused by proteolysis.

In addition to threadfin bream, bigeye snapper has been used as an important raw material for surimi production in Thailand. Owing to its thick and tough skin, it is not consumed directly and therefore is suitable for surimi manufacture.[8] Two species of bigeye snapper, *Priacanthus tayenus* and *P macracanthus*, are normally caught in the Gulf of Thailand and the Indian Ocean. Surimi produced from *P tayenus* has been found to exhibit better gelling properties than that from *P macracanthus*.[9] The differences in gel quality have been determined by both intrinsic and extrinsic parameters. With the over-exploitation of fish resources, the fishing fleet must travel a longer distance, leading to lower-quality raw

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material for surimi production. Consequently, it takes a longer time for handling the fish to shore. The denaturation and degradation of muscle proteins from the two species can occur to different extents. This may contribute to the differences in final surimi gel quality. The objectives of this work were to investigate the physicochemical and chemical changes of *P tayenus* and *P macracanthus* stored in ice over an extended period and to study the changes in gel quality of surimi produced from the fish.

MATERIALS AND METHODS

Chemicals

Adenosine 5'-triphosphate (disodium salt) and β-mercaptoethanol were purchased from Sigma Chemical Co (St Louis, MO, USA). Trichloroacetic acid was purchased from Riedel-deHaen (Seelze, Germany). All chemicals for electrophoresis were obtained from Bio-Rad (Richmond, CA, USA).

Fish samples

Bigeye snapper, *P tayenus* and *P macracanthus*, caught off the Songkhla-Pattani Coast along the Gulf of Thailand were stored in ice and off-loaded within 10–12 h of capture. The fish were transported to the Department of Food Technology, Prince of Songkla University, washed with tap water and separated into two groups, whole fish and beheaded/eviscerated fish. The fish were kept in a styrene foam box containing crushed ice, with a fish/ice ratio of 1:2 (w/w). The fish were placed and distributed uniformly between the layers of ice. The box was kept at room temperature (28–30 °C). To maintain the ice content, melted ice was removed and replaced with an equal amount of ice. Fish were randomly taken every 3 days for analysis and surimi preparation.

pH determination

Fish muscle was homogenised with 10 volumes of deionised water (w/v), and the pH was measured using a pH meter (Eutech Instruments Pte Ltd, Singapore).

Determination of total volatile base (TVB) and trimethylamine (TMA) contents

TVB and TMA contents were determined using the Conway microdiffusion assay as described by Ng.[10] Fish meat (2 g) was extracted with 8 ml of 40 g l^{-1} trichloroacetic acid (TCA). The mixture was filtered using Whatman No 41 paper, and the filtrate was used for analysis. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in the sample.

Determination of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of actomyosin was determined as described by Benjakul *et al.*[7] An aliquot (1 ml) of actomyosin solution (2.5–4 g l^{-1}) in 0.6 mol l^{-1} KCl, pH 7.0 was mixed with 0.6 ml of 0.5 mol l^{-1} Tris-maleate, pH 7.0 and 1 ml of 0.1 mol l^{-1} $CaCl_2$. Deionised water was added to make up a total volume of 9.5 ml. To the prepared solution, 0.5 ml of 20 mmol l^{-1} ATP was added to initiate the reaction. The reaction mixture was incubated for 8 min at 25 °C, and the reaction was terminated by adding 5 ml of chilled 150 g l^{-1} TCA. The reaction mixture was then centrifuged at 3500 × *g* for 5 min. The inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow.[11] The specific activity was expressed as μmol inorganic phosphate (Pi) released mg^{-1} protein min^{-1}. A blank solution was prepared by adding chilled TCA prior to addition of ATP.

Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method described by Morrissey *et al.*[12] Fish muscle (3 g) was homogenised with 27 ml of 50 g l^{-1} TCA. 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 g^{-1} muscle.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The protein pattern of bigeye snapper muscle was analysed by SDS-PAGE according to the method of Laemmli.[13] To prepare the protein sample, 27 ml of 50 g l^{-1} SDS solution heated to 85 °C was added to the sample (3 g). The mixture was then homogenised (IKA Labortechnik, Malaysia) for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve the proteins. The sample was centrifuged at 3500 × *g* for 20 min to remove undissolved debris. Protein concentration was determined according to the method of Lowry *et al*[14] using bovine serum albumin as standard. The SDS-PAGE gel was made of 10% running gel and 4% stacking gel. After separation the proteins were fixed and stained with Coomassie Blue R-250.

Surimi and surimi gel preparation

Fish kept in ice for different times were washed with tap water. The flesh was removed manually and minced to uniformity. 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 the washed mince was filtered with a layer of nylon screen. The washing process was repeated twice. Finally the washed mince was centrifuged (Model CE 21K basket centrifuge, Grandiumpiant, Belluno, Italy) at 700 × *g* for 15 min. The washed mince was kept in ice until used.

The prepared surimi was supplemented with 25 mg g^{-1} salt, and the moisture content was adjusted to 800 mg g^{-1}. The mixture was chopped for 5 min at 4 °C to obtain a homogeneous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 2.5 cm, and both ends of the casing were sealed tightly. One-step heated gels were prepared by heating the sol at 90 °C for 20 min. Two-step heated gels were

prepared by incubating the sol at 40 °C for 30 min, followed by heating at 90 °C for 20 min. The gels were cooled in iced water and stored for 24 h at 4 °C prior to analysis.

