รายงานวิจัยฉบับสมบูรณ์

โครงการ การปรับปรุงคุณภาพผลิตภัณฑ์ประมงและการใช้ประโยชน์สูงสุดของผลพลอยได้จากการแปรรูปสัตว์น้ำ

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สัญญาเลขที่ RTA 5180006

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มหาวิทยาลัยสงขลานครินทร์

อ.หาดใหญ่ จ.สงขลา

สนับสนุนโดยสำนักงานคณะกรรมการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

ขอขอบพระคุณสำนักงานกองทุนสนับสนุนการวิจัย และสำนักงานคณะกรรมการอุดมศึกษา สำหรับการสนับสนุนทุนส่งเสริมกลุ่มวิจัยเรื่อง “การปรับปรุงคุณภาพผลิตภัณฑ์ประมงและการใช้ประโยชน์สูงสุดของผลพลอยได้จากการแปรรูปสัตว์น้ำ” อันก่อให้เกิดองค์ความรู้ใหม่และเทคโนโลยีที่เป็นประโยชน์ต่อการพัฒนาอุตสาหกรรมสัตว์น้ำของไทย รวมทั้งได้สร้างกลุ่มวิจัยทางด้านสัตว์น้ำที่เข้มแข็งเพื่อการพัฒนาประเทศต่อไป

ศาสตราจารย์ ดร.สุทธวัฒน์ เบญจกุล

บทคัดย่อ

จากการศึกษาการปรับปรุงคุณภาพของผลิตภัณฑ์ประมงโดยเน้นศึกษาเกี่ยวกับซูริมิและเจลซูริมิ ของปลาผิวน้ำซึ่งมีเนื้อดำสูง เช่น ปลาแมคเคอเรลหลายชนิด ที่จับในภาคใต้ของประเทศไทย พบว่า ซูริมิจากปลาดังกล่าวสามารถมีความแข็งแรงเจลเพิ่มขึ้นโดยการผสมกับซูริมิจากปลาเนื้อขาว เช่น ปลาจวด หรือการใช้กระบวนการละลายด้วยด่างในการผลิตซูริมิ นอกจากนี้การปรับปรุงเจลซูริมิสามารถกระทำได้โดยการใช้สารประกอบฟีนอลิกที่ผ่านกระบวนการออกซิเดชัน โดยเฉพาะกรดแทนนิก ซึ่งได้จากทางการค้าและจากสารสกัดจากธรรมชาติ โดยเฉพาะไม้เคี่ยม กรดแทนนิกที่ผ่านกระบวนการออกซิเดชัน สามารถเหนี่ยวนำให้เกิดการเชื่อมประสานโปรตีนกล้ามเนื้อในซูริมิ ส่งผลให้โครงข่ายเจลมีความแข็งแรงเพิ่มขึ้น

สำหรับการศึกษาการใช้ประโยชน์จากวัสดุเศษเหลือการแปรรูปสัตว์น้ำ เช่น เครื่องใน หนัง เป็นต้น โดยได้สกัดโปรตีเอสและจำแนกคุณลักษณะของโปรตีเอสจากเครื่องในปลาชนิดต่างๆ ซึ่งประกอบด้วย เปปซิน ทริปซิน โดยเปปซินสามารถใช้ในการย่อยโปรตีนเพื่อผลิตโปรตีนไฮโดรไลเสตจากเนื้อปลา ส่วนทริปซินใช้ผลิตไฮโดรไลเสตจากเนื้อปลาและเจลาติน ซึ่งมีฤทธิ์ทางชีวภาพ เช่นฤทธิ์ต้านอนุมูลอิสระ และฤทธิ์ยับยั้งเอนไซม์ ACEโดยเฉพาะเมื่อใช้ร่วมกับโปรตีเอสจากจุลินทรีย์ทางการค้าภายใต้สภาวะที่เหมาะสม นอกจากนี้มีการศึกษาเกี่ยวกับโปรตีเอสจากพืช เช่น ยางต้นรัก และสารยับยั้งโปรตีเอสจากเมล็ดถั่วต่างๆ เพื่อเป็นแนวทางพัฒนาการใช้ประโยชน์สำหรับการแปรรูปสัตว์น้ำ โดยได้มีการแยกส่วนโปรตีเอสและสารยับยั้งโปรตีเอสโดยใช้ระบบ Aqueous two-phase และ three-phase

จากการพัฒนากระบวนการสกัดคอลลาเจนและเจลาตินจากหนังปลา โดยใช้เปปซินจากปลาเพื่อเพิ่มผลผลิตพบว่า ได้ผลผลิตเพิ่มขึ้น และไม่มีผลต่อสมบัติของคอลลาเจนและเจลาตินที่ได้ เมื่อศึกษาการปรับปรุงสมบัติการเกิดเจลของเจลาตินจากหนังปลา พบว่า การปฏิบัติเบื้องต้นต่อหนังปลาโดยใช้กรดฟอสฟอริกเพื่อเติมหมู่ฟอสเฟตให้กับเจลาตินส่งผลให้ความสามารถในการรวมตัวของโมเลกุลเจลาตินระหว่างการเกิดเจลสูงขึ้น นอกจากนี้ได้มีการศึกษาบทบาทของโปรตีเอสที่อยู่ภายในหนังปลาต่อการย่อยสลายโปรตีนและสมบัติการเกิดเจล พบว่า การป้องกันการย่อยสลายโปรตีน โดยใช้สารยับยั้งโปรตีเอสสามารถรักษาความยาวโซ่ของโมเลกุลเจลาติน ส่งผลให้ความสามารถในการเกิดเจลเพิ่มขึ้น และเจลาตินจากหนังปลาสามารถนำมาใช้เป็นชีววัสดุสำหรับเตรียมฟิล์มบริโภคได้ โดยฟิล์มมีสมบัติป้องกันการส่องผ่านของแสงยูวี

นอกจากนี้ได้แยกและจำแนกจุลินทรีย์จากสัตว์น้ำหมักหลายชนิด โดยเฉพาะสายพันธุ์ใหม่ เช่น *Natrinema gari* sp. Nov จากน้ำปลา และ *Halobacterium piscisalsi* sp. Nov จากปลาร้า โดย *Natrinema gari* BCC 24369 สามารถสลายฮิสตามีนในน้ำปลา ส่วนแบคทีเรียแลกติกที่แยกจากปลาส้ม สามารถใช้ผลิตเป็นกล้าเชื้อเพื่อผลิตปลาส้มที่มีคุณภาพสม่ำเสมอ

ดังนั้นงานวิจัยนี้จึงให้ข้อมูลเพื่อความเข้าใจที่เพิ่มขึ้นเกี่ยวกับการปรับปรุงคุณภาพผลิตภัณฑ์ประมง และการใช้ประโยชน์จากวัสดุเศษเหลือจากการแปรรูปสัตว์น้ำ ส่งผลให้มีการใช้ประโยชน์สำหรับผู้ประกอบการ องค์กร และหน่วยงานที่เกี่ยวข้องในอนาคตเพิ่มมากขึ้น

คำหลัก ผลพลอยได้ สัตว์น้ำ คุณภาพ คอลลาเจน โปรตีเอส เจลลาติน ฟิลม์ ซูริมิ เจล จุลินทรีย์ ฤทธิ์ทางชีวภาพ

สารประกอบฟีนอลิก หนัง เครื่องใน

Abstract

The improvement of fishery products mainly focused on surimi and surimi products was carried out. Pelagic dark-fleshed fish including several kinds of mackerel caught in the southern part of Thailand were used for their gelling property. The gel strengthening of surimi from those species was achieved by blending with surimi from lean fish, e.g. croaker or the use of alkaline aided process. Additionally, the gel properties of surimi could be improved by using the oxidized phenolic compounds, especially tannic acid, both commercial susbtance or natural extract, particularly from kiam wood. Oxidized phenolic compounds were able to induce the cross-linking of muscle proteins in surimi, thereby strengthening the gel network.

Utilization of fish processing byproducts including viscera, skin, etc were investigated. Proteases including pepsin and trypsin from viscera of different fish species were successfully extracted and characterized. Fish pepsin was used to prepare the protein hydrolysate from fish muscle. Fish trypsin was also implemented to produce protein hydrolysate from fish muscle and fish gelatin with bioactivity including antioxiative activity and ACE inhibitory activity. Maximized use of fish protease was accomplished when it was used in conjunction with the selected commercial microbial proteases under the optimal condition. The study of plant protease, especially from the latex of *Calotropis procera*, and protease inhibitor from several legume seeds, was extended to serve as the possible novel processing aids for fish processing. Partitioning using aqueous two phase and three phase systems were successfully implemented to recover those proteases and protease inhibitors.

When collagen and gelatin from fish skin was extracted by the developed method using fish pepsin as the processing aid, the extraction yield was increased and it had no detrimental effect on the properties of resulting collagen and gelatin. Furthermore, the property of gelatin from skin, particularly gelling property was improved by the pretreatment with phosphoric acid prior to extraction. The incorporation of phosphate group mainly contributed to the enhanced interaction of gelatin molecules during gelation process. The role of endogenous protease associated with the skin in protein degradation and gelatin properties was elucidated. The prevention of degradation using the selected protease inhibitor was found to maintain the chain length, thereby improving the properties of resulting gelatin. Fish gelatin was used as a biomaterial for edible film preparation. The film showed the excellent UV light barrier property.

Microorganisms from fermented fishery products were isolated and identified. *Natrinema gari* sp. Nov was isolated from fish sauce, while *Halobaterium piscisalsi* sp. Nov. was isolated from Pla-ra. *Natrinema gari* BCC 24369 was used for histamine degradation in fish sauce. Lactic acid bacteria were also isolated from Pla-som and the selected strain was used as the starter culture for production of Pla-som to ensure the constant quality of finished product.

As a whole, the researches provided the better understanding on the improvement of fishery products and the utilization of fish processing byproducts, in which the further implementation by fish processors or related association/organization can be achieved.

Keywords: byproducts, fish, quality, collagen, protease, gelatin, film, surimi, gel, microorganisms, bioactivity, phenolic compounds, skin, viscera

Chapter 1

Surimi and Improvement of Surimi Gel Properties

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Physicochemical properties and gel-forming ability of surimi from three species of mackerel caught in Southern Thailand

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ABSTRACT

Physicochemical and gelation properties of surimi prepared from three species of mackerel were investigated. The highest whiteness with the lowest redness index corresponding to the lowest myoglobin content especially its oxidised form, metmyoglobin, was found in short-bodied mackerel (*Rastrelliger brachysoma*) surimi ($p < 0.05$). Frigate mackerel (*Auxis thazard*) surimi contained the highest lipid content ($p < 0.05$). The pH of all surimi was in the range of 6.58–6.80. The highest sulfhydryl group and Ca^{2+}-ATPase activity was found in natural actomyosin extracted from short-bodied mackerel surimi ($p < 0.05$). The highest TCA-soluble peptide content was found in frigate mackerel surimi gels ($p < 0.05$). Kamaboko gel of short-bodied mackerel surimi exhibited the highest breaking force with the lowest expressible drip ($p < 0.05$). Heating regime had no effect on deformability of gels from Indian mackerel (*Rastrelliger kanagurta*) and short-bodied mackerel but not for frigate mackerel. The highest metmyoglobin content with the lowest whiteness was found in frigate mackerel surimi gel ($p < 0.05$). Therefore, short-bodied mackerel was the best suited for the production of surimi with superior functional attributes including whiteness and gel-forming ability.

1. Introduction

Dark muscle fish species make up 40–50% of the total fish catch in the world (Hultin & Kelleher, 2000). There is a great interest in using the large quantities of these low value fatty pelagic fish for human food, particularly for surimi production. However, problems faced with producing surimi from small pelagic species, such as sardine and mackerel, is the high content of dark muscle associated with the high content of lipid and myoglobin (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). Those components contribute to the difficulties in making high-quality surimi (Chen, 2002; Ochiai, Ochiai, Hashimoto, & Watabe, 2001). The presence of sarcoplasmic proteins of dark muscle has often been cited as one of the reasons for the poorer gelation characteristics of dark muscle fish compared with light muscle (Haard, Simpson, & Pan, 1994). Sarcoplasmic proteins bind to the myofibrillar proteins and thus interfere with the formation of gels. Recently, Chaijan, Benjakul, Visessanguan, Lee, and Faustman (2007a, 2007b) reported that the interaction between fish myoglobin and natural actomyosin was enhanced at higher ionic strength and temperature and the binding was augmented with increasing incubation times especially in the presence of aldehydes. Hultin and Kelleher (2000), Haard et al. (1994) and Sikorski (1994) reported that small quantities of sarcoplasmic proteins had an adverse effect on the strength and deformability of myofibril protein gels. These proteins may interfere with myosin cross-linking during gel matrix formation because they did not form gels and had poorer water holding capacity. In addition, lipid oxidation seems to be a distinct problem in surimi made from some dark-fleshed fish. Fish muscle lipid oxidation may be the primary factor for limiting storage life, causing the formation of disagreeable flavours and leading to the denaturation of proteins and decreased gelling ability through peroxide formation (Lanier, 2000). Murakawa, Benjakul, Visessanguan, and Tanaka (2003) reported that oxidised lipids can interact with proteins, causing denaturation, polymerisation, changes in functional properties and bring about an adverse effect on the quality of surimi products. Although most of the depot fat is removed when fish are headed, gutted, and skinned, however, a small percentage of membrane phospholipids are present in fish muscle, which are difficult to remove by washing. These phospholipids are highly unsaturated and often in contact with muscle heme iron and are therefore sensitive to deterioration by oxidation (Lanier, 2000). Dark muscle fish also had a high proteolytic activity resulting in the poorer gelation characteristic and the high susceptibility to modori (Shimiza, Toyohara, & Lanier, 1992). Shimiza et al. (1992) also reported that the poor gel-forming properties of muscle from dark-fleshed species is caused by the presence of heat-stable

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proteases, which are active in degrading myosin during heating at temperature ranges of 50–70 °C. Ochiai et al. (2001) suggested that to prepare high-quality surimi and process it into a fish cake of higher gel strength and better whiteness, it was necessary to remove dark muscle as much as possible. However, in the case of red-fleshed fish such as mackerel and sardine, abundant dark muscle is difficult to remove with a meat separator (Ochiai et al., 2001). Hence, the washing process is necessary for colour improvement of products prepared from whole muscle.

Due to the limited fish resources, especially white muscle fish, dark muscle fish have been paid more attention as a potential alternative raw material for surimi production (Wu, Li, Ho, & Jiang, 2000; Chen, Chiu, & Huang, 1997; Jiang, 2000; Kelleher, Hultin, & Wilhelm, 1994). In 2006, the catch of pelagic fish in the Gulf of Thailand was approximately 844.2 metric tons (Department of Fisheries., 2006). Among all dark-fleshed fish species, mackerel was one of the most abundant species caught in Southern Thailand (Department of Fisheries, 2006). Therefore, the use of this small pelagic fish for surimi production is one of a major challenges in transforming underutilised fish protein resources into human foods, particularly protein gel-based products. However, the characteristics of surimi gel depended on the properties of myofibrillar proteins, which were affected by the species and freshness of the fish, as well as on the processing parameters (Niwa, 1992). The strength of gels is species characteristic (Niwa, 1992) and it depends on the method of cooking (Roussel & Cheftel, 1990). Therefore, the objective of this study was to investigate the characteristics and gel-forming ability of surimi from three species of mackerel caught in Southern Thailand.

2. Materials and methods

2.1. Chemicals

Sodium dodecyl sulphate (SDS), dithiothreitol (DTT), β-mercaptoethanol (βME) were purchased from Sigma (St. Louis, MO, USA). Trichloroacetic acid was obtained from Merck (Darmstadt, Germany). Acrylamide, *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and bis-acrylamide were obtained from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

2.2. Fish samples

Frigate mackerel (*Auxis thazard*) with an average weight of 95–110 g, Indian mackerel (*Rastrelliger kanagurta*) with an average weight of 87–90 g and short-bodied mackerel (*Rastrelliger branchysoma*) with an average weight of 80–82 g were caught from Thasala-Nakhon Si Thammarat Coast along the Gulf of Thailand. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat within 20 min. The fish were immediately washed, gutted, filleted, skinned and the whole muscles were collected. To prepare fish mince, fish fillets were minced to uniformity using a meat grinder (a 4 mm hole diameter; Panasonic MK-G20MR, Japan). The muscles were kept on ice during preparation.

2.3. Surimi and surimi gel preparation

To prepare surimi by conventional washing process, fish mince was washed with cold water (4 °C) (Chaijan et al., 2004) using a water/mince ratio of 3:1 (v/w). The mixture was stirred gently for 10 min in a cold room (4 °C) and the washed mince was filtered with a layer of nylon screen. Washing was performed three times. Finally, the washed mince was centrifuged at 700*g* for 15 min using a basket centrifuge (Model CE 21 K, Grandiumpiant, Belluno, Italy). The washed mince was added with 4% sucrose and 4% sorbitol mixed well and frozen using an air-blast freezer. The frozen samples referred to as 'surimi' were kept at −18 °C until used. The storage time was not more than 1 month.

To prepare the gels, the frozen surimi samples were thawed at 4 °C for 3–4 h until the core temperature reached 0 °C. The samples were then cut into small pieces and the moisture content was adjusted to 80% by the addition of iced water. The samples were added with 2.5% (w/w) NaCl and chopped for 5 min in a walk-in cold room at 4 °C to obtain the homogeneous sol. The sol was then stuffed into a polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. The sol was then incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min. This sample was referred to as 'kamaboko gel' (Chaijan et al., 2004). Modori gel was prepared by incubating the paste at 60 °C for 30 min, followed by heating at 90 °C for 20 min (Jiang, 2000). A directly cooked gel was heated at 90 °C for 20 min. After heating, all gels were immediately cooled in iced water for 30 min and stored for 24 h at 4 °C prior to analysis.

2.4. Determination of Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity of natural actomyosin (NAM) from surimi was determined according to the method of Benjakul, Seymour, Morrissey, and An (1997). NAM prepared as described by Benjakul et al. (1997) was diluted to 2.5–8 mg/ml with 0.6 M KCl, pH 7.0. Diluted NAM solution (1 ml) was added to 0.6 ml of 0.5 M Tris–maleate, pH 7.0. The mixture was added with 1 ml of 0.1 M $CaCl_2$. Deionised water was added to make up a total volume of 9.5 ml. A 0.5 ml of 20 mM adenosine 5′-triphosphate (ATP) solution was added to initiate the reaction. The reaction was conducted for 8 min at 25 °C and terminated by adding 5 ml of chilled 15% (w/v) trichloroacetic acid (TCA). The reaction mixture was centrifuged at 3500*g* for 5 min at 25 °C and the inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). The Ca^{2+}-ATPase activity was expressed as μmoles inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP.

2.5. Determination of reactive sulfhydryl (SH) content

Reactive SH content was measured using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) according to the method of Ellman (1959) as modified by Sompongse, Itoh, and Obataka (1996). The NAM sample (0.5 ml, 4 mg/ml) was added to 4.5 ml of 0.2 M Tris–HCl buffer, pH 6.8. Thereafter, 0.5 ml of 0.1% DTNB solution was added into the mixture and subjected to incubation at 40 °C for 25 min. Absorbance was measured at 412 nm using a Shimadzu UV-2100 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, Md., USA). A blank was prepared by replacing the sample with 0.6 M KCl, pH 7.0. SH content was calculated from the absorbance using the molar extinction of 13,600 $M^{-1}cm^{-1}$ and was expressed as mol/10^5 g protein.

2.6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of different surimi and surimi gels were visualised by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution were added to the sample (3 g). The mixture was homogenised for 1 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The sample was centrifuged at 8500*g* for 5 min at room temperature (26–28 °C) using a Biofuge primo centrifuge

(Sorvall, Hanau, Germany). Protein concentration was determined according to the Biuret method (Robinson & Hodgen, 1940), using bovine serum albumin as a standard. Solubilised samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS and 20% glycerol) in the presence or absence of 10% βME, representing reducing or non-reducing conditions, respectively. Samples (20 μg protein) were loaded onto polyacrylamide gels comprising a 10% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. A protein standard (Bio-Rad Laboratories, Inc., Richmond, CA, USA) containing myosin (206 kDa), β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), serum albumin (66.2 kDa) and ovalbumin (45 kDa) was used to estimate the molecular weight of the proteins.

2.7. TCA-soluble peptide

To 2 g of finely chopped samples, 18 ml of 5% TCA were added and homogenised for 2 min using IKA homogeniser at a speed of 11,000 rpm. The homogenate was incubated at 4 °C for 1 h and centrifuged at 8000*g* for 5 min. TCA-soluble peptides in the supernatant were measured according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and expressed as μmole tyrosine/g sample (Morrissey, Wu, Lin, & An, 1993).

2.8. Determination of myoglobin content

The extractable myoglobin content in surimi gels was determined by direct spectrophotometric measurement as described by Benjakul and Bauer (2001). A chopped sample (2 g) was weighed into a 50-ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer, pH 6.8 were added. The mixture was homogenised at 13,500 rpm for 10 s, followed by centrifuging at 3000*g* for 30 min at 4 °C. The supernatant was filtered with Whatman No. 1 filter paper. The supernatant (2.5 ml) was added with 0.2 ml of 1% (w/v) sodium dithionite to reduce the myoglobin. The myoglobin content was determined by direct spectrophotometric measurement at 555 nm. Myoglobin content was calculated from the millimolar extinction coefficient of 7.6 and a molecular weight of 16,110 (Gomez-Basauri & Regenstein, 1992). The myoglobin content was expressed as mg/g sample.

2.9. Determination of metmyoglobin

The oxidation of myoglobin of surimi and surimi gels was determined spectrophotometrically. Metmyoglobin was extracted from surimi and surimi gels by using the same procedure of myoglobin extraction. The ratio of A_{630}–A_{525} was calculated according to Hansen and Sereika (1969). A high A_{630}/A_{525} ratio indicates a high relative proportion of metmyoglobin.

2.10. Lipid extraction

The lipid was extracted by the Bligh & Dyer method (1959). The sample (25 g) was homogenised with 200 ml of a chloroform:methanol:distilled water mixture (50:100:50) at a speed of 9500 rpm for 2 min at 4 °C using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was treated with 50 ml of chloroform and homogenised at 9500 rpm for 1 min. Then, 25 ml of distilled water were added and homogenised again for 30 s. The homogenate was centrifuged at 3000*g* at 4 °C for 15 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA), and transferred into a separating flask. The chloroform phase was drained off into the 125 ml Erlenmeyer flask containing about 2–5 g of anhydrous sodium sulphate, shaken well, and decanted into a round-bottom flask through Whatman No. 4 filter paper. The solvent was evaporated at 25 °C using an EYELA rotary evaporator N-100 (Tokyo, Japan), and the residual solvent was removed by flushing with nitrogen. The total lipid content was expressed as g/100 g sample.

2.11. Texture analysis

Texture analysis of the gels was performed using a TA-XT2i texture analyser (Stable Micro Systems, Godalming, Surrey, UK). Gels were equilibrated and evaluated at room temperature (28–30 °C). Seven 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 spherical plunger (diameter 5 mm, depression speed of 60 mm/min).

2.12. 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 Park (1994) as follows:

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

2.13. Determination of expressible drip

Expressible drip was measured according to the method of Ng (1987). A gel sample with a thickness of 0.5 cm was weighed and placed between two pieces of Whatman filter paper No. 1 at the top and three pieces of the same type of filter paper at the bottom. The standard weight (5 kg) was placed on the top of the sample and maintained for 2 min. The sample was then removed and weighed again. Expressible drip was calculated and expressed as percentage of sample weight.

2.14. Statistical analysis

For each experiment, fishes were considered experimental units, each receiving 1 of 3 treatments in a completely randomized design ($n = 3$). Different lots of surimi were produced from each species of mackerel. For each lot, all parameters studied except for texture analysis were determined in triplicate. For texture analysis, seven replications were conducted. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 8.0 for windows, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Chemical compositions and physicochemical properties of surimi

Colour, pigment, pH and lipid content of surimi prepared from three species of mackerel are shown in Table 1. From the result, the highest whiteness with the lowest redness index was found in the short-bodied mackerel surimi ($p < 0.05$). The lightest colour of short-bodied mackerel surimi was related to the lowest content of myoglobin especially its oxidised form, the metmyoglobin. The myoglobin content of frigate mackerel surimi was 1.61 and 2.66 times higher than those of Indian mackerel surimi and short-bodied mackerel surimi, respectively. After washing with cold water

Table 1
Colour, pigment, lipid content and pH of surimi prepared from three species of mackerel.

Parameter	Indian mackerel	Short-bodied mackerel	Frigate mackerel
Colour			
redness index	0.13 ± 0.01b	−0.18 ± 0.01a	0.16 ± 0.01c
whiteness	52.35 ± 0.36b	59.14 ± 0.43c	50.56 ± 0.36a
Pigment			
myoglobin (mg/g)	4.13 ± 0.09b	2.50 ± 0.11a	6.64 ± 0.31c
relative metmyoglobin (A_{630}/A_{525})	0.44 ± 0.05b	0.25 ± 0.02a	0.65 ± 0.09c
Lipid (g/100 g)	0.78 ± 0.02b	0.53 ± 0.01a	1.00 ± 0.01c
pH	6.67 ± 0.02b	6.80 ± 0.08c	6.58 ± 0.01a

Values are given as mean ± SD from triplicate determinations.
Different letters within the same row indicate significant differences ($p < 0.05$).

three times, some residual myoglobin especially ferric-metmyoglobin associated with muscle component might cause the darkening of surimi. Frigate mackerel surimi contained the highest content of lipids, followed by Indian mackerel surimi and short-bodied mackerel surimi, respectively ($p < 0.05$). Kisia (1996) reported that dark-fleshed fish contained more dark muscle fibres, and more mitochondria, myoglobin, fats, glycogen and cytochromes, than the white-fleshed fish species did. The initial pH of surimi was found in the range of 6.58–6.80. The ultimate pH value of fish muscle is around 6.2–6.6 (Foegeding, Lanier, & Hultin, 1996). Huss (1988) suggested that the initial post-mortem pH of fish varies with species, catching ground, and season. From the results, the lowest pH exhibited the higher relative metmyoglobin content. Kitahara, Matsuoka, Kobayashi, and Shikama (1990) reported that at acidic pHs, the proton-catalysed displacement process was mainly responsible for promoting the autoxidation reaction of the myoglobin. Chaijan, Benjakul, Visessanguan, Lee, and Faustman (2007c) reported that the greatest metmyoglobin formation in sardine muscle was found at pHs of 5–6. The lowest pH value of surimi was found in frigate mackerel. This was possibly due to the high content of dark muscle composed in frigate mackerel flesh. The dark muscle contained more storage glycogen and it underwent changing to lactic acid during post-mortem glycolysis. As a consequence, more glycogen was stored and a lower pH was found in the muscle. In addition, the activity of enzymes converting glycogen into lactic acid might be different between three species. Lactic acid, generated in anoxic conditions from glycogen, is the principal factor in lowering the post-mortem pH in the fish muscles (Sikorski, Kolakowska, & Burt, 1990).

3.2. Reactive SH content and Ca^{2+}-ATPase activity of natural actomyosin (NAM)

Reactive SH content and Ca^{2+}-ATPase activity of NAM extracted from surimi of three fish species are presented in Fig. 1A and B, respectively. From the result, the highest content of SH group was found in NAM extracted from short-bodied mackerel surimi ($p < 0.05$). No difference in SH content was found in NAM extracted from Indian mackerel and frigate mackerel surimi ($p > 0.05$). From the results, some SH groups might localise on the protein surface and they were reactive. A fish myosin molecule contains many SH groups (Buttkus, 1970). SH groups located in the head portion (SH_1 and SH_2) play an essential role in ATPase activity (Kielley & Bradley, 1956). In addition, the presence of the SH group in all surimi was probably due to the partial denaturation of protein after the 3-cycle washing. This resulted in the exposure of some buried SH groups which can undergo oxidation during heat treatment. The presence of SH groups in surimi was necessary to gel strengthening. High temperatures during heating led to further oxidation of

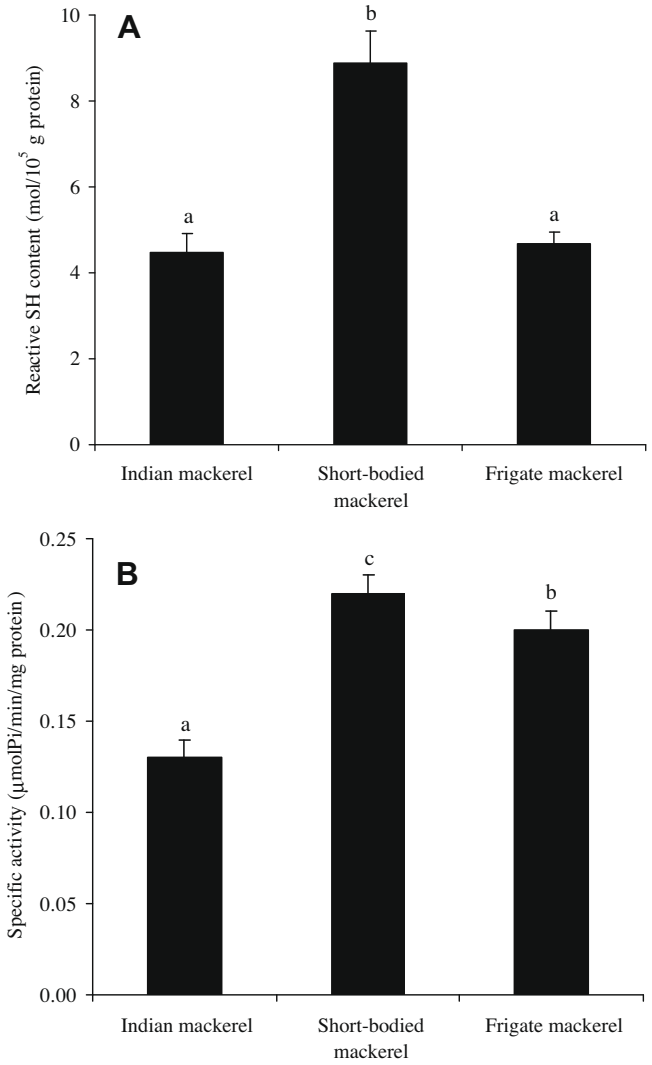

Fig. 1. Reactive SH content (A) and Ca^{2+}-ATPase activity (B) of NAM extracted from surimi prepared from three species of mackerel. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

SH groups with a subsequent disulfide bond formation (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001). SH groups localised in light meromyosin (SH_a) also contribute to oxidation (Sompongse et al., 1996). Angsupanich, Edde, and Ledward (1999) reported that a heat-set myosin gel is primarily stabilised by disulfide bonds and hydrophobic interactions.