Texture analysis

Texture analysis of surimi gels was carried out using a TA-XT2 texture analyser (Stable Micro Systems, Godalming, 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 analyser equipped with a cylindrical plunger (diameter 5 mm, depression speed 60 mm min^{-1}).

Determination of whiteness

Surimi gel colour was determined using a JP7100F colorimeter (Juki Corp, Tokyo, Japan). L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Fujii *et al*:[15]

$$\text{whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

Determination of expressible drip

Expressible drip was measured according to the method of Ng.[16] A gel sample with a thickness of 0.5 cm was weighed and placed between two pieces of Whatman paper No 1 paper at the top and three pieces of Whatman No 1 paper at the bottom. A pressure of 10 kg cm^{-2} was applied on the sample and maintained for 2 min. The sample was removed and weighed. Expressible drip was calculated and expressed as percentage of sample weight.

Statistical analysis

Analysis of variance (ANOVA) was performed and mean comparisons were run by Duncan's multiple-range test.[17]

RESULTS AND DISCUSSION

Changes in pH

Post mortem pH values of *P tayenus* and *P macracanthus* muscle were approximately 6.6–6.7 (Fig 1). Up to 6 days of iced storage, changes in pH were observed only with whole fish samples of both species of bigeye snapper, while muscle pH of pretreated samples remained relatively constant. Although an increase in pH was evident in all conditions tested at the later stage of storage, whole fish most likely exhibited a faster rate of increase than pretreated samples. It should be noted that beheading and evisceration of *P macracanthus* prior to iced storage resulted in no significant changes in pH of fish muscle up to 9 days ($P < 0.05$). In general, *post mortem* changes, particularly glycogen breakdown and anaerobic respiration,

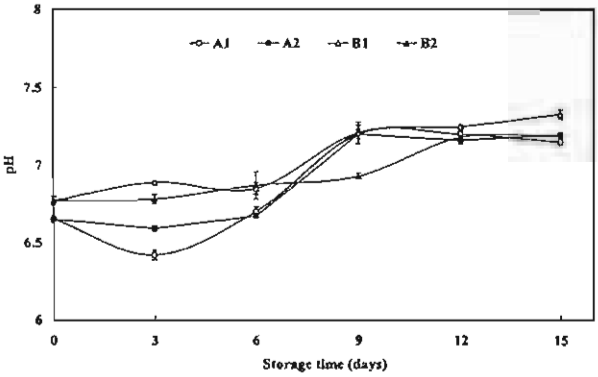

Figure 1. Changes in pH of bigeye snapper muscle during iced storage. A1, *Priacanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated. Vertical bars represent standard deviation from triplicate determination.

lead to the accumulation of lactic acid and a decrease in pH of fish muscle. The increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes. The decomposition of nitrogenous compounds causes an increase in pH in fish flesh.[18] The changes in pH also depend on the liberation of inorganic phosphate and ammonia due to the enzymatic degradation of ATP.[18]

Changes in TVB and TMA

Changes in TVB and TMA content of fish during iced storage are shown in Fig 2. TVB and TMA contents of fish pretreated by beheading and evisceration were generally lower than those of whole fish stored under the same condition ($P < 0.05$). At day 0 the initial TVB content of all fish samples was found to be 5.0–5.4 mg per 100 g, indicating that some changes in nitrogenous compounds occurred prior to iced storage ($P < 0.05$) (Fig 2). TVB comprises mainly TMA and ammonia, which are produced by both microbial and endogenous enzymes. However, no TMA was detected at day 0. TVB and TMA contents of all samples increased slightly during the first 3 days and then increased gradually up to 9 days of iced storage (Fig 2). Marked increases in TVB and TMA were found in all samples kept for more than 9 days in ice, particularly those of *P macracanthus*. Changes in TVB and TMA content of whole samples occurred at a faster rate than those of beheaded/eviscerated samples ($P < 0.05$). Owing to the fact that viscera and gills are major sources of enzymes as well as micro-organisms, removal of these organs presumably resulted in less hydrolysis of nitrogenous compounds. The formation of TVB and TMA is generally associated with the growth of micro-organisms. A number of specific spoilage bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Vibrionaceae*, etc typically use trimethylamine oxide (TMAO) as an electron acceptor in anaerobic respiration, resulting in off-odour and off-flavour due to the formation of TMA.[19,20] Since TMA does not increase much during