The highest activity of Ca^{2+}-ATPase was also found in NAM extracted from short-bodied mackerel ($p < 0.05$; Fig. 1B) indicating the highest integrity of myosin molecule. The lowest Ca^{2+}-ATPase activity was seen in NAM extracted from Indian mackerel surimi ($p < 0.05$). From the results, the stability and integrity of the myosin molecule was dependent on fish species. Foegeding et al. (1996) reported that Ca^{2+}-ATPase activity is a sensitive indicator of myosin denaturation. Roura, Monteccia, Goldemberg, Trucco, and Crupkin (1990) reported that the myofibrillar ATPase activities have been widely used as a measure of actomyosin integrity. From the result, it can be postulated that short-bodied mackerel muscle might contain a number of calcium ions which can activate the myosin ATPase. The calcium ion was necessary for the activity of many enzymes including ATPase and endogenous transglutaminase (TGase). Accordingly, setting at 40 °C or cross-linking ability of myosin induced by TGase could occur to some extent resulting

from the presence of sufficient calcium ion. The setting of surimi is enhanced by sufficient calcium content since endogenous TGase is a Ca^{2+} dependent enzyme (Lee & Park, 1998; Kimura et al., 1991).

3.3. TCA-soluble peptide content of surimi and surimi gels

Generally, no differences in TCA-soluble peptide content of surimi prepared from three species of mackerel were found ($p > 0.05$; Fig. 2). After gel forming, the highest content of TCA-soluble peptide was found in frigate mackerel surimi gels ($p < 0.05$). From the results, the gels of Indian mackerel and short-bodied mackerel prepared with all heating conditions had the same content of TCA-soluble peptide ($p > 0.05$). The TCA-soluble peptide of gels from frigate surimi prepared with all heating regimes was higher than that of its original surimi indicating the proteolysis of frigate surimi occurred to some extent during heat treatment. In general, weakening of surimi gels occurred at temperature ranges of 50–70 °C (Jiang, 2000). This phenomenon, so-called modori, was induced by endogenous heat activated proteases, which can degrade myosin (Jiang, 2000).

3.4. Textural properties of surimi gels

Generally, the kamaboko gel of short-bodied mackerel surimi exhibited the highest breaking force, followed by directly heated gel and modori gel, respectively ($p < 0.05$; Fig. 3A). For Indian mackerel and frigate mackerel, no differences in breaking force were found in surimi gels prepared with and without setting at 40 °C ($p > 0.05$). With the same fish species, setting at 60 °C for 30 min prior to heating at 90 °C for 20 min (modori gel) rendered the gel with the lowest breaking force. This phenomenon occurred to the highest extent in frigate mackerel surimi. From the results, three species of mackerel might have the same level of endogenous TGase with the same activity but the content or activity of proteases might different, which was to be the highest in frigate mackerel. This result was in agreement with the TCA-soluble peptide content which was found to be the highest in Frigate mackerel surimi gels ($p < 0.05$; Fig. 2). As a consequence, setting at a temperature lower than 60 °C (optimum at 40 °C) was one of the ways to enhance the strength of gels prepared from these kinds of fish. The results indicated that protein–protein interactions were established in all cases

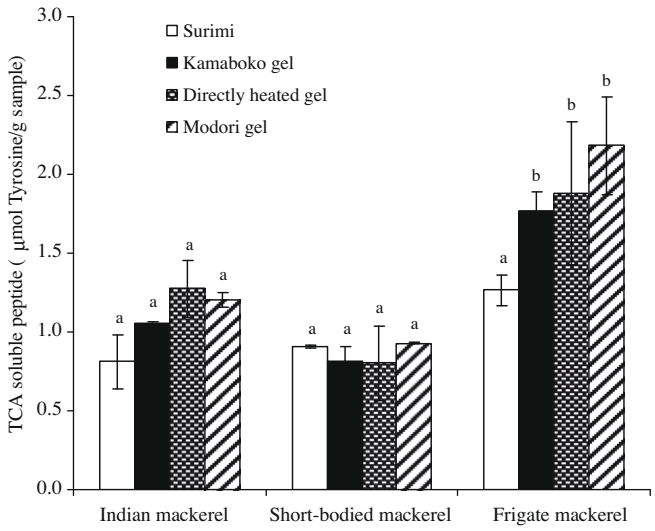

Fig. 2. TCA-soluble peptide content of surimi and surimi gels prepared from three species of mackerel. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

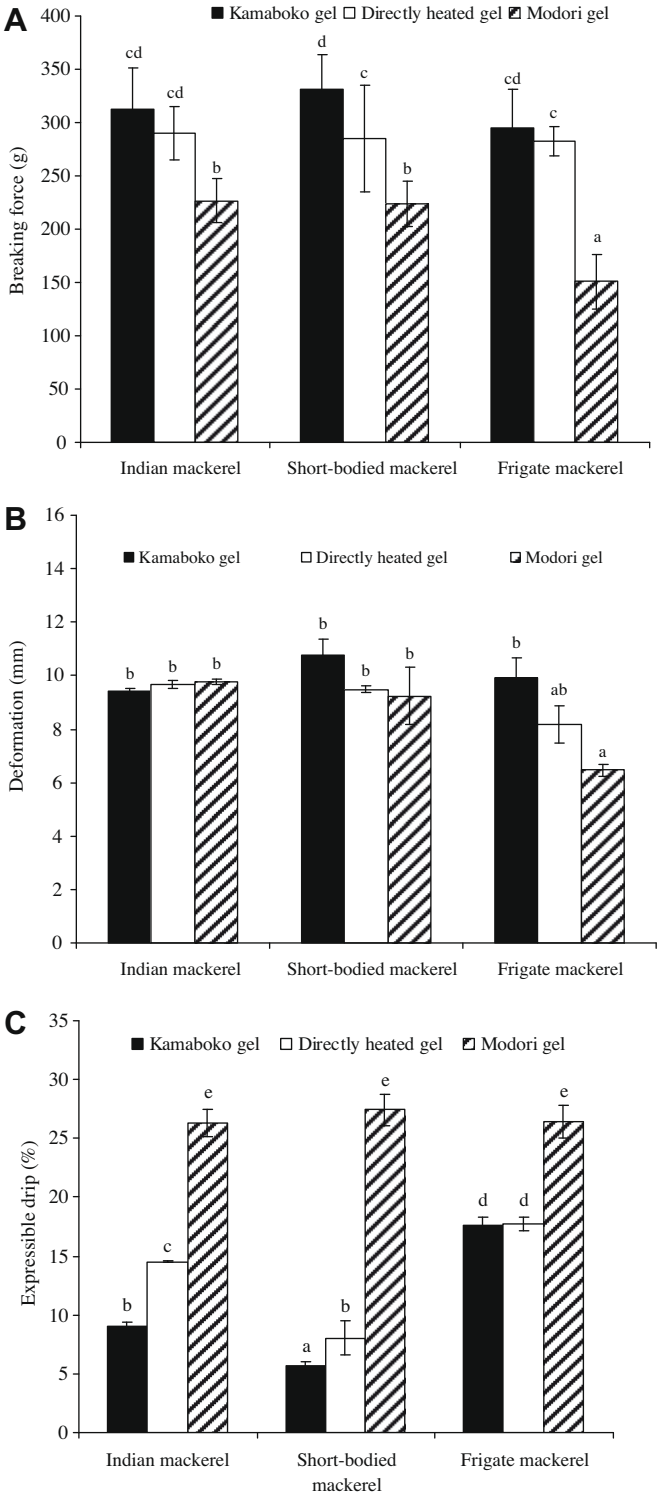

Fig. 3. Breaking force (A), deformation (B) and expressible drip (C) of surimi gels prepared from three species of mackerel. Bars represent the standard deviation from seven determinations for breaking force and deformation or from triplicate determinations for expressible drip. Different letters indicate significant differences ($p < 0.05$).

but a stronger gel was obtained in the kamaboko gel, which strengthened the network previously formed by setting at 40 °C. The gel-forming ability of dark muscle fish meat has been known to be lower than that of ordinary muscle. This apparently resulted from the difference in the unfolding abilities of the myosin between

the muscles. Lo et al. (1991) found that the head portions of the heavy chain of myosin from the dark and ordinary muscles did not differ significantly in the thermostability, but the ordinary muscle myosin had two thermal transition points, at 36 and 57 °C, whereas that of the dark muscle had only one transition point, at 68 °C. The pH of dark-fleshed fish mince decreased rapidly after slaughter, and the gel-forming ability was inhibited once the pH dropped to around its isoelectric point (Chen et al., 1997). Foegeding et al. (1996) reported that the isoelectric point of myosin is approximately pH 5.5. From the results, the pH of frigate mackerel surimi was in the vicinity of the isoelectric point of myosin. Therefore, the gel-forming ability of this surimi was found to be the lowest.

Elasticity of surimi gels from three species of mackerel is shown in Fig. 3B. The heating regime had no effect on deformability of gels from Indian mackerel and short-bodied mackerel ($p > 0.05$) but not for frigate mackerel. The deformation of frigate mackerel surimi gel prepared by setting at 40 °C for 30 min prior to heating at 90 °C for 20 min was higher than those by direct heating at 90 °C for 20 min and setting at 60 °C for 30 min prior to heating at 90 °C for 20 min, respectively ($p < 0.05$).

3.5. Expressible drip of surimi gels

The lowest expressible drip was found in kamaboko gel of short-bodied mackerel indicating the highest water holding capacity ($p < 0.05$; Fig. 3C). With the same fish species, setting at 60 °C prior to heating at 90 °C (modori gel) had the highest expressible drip ($p < 0.05$). This result was in agreement with the result of gel strength which was found to be the lowest in modori gel (Fig. 3A). 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 (Niwa, 1992). From the result, it was noted that the higher the breaking force, the lower the expressible drip. Among all heating regimes and fish species examined, kamaboko gel of short-bodied mackerel surimi exhibited the highest breaking force with the lowest expressible drip ($p < 0.05$).

3.6. Colour of surimi gels

Heating regime had no effect on metmyoglobin formation in surimi gels prepared from three species of mackerel ($p > 0.05$; Fig. 4A). However, the metmyoglobin content of gels depended on fish species (Table 1). From the results, the highest content of metmyoglobin was found in frigate mackerel, followed by Indian mackerel and short-bodied mackerel, respectively ($p < 0.05$). This indicated that myoglobin from frigate mackerel was susceptible to oxidation by heat treatment.

The highest whiteness of gel was found in short-bodied mackerel surimi in all heating conditions, especially kamaboko gel ($p < 0.05$; Fig. 4B). Heating regimes had no effect on whiteness of surimi gel when considered in the same species ($p > 0.05$). This result was in accordance with the content of metmyoglobin formed during heating (Fig. 4A). The lowest metmyoglobin content of gel was found in short-bodied mackerel, followed by Indian mackerel and frigate mackerel, respectively. Also, heating conditions had no effect on metmyoglobin formation of surimi gel when considered in the same species excepted for short-bodied mackerel. This indicated that oxidation of myoglobin during heating occurred in the highest extent in frigate mackerel surimi. The lowest pH together with the highest myoglobin content of frigate mackerel surimi (Table 1) enhanced the oxidation of myoglobin during heating. This corresponded with the darkening of frigate mackerel surimi gel. During heating not only did metmyoglobin formation cause a decreased whiteness of gels but the Maillard browning reaction also affected the colour of gels. Frigate mackerel contained more myoglobin especially its oxidised form, metmyoglobin, and more lipid content. If lipid oxidation took place, the aldehyde generated can be participated in the Maillard reaction (Chaijan, Benjakul, Visessanguan, Lee, & Faustman, 2007b). Hence, the brown pigment can be formed to a greater extent and caused the decreased whiteness eventually.

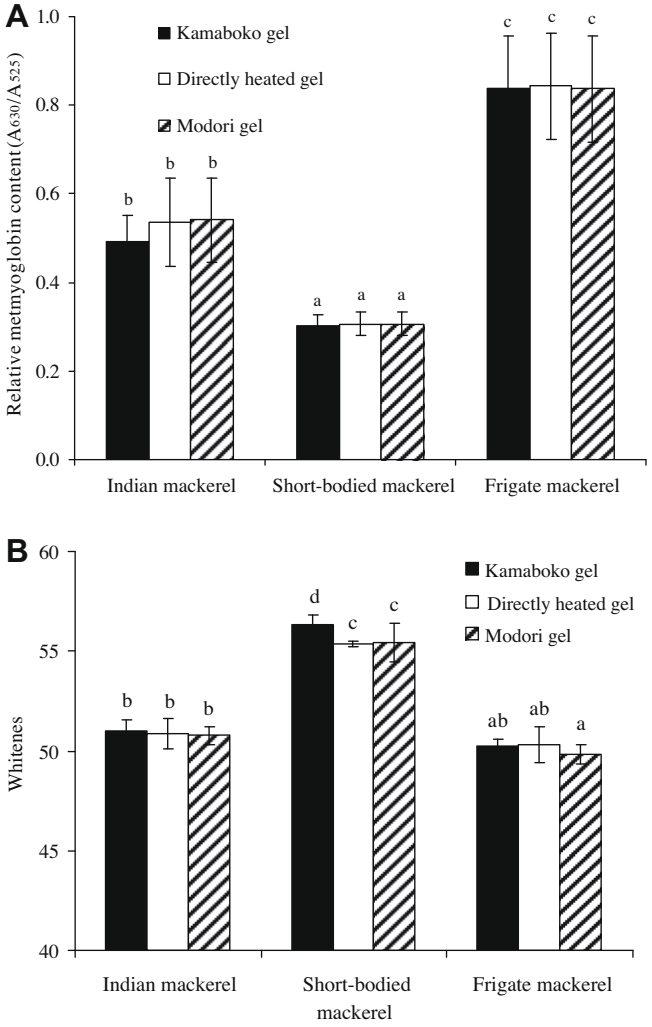

Fig. 4. Relative metmyoglobin content (A) and whiteness (B) of surimi gels prepared from three species of mackerel. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

3.7. Protein patterns of surimi and surimi gels

SDS–PAGE protein patterns of surimi and surimi gels prepared from three species of mackerel under reducing and non-reducing conditions are depicted in Fig. 5A and B, respectively. For surimi, the highest band intensity of myosin heavy chain (MHC; 206 kDa) and actin (45 kDa) was found in short-bodied mackerel in both reducing (Fig. 5A) and non-reducing (Fig. 5B) conditions suggesting the highest integrity of those myofibrillar proteins. However, the polymerisation of proteins as shown by the high-molecular weight-protein occupied in the stacking gel was found in all surimi. This finding indicated the susceptibility to aggregation of mackerel muscle proteins during handling and surimi preparation. After gelation established, the decrease in MHC was noticeable in all gels. The decrease in MHC after heating was due to the polymerisation or degradation depending on heating re-

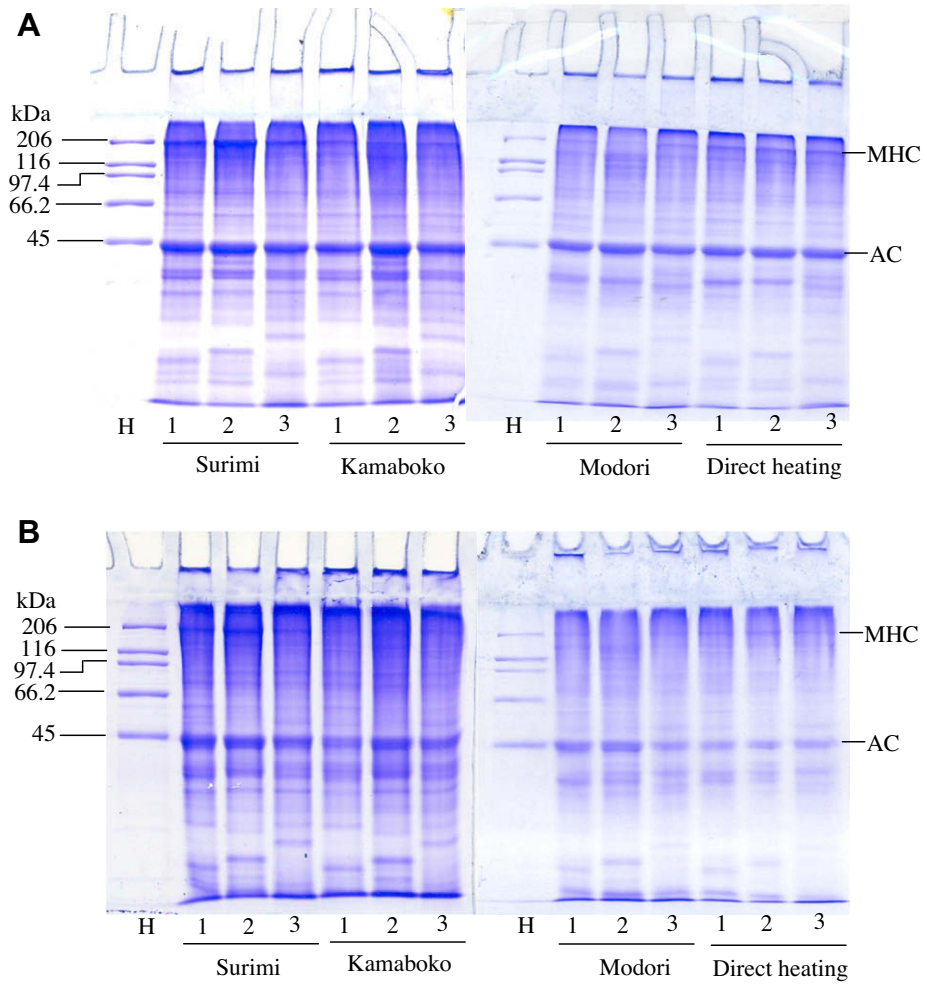

Fig. 5. SDS–PAGE protein pattern of surimi and surimi gels prepared from three species of mackerel under reducing (A) and non-reducing (B) conditions. H: high-molecular-weight marker, 1: Indian mackerel, 2: short-bodied mackerel, 3: frigate mackerel, MHC: myosin heavy chain, AC: actin.

gimes. Setting at 40 °C prior to heating at 90 °C (kamaboko gel) and direct heating at 90 °C enhanced the gel strength of surimi from three species of mackerel (Fig. 3A) indicating the cross-linking of MHC to form the three dimensional network of gel. However, the degradation of MHC appeared in the modori gel of all surimi especially frigate surimi as shown by the decreased breaking force (Fig. 3A). The disappearance of MHC with the occurrence of new protein bands of molecular weight below 206 kDa suggested the degradation of MHC during setting at 60 °C. The polymerised proteins of molecular weight higher than 206 kDa were found in kamaboko and directly heated gels of all surimi. It was noted that under reducing and non-reducing conditions, there was a band with molecular weight above 206 kDa and with the same intensity in all samples. This result indicated that non-disulfide bonds were participating in stabilising the gel structure. When pointed to the actin bands under reducing and non-reducing conditions, the difference in band intensity was found. The fading of the actin band under non-reducing conditions indicated that disulfide bonds could stabilise the aggregate of actin-myofibrillar proteins. The addition of βME resulted in the recovery of actin. Hence, the protein band of 45 kDa was more intense under reducing condition.

4. Conclusion

The physicochemical properties and gel-forming ability of surimi depended on fish species and method of heating. The high-quality of mackerel surimi with the highest gel strength and whiteness was obtained when it was produced from short-bodied mackerel with the setting at 40 °C prior to heating at 90 °C. Short-bodied mackerel surimi had the highest activity of Ca^{2+}-ATPase with the highest content of SH group when compared to those of Indian mackerel and frigate mackerel. Frigate mackerel surimi exhibited the worst gelling property because it contained the highest content of lipid and myoglobin especially its oxidised form, metmyoglobin. In general, the order of gel-forming ability and gel quality was short-bodied mackerel > Indian mackerel > frigate mackerel.

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Gel properties of croaker–mackerel surimi blend

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ABSTRACT

Gel properties of croaker surimi blended with three types of mackerel surimi at different ratios were evaluated. The gel strength of the croaker–mackerel surimi blend was higher than that of the original mackerel surimi ($p < 0.05$). The presence of croaker surimi in the blend resulted in the increase in myosin heavy chain (MHC) band intensity. No differences in deformation of gels were observed in croaker surimi and croaker-short-bodied mackerel blend at all ratios ($p > 0.05$). The addition of short-bodied mackerel surimi into croaker surimi up to a ratio of 1:2 had no effect on whiteness and metmyoglobin content of the gel ($p > 0.05$). Marked decrease in expressible drip and TCA-soluble peptide of gel was noticeable in croaker–frigate mackerel surimi blend ($p < 0.05$). Therefore, the gel properties of croaker–mackerel surimi blend were governed by the type and content of mackerel surimi used.

1. Introduction

Surimi-based gel products represent the most suitable food application for underutilised fish species (Shimizu, Toyohara, & Lanier, 1992). Theoretically, any fish can be used to produce surimi but the rheological characteristics of the surimi gel depend on the properties of myofibrillar proteins, which are affected by the species and freshness of the fish, as well as on the processing parameters, mainly protein concentration, pH, ionic strength, and temperature (Niwa, 1992; Shimizu et al., 1992). The white fish species used in south-east Asia for production of surimi are mainly threadfin bream (*Nemipterus* spp.), bigeye snapper (*Priacanthus* spp.), croaker (*Pennahia* and *Johnius* spp.) and lizardfish (*Saurida* spp.) (Morrissey & Tan, 2000). However, the main problem facing the Thai surimi industry is the supply of raw materials (Morrissey & Tan, 2000). Due to the limited white muscle fish resources caused by the overexploitation of white fish in the Gulf of Thailand, dark muscle fish have been paid more attention as a potential alternative raw material for surimi production (Morrissey & Tan, 2000). Hultin and Kelleher (2000) reported that dark-fleshed fish species make up 40–50% of the total fish catch in the world. In 2006, the catch of pelagic fish in the Gulf of Thailand was approximately 844.2 metric tons (Department of Fisheries, 2006). Among all dark-fleshed fish species, mackerel was one of the most abundant species caught in southern Thailand. The use of this small pelagic fish for surimi production is one way to transform underutilised fish protein resources into human foods, particularly protein gel-based products. Naturally, dark-fleshed fish species contain a high content of dark muscle, comprising a considerable amount of lipids and sarcoplasmic proteins (Sikorski, Kolakowska, & Burt, 1990; Spinelli & Dassow, 1982). The presence of sarcoplasmic proteins and lipids in dark muscle is associated with its poorer gelation characteristics, compared with light muscle (Chen, 2002; Haard, Simpson, & Pan, 1994; Hultin & Kelleher, 2000; Ochiai, Ochiai, Hashimoto, & Watabe, 2001). Sarcoplasmic proteins have an adverse effect on the strength, deformability (Haard et al., 1994) and colour (Chaijan, Benjakul, Visessanguan, & Faustman, 2004; Chen, Chiu, & Huang, 1997) of fish myofibril protein gels. Myoglobin is the predominant pigment protein in the sarcoplasmic fraction of fish dark muscle (Chaijan et al., 2004) and contributes to the lowered whiteness of surimi gel (Chen, 2002). Dark-coloured surimi has a low commercial value because the food applications for which it is suited are limited (Shimizu et al., 1992). The gel-forming ability of dark muscle fish meat has been known to be lower than that of ordinary muscle. This apparently resulted from the difference in the unfolding abilities of the myosin between the muscles (Lo et al., 1991).

A blend of white fish surimi with dark-fleshed fish surimi might be one of the ways to potentially overcome some of the problems caused by the nature of the pelagic species and to encounter the limited white fish resource problems. A high-quality dark fish surimi with improved gel strength and whiteness might be obtained when it is blended with white-fleshed fish surimi. In addition, the reduction of white fish surimi content in the formula of surimi-based products by partial substitution with dark-fleshed surimi could reduce the cost of production. Furthermore, the ratio between white and dark-fleshed fish surimi could be optimised to

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eliminate the adverse effect on gel properties of surimi blend. Therefore, the objective of this study was to investigate the characteristics of gels from the surimi of croaker, a white fish, blended with different types of mackerel surimi at different ratios.

2. Materials and methods

2.1. Chemicals

Sodium dodecyl sulphate (SDS), dithiothreitol (DTT), β-mercaptoethanol (βME) were purchased from Sigma (St. Louis, MO). Trichloroacetic acid was obtained from Merck (Darmstadt, Germany). Acrylamide, *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and bis-acrylamide were obtained from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

2.2. Fish samples

Frigate mackerel (*Auxis thazard*) with an average weight of 95–110 g, Indian mackerel (*Rastrelliger kanagurta*) with an average weight of 87–90 g, short-bodied mackerel (*Rastrelliger branchysoma*) with an average weight of 80–82 g and croaker (*Otolithes ruber*) with an average weight of 80–85 g were caught from Thasala–Nakhon Si Thammarat Coast along the Gulf of Thailand during July 2009. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat within 20 min. The fish were immediately washed, gutted, cleaned, filleted and the whole muscles including both ordinary and dark muscles were collected. To prepare fish mince, fish fillets were minced to uniformity using a mincer (4-mm hole diameter; Panasonic MK-G20MR, Japan). The muscles were kept on ice during preparation.

2.3. Surimi and surimi gel preparation

To prepare surimi by the conventionally washing process, fish mince was washed with cold water (4 °C) (Chaijan et al., 2004) using a washing medium/mince ratio of 3:1 (v/w). The mixture was stirred gently for 10 min in a cold room (4 °C) and the washed mince was filtered with a layer of nylon screen. Washing was performed three times. Finally, the washed mince was centrifuged at 700*g* for 15 min using a basket centrifuge (Model CE 21 K, Grandiumpiant, Belluno, Italy). The washed mince was added with 4% sucrose and 4% sorbitol, mixed well and frozen using a blast freezer. The frozen samples referred to as 'surimi' were kept at −18 °C until used. The storage time was not more than 1 month.

To prepare the gels, the frozen surimi samples were thawed at 4 °C until the core temperature reached 0 °C. The samples were then cut into small pieces and the moisture content of all surimi was adjusted to 80% by the addition of iced water. Croaker surimi was blended with different types of mackerel surimi at a ratio of 1:0, 0:1, 1:1 and 1:2 (w/w). The samples were added with 2.5% (w/w) NaCl and chopped for 5 min in a walk-in cold room at 4 °C, to obtain the 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. The sol was then incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Chaijan et al., 2004). After heating, all gels were immediately cooled in iced water for 30 min and stored for 24 h at 4 °C prior to analysis.

2.4. Texture analysis

Texture analysis of the gels was performed using a TA-XT2i texture analyser (Stable Micro Systems, Godalming, 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 spherical plunger (diameter 5 mm; depression speed 60 mm/min).

2.5. TCA-soluble peptide

To 2 g of finely chopped samples, 18 ml of 5% TCA were added and homogenised for 2 min using an IKA homogeniser at a speed of 11,000 rpm. The homogenate was incubated at 4 °C for 1 h and centrifuged at 8000*g* for 5 min. TCA-soluble peptides in the supernatant were measured according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and expressed as μmol tyrosine/g sample (Morrissey, Wu, Lin, & An, 1993).

2.6. 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 Park (1994), as follows:

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

2.7. Determination of metmyoglobin

The oxidation of myoglobin of surimi gels was determined spectrophotometrically. Metmyoglobin was extracted from surimi gels by using the procedure of Benjakul and Bauer (2001). A chopped sample (2 g) was weighed into a 50-ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer (pH 6.8) were added. The mixture was homogenised at 13,500 rpm for 10 s, followed by centrifuging at 3000*g* for 30 min at 4 °C. The supernatant was filtered with Whatman No. 1 filter paper. The metmyoglobin content was determined by direct spectrophotometric measurement at 630 and 525 nm. The ratio of A_{630} to A_{525} was calculated according to Hansen and Sereika (1969). A high A_{630}/A_{525} ratio indicates a high relative proportion of metmyoglobin.

2.8. Determination of expressible drip

Expressible drip was measured according to the method of Ng (1987). A gel sample with a thickness of 0.5 cm was weighed and placed between two pieces of Whatman No. 1 filter paper at the top and three pieces of the same filter paper at the bottom. The standard weight (5 kg) was placed on the top of the sample and maintained for 2 min. The weight was then removed and the sample weighed again. Expressible drip was calculated and expressed as a percentage of sample weight.

2.9. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of different surimi gels were visualised by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution were added to the sample (3 g). The mixture was homogenised for 1 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The sample was centrifuged at 8500*g* for 5 min at room temperature (26–28 °C) using a Biofuge Primo centrifuge (Sorvall, Hanau, Germany). Protein concentration was determined according to the Biuret method (Robinson & Hodgen, 1940), using bovine serum albumin as a standard. Solubilised samples were mixed at a 1:1

(v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS and 20% glycerol) in the presence of 10% βME, representing reduction. Samples (20 μg protein) were loaded onto polyacrylamide gels comprising a 10% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. A protein standard (Bio-Rad Laboratories, Inc.,) containing myosin (206 kDa), β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), serum albumin (66.2 kDa) and ovalbumin (45 kDa) was used to estimate the molecular weight of the proteins.