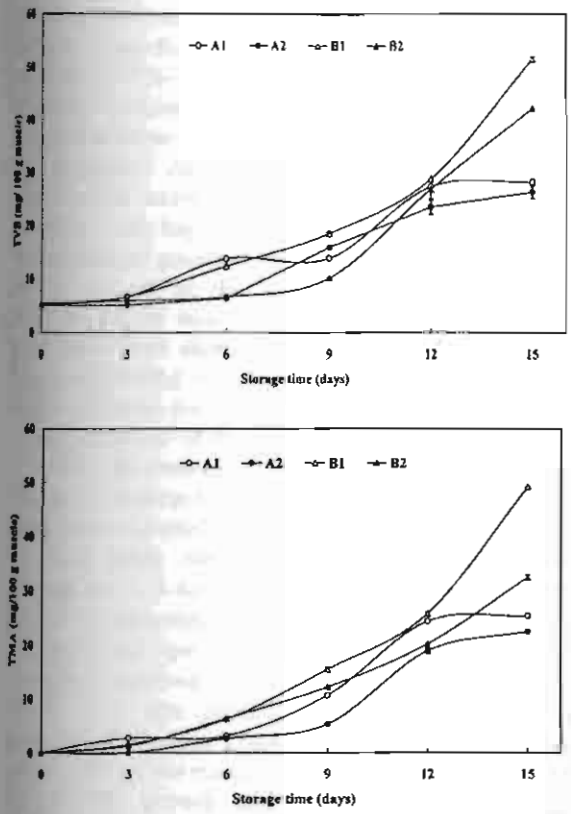

Figure 2. Changes in TVB and TMA in bigeye snapper muscle during iced storage. A1, *Priacanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated. Vertical bars represent standard deviation from triplicate determination.

the early stage of spoilage, it is not considered suitable as a quality index for fish that have been stored in ice for less than 6 days.[21]

When comparing TVB and TMA contents between the two species under the same pretreatment condition, *P macracanthus* muscle showed the higher contents of TVB and TMA, especially after 9 days of storage ($P < 0.05$). The results indicated that *P macracanthus* muscle had a higher degree of spoilage than *P tayenus* muscle. It was postulated that *P macracanthus* possibly contained a higher amount of TMAO and/or TMAO reductase in the muscle. As a consequence, TMA could be formed in *P macracanthus* more efficiently than in *P tayenus*. The amount of TMAO in the fish muscle tissue depends on the species, season, fishing grounds, etc.[19] Additionally, *P macracanthus* probably had a higher count of TMAO reductase-producing bacteria, which can convert TMAO to TMA to a higher extent. Up to 12 days the TVB content of all samples was generally lower than 30 mg per 100 g, which is the limit of acceptability proposed by Connell.[22] However, a much higher content of TVB in *P macracanthus* muscle was detected at day 15, indicating spoilage caused by microorganisms. Nevertheless, no marked increases in TVB and TMA were noted in *P tayenus*. From the results, TMA was found to be a major constituent of TVB, since it represented a comparable content to TVB. Production of TMA could be used as an indicator of bacterial activity.[23] Since all fish were kept in ice, the formation of TVB and TMA was probably mediated by psychrotropic bacteria.[24,25] Pastoriza *et al*[26] found a correlation between TMA content and total viable count (TVC) in hake slices stored in ice under modified atmosphere packaging. From the results, pretreatment of bigeye snapper by beheading and evisceration could retard the spoilage of fish during iced storage.

Changes in TCA-soluble peptides and protein pattern

An increase in TCA-soluble peptides indicated autolytic degradation of fish proteins during iced storage (Fig 3). At day 0, *P tayenus* muscle contained slightly fewer TCA-soluble peptides than *P macracanthus* muscle. This was possibly caused by the more reactive proteolytic activity in the latter muscle. TCA-soluble peptides in *P tayenus* muscle increased gradually up to 15 days of iced storage ($P < 0.05$). For *P macracanthus* muscle, TCA-soluble peptides increased continuously up to day 12, followed by a sharp increase up to day 15 ($P < 0.05$). At days 12 and 15 respectively, TCA-soluble peptides in *P macracanthus* muscle were 1.5–1.7- and 2.9–3.4-fold higher than those observed in *P tayenus* muscle. The marked differences in degradation between the two species were presumed to be due to the differences in proteolytic activities mediated through muscle proteinases, digestive proteinases as well as microbial proteinases. The increase in TCA-soluble peptides in *P macracanthus* muscle at days 12 and 15 of iced storage coincided with the increase in TVB and TMA contents (Fig 2). This suggested that bacterial proteinases were mainly involved in the degradation of muscle proteins, especially when the storage time increased.

When comparing the changes in TCA-soluble

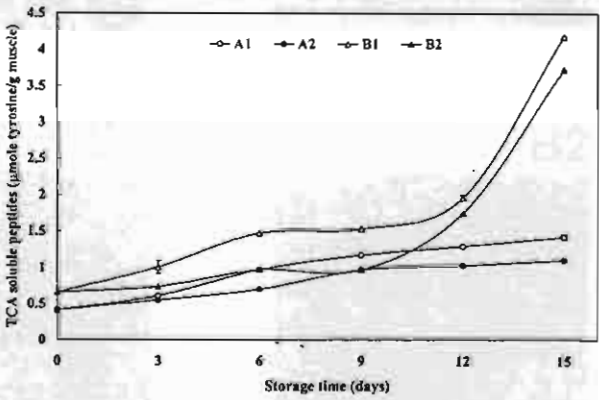

Figure 3. Changes in TCA-soluble peptides in bigeye snapper muscle during iced storage. A1, *Priacanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated. Vertical bars represent standard deviation from triplicate determination.

peptides between whole and beheaded/eviscerated samples, it was found that whole samples had a higher content of TCA-soluble peptides. This indicated that whole fish proteins underwent degradation to a higher extent than beheaded/eviscerated fish proteins. Whole fish comprised the head, gills and internal organs, which are the major sources of proteinases and microorganisms with high proteolytic activity. *Pseudomonas nanoglutinosa* protease was found to hydrolyse fish muscle protein at 0–2°C.[27] Apart from digestive enzymes, which could contaminate muscle, muscle proteinases were shown to cause the degradation of fish muscle during iced storage.[7,28] The degradation of muscle proteins, particularly myosin, resulted in poor gelling properties of the surimi.[5]

SDS-PAGE patterns of muscle proteins indicated a decrease in myosin heavy chain (MHC) bands only in *P macracanthus* whole samples stored for 15 days (Fig 4). This result corresponds with the substantial increase in TCA-soluble peptides at day 15 (Fig 3). In spite of increased TCA-soluble peptides in whole samples of both species, especially at day 15 of storage, no marked decrease in MHC was observed by SDS-PAGE. This was possibly due to the proteolytic degradation of other cytosolic and cytoskeletal proteins present in the muscle during iced storage, caused by microbial growth and structural disintegration. Collagenase is capable of hydrolysing collagen to small peptides, leading to higher amounts of amino acids or

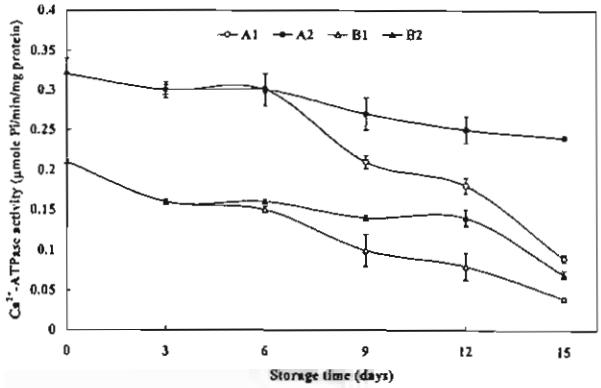

Figure 5. Changes in Ca^{2+}-ATPase of bigeye snapper muscle during iced storage. A1, *Priacanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated. Vertical bars represent standard deviation from triplicate determination.

peptides during extended storage. The solubility of Pacific rockfish muscle collagen increased as the raw muscle texture softened during iced storage.[29] The hydrolysis of fish muscle collagen appears to result from two heat-stable alkaline metalloproteinases.[30] Additionally, it was possible that endogenous proteinases in muscle and microbial proteinases cleaved the proteins extensively into small peptides. As a result, the fragment with high molecular weight could

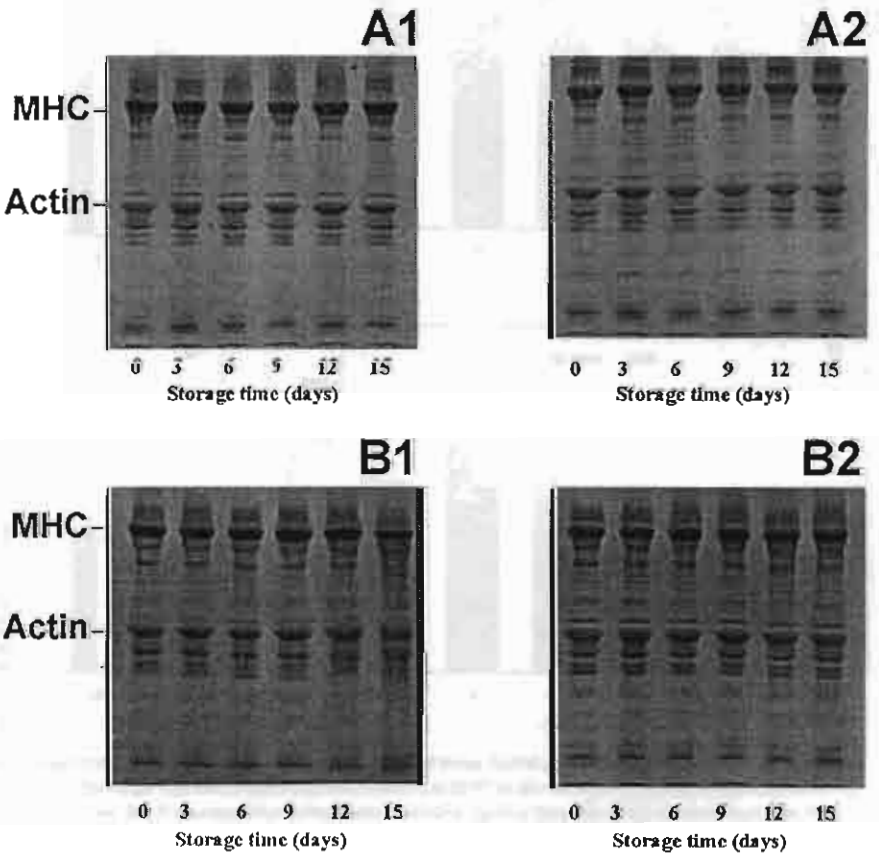

Figure 4. Electrophoretic pattern of bigeye snapper muscle protein during iced storage. A1, *Priacanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated.

not be observed by SDS-PAGE, and small degradation products of MHC could not be retained in the gel. However, no changes in actin were observed in fish muscle throughout 15 days of iced storage. MHC was more susceptible to proteolytic degradation than other muscle proteins, eg actin, troponin and tropomyosin.[7] In comparison with *P tayenus*, *P macracanthus* MHC seemed to be more susceptible to proteolytic degradation during iced storage. *P macracanthus* muscle displayed higher proteolytic activity than *P tayenus* muscle, as shown by autolytic activity assay.[31] The results suggested that pretreatment of *P macracanthus* by beheading and evisceration effectively protected myosin from degradation.