2.10. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 8.0 for Windows, SPSS Inc., Chicago, IL).

3. Result and discussion

3.1. Textural properties

Croaker surimi gel had the highest breaking force when compared with other surimi gels prepared from three species of mackerel ($p < 0.05$; Fig. 1A). When the mackerel surimi was added into croaker surimi at a ratio of 1:1, the gel strength of the blend was significant higher than that of the mackerel surimi alone, except in the case of frigate mackerel, for which the difference was not significant. With increasing content of mackerel surimi in the blend, no differences in breaking force were found in all blends ($p > 0.05$; Fig. 1A). The result indicated that the content of mackerel surimi added had no effect on breaking force up to the ratio of 1:2.

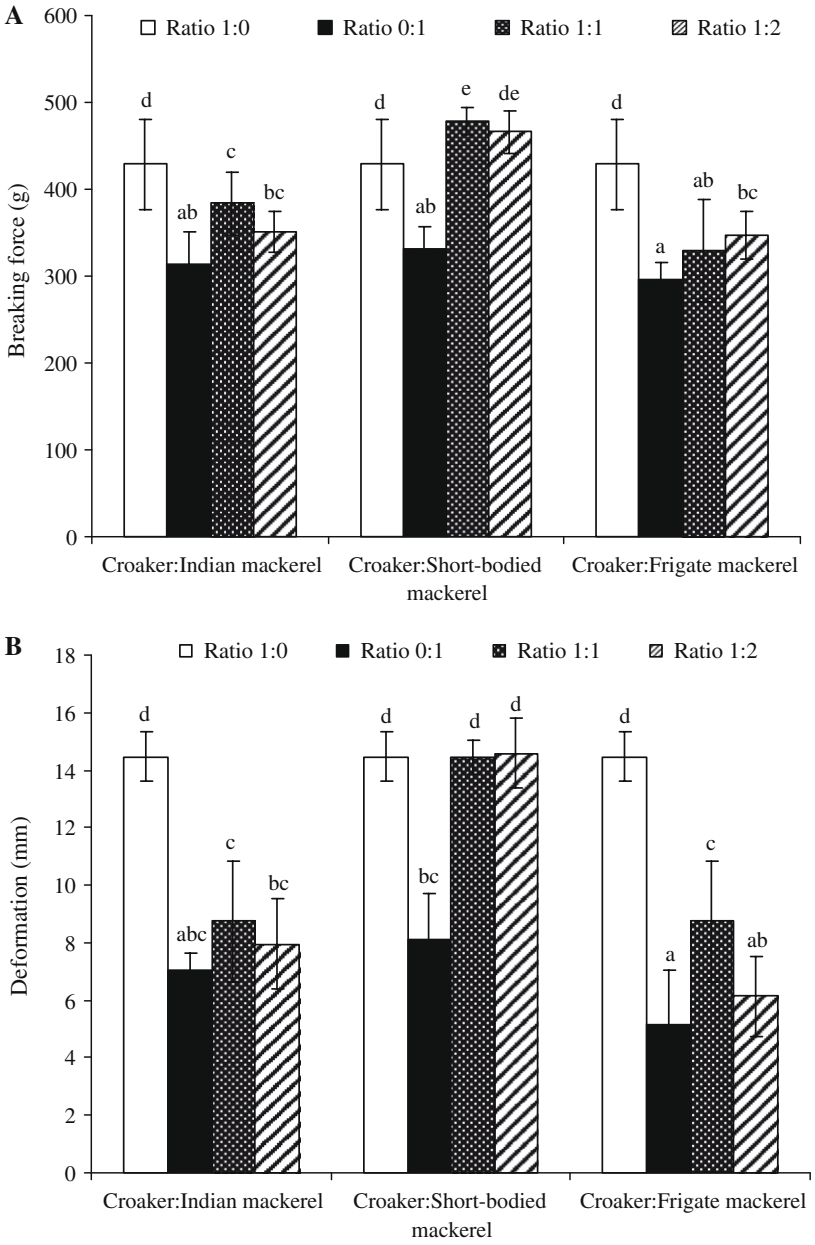

Fig. 1. Breaking force (A) and deformation (B) of gels from croaker surimi blended with different types of mackerel surimi at different ratios. Bars represent the standard deviation from five determinations. Different letters indicate significant differences ($p < 0.05$).

From the result, short-bodied mackerel can be used to blend with croaker at a ratio of 1:2. This blend exhibited similar gel strength to croaker surimi alone. Therefore, the addition of short-bodied mackerel can reduce the cost of production of gel products without any detrimental effects on gel strength. The use of Indian mackerel surimi and frigate mackerel surimi substituted for croaker surimi negatively affected gel strength of croaker-blended formula. The highest deformation of gels was found in croaker surimi and short-bodied mackerel blends ($p < 0.05$; Fig. 1B). Among all mackerel species studied, short-bodied mackerel tended to have the highest deformation ($p < 0.05$; Fig. 1B). With the addition of mackerel surimi into croaker surimi, no differences in deformation of gels were observed in croaker surimi and croaker-short-bodied mackerel blend at all ratios. However, when compared to croaker surimi alone, the presence of Indian mackerel or frigate mackerel in the blend rendered the gels with decreased deformation especially at high ratio of mackerel added ($p < 0.05$; Fig. 1B).

Myosin integrity of croaker (white-fleshed fish) should be higher than that of three species of mackerel (dark-fleshed fish). The extent of denaturation of the myofibrillar proteins was one of factors affecting heat-induced gelling properties of surimi (Lanier, 2000). It was shown that the myosin ATPase activities of fish ordinary myosin are higher than those of dark myosin (Okagaki et al., 2005). Roura, Monteccia, Goldemberg, Trucco, and Crupkin (1990) reported that the myofibrillar ATPase activities have been widely used as a measure of actomyosin integrity. Generally, dark-fleshed fish contained more dark muscle fibres than did white-fleshed fish species (Kisia, 1996). In addition to the difference in composition between dark and white-fleshed fish, dark-fleshed fish contained more lipids, connective tissue, myoglobin, and cytochromes than white-fleshed fish species (Chen et al., 1997; Kelleher, Hultin, & Wilhelm, 1994). Hultin and Kelleher (2000) reported that sarcoplasmic proteins showed a detrimental effect on the strength of myofibril protein gels. In addition, lipid oxidation seems to be a distinct problem in surimi made from some dark-fleshed fish (Lanier, 2000; Wu, Li, Ho, & Jiang, 2000).

When short-bodied mackerel was added into the croaker surimi, the Ca^{2+} and endogenous transglutaminase (TGase) from short-bodied mackerel muscle could enhance the cross-linking of myosin molecule by using the myosin from croaker with a suitable conformation to TGase as a substrate. Setting of surimi is enhanced by sufficient calcium content since endogenous TGase is a Ca^{2+} dependent enzyme (Lee & Park, 1998). From the result, it can be postulated that the myofibrillar proteins, especially myosin from croaker, was an excellent substrate for short-bodied mackerel TGase. Hence, the cross-linking occurred to a higher extent in this blend. However, this phenomenon could not occur in the presence of Indian mackerel and frigate mackerel. This was might be due to the lowered activity of endogenous TGase present in those fish muscles. Lanier (2000) stated that endogenous TGase activity of surimi commonly depended on the fish species. In addition, the present of proteolytic enzymes in the muscle was one of the factors determining the gel strength of surimi. Frigate mackerel surimi could contain a number of active proteases as evidenced by the highest TCA-soluble peptide content in that gel (Fig. 2).

3.2. TCA-soluble peptide

TCA-soluble peptide content of surimi gels is shown in Fig. 2. The highest content of TCA-soluble peptide was found in frigate mackerel surimi gel ($p < 0.05$). This indicated that the proteolysis of frigate surimi occurred to some extent during heat treatment. This resulted in the weakening of the gel, which was in agreement with the lowest breaking force of this gel (Fig. 1A). Among three species of mackerel studied, the most darkening of meat was noticeable in frigate mackerel. Dark muscle fish had a high proteolytic activity resulting in poorer gelation characteristics and high susceptibility to modori (Shimizu et al., 1992). Shimizu et al. (1992) also reported that the poor gel-forming properties of muscle from dark-fleshed species is caused by the presence of heat-stable proteases, which are active in degrading myosin during heating at temperature ranges of 50–70 °C. In general, weakening of surimi gel, so-called modori, was induced by endogenous heat-activated proteases, which can degrade myosin (Jiang, 2000). From the result, the surimi gels of croaker, Indian mackerel and short-bodied mackerel tended to have the same content of TCA-soluble peptide ($p > 0.05$). When croaker surimi was blended with mackerel surimi, changes in TCA-soluble peptide were observed (Fig. 2). The result showed that no difference in TCA-soluble peptide was found in croaker surimi, Indian mackerel surimi and

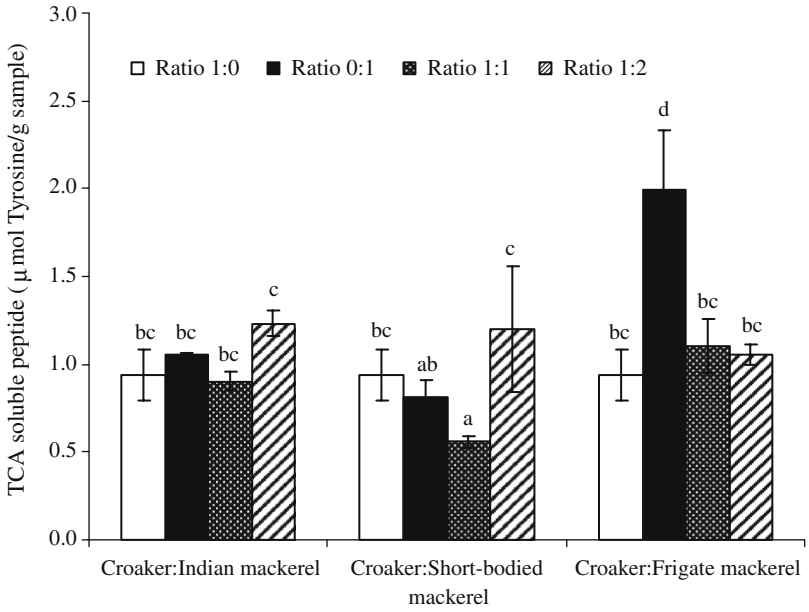

Fig. 2. TCA-soluble peptide content of gels from croaker surimi blended with different types of mackerel surimi at different ratios. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

their blends ($p > 0.05$). The blend of croaker-short-bodied mackerel at a ratio of 1:1 decreased the content of TCA-soluble peptide of gel effectively when compared with croaker surimi gel alone ($p < 0.05$). Further increase in the content of short-bodied mackerel surimi up to 1:2 resulted in increased TCA-soluble peptide, reaching the original content of croaker surimi gel alone ($p > 0.05$). However, the presence of croaker surimi in the blend of frigate surimi resulted in decreased TCA-soluble peptide ($p < 0.05$).

3.3. Whiteness and metmyoglobin content

No significant differences in whiteness of gels from croaker, short-bodied mackerel and their blends were observed ($p > 0.05$; Fig. 3). The addition of short-bodied mackerel surimi into croaker surimi up to ratio 1:2 had no effect on whiteness of the blend. For croaker/Indian mackerel blend, the whiteness of gel prepared by mixing croaker with Indian mackerel at ratios of 1:1 and 1:2 was better than that of Indian mackerel alone ($p < 0.05$). However, the whiteness of the blends at all ratios was lowered when compared to that of croaker surimi gel alone. For croaker/frigate mackerel blend, the whiteness of gel prepared by mixing croaker and frigate mackerel at ratios of 1:1 and 1:2 was better than that of frigate mackerel alone. However, with increasing frigate mackerel surimi content in the blend, decreased whiteness was observed. The oxidation of lipid in frigate mackerel could occur to a greater extent during heat-induced gelation, resulting in the formation of

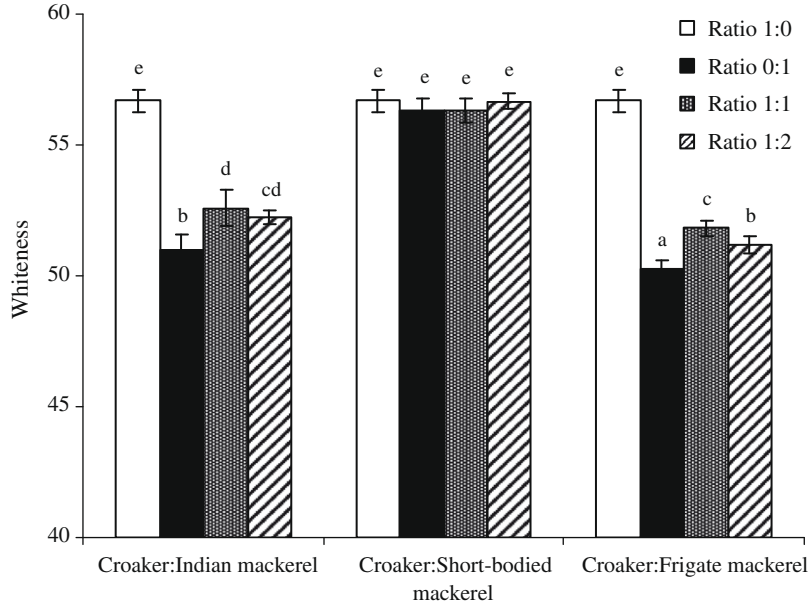

Fig. 3. Whiteness of gels from croaker surimi blended with different types of mackerel surimi at different ratios. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

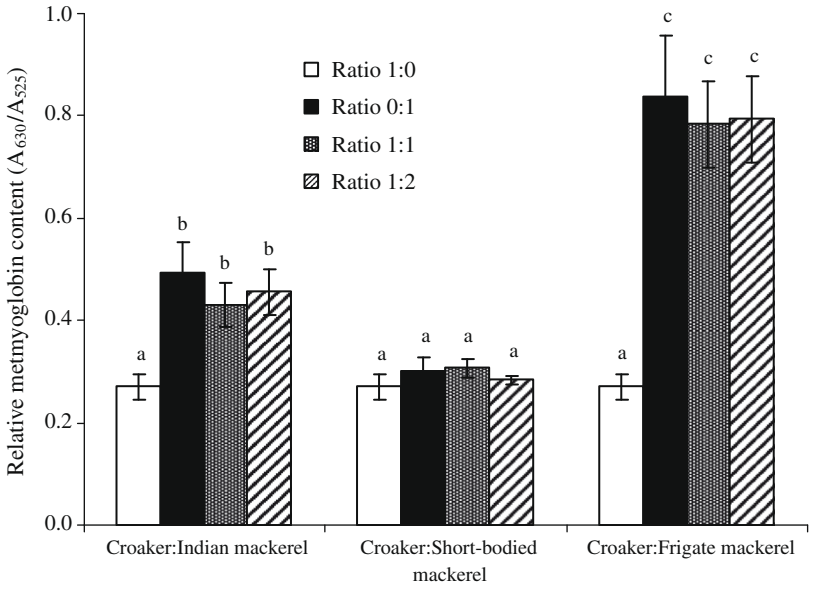

Fig. 4. Relative metmyoglobin content of gels from croaker surimi blended with different types of mackerel surimi at different ratios. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

aldehydes or carbonyl compounds. Those compounds would interact with protein amino groups *via* the Maillard reaction, and any resulting coloured reaction products would lower the whiteness of gel. Chaijan, Benjakul, Visessanguan, Lee, and Faustman (2007) reported that aldehyde lipid oxidation products favoured cross-linking of fish myoglobin with myofibrillar proteins, increased metmyoglobin formation and decreased whiteness of fish myofibrillar proteins *in vitro*.

The presence of frigate mackerel surimi rendered the gel with the highest metmyoglobin content ($p < 0.05$; Fig. 4). The lowest metmyoglobin content of gel was found in croaker surimi and croaker/short-bodied mackerel surimi blend ($p < 0.05$). For croaker/Indian mackerel surimi blend, the higher metmyoglobin content was found in all blends when compared with that found in croaker surimi alone. From the results, it was observed that the lower the metmyoglobin, the greater the whiteness (Figs. 3 and 4). The results suggested that the presence of frigate mackerel and Indian mackerel surimi showed a detrimental effect on colour of the surimi blend.

3.4. Expressible drip

The highest expressible drip of gels was found in frigate mackerel surimi ($p < 0.05$; Fig. 5) indicating that protein network of the gel was lower in water-binding properties (Niwa, 1992). The presence of croaker surimi in Indian mackerel and frigate mackerel surimi, especially at a ratio of 1:1, resulted in decreased expressible drip ($p < 0.05$). From the result, marked decrease in expressible moisture was found in gel from croaker/frigate mackerel surimi blend. Therefore, the water holding capacity of frigate mackerel surimi can be improved by partial substitution with croaker surimi. No significant difference in expressible drip of gels was found in croaker surimi and croaker/short-bodied mackerel blend ($p > 0.05$).

3.5. Protein patterns

SDS–PAGE protein pattern of surimi gels under reducing conditions is depicted in Fig. 6. The highest band intensity of myosin

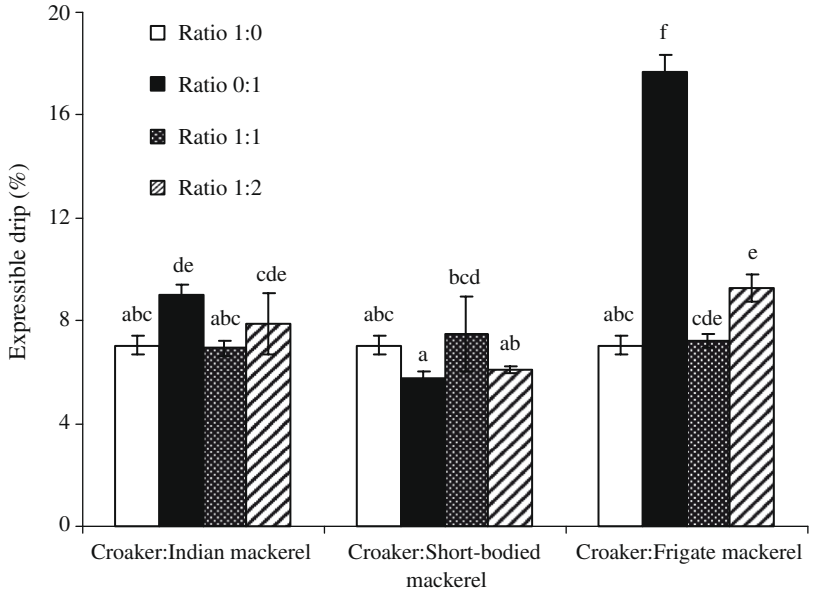

Fig. 5. Expressible drip of gels from croaker surimi blended with different types of mackerel surimi at different ratios. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

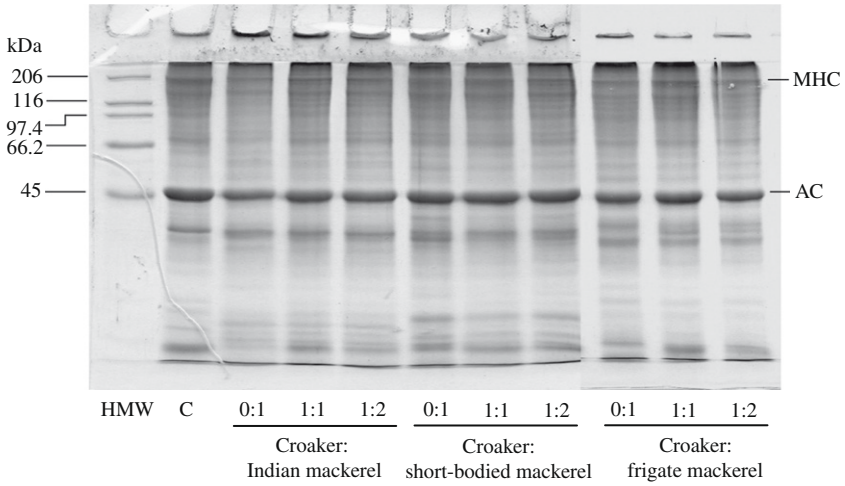

Fig. 6. SDS–PAGE protein pattern of gels from croaker surimi blended with different types of mackerel surimi at different ratios under reducing condition. HMW, high-molecular-weight marker; C, croaker surimi gel; MHC, myosin heavy chain; AC, actin.

heavy chain (MHC; 206 kDa) was found in croaker surimi gel (Fig. 6). Generally, the same intensity MHC band of surimi gels from Indian mackerel, short-bodied mackerel and frigate mackerel was observed. When croaker surimi was blended with those mackerel surimi gels, the increase in MHC band intensity of the blend was noticeable. The lowest intensity of actin band (AC; 45 kDa) was found in Indian mackerel surimi gel. The incorporation of croaker surimi into Indian mackerel surimi resulted in an increased AC band. From the result, the polymerisation of proteins, as shown by the high-molecular weight-protein occupied in the stacking gel with a pale MHC band, was found in all surimi gels.

4. Conclusion

Short-bodied mackerel surimi can be used to blend with croaker surimi up to a ratio of 1:2. This blend exhibited similar colour and textural properties to that of croaker surimi alone. However, the use of Indian mackerel surimi and frigate mackerel surimi substituted for croaker surimi negatively affected gel strength of the blend. Therefore, the blend with superior gel properties depended on the type and the content of dark-fleshed fish used.

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Physicochemical and gelling properties of short-bodied mackerel (*Rastrelliger brachysoma*) protein isolate prepared using alkaline-aided process

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ABSTRACT

This study demonstrated the characteristics and gel properties of short-bodied mackerel (*Rastrelliger brachysoma*) surimi prepared by the conventional washing process and protein isolate prepared by alkaline solubilization process without and with prewashing. A decrease in Ca^{2+}-ATPase activity ($P<0.05$), with changes in the tryptophan fluorescence intensity of natural actomyosin, was observed in protein isolate from alkaline solubilization process. However, higher reactive sulfhydryl (SH) content was found in protein isolate, compared with that in conventional surimi ($P<0.05$). Generally, the higher amounts of lipid and myoglobin in fish mince were removed by using the alkaline-aided process, compared with the conventional process ($P<0.05$). Protein isolate from the alkaline solubilization process formed gels with higher breaking forces than conventional suirmi, especially with 2-step heating (40 °C, 30 min/90 °C, 20 min) ($P<0.05$). The lowest expressible moisture was found in the gel of protein isolate prepared by alkaline solubilization process ($P<0.05$). However, the highest whiteness was found in the conventional surimi gel prepared by 2-step heating ($P<0.05$). Protein isolate prepared by the alkaline solubilization process yielded superior gels compared with the conventional surimi. Therefore, it can be concluded that the gelling properties of surimi or protein isolate are governed by the physicochemical properties of proteins and are affected by processing conditions.

Keywords: Short-bodied mackerel; Surimi; Protein isolate; Alkaline

1. Introduction

Short-bodied mackerel (*Rastrelliger brachysoma*) is a small pelagic fish having large quantities of lipids and myoglobin in the muscle tissue. These components limited the use of this underutilized fish for surimi production. Generally, high-quality surimi with an improved gel strength and whiteness can be obtained when dark muscle is removed as much as possible (Ochiai et al., 2001). However, abundant dark muscle in red-fleshed fish such as mackerel and sardine is difficult to remove with a meat separator (Ochiai et al., 2001). Principally, the washing process is necessary for color improvement and gel strengthening of surimi produced from whole muscle.

Conventional surimi production is aimed to concentrate myofibrillar proteins by removing sarcoplasmic proteins, fat, blood and pigments through continuous washing of the mechanically separated fish mince (Park et al., 1997). A washing process can improve the functionalities and sensory characteristics of fish meat. However, a low yield was obtained when conventional washing was used (Kristinsson et al., 2005). To overcome this problem, a new approach of recovering protein by a pH-shift process, which has been developed by Hultin and Kelleher (2000), can be used. An acid or alkaline solubilizing process potentially overcomes some of the problems caused by the nature of the pelagic species (Undeland et al., 2002). This process consists of isolating the protein component of fish muscle tissue by acid or alkaline and subsequently precipitating all soluble proteins at their isoelectric point (Yongsawatdigul and Park, 2004; Perez-Mateos et al., 2004; Kim et al., 2003). As stated by Rawdkuen et al.

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(2009), the extraction mechanism of these two processes is to solubilize the muscle protein at low and high pH to separate soluble proteins, bone, skin, connective tissue, cellular membranes, and neutral storage lipids through the centrifugation. The solubilized proteins are collected and recovered by isoelectric precipitation to give a highly functional and stable protein isolate (Kristinsson and Ingadottir, 2006). The proteins recovered by this process have good functionality and in some cases better gelation properties than proteins recovered with conventional surimi processing (Kristinsson et al., 2005). Kristinsson et al. (2005) reported that the acid- and alkaline-aided processes of channel catfish muscle gave higher protein recoveries compared with the conventional surimi process. Batista et al. (2007) reported that the global yields of sardine muscle achieved were 77% and 73% for the alkaline and acid solubilization, respectively. Gels prepared from rockfish and Atlantic croaker from proteins solubilized at alkaline pH exhibited better gel quality than those prepared from the acid-aided process (Perez-Mateos et al., 2004; Kim et al., 2003). Chaijan et al. (2006) reported that the alkaline solubilization process in combination with prewashing using a water/mince ratio of 3:1 (w/w) was an alternative means to enhance the removal of myoglobin associated with the muscle tissue and to improve the color of resulting protein isolate. The present study aimed to investigate the effect of alkaline-aided process and conventionally washing process on the physicochemical properties and gel-forming ability of proteins from short-bodied mackerel whole muscle.

2. Materials and methods

2.1. Chemicals

Adenosine 5′-triphosphate (ATP) was purchased from Sigma (St. Louis, MO, U.S.A.). Trichloroacetic acid, sodium chloride and potassium chloride were obtained from Merck (Darmstadt, Germany). Sodium hydroxide and hydrochloric acid were obtained from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

2.2. Fish samples

Short-bodied mackerel (*R. brachysoma*) with an average weight of 70–75 g were caught from Thasala-Nakhon Si Thammarat Coast along the Gulf of Thailand during August 2008. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, the School of Agricultural Technology, Walailak University within 1 h. The fish were immediately washed, gutted, cleaned and filleted. The muscle was kept on ice during preparation and analysis. The pH value of fish muscle was 6.53–6.58.

2.3. Surimi and fish protein isolate preparation

To prepare surimi by the conventional washing process, fish mince was washed with cold water (4 °C) (Chaijan et al., 2004) using a washing media/mince ratio of 3:1 (w/w). The mixture was stirred gently for 10 min in a cold room (4 °C) and the washed mince was filtered with a layer of nylon screen. Washing was performed three times. Finally, to remove water, the washed mince was centrifuged at 700 × *g* for 15 min using a basket centrifuge (Model CE 21K, Grandiumpiant, Belluno, Italy). To prepare the protein isolate, the alkaline solubilization process was used following the method of Undeland et al. (2002). The mince (250 g) was homogenized for 1 min with 2.25 l of cold distilled water (4 °C) using an IKA homogenizer (Selangor, Malaysia). The homogenate was adjusted to the pH of 10.8 using 2N NaOH. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 °C to remove the insoluble materials. The soluble proteins were then precipitated by adjusting the pH to 5.5 using 2N HCl. Precipitated proteins were collected and their pH was adjusted to 7.0 using 2N NaOH. For the prewashing-alkaline process, the mince was prewashed with a cycle of distilled water using a washing media/mince ratio of 3:1 (w/w) prior to alkaline solubilization. The unwashed mince was used as a control. Unwashed mince, conventional surimi and protein isolate prepared by the alkaline-aided process, either without or with prewashing, were added with 4% sucrose and 4% sorbitol, mixed well and frozen using an air-blast freezer. The frozen samples were kept at −18 °C until used. The storage time was not more than 1 month.

2.4. Gel preparation

To prepare the gels, the frozen samples were thawed at 4 °C for 12 h until the core temperature reached 0 °C. The samples were then cut into small pieces and the moisture content was adjusted to 80%. Dry NaCl was added to the samples (2.5%, w/w) and chopped for 5 min in a walk-in cold room at 4 °C to obtain the 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. The sol was then incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Chaijan et al., 2004). The gels were cooled in iced water and stored for 24 h at 4 °C prior to analysis.

2.5. Determination of Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity of natural actomyosin (NAM) from unwashed mince, protein isolates and conventional surimi was determined according to the method of Benjakul et al. (1997). NAM prepared as described by Benjakul et al. (1997) was diluted to 2.5–8 mg/ml with 0.6 M KCl, pH 7.0. Diluted NAM solution (1 ml) was added to 0.6 ml of 0.5 M Tris–maleate, pH 7.0 and 1 ml of 0.1 M $CaCl_2$ was added to this mixture. Deionized water was added to make up a total volume of 9.5 ml. 0.5 ml of 20 mM adenosine 5′-triphosphate (ATP) solution was added to initiate the reaction. The reaction was conducted for 8 min at 25 °C and terminated by adding 5 ml of chilled 150 g/l trichloroacetic acid. The reaction mixture was centrifuged at 3500 × *g* for 5 min and the inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). The Ca^{2+}-ATPase activity was expressed as micromoles inorganic phosphate released per milligram of protein per minute. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP.

2.6. Determination of reactive SH content

Reactive SH content was measured using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) according to the method of Ellman (1959) as modified by Sompongse et al. (1996). NAM sample (0.5 ml, 4 mg/ml) was added to 4.5 ml of 0.2 M Tris–HCl buffer, pH 6.8. Thereafter, 0.5 ml of 0.1% DTNB solution was added into the mixture and subjected to incubation at 40 °C for 25 min. Absorbance was measured at 412 nm using a Shimadzu UV-2100 spectrophotometer (Shimadzu Scientific

Table 1 – Ca^{2+}-ATPase activity, reactive SH content and tryptophan fluorescence intensity of NAM extracted from unwashed mince, conventional surimi and protein isolate prepared by alkaline solubilization process with and without prewashing.