Changes in Ca^{2+}-ATPase activity

A decrease in Ca^{2+}-ATPase activity of extracted actomyosin was generally observed during iced storage, especially after 6 days of storage ($P<0.05$) (Fig 5). For the first 6 days of iced storage, only a slight decrease in Ca^{2+}-ATPase activity was observed in all samples. Thereafter a higher rate of decrease was observed with the actomyosin extracted from whole fish of both species compared with that extracted from pretreated fish. Kamal *et al*[32] reported a decrease in myofibrillar ATPase of sardine muscle during 10 days of iced storage. Ca^{2+}-ATPase is considered as an indicator of myosin integrity.[7,33] Since no marked changes in MHC were observed throughout the storage period, the continuous decrease in Ca^{2+}-ATPase of bigeye snapper natural actomyosin during iced storage was presumed to be due to the structural alteration of native myosin rather than proteolytic degradation. The native conformation of myosin is of primary importance for proper gelation. Maximum gel strength cannot be obtained if myosin is denatured before gelation is initiated.[5,34] The denaturation of myosin was possibly caused by changes in sulphydryl groups through oxidation of SH groups or disulphide interchanges.[7] Benjakul *et al*[35] reported a decrease in sulphydryl groups of natural actomyosin extracted from two species of bigeye snapper during iced storage. The conformational changes of myosin could be indicated by the increase in surface hydrophobicity of actomyosin during iced storage.[7] Therefore the changes in myosin integrity during iced storage directly affected the gelation of surimi. From the results, pretreatment of fish before storage can be another approach to retard the denaturation of myosin during iced storage.

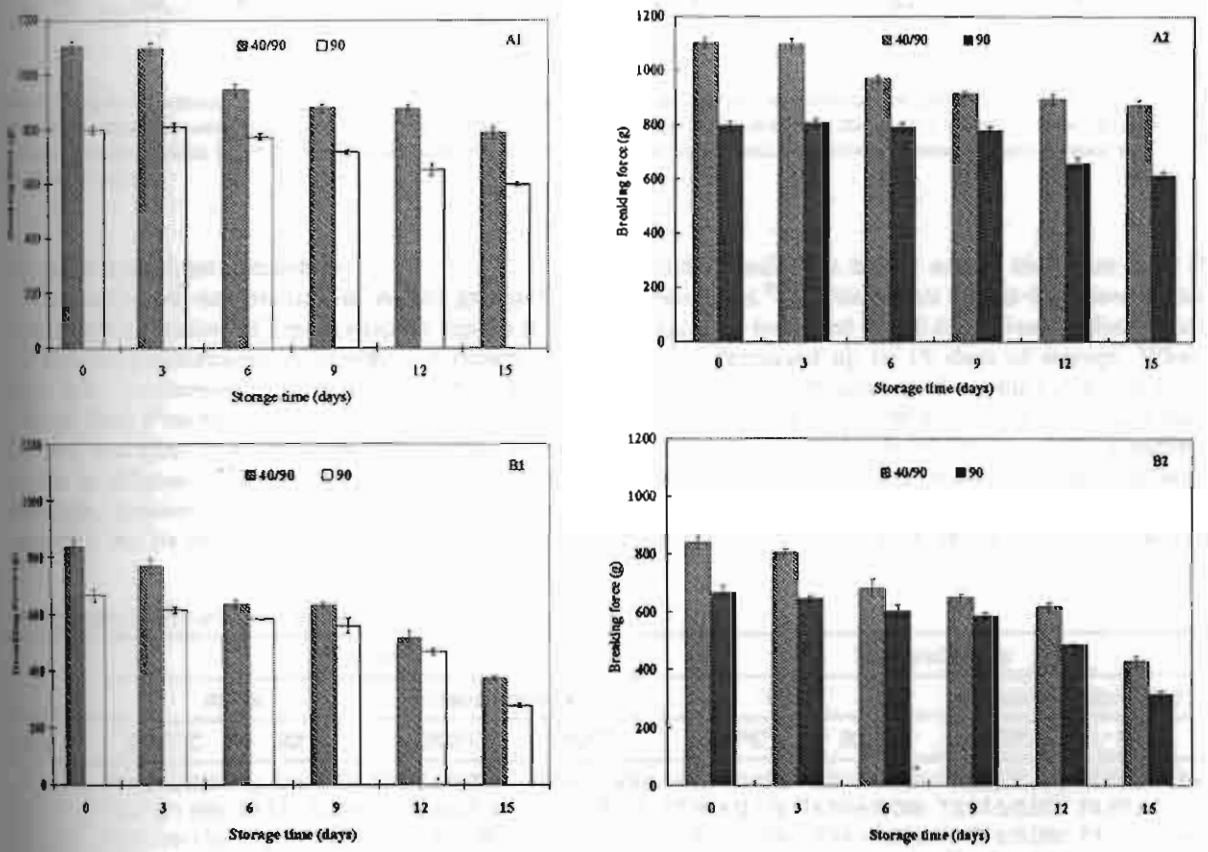

Figure 5. Changes in breaking force of surimi gel prepared from bigeye snapper stored in ice for different times. Surimi gel was prepared by incubating surimi sol at 40°C for 30 min, followed by heating at 90 °C for 20 min. Surimi gel was also prepared by direct heating at 90 °C for 20 min. A1, *Priancanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated. Vertical bars represent standard deviation from triplicate determination.

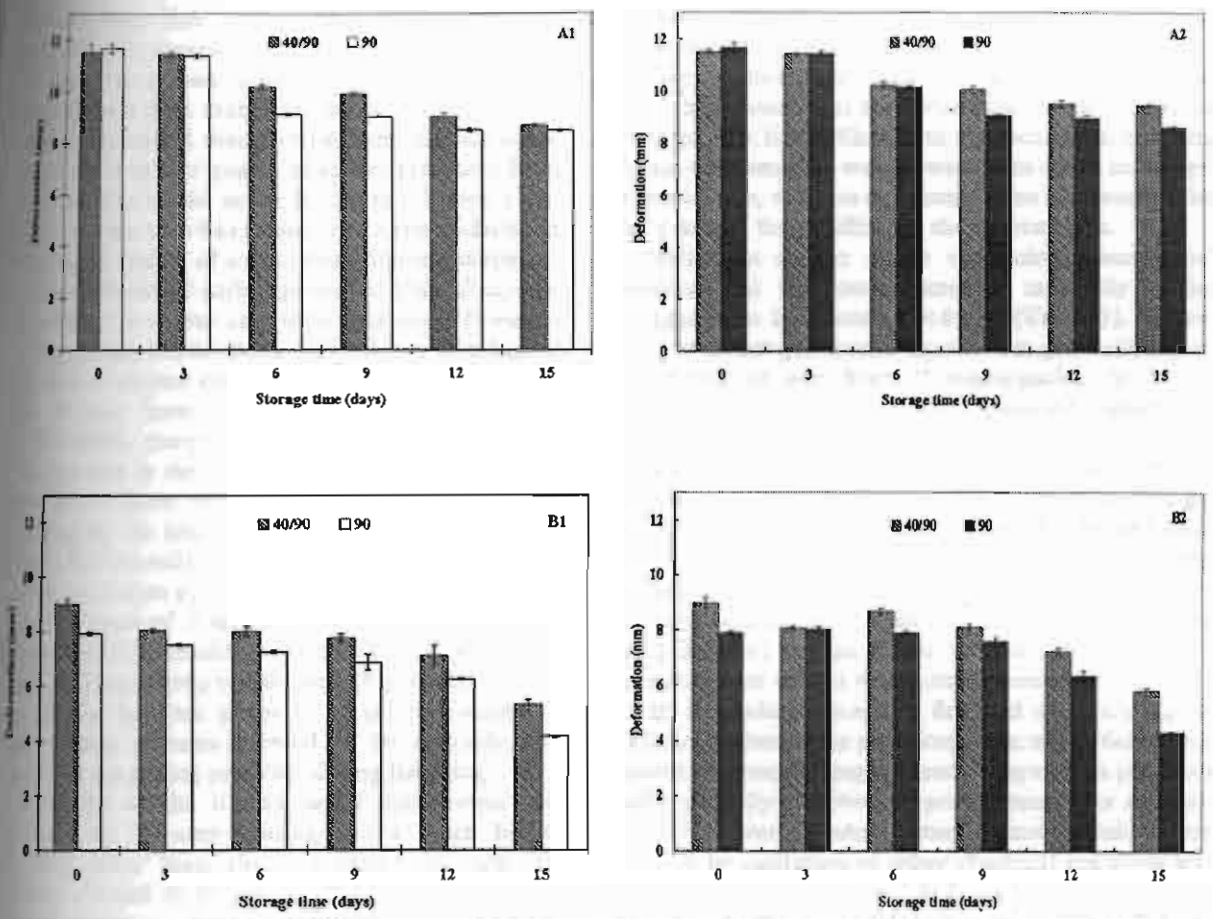

Figure 7. Changes in deformation of surimi gel prepared from bigeye snapper stored in ice for different times. Surimi gel was prepared by incubating surimi sol at 40°C for 30 min, followed by heating at 90°C for 20 min. Surimi gel was also prepared by direct heating at 90°C for 20 min. A1, *Priacanthus tayenus* whole; A2, *P tayenus* beheaded/eviscerated; B1, *P macracanthus* whole; B2, *P macracanthus* beheaded/eviscerated. Vertical bars represent standard deviation from triplicate determination.