Processing	Ca^{2+}-ATPase activity (μmoles inorganic phosphate released/mg of protein/min)	Reactive SH content (mmol/kg)	Tryptophan fluorescence intensity
Unwashed mince	0.16 ± 0.03b	39.1 ± 1.1b	0.18 ± 0.05a
Conventional washing	0.09 ± 0.08ab	14.2 ± 4.0a	0.28 ± 0.02b
Alkaline solubilization without prewashing	0.03 ± 0.01a	55.2 ± 8.2bc	0.19 ± 0.03a
Alkaline solubilization with prewashing	0.02 ± 0.01a	72.2 ± 9.3c	0.18 ± 0.06a

Values are given as mean ± S.D. from triplicate determinations. Different letters in the same column indicate significant differences ($P < 0.05$).

Instruments Inc., Columbia, MD, U.S.A.). A blank was prepared by replacing the sample with 0.6 M KCl, pH 7.0. SH content was calculated from the absorbance using the molar extinction of 13,600 M^{-1} cm^{-1} and was expressed as mmol/kg protein.

2.7. Measurement of tryptophan fluorescence

Tryptophan fluorescence of NAM extracted from unwashed mince, protein isolates and conventional surimi was measured with a Jasco FP-6500 spectrofluorometer (Jasco, Tokyo, Japan) at an excitation wavelength of 280 nm and an emission wavelength of 325 nm according to the method of Chanthai et al. (1996).

2.8. Determination of myoglobin content

The extractable myoglobin content was determined by direct spectrophotometric measurement as described by Benjakul and Bauer (2001). A chopped sample (2 g) was weighed into a 50-ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer, pH 6.8 were added. The mixture was homogenized at 13,500 rpm for 10 s, followed by centrifuging at 3000 × g for 30 min at 4 °C. The supernatant was filtered with Whatman No. 1 filter paper. The myoglobin content was determined by direct spectrophotometric measurement at 525 nm. Myoglobin content was calculated from the millimolar extinction coefficient of 7.6 mM^{-1} cm^{-1} and a molecular mass of 16,110 (Gomez-Basauri and Regenstein, 1992). The myoglobin content was expressed as mg/g sample.

2.9. Lipid extraction

Lipid was extracted by the Bligh and Dyer method (1959). Sample (25 g) was homogenized with 200 ml of a chloroform:methanol:distilled water mixture (50:100:50) at the speed of 9500 rpm for 2 min at 4 °C using an IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was treated with 50 ml of chloroform and homogenized at 9500 rpm for 1 min. Then, 25 ml of distilled water were added and homogenized again for 30 s. The homogenate was centrifuged at 3000 rpm at 4 °C for 15 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CO, U.S.A.), and transferred into a separating flask. The chloroform phase was drained off into the 125 ml Erlenmeyer flask containing about 2–5 g of anhydrous sodium sulfate, shaken very well, and decanted into a round-bottom flask through a Whatman No. 4 filter paper. The solvent was evaporated at 40 °C using an EYELA rotary evaporator N-100 (Tokyo, Japan) and the total lipid content in samples was calculated.

2.10. Texture analysis

Texture analysis of the gels was performed using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, U.K.). Gels were equilibrated and evaluated at room temperature (28–30 °C). Six cylinder-shaped samples with a length of 2.5 cm were prepared and subjected to determination. Breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyzer equipped with a spherical plunger (diameter 5 mm; depression speed 60 mm min^{-1}).

2.11. Determination of whiteness

Color of the gels 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 Park (1994) as follows:

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

2.12. Determination of expressible moisture

Expressible moisture was measured according to the method of Ng (1978). A gel sample with a thickness of 0.5 cm was weighed and placed between two pieces of Whatman filter paper No. 1 at the top and three pieces of the same filter paper at the bottom. The standard mass (5 kg) was placed on the top of the sample and maintained for 2 min. The sample was then removed and weighed again. Expressible moisture was calculated and expressed as percentage of sample weight.

2.13. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test to identify significant differences ($P < 0.05$) among treatments (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 10.0 for windows, SPSS Inc., Chicago, IL, U.S.A.).

3. Results and discussion

3.1. Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of NAM extracted from unwashed mince, conventional surimi and protein isolates is shown in Table 1. The highest activity of Ca^{2+}-ATPase was found in NAM extracted from unwashed mince and conventional surimi

Table 2 – Myoglobin and lipid contents of unwashed mince, conventional surimi and protein isolate prepared by alkaline solubilization process with and without prewashing.

Processing	Myoglobin content (mg/g sample)	Lipid content (%)
Unwashed mince	29.7 ± 3.2c	3.9 ± 0.9c
Conventional washing	11.6 ± 1.3b	2.4 ± 0.7b
Alkaline solubilization without prewashing	7.3 ± 0.1a	0.3 ± 0.0a
Alkaline solubilization with prewashing	14.1 ± 1.0b	0.2 ± 0.1a

Values are given as mean ± S.D. from triplicate determinations. Different letters in the same column indicate significant differences ($P < 0.05$).

($P > 0.05$). With the cold water washing, sarcoplasmic proteins, lipid and unnecessary materials could be removed, resulting in the concentrated myofibrillar proteins (Chaijan et al., 2004). This resulted in the partial denaturation of heavy chain myosin with decreased Ca^{2+}-ATPase activity. Roura et al. (1990) reported that the myofibrillar ATPase activities have been widely used as a measure of actomyosin integrity. Proteins obtained by the alkaline-aided process with and without prewashing had the lower Ca^{2+}-ATPase activity than conventional surimi and unwashed mince ($P < 0.05$). The results suggested that the denaturation of myosin was induced by the alkaline solubilizing process. These results were in agreement with Chaijan et al. (2006) who reported the increase of myosin denaturation in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) by alkaline treatment. However, no differences in Ca^{2+}-ATPase activity were observed between protein isolates with and without prewashing ($P > 0.05$).

3.2. *Reactive SH content*

The reactive sulfhydryl (SH) content of NAM extracted from unwashed mince, conventional surimi and protein isolates is shown in Table 1. The lowest SH content was found in NAM extracted from conventional surimi ($P < 0.05$). Chaijan et al. (2006) reported that alkaline pH could modify the charge of proteins, resulting in the repulsion of molecules with subsequent conformational changes. The decrease in the SH content of NAM extracted from conventional surimi might be due to the oxidation of SH to form disulfide cross-linkage within the NAM molecules during 3-cycle washing. When the alkaline solubilization process with or without prewashing was applied, the SH content of NAM extracted from protein isolates increased ($P < 0.05$). The conformational changes of proteins might take place in the way that buried SH groups could be exposed.

3.3. *Tryptophan fluorescence intensity*

The tryptophan fluorescence intensity of NAM extracted from unwashed mince, conventional surimi and protein isolates is shown in Table 1. The increase in tryptophan fluorescence intensity can be used to indicate the conformational changes of a tertiary structure of proteins (Chanthai et al., 1996). After conventional washing, the tryptophan fluorescence intensity of the NAM increased by approximately 55.6%, when compared with that of unwashed mince. The increase in fluorescence intensity might be associated with the unfolding of NAM with increasing washing cycles. Copeland (1994) reported that when a protein unfolds, amino acid residues that were buried in the hydrophobic interior of the protein become exposed to the polar aqueous solvent. Conversely, no changes in fluorescence intensity of alkaline-treated proteins with and without prewashing were observed ($P > 0.05$). Muscle proteins might undergo conformational changes during alkaline solubilization process. However, from the result, the hydrophobic interaction could occur during precipitation at pI or the refolding of partially denatured proteins took place to some extent. Therefore, the measurable tryptophan residues of NAM extracted from alkaline-treated proteins with and without prewashing were not changed remarkably when compared with that of unwashed mince. The results suggested that the conformational changes of alkaline-treated proteins with and without prewashing might take place in the way, which hydrophobic interaction could be enhanced.

3.4. *Myoglobin content*

The myoglobin content of unwashed mince, conventional surimi and protein isolates is shown in Table 2. The myoglobin content of unwashed mince was 29.72 mg/g sample. The highest myoglobin removal was found in protein isolate prepared by alkaline solubilization process without mince prewashing ($P < 0.05$). The similar myoglobin content of the prewashed alkaline recovered proteins and the conventional surimi was observed ($P > 0.05$). Washing with cold water removed 61% of myoglobin from short-bodied mackerel muscle. Alkaline solubilizing process without and with prewashing removed 75% and 53% of myoglobin, respectively. After the recovery process, retained myoglobin could be co-precipitated with myofibrillar proteins. The lowest content of myoglobin found in alkaline-treated protein without prewashing was probably due to the removal of non-precipitated myoglobin. Yamaguchi et al. (1979) reported that mackerel myoglobin had the isoelectric point of 5.8–5.9. At pH 5.5, used for muscle proteins recovery, some denatured myoglobin might not be precipitated, leading to the lowered myoglobin in the resulting precipitates. At the pH above the pI, myoglobin possesses negative charge, resulting in the solubilization of myoglobin (Yamaguchi et al., 1979). Furthermore, the degradation or the detachment of globin-heme of myoglobin could occur at high pH resulting in the lowered detectable myoglobin content. Chaijan et al. (2007) reported the destruction of heme protein at very acidic or alkaline pHs.

3.5. *Lipid content*

Lipid content of unwashed mince was 3.9%. Cold water washing and pH-shift processing caused the decrease of lipid content in fish muscle. It was found that 2.4%, 0.3% and 0.2% of lipid was retained in the muscle after it was processed with conventional washing, alkaline-aided treatment without and with prewashing, respectively (Table 2). Batista et al. (2007) reported the decrease in fat content in sardine muscle was 65.3% and 51.0% for the proteins recovered after alkaline and acidic solubilization respectively. Kristinsson et al. (2005) reported that the acid- and alkaline-aided processes

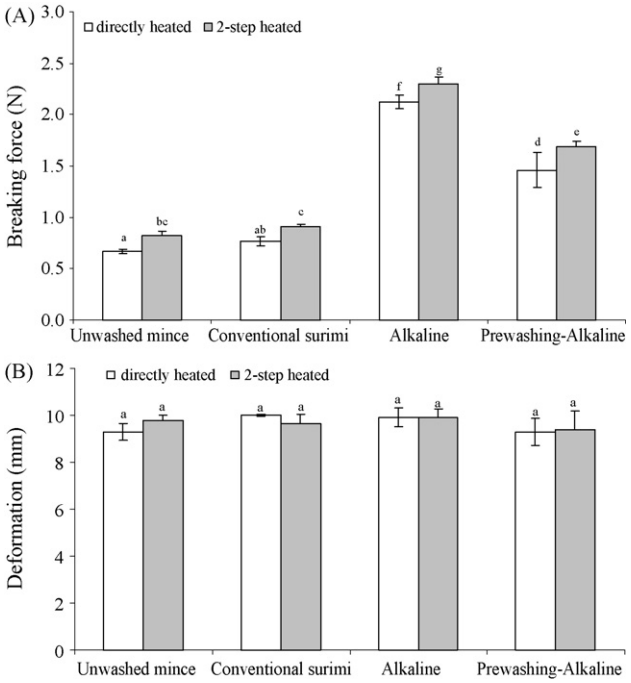

Fig. 1 – Breaking force (A) and deformation (B) of gels prepared from unwashed mince, conventional surimi and protein isolate prepared by alkaline solubilization process with and without prewashing. Bars represent the standard deviation from six determinations. Different letters indicate significant differences ($P < 0.05$).

of channel catfish led to more decrease in lipids compared with the surimi process. The lipid decrease in Atlantic croaker was more than 4 times when using alkaline-aided process compared with conventional process (Kristinsson and Liang, 2006). The higher lipid content of the conventional surimi was noticeable because membrane lipids are retained and a portion of the storage lipids co-aggregates with the proteins during the washing process. The larger decrease for the alkaline-aided process with and without prewashing was obtained because at high pH, the solubilized proteins are separated from the storage lipids and the membrane phospholipids. On centrifugation, these components separated on the basis of density and solubility differences (Kristinsson et al., 2005; Undeland et al., 2002). Kristinsson and Hultin (2003) suggested the higher lipid removal by the alkaline process may be due to the higher emulsification ability of the proteins at alkaline pH. Hultin and Kelleher (2000) also suggested that the first centrifugation step will cause a portion of the membrane phospholipids to sediment in the bottom layer of the centrifuge tube, and also cause significant separation of neutral lipids to the top.

3.6. *Textural properties of gels*

The highest breaking force of gel was found in alkaline-aided protein, followed by the gels of prewashing-alkaline-treated protein, conventional surimi or unwashed mince, respectively ($P < 0.05$; Fig. 1A). From the results, the breaking forces of gels of unwashed mince and surimi were not significantly different ($P > 0.05$). When compared between directly heated and 2-step heated gels, the breaking force of the former was lower than that of the latter ($P < 0.05$). The results indicated that protein-protein interactions were established in both cases but a stronger gel was obtained in the 2-step heated gel, which strengthened the network previously formed by setting at 40 °C. The gel strength of surimi can be increased by setting the surimi sol below 40 °C prior to cooking (Benjakul et al., 2003; An et al., 1996; Kimura et al., 1991). The setting phenomenon has been attributed to endogenous transglutaminase (TGase) activity that induces protein cross-linking and gel strengthening (Perez-Mateos and Lanier, 2006). However, no differences in deformation of gels prepared with different processings and heating conditions were observed ($P > 0.05$; Fig. 1B). From the results, the highest breaking force of alkaline-aided protein isolate gel was probably due to the partial denaturation of protein after alkaline treatment. This resulted in the exposure of some sulfhydryl groups (Table 1) which can undergo oxidation during heat treatment. Hence, the increased gel strength was observed. High temperatures during heating led to further oxidation of sulfhydryl groups with a subsequent disulfide bond formation (Benjakul et al., 2001). Angsupanich et al. (1999) reported that a heat-set myosin gel is primarily stabilized by disulfide bonds and hydrophobic interactions. The protein recovered using the alkaline-aided process with prewashing also had a high content of reactive sulfhydryl groups (Table 1) but the breaking force of that gel was lower than the gel of protein recovered using alkaline-aided process. This was possibly due to the elimination of endogenous transglutaminase by water leaching.

Although the decreased Ca^{2+}-ATPase activity (Table 1) was observed after alkaline treatment, the highest gel strength was still found in the resulting protein isolate. Kristinsson and Hultin (2003) reported that the acid and alkaline unfolding of cod myosin had no impact on the solubility characteristics of myosin refolded at pH 7.5. It is likely due to the fact that the rod portion of the protein was in a native configuration after acid and alkaline treatments (Kristinsson and Hultin, 2003). Generally, gel network development involves two steps. First, interaction of the tail portion of myosin molecules occurs, followed by hydrophobic interaction among the head portions (Sano et al., 1990). A tail-to-tail network could form to a higher extent during thermal gelation of alkaline-treated protein, resulting in the enhanced gel strength. Furthermore, the lipid decrease was one of the factors affecting the increased breaking force of alkaline-aided protein. The presence of lipids may interfere with myosin cross-linking during gel matrix formation because they do not form gels and have poor water holding capacity. The decrease of myoglobin by alkaline solubilization process can enhance the gel strength of protein isolate. Hultin and Kelleher (2000) and Haard et al. (1994) reported that small quantities of sarcoplasmic proteins can have an adverse effect on the strength and deformability of myofibrillar protein gels. Additionally, differences in endogenous transglutaminase activity, which plays a role in protein cross-linking, contribute to the differences in gel strength (Benjakul and Visessanguan, 2003; Kumazawa et al., 1995). From the results, setting occurred to a higher extent in alkaline-treated proteins, compared to unwashed mince and conventional surimi as evidenced by the much higher breaking force. It was postulated that alkaline solubilization process might recover undenatured-active transglutaminase, leading to the higher setting phenomenon. Endogenous transglutaminase activity (evidenced as enhanced strength of cooked gels subjected to 30–40 °C preincubation) was found in alkaline-aided protein from Atlantic menhaden (Perez-Mateos and Lanier, 2006). Undeland et al. (2002) and Perez-Mateos et al. (2004) reported that protein recovered by alkaline solubiliz-

Table 3 – Whiteness and expressible drip of surimi and protein isolate gels from short-bodied mackerel prepared with different heating conditions.

Heating conditions	Processing	Whiteness	Expressible drip (%)
Direct heating[a]	Unwashed mince	59.6 ± 0.4a	29.1 ± 1.5c
	Conventional washing	65.9 ± 0.2f	26.9 ± 0.6abc
	Alkaline solubilization without prewashing	60.4 ± 0.2b	26.7 ± 0.2abc
	Alkaline solubilization with prewashing	59.8 ± 0.1a	28.1 ± 1.1c
2-Step heating[a]	Unwashed mince	62.1 ± 0.4d	27.2 ± 1.7bc
	Conventional washing	68.1 ± 0.1g	23.9 ± 0.6ab
	Alkaline solubilization without prewashing	63.1 ± 0.3e	21.0 ± 1.5a
	Alkaline solubilization with prewashing	61.1 ± 0.1c	25.6 ± 0.5abc

Values are given as mean ± S.D. from triplicate determinations. Different letters in the same column indicate significant differences ($P < 0.05$).

[a] Directly heated gels were prepared by heating the sol at 90 °C for 20 min. 2-Step heated gels were prepared by incubating the sol at 40 °C for 30 min, followed by heating at 90 °C for 20 min.

ing process rendered the gel with the better gel properties, compared with conventional process.

3.7. *Color of gels*

Whiteness of gels of unwashed mince, conventional surimi and protein isolates is shown in Table 3. The highest whiteness was found in surimi gel prepared by the conventional washing method, especially with 2-step heating ($P < 0.05$). Perez-Mateos and Lanier (2006) reported that gels of conventional surimi exhibited a higher whiteness than those of the alkaline-aided protein. The similar result was reported by Chaijan et al. (2006) for the gel prepared from mackerel (*R. kanagurta*) muscle. The lower whiteness of gels of protein isolates with and without prewashing was possibly due to the oxidation of myoglobin in the recovered proteins, which was catalyzed by the alkaline pH. Chaijan et al. (2007) reported that alkaline and acid conditions accelerated autoxidation of sardine myoglobin. The result also showed that higher whiteness was found in the 2-step heated gels than that in directly heated gels.

3.8. *Expressible moisture of gels*

The lowest expressible moisture was found in the gel of alkaline-aided protein with 2-step heating (Table 3). In general, the lower expressible moisture was coincidental with the increased breaking force (Fig. 1A). High expressible moisture found in gel of unwashed mince was possibly due to the poor gel network of unwashed mince which contained sufficient foreign materials such as lipids and blood. Therefore, gel matrixes could not retain water, leading to high water releases. Directly heated gels showed higher expressible moisture than the 2-step heated gels, indicating that protein network of the former was lower in water binding properties. 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 (Niwa, 1992).

4. Conclusions

Isolation of fish muscle proteins using the alkali-aided process was a major challenge to transform the underutilized fish protein resources into protein-rich human foods. Marked decreases in myoglobin and lipid contents were found in alkaline-aided protein, leading to the improved gel strength of short-bodied mackerel protein. However, the alkaline solubilizing process induced the denaturation of the muscle proteins as evidenced by the decrease in the Ca^{2+}-ATPase activity with the changes in the tryptophan fluorescence intensity. Generally, protein recovered by the alkaline solubilizing process produced a gel superior to that from the conventional washing process.

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Effect of oxidised phenolic compounds on the gel property of mackerel (*Rastrelliger kanagurta*) surimi

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ABSTRACT

The effects of different oxidised phenolic compounds (ferulic acid, OFA; tannic acid, OTA; catechin, OCT and caffeic acid, OCF) at different levels (0–0.60% of protein content) on the properties of gels from mackerel (*Rastrelliger kanagurta*) surimi were investigated. Gels with addition of 0.40% OFA, 0.50% OTA, 0.50% OCF or 0.10% OCT had increases in breaking force by 45%, 115%, 46.1% and 70.3% and in deformation by 12.2, 27.5, 28.1 and 28.4%, respectively, compared with the control (without addition of oxidised phenolics). Lowered expressible moisture content without any change in the whiteness of resulting gels was found. Slightly lower myosin heavy chain (MHC) band intensity of gels added with oxidised phenolics at the optimal level was noticeable compared with that of the control. A sensory evaluation study indicated that addition of oxidised phenolic compounds had no negative impact on the colour and taste of the resulting gels ($P > 0.05$). Gels with addition of all oxidised phenolics had a finer matrix with smaller strands.

1. Introduction

Phenolic compounds are a diverse group of chemicals possessing one or more aromatic rings to which at least one hydroxyl group is attached. Phenolic compounds are produced as secondary metabolites by most plants and probably function as natural antimicrobial agents and inhibitors of pre-harvest seed germination (O'Connell and Fox, 2001). These compounds generally have an *ortho*-diphenol (or a 1-hydroxy-2-methoxy) structure (Strauss & Gibson, 2004).

Surimi is a Japanese term which can be defined as washed fish mince. With the washing process, myofibrillar proteins, which mainly contribute to gel formation, are concentrated in the resulting surimi (Benjakul, Visessanguan, & Tueksuban, 2003). Thailand is one of the largest surimi producers in southeast Asia. About 16 surimi factories are located in Thailand, with a total production of 96,500–1,13,500 metric tons per year of which 80% is exported to Japan and Korea and the remainder to Singapore and other countries (Hong & Eong, 2005). In general, lean fish have been used for surimi production in Thailand. Due to the limited fish resources, especially lean fish, dark flesh fish have been paid more attention as a potential alternative raw material for surimi production, due to its high potential for capture and low price (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). However, a problem with producing surimi from dark flesh fish, such as mackerel, is the high content of dark muscle associated with the high content of lipid and myoglobin. This results in difficulties in making high quality surimi as evidenced by poor gel forming ability of those species (Chaijan et al., 2004). To increase the gel strength of surimi, various food grade ingredients have been used but the addition of these ingredients poses adverse effects on the surimi gel, particularly off flavour or off colour (Rawdkuen & Benjakul, 2008). Bovine plasma protein has been prohibited due to mad cow disease, while egg white is associated with allergy problems. Therefore, alternative food-grade ingredients are still needed to increase the gel strength of surimi, particularly those produced from dark flesh fish.

Naturally derived plant phenolic compounds, especially in the oxidised form, have been shown to be the potential protein cross-linker (Rawel, Rohn, Kruse, & Kroll, 2002). Delcour et al. (1984) found the formation of a haze in beer due to protein–phenolic compound interactions. Interactions of different phenolic acids and flavonoids with soy proteins were reported by Rawel, Czajka, Rohn, and Kroll (2002). Plant phenols at pH 8 increased the bloom strength of gelatin gel (Strauss & Gibson, 2004). Addition of phenolic compound in combination with 0.1 M NaCl at pH 8.5 resulted in the improved gel properties of canola protein (Rubino, Arntfield, Nadon & Bernatsky 1996). Addition of phenolic compounds at very low amounts might

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have no negative effect on colour of the resulting gel from dark flesh fish surimi, which is generally dark in colour. Nevertheless, the information regarding the effect of phenolic compounds on the gel property of dark flesh fish surimi is very scarce. Thus, this study aimed to investigate the effect of oxidised phenolic compounds, including ferulic acid, tannic acid, catechin and caffeic acid on the properties of mackerel surimi gel.

2. Materials and methods

2.1. Chemicals

Ferulic acid (FA), tannic acid (TA) and β-mercaptoethanol (βME) were obtained from Sigma (St. Louis, MO, USA). Caffeic acid (CF) and catechin (CT) were purchased from Fluka (Buchs, Switzerland). All phenolic compounds used were of analytical grade. Sodium dedocyl sulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Fish sample

Mackerel (*Rastrelliger kanagurta*) with an average weight of 85–90 g were caught from Songkhla coast along the Gulf of Thailand during March–April, 2008, stored in ice and off-loaded approximately 36 h after capture. Upon arrival at the dock in Songkhla, the fish were iced with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. The fish were immediately washed and drained before using for surimi preparation.

2.3. Surimi preparation

Surimi was prepared according to the method of Benjakul and Visessanguan (2003) with slight modifications. Mackerel skin and bones were removed manually and the flesh was minced to uniformity using a mincer with a hole diameter of 5 mm. The mince was then washed with cold water (5 °C) at a mince/water ratio of 1:3 (w/w). The mixture was stirred gently for 4 min and washed mince was filtered with a layer of nylon screen. The washing process was repeated twice. For the third washing, cold 0.5% NaCl solution was used. Finally, the washed mince was subjected to centrifugation using a Model CE 21K basket centrifuge (Grandiumpiant, Belluno, Italy) with a speed of 700 × *g* for 10 min. To the washed mince, 4% sucrose and 4% sorbitol were added and mixed well. The mixture (500 g) was packed in a polyethylene bag and frozen using an air-blast freezer (−20 C). The chemical composition of the resulting surimi was determined according to the AOAC method (AOAC, 1999). Surimi contained 78.41% moisture, 14.0% protein and 0.30% lipid. The pH of the surimi was 6.8. Gel testing was performed within one week of frozen storage.

2.4. Effect of oxidised phenolic compounds on the properties of surimi gels

2.4.1. Preparation of oxidised phenolic solutions

Four phenolic compounds including ferulic acid, tannic acid, caffeic acid and catechin were dissolved according to the method of Strauss and Gibson (2004) with slight modifications. Phenolic solution (100 ml; 1% w/v) was adjusted to pH 8 using 6 N NaOH or 6 N HCl. The prepared solution was placed in a temperature-controlled water bath (40 °C) and subjected to oxygenation for 1 h by bubbling the solution with oxygen to convert the phenolic compounds to quinone. After being oxygenated for 1 h, the solution was then adjusted to pH 7 by using 6 N HCl and was referred to as 'oxidised phenolic compound'.

2.4.2. Surimi gel preparation

To prepare the gel, frozen surimi was tempered for 30 min in running water (26–28 °C) until the core temperature reached 0–2 °C. The surimi was then cut into small pieces with an approximate thickness of 1 cm. The surimi was placed in a mixer (National Model MK-K77, Tokyo, Japan). The moisture content was adjusted to 80% and 2.5% salt was added. Different oxidised phenolic compounds at various concentrations (0%, 0.10%, 0.20%, 0.30%, 0.40%, 0.50% and 0.60% of protein content of surimi) were added. The mixture was chopped for 4 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. Sols were incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Benjakul & Visessanguan, 2003). The control gels were prepared by adding the same volume of distilled water (pH 7) as that of oxidized phenolic solutions. All gels were cooled in iced water and stored overnight at 4 °C prior to analyses.

2.4.3. Texture analysis

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

2.4.4. Determination of expressible moisture content

Expressible moisture content was measured according to the method of Benjakul, Visessanguan, and Srivilai (2001) with slight modifications. Gel samples were cut to a thickness of 5 mm, weighed (*X*) and placed between 3 sheets of Whatman paper No. 4 at the bottom and 2 sheets on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The sample was then removed from the papers and weighed again (*Y*). Expressible moisture content was calculated using the following equation:

$$\text{expressible moisture content (\%)} = 100\,[(X - Y)/X].$$

2.4.5. Determination of whiteness

The colour of the surimi gels was determined using a JP7100F colorimeter (Juki Corporation, Tokyo, Japan). L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Lanier, Hart, and Martin (1991) as follows:

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

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

Protein patterns of surimi gels were analysed by SDS-PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85 °C were added to the sample (3 g). The mixture was then homogenised using a homogeniser (IKA Labortechnik, Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500 × *g* for 20 min to remove undissolved debris. The samples (20 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.4.7. Protein determination

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

2.4.8. Sensory evaluation

Mackerel gels containing oxidised phenolic compounds at optimal levels were evaluated for colour, taste, texture, odour and overall liking by 30 non-trained panelists, in comparison with the control gel (without oxidised phenolic compound). A nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation (Meilgaard, Civille, & Carr, 1990).

2.4.9. Scanning electron microscopy (SEM)

The microstructure of the surimi gels was observed using SEM. Gels containing oxidised phenolic compounds and the control gel (without oxidised phenolic compound) with a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50%, 70%, 80%, 90% and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 10 kV.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed and the mean comparisons were carried out by Duncan's multiple range tests (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 10.0 for Windows: SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Effect of oxidised phenolic compounds on the properties of surimi gels

Breaking force and deformation of gels from mackerel surimi with the addition of different oxidised phenolic compounds at various levels are shown in Fig. 1. Breaking force and deformation of gels increased as the oxidised phenolic compounds were added up to a particular level ($P < 0.05$). Gels with addition of 0.40% oxidised ferulic acid (OFA) or 0.50% oxidised caffeic acid (OCF) had increases in breaking force by 45% or 46.1% and in deformation by 12.2% or 28.1%, respectively, compared with that of the control ($P < 0.05$). For gels with addition of 0.50% oxidised tannic acid (OTA) or 0.10% oxidised catechin (OCT), the breaking force was increased by 115.0% or 70.4% and deformation was increased by 27.5% or 28.4%, respectively. Nevertheless, the continuous decreases in both breaking force and deformation were noticeable when all oxidised phenolic compounds at the higher levels were added ($P < 0.05$). The results revealed that oxidised phenolic compounds at the optimum concentration were effective in increasing gel strength of mackerel surimi. Prigent et al. (2003) found that phenolic compounds can interact with proteins via non-covalent interactions and via covalent interactions. Two types of complexation mechanisms can be distinguished, monodentate and multidentate mechanisms (Haslam, 1989). The multidentate mechanism generally requires a much lower phenolic compound/protein molar ratio and thus a lower concentration of phenolic compound is needed. For the "monodentate" mechanism, a phenolic compound interacts with only one protein site and a higher concentration of phenolic compound is required. OTA, OFA and OCF at higher levels (0.50%, 0.40% and 0.50%, respectively) were required to increase the breaking force and deformation of surimi gels, while OCT at a lower level (0.10%) effectively increased both the breaking force and deformation of surimi gels. The larger the phenolic compound or the more binding sites the phenolic compound possesses, the stronger the association with proteins is expected (Hagerman, Rice, & Ritchard, 1998). Proanthocyanidin trimers bind more tightly to BSA than proanthocyanidin dimers (Artz, Bishop, Dunker, Schanus, & Swanson, 1987). In the present study, the highest breaking force was observed when 0.5% OTA was added ($P < 0.05$). OTA has a greater number of hydroxyl groups attached to the aromatic benzene ring compared to others. As a consequence, a larger amount of quinone could be formed via an oxidation process. Quinones were reported to be able to cross-link the protein molecules (Strauss & Gibson, 2004). The decreased breaking force and deformation of surimi gel with increasing concentrations of phenolic compounds might be associated with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking. De Freitas and Mateus (2001) found that the high concentration of phenolic compounds showed lower efficiency in interacting with proteins.