Changes in surimi gel properties

Breaking force and deformation of surimi gels prepared from both species of bigeye snapper kept in ice with different pretreatment conditions are shown in Figs 6 and 7 respectively. At death (day 0), surimi produced from *P tayenus* exhibited a higher breaking force than that from *P macracanthus* ($P < 0.05$). This was due to differences in the inherent properties of actomyosin. Actomyosin from *P tayenus* underwent aggregation via hydrophobic interaction and/or disulphide bonds to a higher extent than that from *P macracanthus*.[9] Breaking force of gels decreased as the storage time increased ($P < 0.05$). Breaking force of all samples decreased up to 15 days of storage. When considering the gel quality of surimi produced from fish stored for up to 12 days in ice, it was found that surimi prepared from whole fish had only a slightly lower breaking force than that from beheaded/eviscerated fish at the same period of storage. However, marked differences in breaking force between surimi

Table 1. Whiteness of surimi gels from bigeye snapper during iced storage[a]

	P tayenus				P macracanthus			
	Whole		*Beheaded/eviscerated*		*Whole*		*Beheaded/eviscerated*	
Storage time (days)	*40/90°C*	*90°C*	*40/90°C*	*90°C*	*40/90°C*	*90°C*	*40/90°C*	*90°C*
0	77.22±0.20a	79.79±0.23a	77.22±0.20a	79.79±0.23a	74.83±0.42a	75.61±0.08a	74.83±0.42a	75.61±0.08a
3	74.81±1.69b	78.57±0.29b	74.83±0.24b	78.97±0.35b	72.35±0.12b	74.50±0.08b	73.81±0.95b	74.47±0.10b
6	74.07±0.11c	75.56±0.25c	74.52±0.17cd	75.21±0.17c	72.81±0.35b	73.94±0.39c	72.72±0.09c	73.39±0.34c
9	73.08±0.28d	74.28±0.24d	74.30±0.14d	75.02±0.14d	71.54±0.17c	72.10±0.08d	69.23±0.24d	70.95±0.52d
12	71.80±0.23e	73.04±0.18e	73.56±0.10e	74.35±1.02e	70.84±0.36d	71.31±0.38e	68.88±0.04d	69.23±0.32e
15	71.13±0.23f	72.21±0.18f	73.00±0.18f	74.03±1.53f	69.11±0.26e	70.23±0.52f	68.17±0.06e	67.61±0.64f

[a] Values are mean±standard deviation ($n=5$). Values with the same letter within a column are not significantly different ($P > 0.05$).

produced from fish under different pretreatment conditions were observed at day 15 ($P < 0.05$). Surimi produced from pretreated fish exhibited a much higher breaking force than that from whole fish. From the results it was noted that pretreatment did not significantly affect the gel quality of surimi produced from bigeye snapper stored in ice for up to 12 days. Conversely, storage time was found to be a crucial factor in reducing gel quality of surimi from bigeye snapper.

Breaking force of surimi produced from *P tayenus* decreased at a slower rate than that from *P macracanthus*. At day 15, breaking force of two-step heated gels from *P tayenus* decreased to 79.6–81.3%, while that of gels from *P macracanthus* decreased to 51.6–73.36%, compared with the values at day 0. This was due to the fact that *P macracanthus* underwent much faster deterioration than *P tayenus*, as observed by the higher contents of TVB and TMA (Fig 2). Additionally, myofibrillar proteins of *P macracanthus* were more prone to denaturation and degradation than those of *P tayenus*, as shown by the higher content of TCA-soluble peptides (Fig 3) and lower Ca^{2+}-ATPase activity respectively (Fig 5). Apart from the inferior inherent property of gel formation,[9] *P macracanthus* proteins seemed to be unstable and easily lost the gelling property during handling.

From the results it was noted that surimi gels prepared by two-step heating had a much higher breaking force than those prepared by one-step heating. Surimi from bigeye snapper exhibited the high-temperature setting phenomenon, probably caused by endogenous transglutaminase. Bigeye snapper muscle contained transglutaminase, which could be inhibited by EDTA and *N*-methylmaleimide (Benjakul S and Visessanguan W, unpublished). Transglutaminase was also found in fish muscle and mediated the formation of cross-linked myosin heavy chain.[36,37] Large differences in breaking force between one-step and two-step heated gels were found in surimi produced from *P tayenus* kept throughout 15 days of iced storage, while smaller differences were observed in surimi prepared from *P macracanthus*, particularly after 3 days of storage. The results implied that *P macracanthus* possibly possessed endogenous transglutaminase with a lower activity and stability and/or unsuitable protein conformations for non-disulphide covalent cross-linking.

Surimi gels from *P tayenus* showed higher deformation than those from *P macracanthus* in all conditions studied ($P < 0.05$). Similar to the changes in breaking force, pretreatment was shown not to affect surimi gel deformation, whereas the storage time was found to be an essential factor affecting the deformation.