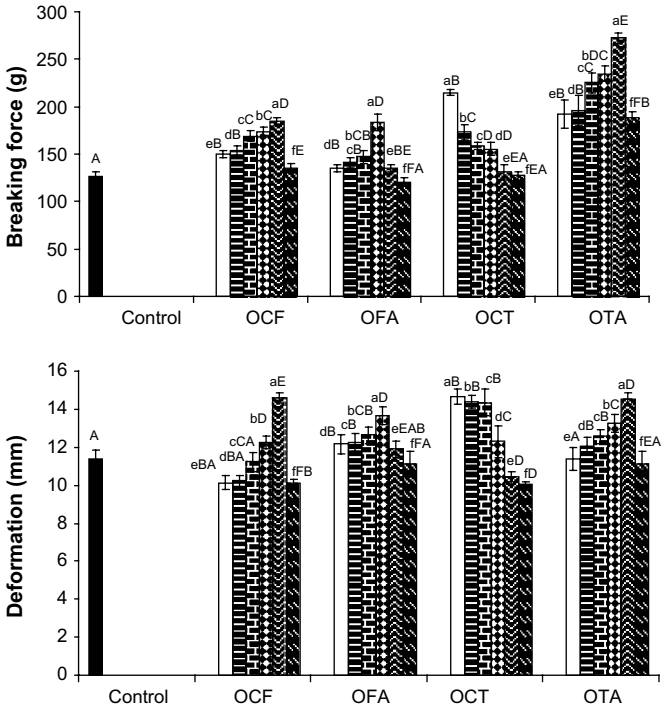

Fig. 1. Breaking force and deformation of gels from mackerel surimi with addition of oxidised phenolic compounds at different levels. OCF, OFA, OCT and OTA represent oxidised caffeic acid, ferulic acid, catechin and tannic acid, respectively. ■, 0%; □, 0.10%; ▤, 0.20%; ▦, 0.30%; ◘, 0.40%; ▨, 0.50%; ▧, 0.60%. Bars represent the standard deviation ($n = 5$). Different capital letters on the bars within the same oxidised phenolic compounds together with the control indicate the significant differences ($P < 0.05$). The different letters on the bars within the same levels of oxidised phenolic compounds indicate the significant differences ($P < 0.05$).

3.2. Effect of oxidised phenolic compounds on the expressible moisture content of surimi gels

The expressible moisture content of surimi gels with the addition of different oxidised phenolic compounds at various levels is shown in Table 1. When OFA, OCT, OCF or OTA at the optimal levels were added, the expressible moisture content of mackerel surimi gels significantly decrease, compared to that of controls ($P < 0.05$).

Table 1
Expressible moisture content and whiteness of gels from mackerel surimi added with various oxidised phenolic compounds at different levels.

Oxidised phenolic compounds	Amount added (%)	Expressible moisture content (%)	Whiteness
Control	–	17.25 ± 1.69a*	65.19 ± 1.12a*
OCF	0.1	12.43 ± 0.22bA**	65.12 ± 0.47aA**
	0.2	12.03 ± 0.68bA	65.10 ± 0.92aA
	0.3	9.79 ± 0.28cA	65.08 ± 0.57aA
	0.4	8.81 ± 1.05dA	65.05 ± 0.59aA
	0.5	7.12 ± 0.76eA	65.00 ± 0.23aA
	0.6	14.23 ± 0.21fA	64.76 ± 0.66bA
OFA	0.1	11.76 ± 0.31bB	65.20 ± 0.48aA
	0.2	8.16 ± 1.06cB	65.18 ± 0.37aA
	0.3	7.99 ± 0.63cB	65.15 ± 0.27aA
	0.4	7.76 ± 0.92cB	65.15 ± 0.35aA
	0.5	8.33 ± 0.32cB	65.10 ± 0.72aA
	0.6	12.32 ± 0.31dB	65.00 ± 0.12aB
OCT	0.1	7.10 ± 0.74bC	65.16 ± 0.54aA
	0.2	7.63 ± 0.47bC	65.16 ± 0.63aA
	0.3	8.44 ± 0.75cC	65.12 ± 0.70aA
	0.4	9.16 ± 1.06dC	65.04 ± 0.45aA
	0.5	9.80 ± 0.50dC	65.01 ± 0.67aA
	0.6	10.79 ± 0.99eC	64.86 ± 0.26bA
OTA	0.1	8.46 ± 0.20bD	65.14 ± 0.54aA
	0.2	4.59 ± 0.91cD	65.13 ± 0.22aA
	0.3	3.67 ± 0.51dD	65.04 ± 0.47aA
	0.4	3.54 ± 0.22dD	65.03 ± 0.32aA
	0.5	3.26 ± 1.00dD	65.00 ± 0.97aA
	0.6	10.38 ± 0.21eDC	64.73 ± 0.21bA

Values are mean ± standard deviation ($n = 5$).
* Different letters in the same column within the same oxidised phenolic compound together with the control indicate the significant differences ($P < 0.05$).
** Different capital letters in the same coloumn within the same level of oxidised phenolic compounds used indicate the significant differences ($P < 0.05$).

The lowest expressible moisture content was observed in gels with addition of 0.50% OTA. This indicated that the water-holding capacity of surimi gels could be improved with the addition of phenolic compounds at optimal levels. During setting at 40 °C, proteins underwent some denaturation and aligned themselves gradually to form the network, which can imbibe water (Benjakul & Visessanguan, 2003). When the optimal level of oxidised phenolic compound was added, the cross-linking of proteins could be enhanced, resulting in the formation of a stronger network with greater water holding capacity. In general, the water holding capacity of gel with addition of oxidised phenolic compounds varied with the type of phenolics.

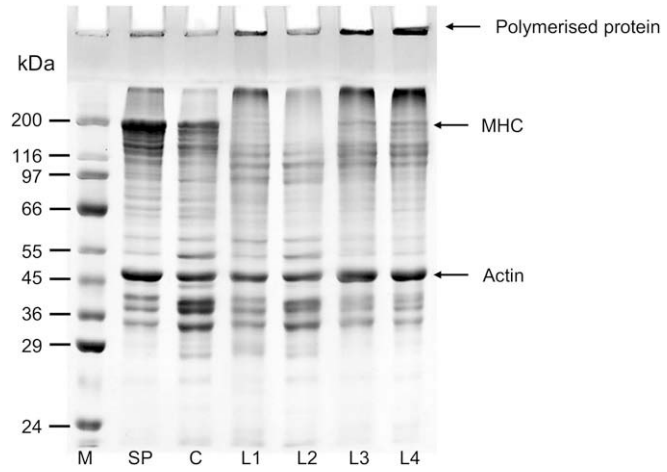

Fig. 2. SDS-PAGE patterns of protein in gels from mackerel surimi with the addition of various oxidised phenolic compounds at the selected levels. MHC, myosin heavy chain; M, marker; SP, surimi paste; C, control (without oxidised phenolic addition); L1, 0.50% OCF, oxidised caffeic acid; L2, 0.40% OFA, oxidised ferulic acid; L3, 0.10% OCT, oxidised catechin; L4, 0.50% OTA, oxidised tannic acid.

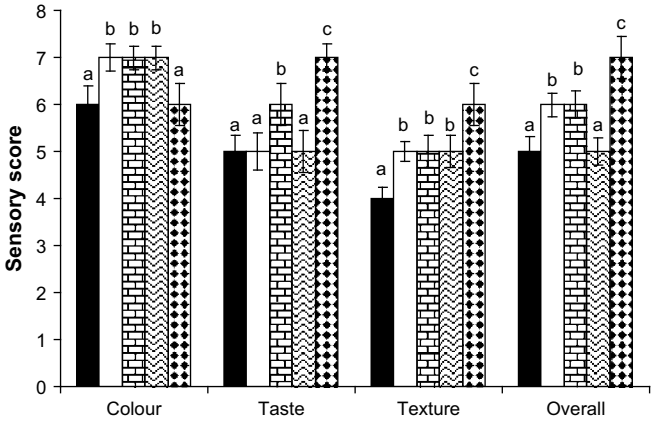

Fig. 3. Likeness score of surimi gels from mackerel without and with the addition of different oxidised phenolic compounds at selected levels. C, control (without oxidised phenolic addition); OCF, oxidised caffeic acid; OFA, oxidised ferulic acid; OCT, oxidised catechin; OTA, oxidised tannic acid. ■, control; □, 0.50% OCF; ▤, 0.40% OFA; ▨, 0.10% OCT; ◈, 0.50% OTA. Bars represent standard deviation ($n = 30$). Different letters on the bars within the same sensory attribute indicate significant differences ($P < 0.05$).

3.3. Effect of oxidised phenolic compounds on the whiteness of surimi gels

No changes in whiteness were observed in surimi gels with addition of all four oxidised phenolic compounds at optimal levels, compared with the control ($P > 0.05$). When oxidised phenolic compounds at a level of 0.6% were added, all gels except that with OFA added had a decrease in whiteness ($P < 0.05$) (Table 1). Phenolic compounds were responsible for discoloration in cheese products (O'Connell & Fox, 2001). However, mackerel fish has a high content of dark muscle associated with a high content of myoglobin. This results in a natural dark colour of mackerel surimi (Chen, 2002). Thus, oxidised phenolic compounds at the optimum level could be used in mackerel surimi to improve the gel strength without any effect on the whiteness of the surimi gel.

3.4. Effect of oxidised phenolic compounds on the protein patterns of surimi gels

The protein patterns of surimi gels without and with the addition of different oxidised phenolic compounds at the optimum level yielding the highest breaking force and deformation are depicted in Fig. 2. Surimi paste contained MHC and actin as the major proteins. Decrease in MHC band intensity was found in the control gel (without addition of oxidised phenolic compounds), when compared with that observed in sol. The result suggested that the formation of cross-links stabilised by non-disulphide covalent bonds took place, especially during setting. MHC was most susceptible to cross-linking during setting (Benjakul, & Visessanguan, 2003). Benjakul and Visessanguan (2003) reported the decrease in MHC of surimi gel from bigeye snapper, particularly when the setting was implemented. No MHC band remained in gels with addition of 0.50% OCF or 0.40% OFA. For gels with addition of 0.10% OCT or 0.5% OTA, the MHC band disappeared almost completely. The result suggested that MHC was cross-linked by oxidised phenolic compounds to a high extent via non-disulphide covalent bonds. Coincidental occurrence of polymerised proteins in

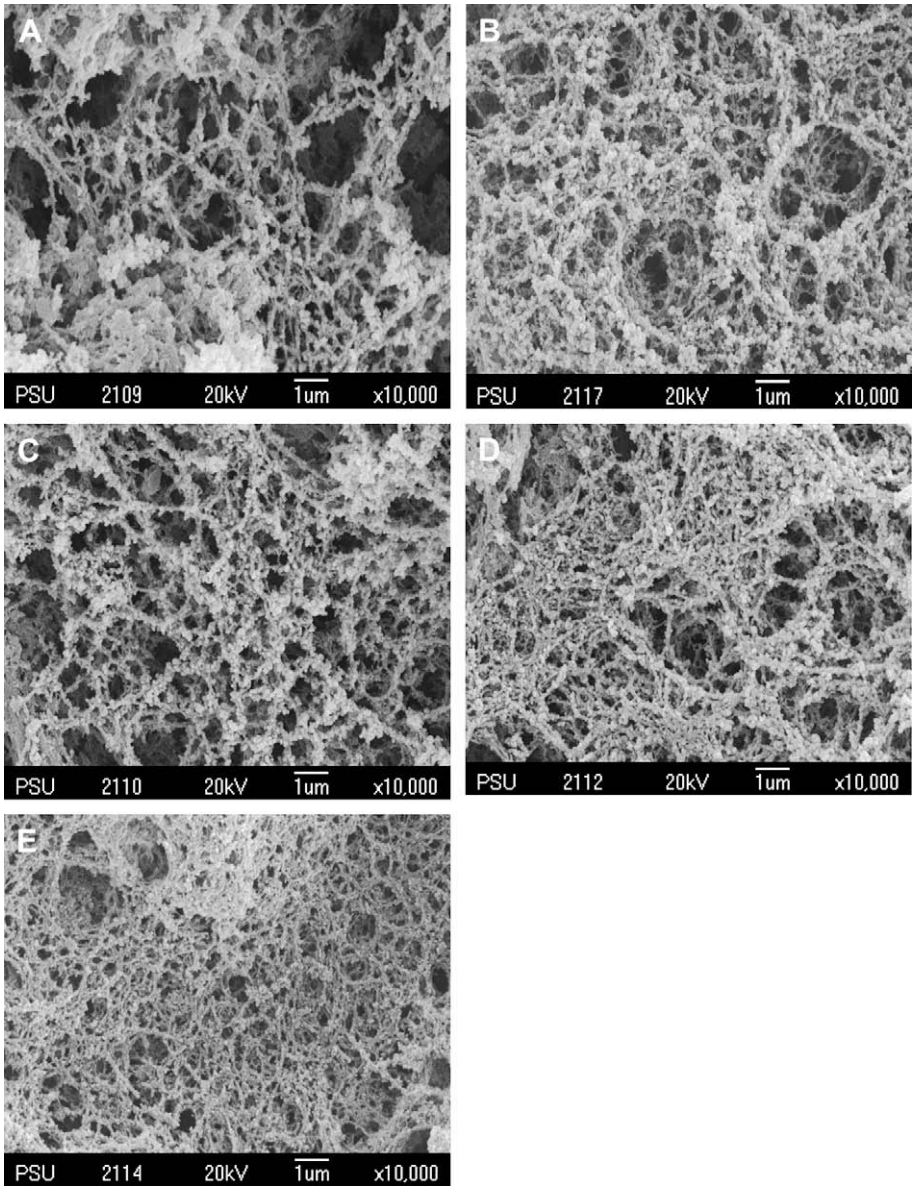

Fig. 4. Electron microscopic images of gels from mackerel surimi without and with the addition of different oxidised phenolic compounds at the selected levels. OCF, oxidised caffeic acid; OFA, oxidised ferulic acid; OCT, oxidised catechin; OTA, oxidised tannic acid. A, control; B, 0.50% OCF; C, 0.40% OFA; D, 0.10% OCT; E, 0.50% OTA (magnification: 10,000×).

the stacking gel was noticeable. No marked changes in actin band intensity were observed between the control gel and those with addition of 0.10% OCT or 0.50% OTA. However, lower band intensity of actin was found in gels with addition of 0.5% OCF or 0.4% OFA. Due to the slight differences in protein patterns among gels with addition of different oxidised phenolic compounds, the protein molecules might be cross-linked differently in term of degree and site/domains of interaction induced by oxidised phenolic compounds. Additionally, dark flesh fish were reported to possess high autolytic activity (Shimizu, Toyohara, & Lanier, 1992), which is associated with the poor gel properties. Kroll, Rawel, and Rohn (2003) reported that the interactions between phenolic compounds and proteins may lead to a decrease of protein digestibility, by blocking the substrate and/or inhibiting certain proteases. Covalent modification of proteins by phenolic oxidation products generated at alkaline pH was reported extensively (Rawel, Czajka, et al., 2002; Rawel, Rohn, et al., 2002). It was postulated that oxidised phenolic compounds might partially lower the proteolysis caused by endogenous proteinases. Cross-linked proteins were more likely less susceptible to proteolysis. This might be associated with gel strengthening in addition to enhanced protein cross-linking.

3.5. Effect of oxidised phenolic compounds on the sensory properties of surimi gels

Likeness scores of the control gels (without oxidised phenolic compounds) and those containing oxidised phenolic compounds at selected levels are depicted in Fig. 3. Addition of oxidised phenolic compounds had no negative impact on the colour and taste of the resulting gels. The addition of oxidised phenolic compounds at selected levels yielded the gel with the higher texture score, compared with that of control ($P < 0.05$). This was coincidental with the increased breaking force and deformation in the surimi gels with addition of oxidised phenolic compounds (Fig. 1). Among surimi gels with addition of different phenolic compounds, that containing 0.5% OTA had the highest texture score ($P < 0.05$). However, no differences in texture score were found between the gels with addition of 0.40% OFA, 0.10% OCT and 0.50% OCF

($P > 0.05$). Phenolic compounds play a major role in the sensory attributes of many food products (O'Connell and Fox, 2001). The role of phenolic compounds in astringency has been established and is thought to be associated with the precipitation of salivary glycoproteins and mucopolysaccharides onto the tongue, resulting in the development of a feeling of constriction, roughness and dryness on the palate (Haslam & Lilley, 1988). The addition of tea extracts containing a high proportion of polyphenols to sherbet mixes, yoghurt and acidified milk drinks was reported (Ioki & Suzuki, 1992). Addition of 0.10% OCT or 0.50% OCF had no effect on taste score ($P > 0.05$). Furthermore, gels with addition of 0.40% OFA or 0.50% OTA showed the highest taste score ($P < 0.05$). For the colour score, no differences were found between the control gel and that containing 0.50% OTA ($P > 0.05$). Moreover, the addition of 0.40% OFA, 0.10% OCT or 0.50% OCF yielded a gel with increased colour score ($P < 0.05$). The results suggested that the addition of oxidised phenolic compounds at very low levels had no adverse effect on the colour of the resulting gel. Among all the gel samples with added oxidised phenolic compounds, those containing 0.5% OTA had the highest overall likeness score, followed by those with 0.40% OFA or 0.50% OCF. Gel with 0.1% OCT exhibited a similar overall likeness score to the control gel ($P > 0.05$). No differences in odour score were found between the control gel and those with the oxidised phenolic compounds ($P > 0.05$) (data not shown). Therefore, the addition of oxidised phenolic compounds at low concentration in this study did not negatively affect the sensory properties of mackerel surimi gel.

3.6. Effect of oxidised phenolic compounds on the microstructure of surimi gels

Microstructure of control gel (without oxidised phenolic compounds) (A), gel with 0.50% OCF (B), 0.40% OFA (C), 0.10% OCT (D) and 0.50% OTA (E) are illustrated in Fig. 4. Surimi gels containing all oxidised phenolic compounds had a finer and continuous matrix than the control. This suggested that oxidised phenolic compounds might induce the cross-linking of protein, in which the protein filaments could be formed. Those filaments further underwent polymerization effectively, leading to a gel network with a fibrillar structure. Among four oxidised phenolic compounds, the gel with 0.50% OTA added possessed a more ordered fibrillar structure with finer strands and high capacity of imbibing water. This might be attributed to the greater number of binding sites in OTA, which in turn caused a higher aggregation. The finer and more ordered structure of the gel with OTA added correlated with the highest breaking force and deformation (Fig. 1) as well as the lowest expressible moisture content (Table 1).

4. Conclusions

The type and concentration of oxidised phenolic compounds had varying influences on mackerel surimi gels. OTA, at a level of 0.5%, exhibited a gel strengthening effect without affecting the whiteness of mackerel surimi gels. Therefore, the use of oxidised phenolic compounds can be a suitable option to improve the gel strength of surimi manufactured from dark flesh fish.

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Effect of oxidised tannic acid on the gel properties of mackerel (*Rastrelliger kanagurta*) mince and surimi prepared by different washing processes

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ABSTRACT

Effect of oxidised tannic acid (OTA) at different levels (0, 0.25, 0.50 and 0.75% of protein content) on the gel properties of mackerel (*Rastrelliger kanagurta*) mince and surimi prepared by different washing processes was investigated. Breaking force and deformation of gels varied with washing processes and concentrations of OTA. The gel of alkaline-saline washing process surimi (ASWPS) added with 0.25% OTA had the increases in breaking force and deformation by 166.2 and 45.9%, respectively, compared with that of conventional washing process surimi (CWPS) without OTA addition. Those increases were associated with the lowered expressible moisture content. Electrophoretic studies revealed that the greater polymerisation was found in ASWPS added with 0.25% OTA. Slight retention of myosin heavy chain (MHC) with lowered trichloroacetic acid (TCA) soluble peptide contents was observed in ASWPS gel added with 0.25% OTA, suggesting the decreased degradation induced by indigenous proteases. The microstructure of ASWPS gels became more ordered, compact and denser with the addition of 0.25% OTA. The use of OTA in conjunction with alkaline-saline washing process could improve the properties of gel from mackerel surimi without adverse effect on sensory properties.

1. Introduction

Surimi technology has been widely developed to improve the gelling properties of fish mince. Basically surimi is produced by repeatedly washing the mechanically separated fish mince with chilled water (5–10 °C) until most of the water-soluble protein is removed. The washing procedure is of great importance for surimi quality, not only for removing fat and undesirable materials, such as blood, pigments and odourous substances but also for increasing the concentration of myofibrillar proteins, the major proteins contributing to gel formation (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). In general, lean fish have been used for surimi production worldwide. Due to the limited fish resources, dark muscle fish have been paid more attention as a potential alternative raw material for surimi production. However, problem faced with producing surimi from those dark fleshed species, such as mackerel (*Rastrelliger kanagurta*), is the high content of dark muscle associated with high content of lipid and myoglobin, resulting in the difficulties in making high quality surimi (Chen, 2002). Due to the lowered pH of dark fleshed fish during postmortem handling or storage, the gel forming ability decreases gradually. To alleviate this problem, alkaline leaching has been developed to raise the pH of muscle and to increase the efficacy in removing sarcoplasmic proteins, lipid, pigments, etc. (Shimizu, 1965). Shimizu (1965) also reported that surimi produced by alkaline leaching exhibited higher breaking force and deformation, when compared with surimi produced by conventional method.

To enhance the gel strength of surimi or fish mince, various food-grade ingredients and cross-linking enzymes such as microbial transglutaminase have been used (Benjakul & Visessanguan, 2003; Benjakul, Visessanguan, & Chantarasuwan, 2004; Benjakul, Visessanguan, Tueksuban, & Tanaka, 2004). However, the addition of some ingredients poses the adverse effects on the surimi gel, particularly on its flavour or colour. Addition of the bovine plasma protein has been prohibited due to the mad cow disease, while egg white is associated with allergy problems. Hence, the need of natural additives with an ability of protein cross-linking has been paid increasing attention for the surimi industry.

Polyphenols are the natural compounds which are abundant in the plants. They can be classified into two forms, hydrolysable polyphenols and condensed polyphenol (Shahidi & Naczk, 2004). Tannins, also referred to as tannic acid (TA), belong to the first group and have a structure consisting of a central carbohydrate (glucose) and 10 galloyl groups (Lopes, Schulman, & Hermes-Lima, 1999). Red wine, coffee, chocolate, tea, sorghum, spinach and fruits (Bananas, grapes and persimmons) are the different kinds of foods containing tannins (Lopes et al., 1999; Naczk & Shahidi, 2004).

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Tannins can be used as a food additive with the range of 10–400 mg/l, depending on the type of food to which it is added (Chen & Chung, 2000). Tannins contain sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with the proteins and other macromolecules. The interactions between phenolic compounds and proteins play a very important role in the processing of certain food products. Phenols may be oxidised easily, in an alkaline solution, to their corresponding quinones (Hurrell & Finot, 1984). The quinone, a reactive electrophilic intermediate, can readily undergo attack by nucleophiles such as lysine, methionine, cysteine and tryptophan residues in a protein chain (Hurrell & Finot, 1984). The formation of rigid molecular structures by reactions of ortho-quinones with proteins has been demonstrated by Strauss and Gibson (2004). Interactions of different phenolic acids and flavonoids with soy proteins were reported by Rawel, Czajka, Rohn, and Kroll (2002). Significant increase in the gel strength of bigeye snapper surimi was found when oxidised phenolic compounds were added (Balange & Benjakul, 2009). Among the all used oxidised phenolic compounds, the oxidised tannic acid (OTA) exhibited the highest gel strengthening effect, compared with oxidised ferulic acid, catechin and caffeic acid. The use of tannic acid in combination with appropriate washing process would be an effective means to fully improve the gel property of surimi from dark fleshed fish. However, no information regarding the effect of tannic acid on the properties of surimi gel of mackerel prepared by different washing processes has been reported. Therefore, this study aimed to investigate the effect of oxidised tannic acid (OTA) as a protein cross-linker on the textural, physical and sensory properties of mackerel (*R. kanagurta*) unwashed mince and surimi prepared by conventional washing process (CWPS) and alkaline-saline washing process (ASWPS).

2. Materials and methods

2.1. Chemicals

Tannic acid (TA), L-tyrosine and β-mercaptoethanol (βME) were obtained from Sigma (St. Louis, MO, USA). Sodium dedocyl sulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA). Sodium chloride, tricholoroacetic acid and ethanol were obtained from Merck (Darmstadt, Germany).

2.2. Fish sample

Mackerel (*R. kanagurta*) with an average weight of 85–90 g were caught from Songkhla coast along the Gulf of Thailand, stored in ice and off-loaded approximately 36 h after capture. Upon the arrival to the dock in Songkhla province, fish were iced with a fish/ice ratio of 1:2 (w/w) and transported to the laboratory within 1 h. The fish were immediately washed and drained. The flesh was removed manually and used for the preparation of mince and surimi.

2.3. Preparation of mince and surimi by different washing processes

2.3.1. Preparation of unwashed mince

Mince was prepared according to the method of Benjakul and Visessanguan (2003) with a slight modification. The flesh was minced to uniformity using a mincer with a hole diameter of 5 mm. This was referred to as "Unwashed mince". Cryoprotectants (4% sucrose and 4% sorbitol) were added to the unwashed mince and mixed well. Sample (0.5 kg) was packed in polyethylene bag, sealed and frozen using an air-blast freezer at −18 °C.

2.3.2. Preparation of surimi by conventional and alkaline-saline washing processes

Surimi was prepared by conventional washing process according to the method of Chaijan et al. (2004) with a slight modification. Mince was suspended in cold water (5 °C) at a mince/water ratio of 1:3 (w/w). The mixture was stirred gently for 4 min and washed mince was filtered with a layer of nylon screen (Material: Nylon 1010, nylon 66, polyamide and polyester fiber) (Butterfly, Lao Hah Seng Lee Co., Ltd., Bangkok, Thailand). The washing process was repeated twice. For the third washing, cold 0.5% NaCl solution was used. Finally, the washed mince was subjected to centrifugation using a Model CE 21K basket centrifuge (Grandiumpiant, Belluno, Italy) with a speed of 700 × *g* at 4 °C for 10 min. Washed mince was added with cryoprotectants, packaged and frozen as previously described. This was referred to as "Conventional washing process surimi" (CWPS).

For alkaline-saline washing process, surimi was prepared according to the method of Shimizu (1965) with a slight modification. The mince was suspended in cold (5 °C) alkaline-salt solution (0.15% NaCl in 0.2% $NaHCO_3$) at a mince/solution ratio of 1:4 (w/w). The mixture was stirred gently for 15 min and washed mince was filtered with a layer of nylon screen. The washing process was repeated twice. For the third washing, cold 0.5% NaCl solution was used. Finally, the washed mince was subjected to centrifugation using a Model CE 21K basket centrifuge with a speed of 700 × *g* at 4 °C for 10 min. To the washed mince, cryoprotectants were added. The mixed sample was packaged and frozen as mentioned before. This was referred to as "Alkaline-saline washing process surimi" (ASWPS).

The stabilised mince and surimi were stored at −18 °C not longer than 2 months. All samples were subjected to analysis of protein patterns using SDS-PAGE.

2.4. Effect of oxidised tannic acid (OTA) on gel properties of mince and surimi prepared by different washing processes

2.4.1. Preparation of OTA solution

Tannic acid was dissolved in distilled water as per the method of Strauss and Gibson (2004) with a slight modification. The solution (100 ml; 1% w/v) was adjusted to pH 8 using 6 N NaOH or 6 N HCl. The prepared solution was placed in a temperature-controlled water bath (40 °C) and subjected to oxygenation for 1 h by bubbling the solution with oxygen with the purity of 99.5–100% (TTS Gas Agency, Hat Yai, Songkhla, Thailand) to convert tannic acid to quinone. The solution was adjusted to pH 7 by using 6 N HCl and referred to as 'oxidised tannic acid' (OTA).

2.4.2. Surimi gel preparation

To prepare the gels, frozen mince and surimi prepared by different washing processes, were tempered for 30 min in running water (26–28 °C) until the core temperature reached 0–2 °C. The mince and surimi was then cut into small pieces with an approximate thickness of 1 cm and placed in a mixer (National Model MK-K77, Tokyo, Japan). The moisture was adjusted to 80% and 2.5% sodium chloride was added. OTA at various concentrations (0, 0.25, 0.50 and 0.75% of protein content) was added into the sols. The mixture was chopped for 4 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 casing were sealed tightly. Sols were incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Benjakul, Visessanguan, & Tueksuban, 2003). All gels were cooled in iced water and stored overnight at 4 °C prior to analyses.

2.4.3. Measurement of gel properties

2.4.3.1. Texture analysis. Texture analysis of gels was performed using a texture analyser Model TA-XT2 (Stable Micro Systems, Surrey, England) according to the method of Benjakul et al. (2003). Gels were equilibrated and tested at room temperature. Five cylinder-shaped samples of 2.5 cm in length were prepared. The breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyser equipped with a spherical plunger (5 mm diameter). The probe was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed (60 mm min^{-1}) until the puncture occurred. The force in gram (g) required to puncture into the gel (breaking force) and the distance (in mm) at which the ball probe punctured into the gel (deformation) were recorded.

2.4.3.2. Determination of expressible moisture content. Expressible moisture content was measured according to the method of Benjakul, Visessanguan, and Srivilai (2001) with a slight modification. Gel samples were cut into a thickness of 5 mm, weighed (X) and placed between 3 pieces of Whatman paper No. 4 at the bottom and 2 pieces on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The samples were then removed from the papers and weighed again (Y). Expressible moisture content was calculated using the following equation:

$$\text{Expressible moisture content}(\%) = 100[(X - Y)/X]$$

2.4.3.3. Determination of whiteness. Colour of gels was determined in triplicate using a JP7100F colourimeter (Juki Corporation, Tokyo, Japan). CIE L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Lanier, Hart, and Martin (1991) as follows:

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

2.4.3.4. SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Protein patterns of gels were analysed under reducing condition, in comparision with unwashed mince and surimi prepared by different washing processes by SDS-PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85 °C were added to the sample (3 g). The mixture was then homogenised using a homogeniser (IKA Labortechnik, Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500 × g for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson & Hodgen, 1940) using bovine serum albumin as a standard. The sample was then mixed with sample buffer (4 ml of 10% SDS, 2 ml of glycerol, 1 ml of β-mercaptoethanol, 2.5 ml of 0.5 M Tris–HCl (pH 6.8), and 0.03 g Bromophenol blue) at 1:1 ratio (v/v). The samples (20 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.4.3.5. Solubility determination. Solubility of protein in surimi gel was determined as described by Benjakul et al. (2001). Finely chopped gel sample (1 g) was solubilised with various solvents including 0.6 M KCl (S_1); 20 mM Tris–HCl, pH 8.0 (S_2); 20 mM Tris–HCl, pH 8.0 containing 1% SDS (S_3); 20 mM Tris–HCl, pH 8.0 containing 1% SDS and 8 M urea (S_4) and 20 mM Tris–HCl, pH 8.0 containing 1% SDS, 2% β-mercaptoethanol and 8 M urea (S_5). The mixture was homogenised for 1 min, boiled for 2 min and stirred for 4 h at room temperature (28–30 °C) using a magnetic stirrer (IKA-Werke, Staufen, Germany). The mixture was centrifuged at 10,000 × g for 30 min at 25 °C using a centrifuge (Sorvall Model RC-B plus, Newtown, CT, USA). Two millilitres of 50% (w/v) cold trichloroacetic acid (TCA) were added to 10 ml of supernatant. The mixture was kept at 4 °C for 18 h prior to centrifugation at 10,000 × g for 20 min. The precipitate was washed with 10% (w/v) TCA, followed by solubilising in 0.5 M NaOH. Protein concentration was determined by the Biuret method (Robinson & Hodgen, 1940). Solubility of protein in surimi samples was expressed as the percentage of total protein in surimi. To completely solubilise the total proteins, gels were solubilised directly in 0.5 M NaOH.

2.4.3.6. Determination of TCA-soluble peptides. To 2 g of finely chopped gel samples, 18 ml of 5% TCA were added and the mixture was homogenised at a speed of 11,000 rpm for 2 min using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was incubated at 4 °C for 1 h and centrifuged at 8000 × g for 5 min (25 °C) using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). TCA-soluble peptide content in the supernatant was measured according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and expressed as mmol tyrosine/g sample.

2.4.3.7. Scanning electron microscopy (SEM). Microstructure of gels from unwashed mince, CWPS and ASWPS without and with OTA at the optimum level was determined using SEM. Samples with a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 10 kV.

2.4.3.8. Sensory evaluation. ASWPS gels without and with 0.25% OTA were evaluated for colour, appearance, odour, taste, texture and overall liking by 30 non-trained panelists. A nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely, was used for sensory evaluation (Meilgaard, Civille, & Carr, 1990).

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range tests (Steel & Torrie, 1980). To compare the differences in sensorial data, T-test was used. Analysis was performed using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL, USA).

3. Results and discussion

3.1. Protein patterns of unwashed mince and surimi prepared by different washing processes

Differences in protein patterns were noticeable between unwashed mince and surimi prepared from different washing processes (Fig. 1). The lowest myosin heavy chain (MHC) and actin band intensity was found in unwashed mince. Nevertheless, the higher band intensity of proteins with molecular weight of 39, 37 and 32 kDa was observed in unwashed mince. Higher MHC and

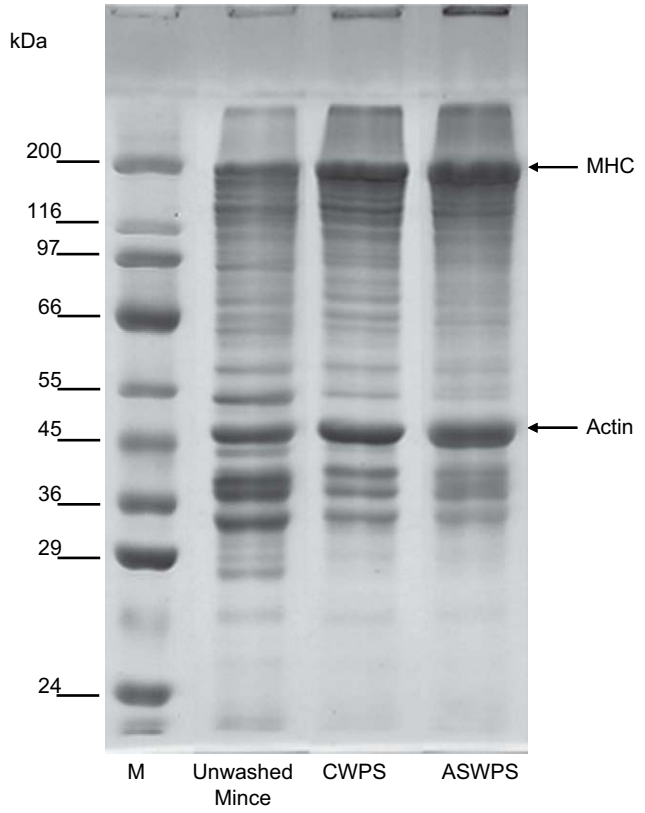

Fig. 1. SDS-PAGE patterns of proteins of unwashed mackerel mince and surimi from different washing processes. MHC, myosin heavy chain; M, protein markers; CWPS, conventional washing process surimi; ASWPS, alkaline-saline washing process surimi.

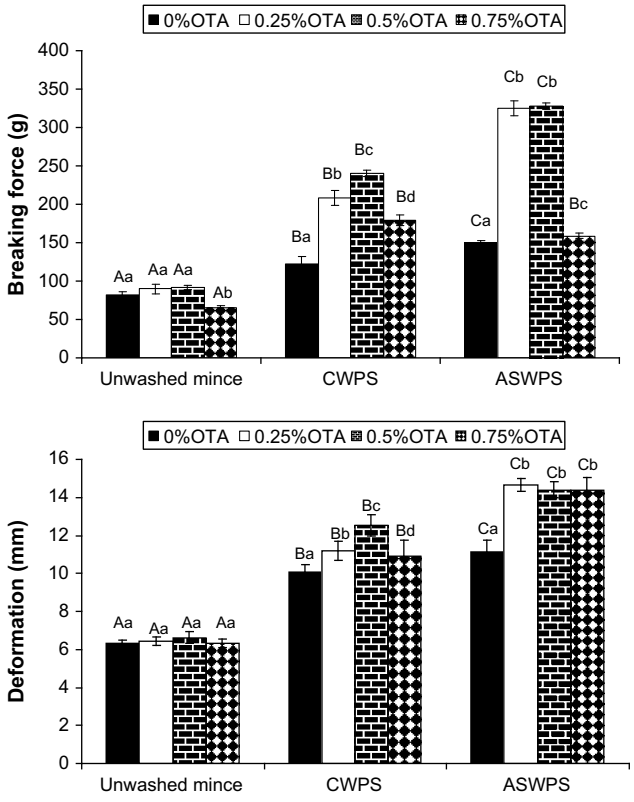

Fig. 2. Breaking force and deformation of gels from unwashed mackerel mince and surimi from different washing processes. CWPS and ASWPS represent conventional washing process surimi and alkaline-saline washing process surimi, respectively. Bars represent the standard deviation from five determinations. Different letters on the bars within the same washing treatment indicate the significant differences ($P < 0.05$). The different capital letters on the bars within the same levels of OTA added indicate the significant differences ($P < 0.05$).

actin band intensity was noticeable in ASWPS, compared with CWPS. The increase in MHC band intensity in ASWPS must be attributed to the effective removal of interfering and low molecular weight components, especially sarcoplasmic proteins. Protein with the molecular weight of 55 kDa almost disappeared in ASWPS and the lower amount of this protein was retained in CWPS, in comparison with that found in unwashed mince. For proteins with the molecular weight ranging from 55 to 120 kDa, slightly lower band intensity was observed in ASWPS. Sarcoplasmic proteins are soluble in water and salt solutions of low ionic strength of 0.05 (Govindan, 1985). Solubility of sarcoplasmic proteins of dark fleshed species is increased in "alkaline-saline leaching" solution (Shimizu, 1965). The greater removal of sarcoplasmic proteins from the mince by alkaline-saline washing resulted in the higher concentration of myofibrillar proteins including MHC and actin, compared with typical washing process as evidenced by the larger band intensity of those proteins. After washing, the proteins with molecular weight lower than 35 kDa almost disappeared.

3.2. Effect of OTA on breaking force and deformation of gel from unwashed mince and surimi prepared by different washing processes

Different breaking force and deformation were observed between gels from unwashed mince, CWPS and ASWPS ($P < 0.05$) (Fig. 2). ASWPS showed the highest breaking force and deformation, followed by CWPS and unwashed mince, respectively. The higher MHC content of ASWPS (Fig. 1) might be associated with higher gel forming ability because it has been established that myofibrillar proteins, mainly MHC, contribute to gel formation (Benjakul & Visessanguan, 2003). Therefore, washing process, particularly alkaline-saline washing, played a role in the improvement of gel strength of mackerel surimi. Shimizu, Machida, Kawasaki, and Kaguri (1979) reported that Pacific mackerel mince washed by alkaline-saline leaching method had the increase in gel strength by 10-fold, compared with unwashed mince. Only a two to three fold increase in gel strength was found in mince washed by conventional method.

Addition of OTA at various levels resulted in varying breaking force and deformation of resulting gels (Fig. 2). For unwashed mince, the addition of 0.25 and 0.5% OTA had no impact on breaking force of resulting gel ($P > 0.05$). OTA might preferably interact with sarcoplasmic proteins instead of myofibrillar proteins. However, the decrease in breaking force was found in unwashed mince gel added with 0.75% OTA ($P < 0.05$). The larger aggregate of sarcoplasmic protein formed, when 0.75% OTA was added, might impede the interaction between myofibrillar proteins. This led to the slight decrease in breaking force of resulting gel. Addition of OTA up to 0.75% had no effect on deformation of the gel from unwashed mince ($P > 0.05$). For CWPS, breaking force increased when OTA was added up to 0.5% ($P < 0.05$). Thereafter, the decrease was found in gel added with 0.75% OTA ($P < 0.05$). The similar result was observed for deformation. CWPS gel added with 0.5% OTA had the increases in breaking force and deformation by 96.7 and 24.8%, respectively, compared with that without OTA addition.

Gels from ASWPS added with 0.25% OTA showed the increases in breaking force and deformation by 166.2 and 45.9%, respectively, compared with those of gel from CWPS without OTA. No differences in breaking force and deformation were noticeable between

gels added with 0.25 and 0.5% OTA ($P > 0.05$). The result suggests that OTA addition had the synergistic effect on gel strengthening with alkaline-saline washing process. ASWPS gel added with 0.75% OTA had the lower breaking force than those added with 0.25% or 0.5% OTA ($P < 0.05$), while gel with 0.75% OTA showed similar deformation to those with lower OTA levels ($P > 0.05$). The decreased breaking force and deformation with increasing concentrations of OTA in the present study might be associated with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking. The lower solubility of large phenolic compounds at high concentration causes the difficulty to interact with proteins (De Freitas & Mateus, 2001). In addition, the size of the phenolic compound can decrease its conformational flexibility, which is observed to be an important parameter in protein–phenolic compound interactions (Frazier, Papadopoulou, Mueller-Harvey, Kissoon, & Green, 2003).

Alkaline-saline washing process could remove most interfering components including myoglobin, lipid and other impurities more effectively than conventional washing process. This possibly facilitated the better interaction of OTA with MHC. Tannic acid (TA) has a number of hydroxyl groups attached to the aromatic benzene ring which provide more binding cites for proteins (Lopes et al., 1999). Balange and Benjakul (2009) reported an increased breaking force and deformation of bigeye snapper surimi with the addition of oxidised tannic acid. Therefore, 0.5 and 0.25% were the optimal levels of OTA for CWPS and ASWPS, respectively.

3.3. Effect of OTA on expressible moisture content of gels from unwashed mince and surimi prepared by different washing processes

For CWPS gel, the lowest expressible moisture content was found with the addition of 0.5% OTA ($P < 0.05$) (Table 1). The lowest expressible moisture content was found in ASWPS gel added with 0.25 or 0.50% OTA ($P < 0.05$). The decreases in expressible moisture contents were in accordance with the increased breaking force and deformation of resulting surimi gels (Fig. 2). The higher expressible moisture contents were observed in the gels prepared from unwashed mince, irrespective of the levels of OTA incorporated (Table 1). The result suggested that the formation of stronger network induced by OTA might imbibe more water.

Table 1
Expressible moisture content and whiteness of gels of unwashed mackerel mince and surimi prepared by different washing processes and added with OTA at different levels.

Samples	OTA amount added (%)	Expressible[a] moisture content (%)	Whiteness[a]
Unwashed mince	0	17.43 ± 0.78aB	61.21 ± 0.67aB
	0.25	17.04 ± 0.21aB	60.42 ± 0.21bB
	0.50	17.12 ± 0.62aB	59.15 ± 0.07cB
	0.75	18.02 ± 0.13bB	57.72 ± 0.36dB
CWPS	0	14.67 ± 1.09aA	65.87 ± 0.58aA
	0.25	4.84 ± 0.76bA	65.69 ± 0.19aA
	0.50	3.35 ± 0.82 cA	65.54 ± 0.44aA
	0.75	12.30 ± 0.73dA	63.22 ± 0.16bA
ASWPS	0	11.14 ± 0.79aC	67.95 ± 0.46aC
	0.25	3.69 ± 0.43bC	63.66 ± 0.46bC
	0.50	3.14 ± 0.27bCA	61.36 ± 0.51cC
	0.75	7.62 ± 0.32cC	59.82 ± 0.44dC

Different letters in the same column within the same sample indicate the significant differences ($P < 0.05$). Different capital letters in the same column within the same level of OTA added indicate the significant differences ($P < 0.05$).
[a] Mean ± SD ($n = 3$).

3.4. Effect of OTA on whiteness of gels from unwashed mince and surimi prepared by different washing processes

Gels prepared from unwashed mince had the lowest whiteness, compared with those from CWPS and ASWPS (Table 1). Gel from ASWPS had the higher whiteness than that from CWPS ($P < 0.05$). This indicates that alkaline-saline washing process was more effective in removing pigments, especially myoglobin and hemoglobin from the mince than typical washing process. The decreases in whiteness of gels from unwashed mince and ASWPS were observed as OTA levels increased ($P < 0.05$) (Table 1). These results are in agreement with O'Connell and Fox (2001) who reported that phenolic compounds were responsible for darkening in cheese products. For CWPS gels, OTA in the range of 0–0.5% had no impact on the whiteness ($P > 0.05$). However, the addition of 0.75% OTA resulted in the decrease in whiteness ($P < 0.05$). From the result, the addition of OTA caused the decrease in whiteness of resulting gels. Since the plant phenolic compounds naturally have dark colour, the addition of those compounds may cause the darkening of the final products.

3.5. Effect of OTA on protein patterns of gels from unwashed mince and surimi prepared by different washing processes

Protein patterns of gels from unwashed mince, CWPS and ASWPS without and with addition of OTA at the optimum level yielding the highest breaking force and deformation are depicted in Fig. 3. For unwashed mince gel, that containing 0.5% OTA, which had non-significant increase in both breaking force and deformation, was also determined for protein pattern. MHC completely disappeared in the control gel samples (without OTA addition). Actin was found to be the dominant protein in the gel, suggesting that actin was more resistant to proteolysis or could not be polymerised during gelation. The result was in agreement with Benjakul, Seymour, Morrissey, and An (1997) who reported that actin in Pacific whiting muscle was more resistant to proteolysis than MHC. Slight retention of MHC and the increase in actin band intensity were observed in the gels of CWPS and ASWPS added with OTA at

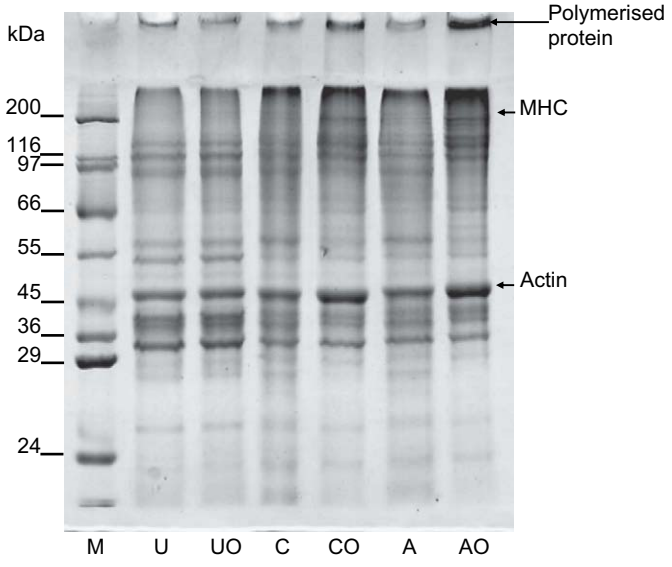

Fig. 3. SDS-PAGE patterns of proteins in gels from unwashed mackerel mince and surimi from different washing processes. MHC, myosin heavy chain; M, protein markers; U, unwashed mince gel; UO, unwashed mince gel with 0.50% OTA; C, CWPS gel; CO, CWPS gel with 0.50% OTA; A, ASWPS gel; AO, ASWPS gel with 0.25% OTA.

the optimum level. The result suggests that OTA might be able to inhibit the degradation of MHC to some extent, as evidenced by the more retained MHC and the lower band intensity of peptides with molecular weight lower than 34 kDa. Benjakul et al. (2003) reported that degradation of muscle proteins, especially MHC, in both washed and unwashed lizardfish mince, occurred at temperatures ranging from 60 to 65 °C. Thus it was postulated that OTA may protect myofibrillar proteins of surimi by binding with indigenous proteases, leading to the loss in their activity. Kroll, Rawel, and Rohn (2003) reported that the interactions between phenolic compounds and proteins may result in inhibiting certain proteases.

For gels of CWPS and ASWPS added with optimal OTA level, a large amount of polymerised proteins as appeared on stacking gel was noticeable (Fig. 3). It indicated that non-disulphide covalent bonds were formed to a higher extent when OTA was incorporated. Protein cross-links might be more resistant to proteolysis caused by indigenous proteases. Additionally, the cross-links mainly contributed to the increases in gel strength of surimi added with OTA at optimal level. Cao, Fu, and He (2007) and Ou, Wang, Tang, Huang, and Jackson (2005) also reported the polymerisation of protein molecules as a possible subsequent reaction of different proteins with phenolic substances.

3.6. Effect of OTA on degradation of gels from unwashed mince and surimi prepared by different washing processes

Protein degradation of gels from unwashed mince, CWPS and ASWPS added without and with OTA at the optimum concentration was monitored as TCA-soluble peptide content (Fig. 4). The highest TCA-soluble peptide content was observed in gels of unwashed mince ($P < 0.05$). This result was in accordance with the lowest gel strength in the unwashed mince gel (Fig. 2). The degradation occurred during heat-induced gelation is considered to result from the action of indigenous proteinases (An, Weerasinghe, Seymour, & Morrissey, 1994; Visessanguan, Menino, Kim, & An, 2001). ASWPS gel had the lower TCA-soluble peptide content than CWPS ($P < 0.05$). In the presence of OTA, the decreases in TCA-soluble peptide content were found in both ASWPS and CWPS gels ($P < 0.05$). It was, therefore, concluded that TCA-soluble peptide formation was slightly inhibited by addition of OTA. This was in agreement with the more retained MHC and actin in gels added with OTA (Fig. 3).

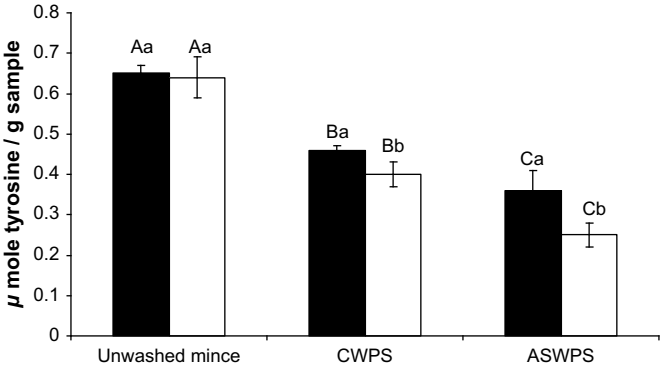

Fig. 4. TCA-soluble peptide content of gels of unwashed mackerel mince and surimi from different washing processes added without and with OTA at optimum level. CWPS, conventional washing process surimi; ASWPS, alkaline-saline washing process surimi. Bars represent the standard deviation from triplicate determinations. Different letters on the bars within the same washing treatment indicate the significant differences ($P < 0.05$). The different capital letters on the bars within the same levels of OTA added indicate the significant differences ($P < 0.05$). ■ without OTA; □ with OTA (0.50% for CWPS and unwashed mince; 0.25% for ASWPS).

3.7. Effect of OTA on solubility of gels from surimi prepared by different washing processes

Solubility of gels from, ASWPS and CWPS added with OTA at the optimum level in different solubilising solutions is shown in Fig. 5. Solubility was found to be lower than 20% in all gels with and without OTA when solubilised with 0.6 M KCl (S_1) and 20 mM Tris–HCl (pH 8.0) (S_2). Native myofibrillar proteins are normally soluble in high ionic strength buffer (Suzuki, 1981). The decrease in solubility suggests the formation of protein aggregates during gelation process. During heating, proteins underwent denaturation and aggregation to form a three dimensional structure (Stone & Stanley, 1992). When the gels were solubilised in 20 mM Tris–HCl (pH 8.0) containing 1% SDS (S_3), solubility was increased up to 34.9 and 31.1% in the control gels of CWPS and ASWPS, respectively, while gels of CWPS and ASWPS added with OTA at the optimum level had the increases in solubility by 29.5% and 27.6%, respectively. SDS is capable of destroying hydrogen and some hydrophobic interactions (Hamada, 1992). Further increases in solubility were observed in S_4, containing urea and SDS, indicating the presence of hydrophobic and hydrogen bonds in surimi gels. Hydrogen bonds might involve in the interactions between hydroxyl groups of phenolic compounds and the nitrogen or oxygen of lysine, arginine, histidine, asparagine, glutamine, serine, threonine, aspartic acid, glutamic acid, tyrosine, cysteine and tryptophan as hydrogen acceptor (Prigent, 2005). Protonation of quinone could take place to some extent after neutralisation. As a consequence, hydroxyl groups could be regenerated partially and interacted with proteins via hydrogen bonding. Hydrophobic interactions may occur between phenolic compounds and hydrophobic amino acids such as alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine and glycine residues (Prigent, 2005). CWPS and ASWPS gels added with OTA at the optimum level had the lower solubility than the control gels when S_4 was used. This suggests that covalent bonds were formed in gels added with phenolic compounds. When the samples were solubilised in S_5, containing urea, SDS and βME, it was noticeable that a higher increase in solubility was observed, indicating the presence of disulphide bonds in the gel. The S–S interchanges and disulphide interchange (SH–SS) between the protein molecules involve in the

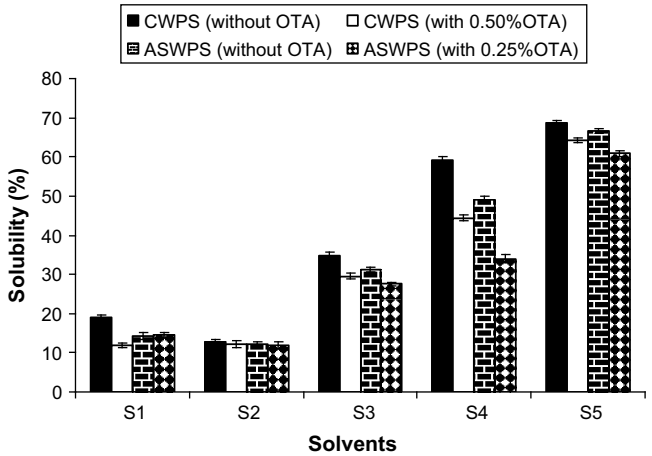

Fig. 5. Solubility of mackerel surimi gels from different washing processes added without and with OTA at optimum level. Samples were solubilised in different solvents and soluble protein was determined by the biuret assay. 0.6 M KCL (S_1); 20 mM Tris–HCl pH 8.0 (S_2); 20 mM Tris–HCl, pH 8.0, containing 1% SDS (S_3); 20 mM Tris–HCl, pH 8.0, containing 1% SDS and 8 M urea (S_4) and 20 mM Tris–HCl, pH 8.0, containing 1% SDS, 8 M urea and 2% β-mercaptoethanol (S_5). CWPS, conventional washing process surimi; ASWPS, alkaline-saline washing process surimi. Bars represent the standard deviation from triplicate determinations.

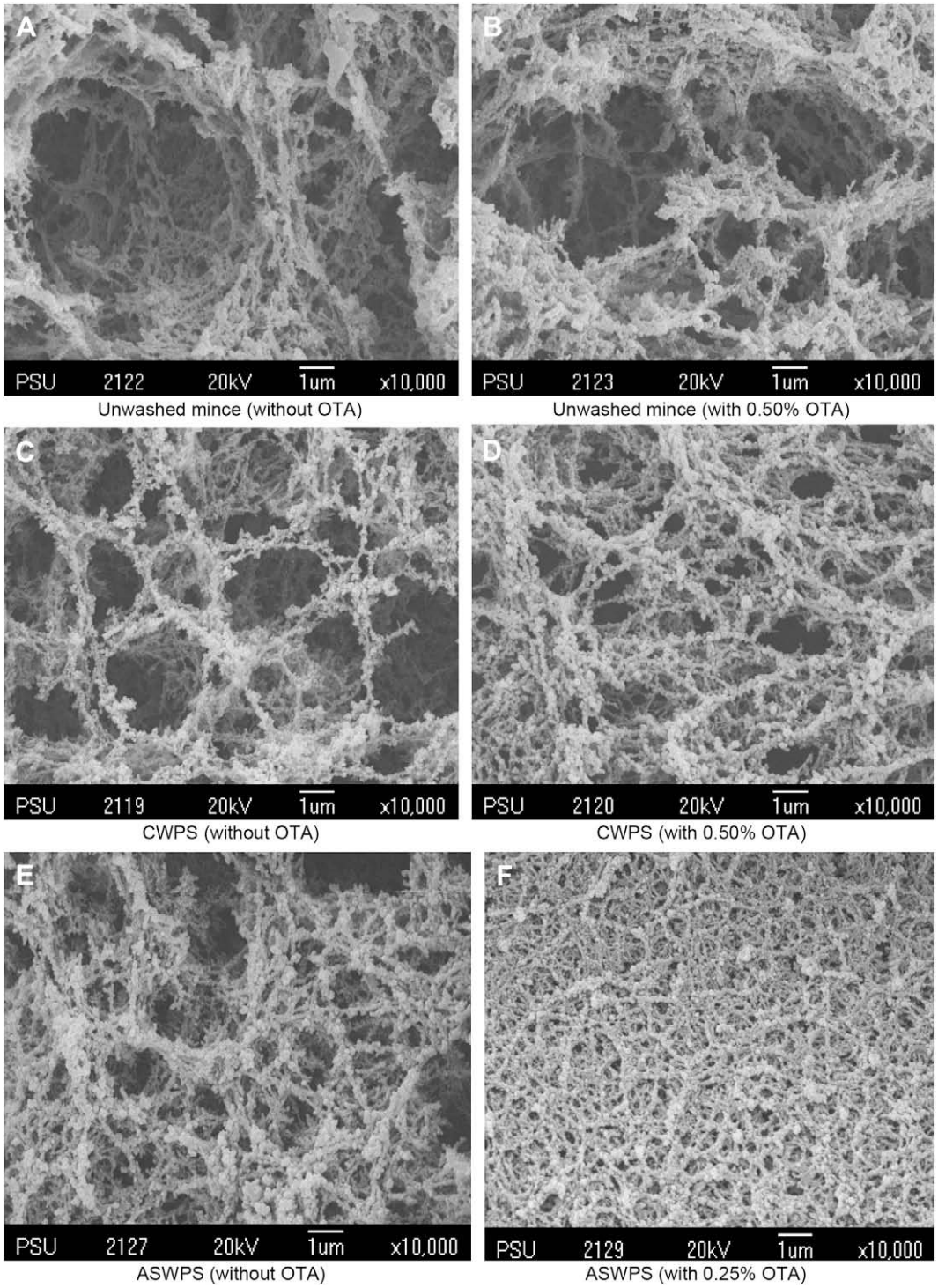

Fig. 6. Electron microscopic images of gels of unwashed mackerel mince and surimi from different washing processes added without and with OTA at optimum level. CWPS, conventional washing process surimi; ASWPS, alkaline-saline washing process surimi. (Magnification: 10,000×).

development of gel structure (Itoh, Yoshinaka, & Ikeda, 1979). When quinones attached to protein molecules, the conformational changes of protein possibly occurred in the way that favoured the oxidation of sulphydryl groups, leading to the increases in disulphide bond formation. Gels added with OTA had a slightly lower solubility in S_4, compared with the control gels. Thus, some non-disulphide covalent bonds induced by quinone, oxidised phenolic, could play a partial role in gel strengthening. It was reported that quinone probably attached to amino groups of protein molecules, in which the subsequent intermolecular cross-linking could be formed (Strauss & Gibson, 2004).