Whiteness is one factor determining surimi gel quality. Gel whiteness decreased markedly as the storage time increased ($P < 0.05$) (Table 1). Whiteness of surimi gels from *P tayenus* was generally higher than that of gels from *P macracanthus* ($P < 0.05$) (Table 1). During iced storage, oxidised pigments in fish muscle, particularly metmyoglobin or methaemoglobin, possibly adducted to muscle and could not be totally removed by washing. As a result, surimi gel produced from fish kept for a longer time showed some discolouration. Since myoglobin is retained by the intracellular structure,[38] it most likely contributes to the appearance more than haemoglobin does during handling and storage. When comparing the effect of pretreatment on gel whiteness, surimi gels produced from beheaded/eviscerated fish had a slightly higher whiteness than those produced from whole fish. Some blood and internal organs containing various pigments were partially removed by pretreatment. As a consequence, fewer pigments remained and discolouration caused by oxidation or other chemical reactions was reduced. However, the whiteness of surimi from pretreated *P macracanthus* was lower than that of surimi from whole fish when fish were stored for more than 9 days (Table 1). The discrepancy was presumably related to the high protein degradation found in pretreated *P macracanthus* muscle. Although fish were gutted, substantial amounts of blood remained in the muscle.[39] Therefore protein degradation may allow liberation of haemoglobin as well as myoglobin, which are susceptible to oxidation and could also accelerate lipid oxidation. Evisceration results in exposing the belly area and cut surfaces to the air, thereby rendering them more susceptible to oxidation and discolouration.[19] Furthermore, evisceration has been known to increase lipid oxidation and the oxidation of pigments in some fish species. Oxidised

Table 2. Expressible moisture of surimi gels from bigeye snapper during iced storage[a]

Storage time (days)	P tayenus				P macracanthus			
	Whole		Beheaded/eviscerated		Whole		Beheaded/eviscerated	
	40/90°C	90°C	40/90°C	90°C	40/90°C	90°C	40/90°C	90°C
0	0.92±0.05e	0.98±0.05f	0.92±0.05e	0.98±0.05d	1.14±0.04f	1.28±0.03e	1.14±0.04e	1.28±0.03d
3	1.01±0.08e	1.11±0.05e	1.03±0.02d	1.04±0.03cd	1.21±0.02e	1.35±0.06d	1.16±0.04e	1.29±0.02d
6	1.13±0.09de	1.17±0.05d	1.11±0.06c	1.11±0.07c	1.29±0.01d	1.37±0.02d	1.25±0.02d	1.32±0.02d
9	1.20±0.08c	1.25±0.05c	1.12±0.07c	1.15±0.02c	1.45±0.05c	1.57±0.02c	1.32±0.02c	1.52±0.02c
12	1.34±0.02b	1.59±0.01b	1.29±0.03b	1.30±0.02b	1.30±0.02b	1.76±0.02b	1.55±0.05b	1.70±0.04b
15	1.65±0.02a	1.89±0.01a	1.34±0.05a	1.43±0.01a	1.43±0.04a	1.92±0.03a	1.77±0.05a	1.89±0.02a

[a] Values are mean ± standard deviation ($n = 5$). Values with the same letter within a column are not significantly different ($P > 0.05$).

them proteins and lipid oxidation products would subsequently bind to myofibrillar proteins, leading to the changes in whiteness. Although gels prepared by two-step heating had a higher breaking force and deformation, they showed a lower whiteness compared with those prepared by direct heating. From the results, both pretreatment and storage time directly affected the whiteness of surimi gels from both species of bigeye snapper.

Expressible drip of surimi from both species increased with increasing storage time of fish ($P < 0.05$) (Table 2). *P macracanthus* surimi gel generally exhibited higher expressible drip than *P tayenus* surimi gel. When fish were kept for a longer time, proteins were more degraded and lost their functionality, including gelation as well as water-holding capacity. As a result, less water was imbibed in the gel network, leading to higher drip. No marked effects of pretreatment on expressible drip were observed among surimi gels prepared from pretreated or whole fish stored for up to 6 days. However, pretreatment showed a marked effect on water holding in surimi gel from *P tayenus* stored for more than 9 days, but had no effect on surimi from *P macracanthus*. This was possibly associated with the protein denaturation and degradation in *P macracanthus*, as indicated by the decreased Ca^{2+}-ATPase (Fig 5) and increased TCA-soluble peptides (Fig 3). In addition, higher lipid and pigment oxidation of pretreated *P macracanthus* stored for a longer time presumably occurred, leading to the loss of protein functionality, especially water-holding capacity. When comparing the gels prepared by direct heating and two-step heating, it was generally found that the two-step heating process led to lower expressible drip than the direct heating process, suggesting that more water was retained in the gel matrix. However, higher expressible drip was found in two-step heated gels of surimi produced from pretreated *P macracanthus* stored for more than 12 days. During direct heating, rapid unfolding of proteins results in more intense coagulation. More water is released from the gel, and the protein dispersion becomes very uneven.[34] Generally, an increase in expressible drip in surimi gels correlated with a decrease in gel strength. Those changes became more intense with surimi produced from bigeye snapper kept in ice for a longer time.

CONCLUSION

Prolonged iced storage of fish resulted in substantial decreases in gel strength and whiteness of surimi from both species of bigeye snapper. Beheading and evisceration of fish prior to storage improved storage stability of fish muscle but had no significant effect on gelling ability. Loss of gel-forming ability of fish muscle during iced storage was mainly caused by denaturation of muscle proteins and to a lesser extent by proteolytic degradation.

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Chapter 2

Proteolysis and setting phenomenon of surimi from two species of bigeye snapper, *Priancathus tayenus* and *Priancanthus macracanthus*