3.8. Effect of OTA on microstructure of gels from unwashed mince and surimi prepared by different washing processes

Microstructures of gels from unwashed mince and surimi prepared by different washing processes are shown in Fig. 6. The control gel of ASWPS exhibited better gel network with less void as

Table 2
Likeness score of alkaline-saline washing process surimi (ASWPS) gels from mackerel added without and with 0.25% OTA.

Attributes	Likeness score[a]	
	ASWPS (without OTA)	ASWPS (with 0.25% OTA)
Colour	6.96 ± 0.12a	6.96 ± 0.13a
Appearance	6.62 ± 0.15a	6.50 ± 0.14a
Odour	6.48 ± 0.12a	6.41 ± 0.36a
Taste	7.09 ± 1.02a	7.18 ± 0.89a
Texture	6.19 ± 0.12a	7.28 ± 0.95b
Overall	6.67 ± 0.15a	6.87 ± 0.21a

Different letters in the same row indicate significant differences ($P < 0.05$).
[a] Mean ± SD ($n = 30$).

compared to those of CWPS and unwashed mince. This was mainly due to the removal of most interfering components for gelation by alkaline-saline washing process, which resulted in the increased concentration of myofibrillar proteins. Those myofibrillar proteins could undergo the aggregation more effectively in the presence of OTA, which induced the protein cross-linking, to yield the more compact and dense gel network.

When OTA was incorporated into the gel, the finer structure with the smaller voids was found in all gels, compared with gels without OTA. Very loose network with larger voids was observed in the unwashed mince gels, irrespective of OTA addition. This was coincidental with the lowest gel strength (Fig. 2) and highest expressible moisture content (Table 1) obtained in the gel of unwashed mince. Gels of CWPS added with 0.50% OTA exhibited the finer and ordered gel network with smaller voids, when compared with the control gel. The compact and dense gel network with finer strand was observed in the gels of ASWPS when 0.25% OTA was added.

3.9. Effect of OTA on sensory properties of ASWPS gels

Likeness scores of the ASWPS gels without and with 0.25% OTA addition are shown in Table 2. No differences in likeness scores for colour, appearance, odour, taste and overall were noticeable between gels without and with 0.25% OTA ($P > 0.05$). Although the addition of 0.25% OTA lowered the whiteness of ASWPS gel (Table 1), it showed no effect on colour likeness ($P > 0.05$). For texture likeness, gel containing 0.25% OTA had the higher score than that without OTA ($P < 0.05$). This was coincidental with the increased breaking force and deformation in the ASWPS gels added with 0.25% OTA (Fig. 2). Phenolic compounds play a major role in the sensory attributes of many food products (O'Connell & Fox, 2001). The addition of tea-extracts containing a high proportion of polyphenols to sherbet mixes, yoghurt and acidified milk drinks was reported (Ioki & Suzuki, 1992). Thus, OTA, at optimum level can be used in mackerel surimi to improve the gel strength without causing the negative effect on sensory property.

4. Conclusion

The addition of OTA in the mackerel surimi at optimum level enhanced the interaction between myofibrillar proteins, which was associated with the formation of an ordered gel microstructure with finer strands. Thus, oxidised tannic acid showed the synergistic effect with alkaline washing process in improving the gel properties of mackerel surimi without any adverse effect on sensory properties.

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Original article

Use of kiam wood extract as gel enhancer for mackerel (*Rastrelliger kanagurta*) surimi

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Summary Kiam (*Cotylelobium lanceotatum* craih) wood was extracted using water or ethanol. Water kiam wood extract (WKWE) and ethanolic kiam wood extract (EKWE) contained tannin at levels of 251.90 and 456.30 mg g^{-1} of dry extract, respectively. Effects of WKWE and EKWE at different levels (0–0.60% of protein content) on the properties of gels from mackerel (*Rastrelliger kanagurta*) surimi were investigated in comparison with commercial tannin (CT). Gels added with 0.30% WKWE, 0.15% EKWE or 0.30% CT had the increases in breaking force by 134.81%, 136.09% and 121.34% and in deformation by 52.60%, 54.96% and 33.53%, respectively, compared with the control (without addition of extracts or CT). The lowered expressible moisture content and the formation of cross-linked myosin heavy chain were also observed in surimi gels incorporated with those additives. Thus, the extract of kiam wood can be used as surimi gel enhancer without affecting its sensory properties.

Keywords Cross-linking, gelation, kiam wood, mackerel, quinone, surimi, tannin.

Introduction

Surimi gel is a three-dimensional myofibrillar protein network. The textural properties developed during gelation are normally expressed in terms of gel strength, which is the basic parameter for determining the quality and price of surimi (Benjakul *et al*., 2004a). In general, the lean fish have been used for the surimi production but overexploitation of the lean fish has resulted in the insufficiencies of those species as raw material. The use of under-utilised small pelagic fish species, such as sardine and mackerel could be a better alternative for the lean fish but their use for surimi production is limited mainly due to the large quantity of lipids and myoglobin in the muscle tissue (Chaijan *et al*., 2004). Furthermore, pelagic fish has been found to possess the high proteolytic activity, which is associated with gel softening. To alleviate the problem, protein additives have been widely used to enhance the gel strength of the surimi via inhibition of proteolysis caused by an endogenous proteinase (Benjakul *et al*., 2004b). To strengthen the gel, the cross-linking enzyme such as microbial transglutaminase has been used (Tammatinna *et al*., 2007). Recently, the interactions between phenolic compounds and proteins have been paid more attention in the processing of certain food products. There have been a few studies describing the cross-linking ability of phenolic compounds with proteins (Rawel *et al*., 2002; Strauss & Gibson, 2004). Balange & Benjakul (2009) reported a significant increase in the gel strength of bigeye snapper surimi when commercial phenolic compounds in oxidised forms were added. Among all oxidised phenolic compounds used, oxidised tannic acid (OTA) exhibited the highest gel strengthening effect, compared with oxidised ferulic acid, oxidised catechin and oxidised caffeic acid (Balange & Benjakul, 2009).

Tannins are polyphenolic compounds occurring in the barks, woods and fruits of many kinds of plants. Extraction of tannins from the bark of different trees has been carried out (Yazaki & Collins, 1994; Fradinho *et al*., 2002). Kiam (*Cotylelobium lanceotatum* craih) trees are very common in the southern part of Thailand. Pieces of wood from the kiam tree have been traditionally submerged in sugar palm sap to prevent or retard microbial fermentation (Chanthachum & Beuchat, 1997). The kiam wood is either burned for energy production or simply disposed. The preparation of kiam extract containing phenolic compounds could increase the value of those wood and the novel natural additives can be applied in food industry, especially surimi industry.

However, there is a little information on the utilisation of kiam wood extract as the cross-linking agents in

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food proteins, particularly myofibrillar proteins. Therefore, the objectives of this research were to extract and quantify tannin in the wood of kiam (*C. lanceotatum* craih) and to use the extracts as gel strengthener in surimi from mackerel (*Rastrelliger kanagurta*).

Materials and methods

Chemicals

Tannic acid (commercial tannin; CT), sodium hydroxide, hydrochloric acid, sodium carbonate, $CuSO_4 \cdot 5H_2O$, bovine serum albumin, Folin-Ciocalteu reagent and β-mercaptoethanol were obtained from Sigma (St Louis, MO, USA). Sodium dedocyl sulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA). Sodium chloride, trichloroacetic acid and ethanol were obtained from Merck (Darmstadt, Germany).

Preparation of kiam wood extracts

Collection and preparation of kiam wood

The kiam wood was obtained from the forest of the Phattalung province in the Southern Thailand. The tree was about 15–20 years old and harvested in the month of June 2008. The tree was cut by using a sawing machine; the leaves and branches were separated manually by cutting and the trunk was kept for sun drying for 3 months. The trunk was chopped into smaller flakes of wood and then dried in an oven at 70 °C for 8 h and cut into pieces with an average size of 1.5×1.5 cm^2 and referred to as 'Intact form'. Those pieces were ground using a portable grinding machine (Spong-90, Leeds, UK) with a sieve size of 6 mm. The resulting ground bark was referred to as 'Coarse form'. This coarse form was then subjected to a blender (National Model MK-K77, Tokyo, Japan) and finally sieved using a stainless steel sieve of 80 mesh size. The obtained powder was referred to as 'Fine form'.

Extraction of crude phenolics from kiam wood

Extraction using ethanol

Three different forms of kiam wood were extracted according to the method of Santoso *et al.* (2004) with slight modifications. The kiam wood samples (10 g) were mixed with 150 mL of absolute ethanol. The mixtures were stirred at room temperature (28–30 °C) using a magnetic stirrer (IKA-Werke, Staufen, Germany) for 3 h. The mixture was then centrifuged at 5000 ***g*** for 10 min at room temperature using a Sorvall Model RC-B plus centrifuge (Newtown, CT, USA). The supernatant was filtered using Whatman filter paper No. 1 (Whatman Schleicher & Schuell, Maidstone, UK). The filtrate was then evaporated at 40 °C using Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd, Tokyo, Japan). The volume was made to 10 mL with ethanol in a volumetric flask. With different forms of wood used for extraction, the corresponding extracts were referred to as 'ethanolic intact wood extract', 'ethanolic coarse wood extract' and 'ethanolic fine wood extract'.

Extraction using water

Three different forms of kiam wood were extracted following the method of Chanthachum & Beuchat (1997) with slight modifications. Kiam (10 g) was mixed with 80 mL of distilled water. The mixtures were stirred continuously at 70 °C for 2 h. The mixtures were allowed to stand until the temperature decreased to room temperature. The mixtures were then centrifuged at 5000 ***g*** for 10 min at room temperature using a Sorvall Model RC-B plus centrifuge (Newtown, CT, USA). The supernatant was filtered using Whatman filter paper No 1. The filtrate was then evaporated on a hot plate (EGO, Model-18715, 1500w, Germany). The volume was made to 10 mL with distilled water in a volumetric flask. With different forms of wood used for extraction, the corresponding extracts were referred to as 'water intact wood extract', 'water coarse wood extract' and 'water fine wood extract', respectively.

Determination of total phenolic compounds in different kiam wood extracts

Quantification of total phenolic compounds in different kiam wood extracts was carried out according to the method of Slinkard & Singleton (1977). The extract (0.5 mL) was mixed with 0.5 mL of distilled water. Thereafter, 0.5 mL of Folin–Ciocalteu reagent (1:1 with water) and 2.5 mL of 2% sodium carbonate solution were added. The reaction mixture was mixed thoroughly and placed in dark for 40 min and the absorbance was recorded at 725 nm. The total phenolic content was calculated from the standard curve of tannins (0–0.1 mg mL^{-1}) and expressed as mg tannin per gram dry kiam after blank substraction. Blank for each extract was prepared in the same manner, except that distilled water was used instead of Folin–Ciocalteu reagent.

Reverse-phase HPLC of different kiam wood extracts

Qualitative analysis of kiam wood extracts was performed using an HPLC equipped with VWD detector following the method of Tian *et al.* (2009) with slight modifications. The HPLC system consisted of an Agilent 1100 series HPLC (Alginet, Wilmington, DE,

USA),quaternary pump with seal wash option, degasser, solvent, cabinet and preparative autosampler with thermostat equipped with a diode array detector. The separation was performed on a column (Hypersil ODS C18 4.0 × 250 mm, 5 μm, Cole-Parmer, Hanwell, London, UK). HPLC conditions were as follows: mobile phase: 0.4% Formic acid: Acetonitrile (85:15), flow rate: 0.8 mL min^{-1}; temperature; 25 °C. The detection was carried out at 280 nm. The concentration of extracts was 25 mg mL^{-1} and each injection volume was 20 μL. Standard tannin was used for peak identification.

Preparation of surimi by alkaline saline washing process

Mackerel (*R. kanagurta*) with an average weight of 85–90 g were caught during September–October, 2008, from Songkhla coast along the Gulf of Thailand, stored in ice and off-loaded approximately 36 h after capture. Fish were iced with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, fish were immediately washed and used for surimi preparation.

Surimi was prepared according to the method of Shimizu (1965) with a slight modification. Mackerel flesh was removed manually and minced to uniformity using a mincer with a hole diameter of 5 mm. The mince was then suspended in four volumes of cold (5 °C) washing solution (0.15% NaCl in 0.2% $NaHCO_3$). The mixture was stirred gently for 15 min and washed mince was filtered with a layer of nylon screen (Butterfly, Lao Hah Seng Lee Co., Ltd, Bangkok, Thailand). The washing process was repeated twice. For the third washing, cold 0.5% NaCl solution was used as washing medium. Finally, the washed mince was subjected to centrifugation using a Model CE 21K basket centrifuge (Grandiumpiant, Belluno, Italy) with a speed of 700 ***g*** at 4 °C for 10 min. To the washed mince, 4% sucrose and 4% sorbitol were added, mixed well and frozen using an air-blast freezer. Resulting surimi was kept at –20 °C for no longer than 1 month.

Effect of oxidised kiam wood extracts on the property of mackerel surimi gel

Preparation of oxidised kiam wood extracts

Kiam wood extracts, either WKWE or EKWE, containing the highest tannin content were oxidised according to the method of Strauss & Gibson (2004) with slight modifications. The extracts (100 mL; 1% w/v) were adjusted to pH 8 using 6 M NaOH or 6 M HCl and placed in a temperature-controlled water bath (Memmert, Schwabach, Germany) at 40 °C. The extracts with pH 8 were subjected to oxygenation for 1 h by bubbling with oxygen. Subsequently, the solutions were adjusted to pH 7 by using 6 M HCl. Commercial tannin (CT) was also oxidised in the same manner. Oxidised kiam wood extracts and oxidised CT were used as the additives in surmi gels.

Surimi gel preparation

To prepare surimi gels, frozen surimi was tempered for 30 min in running water (26–28 °C) until the core temperature reached 0–2 °C. The surimi was then cut into small pieces with an approximate thickness of 1 cm and placed in a mixer (National Model MK-K77, Tokyo, Japan). The moisture was adjusted to 80% and 2.5% salt was added. WKWE, EKWE or CT (pH 7.0) at various concentrations (0%, 0.15%, 0.30%, 0.45% and 0.60% of protein content) was added into the sols. The mixtures were chopped for 4 min at 4 °C to obtain homogeneous sols. The sols were then stuffed into polyvinylidine casings with a diameter of 2.5 cm and both ends of casings were sealed tightly. Sols were incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Benjakul *et al.*, 2003). All gels were cooled in iced water and stored for overnight at 4 °C prior to analyses.

Measurement of gel properties

Textural analysis

Textural analysis of gels was performed using a texture analyser Model TA-XT2 (Stable Micro Systems, Surrey, UK). Gels were equilibrated and tested at room temperature. Five cylinder-shaped samples of 2.5 cm in length were prepared. The breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyser equipped with a spherical plunger (5 mm diameter). The probe was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed (60 mm min^{-1}) until the puncture occurred. The force in gram (g) required to puncture into the gel (breaking force) and the distance (in mm) at which the ball probe punctured into the gel (deformation) were recorded.

Determination of expressible moisture content

Expressible moisture content was measured according to the method of Benjakul *et al.* (2001) with a slight modification. A gel sample with a thickness of 0.5 cm was weighed (X) and placed between two pieces of Whatman paper No. 4 (Whatman Schleicher & Schuell, Maidstone, UK) at the top and three pieces at the bottom. The standard weight (5 kg) was placed at the top and held for 2 min. The sample was then removed from the papers and weighed again (Y). Expressible

moisture content was calculated using the following equation:

$$\text{Expressible moisture content}(\%) = 100[(X - Y)/X]$$

Determination of whiteness

Colour of gels was determined using a JP7100F colourimeter (Juki Corporation, Tokyo, Japan). L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Lanier *et al.* (1991) as follows:

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

SDS–polyacrylamide gel electrophoresis

Protein patterns of gels were analysed by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 mL of 5% (w/v) SDS solution heated to 85 °C were added to the gel samples (3 g). The mixture was then homogenised using a homogeniser (IKA Labortechnik, Selangor, Malaysia) at a speed of 11 000 r.p.m. for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500 ***g*** for 20 min to remove undissolved debris. Protein concentration was determined by the Biuret method (Robinson & Hodgen, 1940), using bovine serum albumin as a standard. The samples (20 μg protein) were loaded into the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie brilliant blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid for 15 min, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid for 3 h.

Sensory evaluation

Mackerel surimi gels without and with WKWE, EKWE or CT at the optimal level were evaluated for colour, appearance, odour, taste, texture and overall liking by 30 non-trained panelists. A nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation (Meilgaard *et al.*, 1990).

Scanning electron microscopy

Microstructure of gels was determined using scanning electron microscopy. Mackerel surimi gels, without and with WKWE, EKWE or CT at the optimal level were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50%, 70%, 80%, 90% and 100% (v/v). Dried samples were mounted on a bronze stub, and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 10 kV.

Statistical analysis

The experiments were run in triplicate. All chemical analyses were performed in triplicate. For physical analyses, e.g. expressible moisture, whiteness and textural properties, at least five determinations were conducted for each sample. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range tests (Steel & Torrie, 1980). *T*-test was used for pair comparison. Analysis was performed using a SPSS package (SPSS 10.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results and discussion

Composition of different kiam wood extracts

Among three different forms of kiam wood, the fine form showed the highest yield. Ethanolic and water extract of fine wood had the total phenolic content of 498.44 and 198.99 mg tannin per g dry kiam wood extract, respectively (Table 1). For all forms of kiam wood used, ethanolic extracts contained the higher total phenolic content than did the water extracts ($P < 0.05$).

Water and ethanolic extracts from finely ground kiam wood were analysed by HPLC-DAD and tannin was found as the major component (data not shown). Bark of different trees mainly contains tannins (Yazaki & Collins, 1994; Fradinho *et al.*, 2002). Water extract contained the lower tannin (251.90 mg tannin per g of dry kiam wood extract) than ethanolic extract (456.30 mg tannin per g of dry kiam wood extract).

Table 1 Total phenolic contents of different kiam wood extracts

	Total phenolic content (mg tannin per g of dry kiam wood extract)		
Extraction media	**Intact form**	**Coarse form**	**Fine form**
Water	29.33 ± 0.49aB	99.22 ± 0.41bB	198.99 ± 0.21cB
Ethanol	78.83 ± 0.22aA	249.14 ± 0.57bA	498.44 ± 0.98cA

Different letters in the same row indicate the significant differences ($P < 0.05$). Different capital letters in the same column indicate the significant differences ($P < 0.05$). Values are means ± standard deviations (n=3).

This was in agreement with the lower total phenolic content in the former. The result indicated that kiam wood extract was an important source of tannin. Both ethanolic kaim wood extract (EKWE) and water kaim wood extract (WKWE) were used for improving the gel properties of surimi from mackerel, in comparison with CT.

Effect of kiam wood extracts on mechanical properties of mackerel surimi gel

Breaking force and deformation of mackerel surimi gels added with EKWE, WKWE or CT at different levels are depicted in Fig. 1. Breaking force and deformation of gels increased as EKWE, WKWE or CT were added up to a particular level ($P < 0.05$). Gels added with 0.30% WKWE or CT had the increases in breaking force by 134.81% or 121.34% and in deformation by 52.60% or 33.53%, respectively, compared with that of the control ($P < 0.05$). For gels added with 0.15% EKWE, breaking force and deformation increased by 136.09% and 54.96%, respectively. Nevertheless, the continuous decreases in both breaking force and deformation were noticeable when both extracts and CT at the greater levels were added ($P < 0.05$). The higher concentration of phenolic compound with larger size results in its lower solubility, which in turn affects its interaction with proteins (De Freitas & Mateus, 2001). In addition, the size of the phenolic compound can decrease its conformational flexibility, which is observed to be an important parameter in protein–phenolic compound interactions (Frazier *et al.*, 2003). The decreased breaking force and deformation with increasing concentrations of the extracts or CT in the present study might be associated with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking. At a level of 0.15%, gel added with EKWE showed the higher breaking force and deformation as compared with gel added with WKWE or CT ($P < 0.05$). Water is highly polar solvent as compared with ethanol and is able to extract highly polar tannins, whereas the ethanol extracts the tannins with weaker polarity (Tian *et al.*, 2009). The major chemical constituents in the bark or wood from different trees were reported to be tannin with small amount of lignin (Yazaki & Collins, 1994; Fradinho *et al.*, 2002). Tannic acid has a number of hydroxyl groups attached to the aromatic benzene ring which provide more binding cites for proteins (Lopes *et al.*, 1999). Balange & Benjakul (2009) reported the increases in breaking force and deformation of bigeye snapper surimi with the addition of oxidised tannic acid. It was noted that surimi gel added with WKWE and EKWE at their optimal concentrations had the higher breaking force and deformation as compared with those of gel added with CT ($P < 0.05$). Therefore, the optimal levels of WKWE, EKWE and CT were 0.3%, 0.15% and 0.15%, respectively.

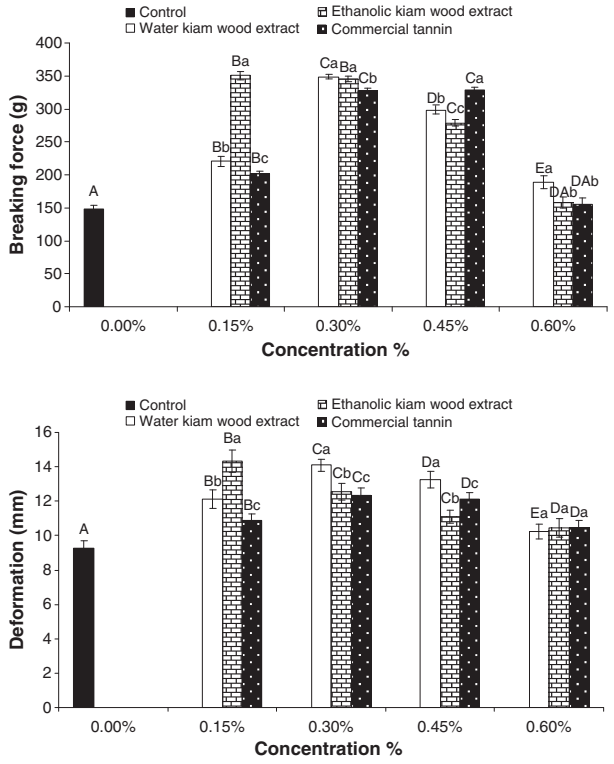

Figure 1 Breaking force and deformation of gels from mackerel surimi added with extracts or commercial tannin (CT). Bars represent the standard deviation ($n = 3$). Different capital letters on the bars within the same additives together with the control indicate the significant differences ($P < 0.05$). The different letters on the bars within the same levels of additives indicate the significant differences ($P < 0.05$).

Effect of kiam wood extracts on expressible moisture content of mackerel surimi gel

The lowest expressible moisture content of mackerel surimi gel was found when WKWE, EKWE or CT at the optimal level were added ($P < 0.05$) (Table 2). The increases in expressible moisture content were found in surimi gels added with WKWE, EKWE or CT above the optimal level ($P < 0.05$). The decreases in expressible moisture contents of surimi gel added with both extracts or CT were in accordance with the increased breaking force and deformation of resulting surimi gels (Fig. 1). At the optimal level, the cross-linking of proteins in the mackerel surimi gels could be enhanced. This resulted in the formation of stronger network with greater water holding capacity. Among the extracts and CT, EKWE at a level of 0.15% yielded the gel with the lowest expressible moisture content. This reconfirmed that EKWE addition resulted in gel strengthening. As a result, gel network with capability of imbibing water could be obtained.

Table 2 Expressible moisture content and whiteness of gels from mackerel surimi added with water and ethanolic kiam wood extracts or commercial tannin at different levels

Oxidised phenolic compounds	Amount added (%)	Expressible moisture content (%)	Whiteness
Control	0	9.48 ± 0.59a	68.15 ± 0.36a
WKWE	0.15	6.69 ±0.23bA	62.36 ± 0.46bA
	0.30	4.14 ± 0.45cA	60.76 ± 0.51cA
	0.45	5.62 ± 0.22dA	57.12 ± 0.44dA
	0.60	10.53 ± 0.73eA	55.07 ± 0.44eA
EKWE	0.15	3.16 ± 0.33bB	66.36 ± 0.46bB
	0.30	4.84 ± 0.37cB	64.76 ± 0.51cB
	0.45	6.12 ± 0.42dB	63.12 ± 0.44dB
	0.60	9.38 ± 0.59eB	63.07 ± 0.44dB
CT	0.15	7.56 ± 0.43bC	64.16 ± 0.46bC
	0.30	5.14 ± 0.57cC	62.26 ± 0.51cC
	0.45	5.02 ± 0.32cC	61.65 ± 0.51dC
	0.60	10.57 ± 0.59eA	60.12 ± 0.44eC

Different letters in the same column within the same additive together with the control indicate the significant differences ($P < 0.05$). Different capital letters in the same coloumn within the same level of additive used indicate the significant differences ($P<0.05$). Values are means ± standard deviations ($n = 3$).

Effect of kiam wood extracts on whiteness of mackerel surimi gel

The decreases in whiteness of mackerel surimi gels were observed as the levels of the extracts or CT were increased ($P < 0.05$) (Table 2). These results were in agreement with O'Connell & Fox (2001) who reported that phenolic compounds were responsible for discolouration in the cheese products. However, the decrease in whiteness was more pronounced in the mackerel surimi gels with the addition of WKWE ($P < 0.05$). Evaporation of water extract at high temperature for a long time enhanced the darkening of water extract. Pansera *et al.* (2004) used the process of hydrosolubilisation at 100 °C for the extraction of tannin and found that the extraction process at high temperature motivates a hydro cracking of sugar and other organic compounds with darkening of the final product. From the result, surimi gel added with EKWE at a level of 0.15% had a slight decrease in whiteness.

Effect of kiam wood extracts on the protein pattern of mackerel surimi gel

Protein patterns of gels added with WKWE, EKWE or CT at different concentrations are shown in Fig. 2. Surimi paste contained MHC and actin as the major proteins. Decrease in MHC band intensity was found in the control gel (without addition of extracts or CT), when compared with that observed in surimi paste.

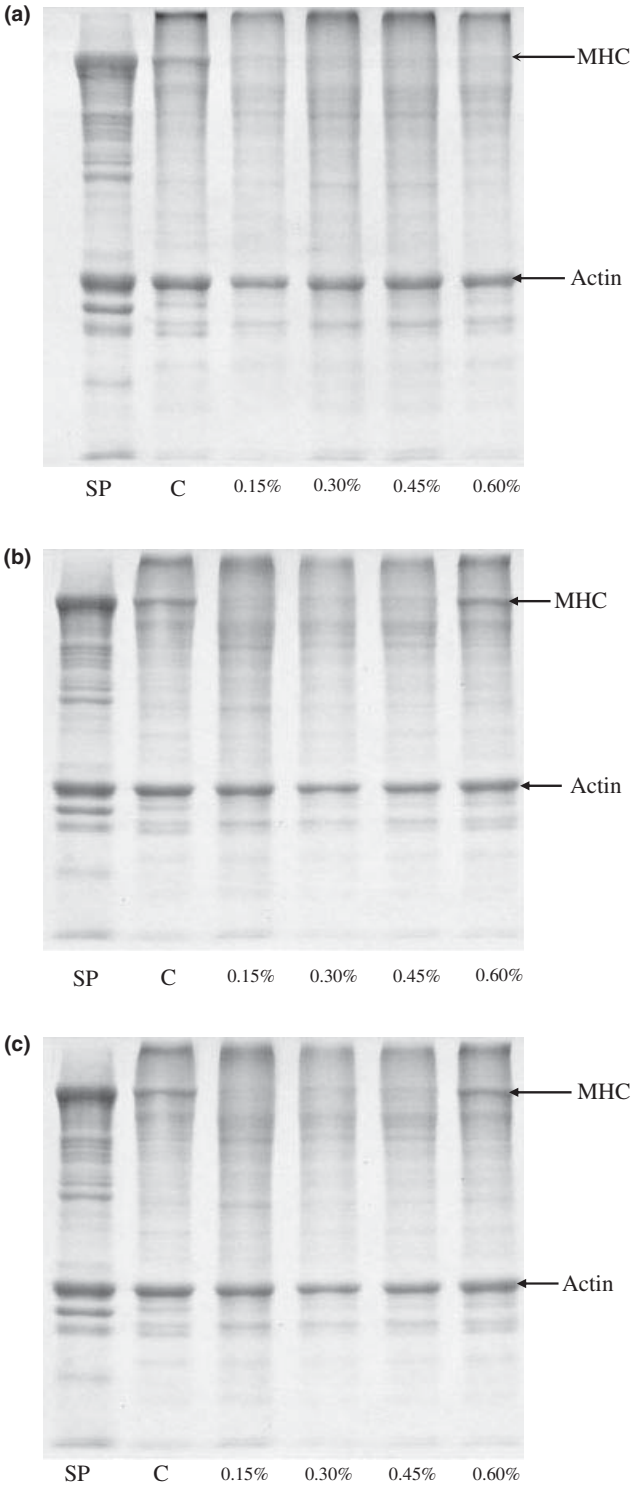

Figure 2 SDS–PAGE patterns of protein in gels from mackerel surimi added with water kiam wood extract (a), ethanolic kiam wood extract (b) or commercial tannin (c) at different concentrations. SP, surimi paste; MHC, myosin heavy chain; C, control gel (without extracts/CT solution).

The result suggested that the formation of cross-linking stabilised by non-disulphide covalent bond took place, especially during setting. MHC was most susceptible to cross-linking during setting (Benjakul & Visessanguan, 2003). Benjakul & Visessanguan (2003) reported the decrease in MHC of surimi gel from bigeye snapper, particularly when the setting was implemented.

For surimi gels added with WKWE, EKWE or CT at the different concentrations, MHC band intensity decreased significantly as compared with the control gel (Fig. 2a–c, respectively). No MHC band was found in all gels added with the extracts or CT ranging from 0.15% to 0.45%. The results suggested that MHC was cross-linked by oxidised phenolic compounds effectively via non-disulphide covalent bond. The disappearance of MHC was in accordance with the increases in breaking force and deformation of mackerel surimi gels added with the extracts or CT at the optimum level (Fig. 1). Ou *et al.* (2005) and Cao *et al.* (2007) also reported the polymerisation of protein molecules as a possible subsequent reaction of different proteins with phenolic substances. Multi-functional groups of tannin possessed a higher potential to bind or attach to protein molecules, in which the alteration of protein conformation could be more enhanced. The results were in agreement with Balange & Benjakul (2009) who reported that OTA even at a low level (0.05%) caused the denaturation of myosin as evidenced by the exposure of hydrophobic domain and sulphydryl groups. Phenols may be oxidised with ease, in an alkaline solution, to their corresponding quinones (Hurrell & Finot, 1984). The quinone, a reactive electrophilic intermediate, can readily undergo attack by nucleophiles such lysine, methionine, cysteine and tryptophan residues in a protein chain (Hurrell & Finot, 1984). For gels added with 0.6% CT or 0.6% EKWE, MHC band was more retained. This suggested that the lower ability of those EKWE or CT in cross-linking proteins, especially MHC. This was in agreement with the lower gel strengthening effect of the extract or CT at the level above the optimal one. This was mainly due to the self-aggregation of quinones at the higher levels. Due to the lower content of tannin in WKWE, a level of 0.6% water extract might not be sufficient to induce the self-aggregation. As a result, the polymerisation of MHC induced by WKWE at such a level could take place effectively.

Effect of kiam wood extracts on the sensory properties of mackerel surimi gel

Likeness scores of the control gels (without extracts or CT) and those added with the extracts or CT at the optimal level are depicted in Fig. 3. Addition of CT and

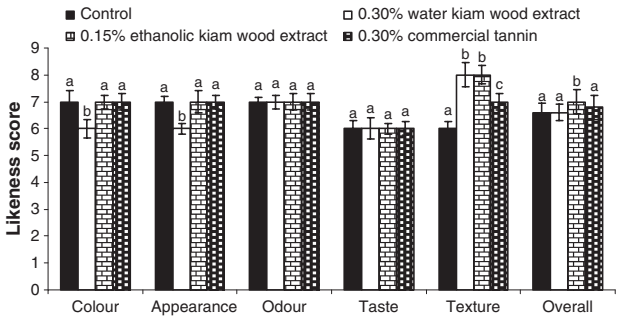

Figure 3 Likeness score of surimi gels from mackerel added without and with water and ethanolic kiam wood extracts or commercial tannin at selected levels. Bars represent standard deviation ($n = 30$). Different letters on the bars within the same sensory attribute indicate significant differences ($P < 0.05$).

EKWE had no negative impact on the colour, appearance, odour and taste of resulting gels. However, the addition of WKWE resulted in significantly lower likeness score for the appearance and colour, compared with other treatments including the control ($P < 0.05$). Dark colour of WKWE most likely contributed to the darker colour of resulting gels. Pansera *et al.* (2004) used the process of hydrosolubilisation at 100 °C for the extraction of tannin and found that the extraction process at high temperature resulted in the darkening of the final product. Nevertheless, addition of WKWE, EKWE or CT yielded the gel with the higher texture score, compared with that of control ($P < 0.05$). This was coincidental with the increased breaking force and deformation in the surimi gels added with those extracts or CT (Fig. 1). Among all surimi gels tested, those containing 0.15% EKWE or 0.3% WKWE had the highest texture score ($P < 0.05$). Phenolic compounds play a major role in the sensory attributes of many food products (O'Connell & Fox, 2001). The addition of tea-extracts containing a high proportion of polyphenols to sherbet mixes, yoghurt and acidified milk drinks was reported (Ioki & Suzuki, 1992). Surmi gel containing 0.15% EKWE exhibited the highest overall likeness score. Quinone in the extracts or CT underwent cross-linking with myofibrillar protein or self-aggregation. As a result, no quinones were available for binding with salivary proteins. Thus they could not have the impact on taste or flavour of surimi gel. Therefore, the addition of extracts or CT at low concentration did not negatively affect the overall sensory properties of resulting mackerel surimi gel.

Effect of kiam wood extracts on microstructure of mackerel surimi gel

Microstructures of control gel (A), gel added with 0.30% WKWE (B), gel added with 0.15% EKWE (C)

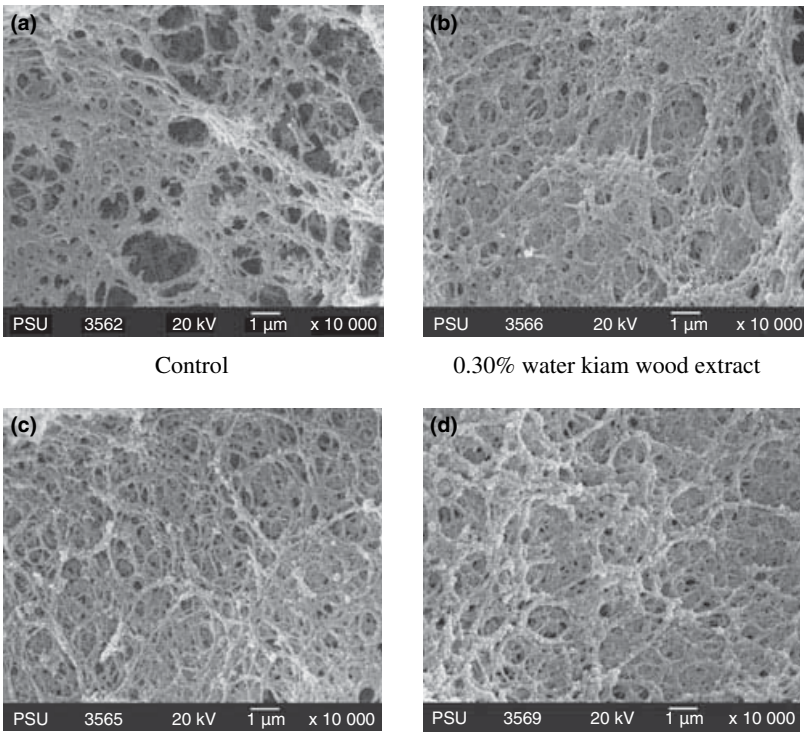

Figure 4 Electron microscopic images of gels from mackerel surimi added without (a) and with 0.30% water kiam wood extract (b), 0.15% ethanolic kiam wood extract (c) or 30% commercial tannin (d) (Magnification: 10 000×).

and gel added with 0.30% CT (D) are illustrated in Fig. 4. Surimi gels containing both extracts or CT had finer and more continuous matrix than the control gel. This suggested that oxidised phenolic compounds in the extract might induce the cross-linking of proteins, in which the filamental network could be formed orderly. In the presence of oxidised phenolic compounds, mostly quinone, the inter-junctions between fibrillar protein molecules could be enhanced. This led to the formation of finer gel matrix. Among all gel, the gel added with 0.15% EKWE possessed more ordered fibrillar structure with larger strands and had high capacity of imbibing the water (Table 2). The presence of tannin with a greater amount in the EKWE was more likely associated with a higher aggregation between protein molecules. This was in agreement with the markedly improved gel strength of mackerel surimi added with EKWE. Additionally, this might lead to the increase in overall liking score of this gel (Fig. 3).

Conclusions

Ethanolic kiam wood extract had a potential in strengthening the gel of mackerel surimi when the optimum level (0.15%) was introduced. Addition of ethanolic extract had no detrimental effect on sensory properties of surimi gel. Furthermore, ethanolic kiam wood extract was more effective than costly commercial tannin. Thus, the extract from kiam wood can be used as a natural gel enhancer for surimi industry.

Acknowledgments

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Cross-linking activity of oxidised tannic acid towards mackerel muscle proteins as affected by protein types and setting temperatures

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ABSTRACT

Cross-linking activity of oxidised tannic acid (OTA) at different levels (0–0.3% of protein content) towards natural actomyosin (NAM), sarcoplasmic protein (SP) and NAM/SP (65:35) mixture from mackerel (*Rastrelliger kanagurta*) muscle incubated at different temperatures for 30 min was investigated. NAM solution showed an increase in turbidity, surface hydrophobicity and disulphide bond contents as OTA added increased up to 0.2%. The higher aggregate formation of NAM solution containing 0.2% OTA was found when incubated at 40 °C, compared with at room temperature (26–28 °C). The lower aggregation of NAM was noticeable in the presence of SP, which was more preferably cross-linked by OTA via weak bonds. Thus, SP showed the interfering effect on NAM cross-linking induced by OTA. Myosin heavy chain (MHC) band intensity was decreased and a highly ordered dense protein network of NAM was obtained when 0.2% OTA was incorporated. Conversely, coagulation was formed in the NAM/SP mixture added with 0.2% OTA. Thus, the cross-linking efficiency of OTA varied with the type of muscle protein and setting temperature.

1. Introduction

Gel-forming ability is one of the most important attributes of fish mince and surimi. It can be affected by both intrinsic and extrinsic factors including species, freshness, endogenous enzymes, additives as well as cooking procedure (Benjakul, Visessanguan, & Chantarasuwan, 2004a, 2004b; Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001; Benjakul, Visessanguan, & Tueksuban, 2003; Benjakul, Visessanguan, Tueksuban, & Tanaka, 2004). Three major groups of proteins are found in the fish muscles: water-soluble sarcoplasmic proteins (about 30 wt.%) consisting of albumins, myoglobin and enzymes; salt-soluble myofibrillar proteins (60–70 wt.%) containing principally myosin, actin and a smaller amount of tropomyosin and troponin; and insoluble stromal proteins representing 3–10% of total proteins (Suzuki, 1981). Myosin is an important myofibrillar protein, mainly responsible for fish gel formation (Niwa, 1992). It is the most abundant myofibrillar component, constituting approximately 40–60% of total protein content. Myosin consists of six polypeptide subunits, two myosin heavy chains (MHCs) and four light chains arranged into an asymmetrical molecule with two pear-shaped globular heads attached to a long helical rod-like tail (Xiong, 1997). On the other hand, sarcoplasmic proteins, which possess a relatively simple globular structure, have poor gelling ability and contribute very little to food texture (Xiong, 1997). Fish mince generally have poorer gel-forming ability, compared with washed mince due to the presence of sarcoplasmic proteins, lipid and pigments (Balange & Benjakul, 2009b). To improve the properties of gel from mince and washed mince, many approaches have been implemented. "Setting" is a well known phenomenon in the surimi paste during the incubation at temperatures lower than 40 °C. During setting, the myosin network is formed due to the cross-linking induced by endogenous transglutaminase (TGase) (Seki, Uno, & Lee, 1990). Additionally, microbial transglutaminase (MTGase) has shown potential in increasing the gel strength of surimi by inducing non-disulphide covalent bond formation (Benjakul, Phatcharat, Tammatinna, Visessanguan, & Kishimura, 2008), whereas protein additives have been widely used to alleviate the softening (modori) induced by endogenous thermostable proteinases (Benjakul et al., 2004a, 2004b; Benjakul, Visessanguan, Tueksuban, et al., 2004). However, some additives such as bovine plasma protein, porcine plasma protein and egg white have been prohibited due to the safety concern. Additionally, the use of cross-linking enzymes, especially MTGase, is still costly in surimi manufacturing. Therefore, the novel and cheap additives capable of improving gel quality of mince or surimi has been paid increasing attention.

Polyphenol compounds are abundant in plants (Shahidi & Naczk, 2004). The interactions between phenolic compounds and proteins play an essential role in the processing of certain food products. Tannin can be used as a food additive with the range of 10–400 mg/l, depending on the type of food to which it is added

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(Chen & Chung, 2000). Tannin contains sufficient hydroxyls and other groups such as carboxyls to form strong complexes with the proteins and other macromolecules (Kroll, Rawel, & Rohn, 2003). Recently, Balange and Benjakul (2009a, 2009b, 2009c) found the increase in gel strength of mackerel and bigeye snapper surimi with the addition of oxidised tannic acid (OTA). Nevertheless, OTA had no gel strengthening effect on unwashed mince (Balange & Benjakul, 2009b). OTA may react more effectively with sarcoplasmic proteins in unwashed mince, thereby being less available in cross-linking myosin heavy chain. However, the reactivity of OTA towards fish myofibrillar proteins and sarcoplasmic proteins has not yet been elucidated. Therefore, the objectives of this research were to compare the cross-linking ability of OTA towards natural actomyosin (NAM) and sarcoplasmic protein (SP) from mackerel muscle and to investigate the impact of OTA on physicochemical changes of both proteins at different setting temperatures.

2. Materials and methods

2.1. Chemicals/fish

Potassium chloride and sodium chloride were purchased from Merck (Darmstadt, Germany). Tannic acid, β-mercaptoethanol (βME), 8-anilino-1-naphthalenesulphonic acid (ANS), guanidine thiocyanate, sodium hydrogen sulphite and Tris-maleate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was obtained from Wako Pure Chemical Industries (Tokyo, Japan). Sodium dedocylsulfate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (TEMED) and all chemicals for electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Mackerel (*Rastrelliger kanagurta*) with an average weight of 85–90 g, caught from Songkhla coast along the Gulf of Thailand during January–February, 2009, was stored in ice and off-loaded approximately 36 h after capture. Fish were stored in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, fish were washed using tap water. Subsequently, fish were filleted and the ordinary muscle was collected. The flesh was minced using a mincer with a hole diameter of 0.5 cm. The mince was then used for extraction of natural actomyosin (NAM) and sarcoplasmic protein (SP).

2.2. Preparation of NAM

For the preparation of NAM, fish mince (10 g) was homogenised in 100 ml of chilled 0.6 M KCl, pH 7.0 for 4 min at a speed of 11,000 rpm using a homogeniser (IKA Labortechnik, Selangor, Malaysia) (Benjakul, Seymour, Morrissey, & An, 1997). Overheating during extraction was avoided by keeping the sample in an iced container and each 20 s of homogenisation was followed by a 20 s rest interval. The homogenate was centrifuged at 5000×*g* for 30 min at 4 °C. Three volumes of chilled water (0–2 °C) were added to precipitate NAM, which was then collected by centrifuging at 5000×*g* for 20 min at 4 °C using a refrigerated centrifuge (Avanti-J-E Centrifuge, Beckman Coulter Inc., Fullerton, CA, USA). The pellets were then dissolved by gradually stirring in an equal volume of chilled 0.6 M KCl, pH 7.0 for 30 min at 4 °C.

2.3. Preparation of SP

SP was prepared according to the method of Benjakul et al. (2004a, 2004b) and Benjakul, Visessanguan, Tueksuban, et al. (2004). Fish mince was homogenised with two volumes of extraction buffer (20 mM Tris–HCl, pH 7.5) using a homogeniser at a speed of 11,000 rpm for 2 min. The homogenate was centrifuged at 16,000×*g* for 20 min at 4 °C using a refrigerated centrifuge. The supernatant obtained was further centrifuged at 18,000×*g* for 60 min at 4 °C. The final supernatant was referred to as "SP: sarcoplasmic protein".

2.4. Preparation of oxidised tannic acid (OTA)

Tannic acid was dissolved in distilled water according to the method of Strauss and Gibson (2004). The pH of solution (100 ml; 1% w/v) was adjusted to 8 with 6 M NaOH or 6 M HCl. The prepared solution was placed in a water bath (40 °C) and oxygenated for 1 h by bubbling the solution with oxygen having a purity of 99.5–100% (TTS Gas Agency, Hat Yai, Songkhla, Thailand) to convert tannic acid to quinone. The solution was then adjusted to pH 7 with 6 M HCl and referred to as "oxidised tannic acid (OTA)".

2.5. Study on the cross-linking ability of OTA towards mackerel muscle proteins at different temperatures

NAM, SP, and NAM/SP (65:35) mixture were diluted to obtain the protein concentration of 5 mg/ml. NAM (65%) was also prepared by mixing NAM with 20 mM Tris–HCl (pH 7.5) at ratio of 65:35 (v/v). To these protein solutions, OTA at various concentrations (0%, 0.1%, 0.2% and 0.3% of the protein) was added. The resulting mixtures were incubated in a temperature-controlled water bath (Memmert, D-91126, Schwabach, Germany) at 40 °C or at room temperature (26–28 °C) for 30 min, followed by cooling in iced water for 30 min. The prepared samples were then subjected to analyses.

2.5.1. Turbidity measurement

Different protein solutions (5 mg protein/ml) were placed in the cuvette and the turbidity was measured by monitoring the absorbance at 660 nm (Benjakul et al., 2001).

2.5.2. Protein solubility

Different protein solutions with different treatments were centrifuged at 3500×*g* for 20 min at 4 °C to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson & Hodgen, 1940) using bovine serum albumin as a standard. Solubility of protein in the samples was expressed as the percentage of protein in the supernatant, relative to that found in the initial solution (5 mg protein/ml).

2.5.3. Surface hydrophobicity

Surface hydrophobicity of all samples with different treatments was determined as described by Benjakul et al. (2001). Different solutions were diluted to obtain the final protein concentration of 0.1%, 0.2%, 0.3% and 0.5% (w/v) using 10 mM phosphate buffer, pH 6.0 containing 0.6 M NaCl. To the diluted protein solution (2 ml), 20 μl of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0 was added. The fluorescence intensity of ANS-conjugates was measured at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. Blanks were prepared for all samples containing different protein contents by adding 0.1 M phosphate buffer, pH 7.0, instead of ANS solution. Net fluorescence intensity of protein solutions at each concentration was obtained after blank substraction. For each sample, the initial slope of the plot of fluorescence intensity versus protein concentration was referred to as SoANS.

2.5.4. Determination of total sulphydryl groups and disulphide bond contents

Determination of total sulphydryl group content was done using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) as per the meth-

od of Ellman (1959) as modified by Benjakul et al. (2001). To 1.0 ml of sample solutions, 9 ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM ethylenediaminetetraacetic acid (EDTA) were added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB was added and incubated at 40 °C for 25 min. Absorbance at 412 nm was then measured. A sample blank of each sample was conducted in the same manner except that distilled water was used instead of DTNB. The sulphydryl group content was calculated using the extinction coefficient of 13,600 M^{-1} cm^{-1}.

Determination of disulphide bond content was carried out by using 2-nitro-5-thiosulphobenzoate (NTSB) (Thannhauser, Konishi, & Scheraga, 1987). To 0.5 ml of sample solution, 3.0 ml of freshly prepared NTSB assay solution was added. The mixture was incubated in the dark at room temperature (26–28 °C) for 25 min. A sample blank of each sample was prepared in the same manner but the distilled water was used instead of NTSB assay solution. Absorbance at 412 nm was measured. Disulphide bond content was calculated using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.5.5. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of different protein solutions added with OTA at various concentrations and incubated at different temperatures were analysed under reducing condition by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 9 ml of 5% (w/v) SDS solution heated to 85 °C were added to the sample (1 ml). The mixture was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500×*g* for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson & Hodgen, 1940) using bovine serum albumin as a standard. The sample was then mixed with sample buffer (4 ml of 10% SDS, 2 ml of glycerol, 1 ml of β-mercaptoethanol, 2.5 ml of 0.5 M Tris–HCl (pH 6.8), and 0.03 g Bromophenol blue) at 1:1 ratio (v/v). The samples (20 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protein II unit (Bio-Rad Laboratories Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.5.6. Transmission electron microscopy (TEM)

Selected protein solutions were diluted to 0.2 mg/ml with 50 mM potassium phosphate buffer containing 0.6 M KCl (pH 7). A drop of sample was fixed for 5 min on carbon-coated grid, negatively stained with 4% uranyl acetate for 5 min and washed with distilled water until the grid was cleaned. The specimens were visualised using a JEOL JEM-2010 transmission electron microscope (JEOL Ltd., Tokyo, Japan) (×80,000) at an accelerating voltage of 160 kV.

2.6. Statistical analysis

The experiments were run in triplicate. Analysis of variance (ANOVA) was performed and the mean comparisons were carried out by Duncan's multiple range tests (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 10.0 for windows: SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Turbidity

Changes in turbidity of different protein solutions in the absence and in the presence of OTA at different concentrations after incubation at room temperature (26–28 °C) or at 40 °C for 30 min are shown in Fig. 1A and B, respectively. After incubation at both temperatures, the NAM solution became more turbid, compared with the control (without OTA), as OTA at the level of 0.1% and 0.2% was added ($P < 0.05$). Nevertheless the lower turbidity was found when OTA at a level of 0.3% was incorporated ($P < 0.05$). Increased turbidity of NAM solution indicated the formation of the protein aggregate induced by OTA added. Absorbance reading is commonly used to monitor the extent of protein aggregates (Benjakul et al., 1997; Yarnpakdee, Benjakul, Visessanguan, & Kijroongrojana, 2009). At all levels of OTA incorporated, NAM solutions incubated at 40 °C became more turbid, compared with those incubated at room temperature ($P < 0.05$). In the absence of OTA, the higher turbidity was also found in the NAM solution incubated at higher temperature ($P < 0.05$). The results indicated that OTA likely functioned as a protein cross-linker, as evidenced by the increase in turbidity, especially when OTA at the 0.2% level was used. Multifunctional groups of OTA possessed a higher potential to bind or attach to protein molecules, in which a large protein aggregate could be formed (Balange & Benjakul, 2009b).

When NAM solution was incubated at 40 °C, hydrophobic domains as well as reactive groups were more exposed and underwent aggregation readily. Unfolding of protein molecules was due to the instability of hydrogen bonds at higher temperature, exposing the greater numbers of hydrophobic portions (Niwa, 1992). As a consequence, the aggregate could be formed via hydrophobic interactions. In the presence of OTA, quinone, an electrophilic group, in OTA was able to interact with the unfolded proteins, mainly via the amino group, a nucleophilic counterpart. However, the turbidity of NAM solution at both incubation temperatures decreased with the addition of 0.3% OTA ($P < 0.05$). This might be associated with self-aggregation of OTA, resulting in the loss in capability of protein cross-linking. These results were in agreement with Balange and Benjakul (2009a) who reported that the lower breaking force of mackerel surimi gel was obtained when the excessive amount of OTA was added.

At both incubation temperatures, the SP solution had the higher turbidity when OTA ranging from 0.1% to 0.3% was added, compared with the control ($P < 0.05$). Nevertheless, at the same incubation temperature, no differences in turbidity were found between SP solution incorporated with OTA at all levels tested ($P > 0.05$). Generally, the absorbance of SP solution incubated at both temperatures was much lower than that of NAM solutions, irrespective of OTA addition. The results suggested that sarcoplasmic protein underwent aggregation to a lower extent than NAM did. Additionally, the SP aggregate formed might have the smaller size than the NAM aggregate. However, OTA was able to induce cross-linking of SP to some degree as shown by the increased turbidity. Turbidity of SP solution was higher when incubated at 40 °C. Unfolding of SP incubated at 40 °C might favour the cross-linking of SP induced by OTA. OTA in the range of 0.1–0.3% had no differences in cross-linking activity as indicated by the similar turbidity of NAM solution. The result suggested that the level of OTA above 0.1% might be excessive for a limited number of reactive groups, mainly amino groups of SP.

For NAM/SP mixture (65:35) incubated at both temperatures, no changes in turbidity were observed when OTA at all levels was added ($P > 0.05$). SP, which had the smaller size, might be more prone to cross-linking induced by OTA. As a result, the cross-linking of NAM in the mixture induced by OTA became lowered. When NAM solution at the same amount (65%) found in NAM/SP mixture was used, the similar result was obtained, compared with NAM solution at higher protein concentration (100%) for both temperatures. This further confirmed the interfering effect of SP on NAM aggregation induced by OTA. The results were in agreement with Balange and Benjakul (2009b) who reported no

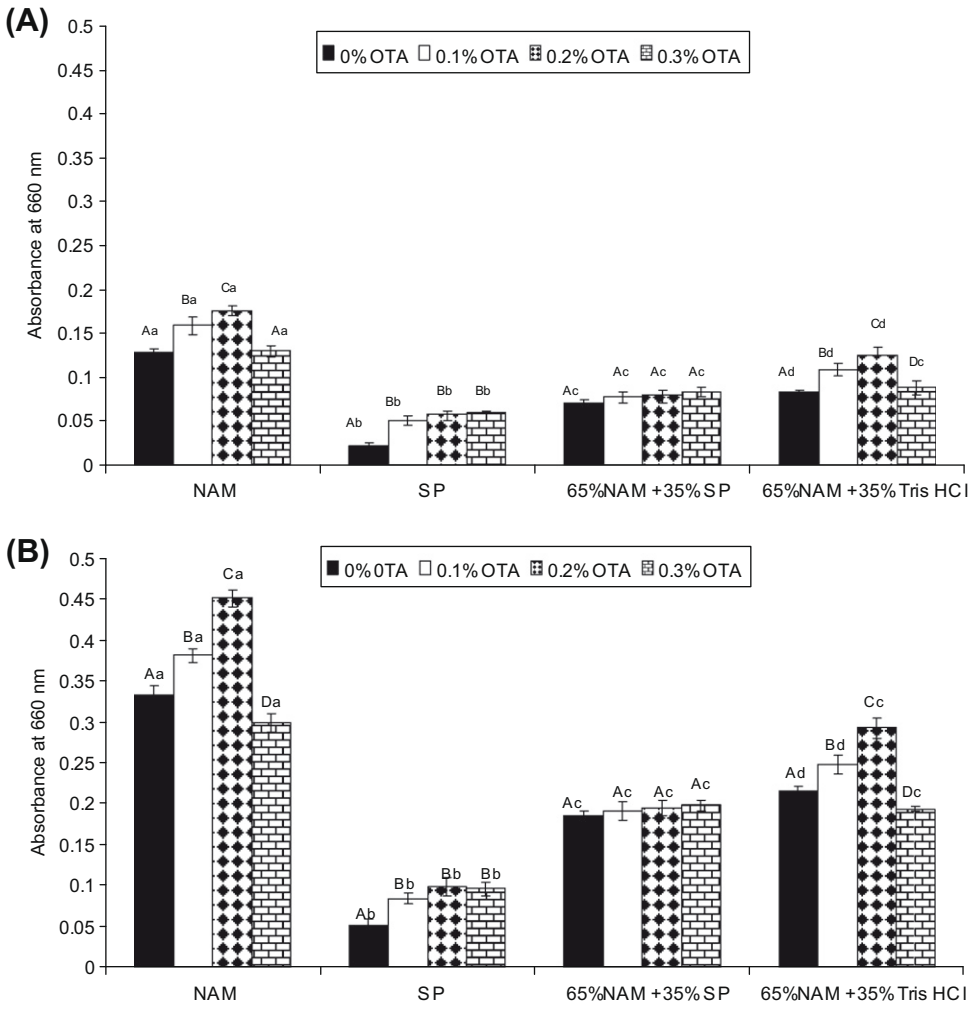

Fig. 1. Turbidity of different protein solutions without and with OTA at different concentrations after incubation at room temperature (26–28 °C) (A) or at 40 °C (B) for 30 min. NAM, SP and OTA represent natural actomyosin, sarcoplasmic protein and oxidised tannic acid, respectively. Different capital letters on the bars within the same protein solution indicate the significant differences ($P < 0.05$). The different letters on the bars within the same levels of OTA indicate the significant differences ($P < 0.05$).

changes in the gel strength of mackerel mince with the addition of OTA at levels ranging from 0% to 0.75%. Therefore, gel strengthening effect of OTA can be found in surimi rather than mince, which contains a larger amount of sarcoplasmic proteins.

3.2. Protein solubility

Solubility of different protein solutions in the absence and presence of OTA at various levels after incubation at room temperature or at 40 °C for 30 min is shown in Fig. 2A and B, respectively. The solubility of NAM solution at both incubation temperatures decreased as the concentration of OTA increased up to 0.2%, compared with that of the control ($P < 0.05$). The decrease in solubility, suggesting the formation of protein aggregates, was in accordance with the increased turbidity (Fig. 1). A slight increase in solubility was found when 0.3% OTA was incorporated compared with that of solution added with 0.1% or 0.2% OTA, regardless of incubation temperatures. During heating, proteins underwent denaturation and aggregation to form a three dimensional structure (Stone & Stanley, 1992). At the same level of OTA used, the lower solubility was observed in NAM solutions added with OTA and incubated at 40 °C, compared with those incubated at room temperature. Proteins most likely underwent conformational changes to a higher extent at 40 °C. Hydrophobic interactions might occur between phenolic compounds and hydrophobic amino acids such as alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine and glycine residues (Prigent, 2005). Furthermore, with increasing exposed reactive groups for cross-linking, the quinones in OTA might induce the formation of strong non-disulphide covalent bonds between NAM molecules to a higher extent. This result was in accordance with the higher turbidity of NAM solution when incubated at 40 °C, compared with at room temperature (Fig. 1).

SP solution, incubated at both incubation temperatures with the addition of OTA ranging from 0.1% to 0.3%, had the lower solubility compared with that of the control ($P < 0.05$). Nevertheless, no differences in solubility were found between samples when OTA at all concentrations range was added ($P > 0.05$). When incubated at 40 °C, SP solutions had the lower solubility, compared with those incubated at room temperature, regardless of OTA addition. For NAM/SP mixture, no changes in solubility were noticeable when OTA was incorporated, irrespective of OTA levels used ($P > 0.05$). The result was in agreement with that of turbidity, in which OTA addition had no impact on turbidity of NAM/SP mixture. This confirmed that SP most likely exhibited the interfering effect on aggregation of NAM induced by OTA. OTA at all concentrations used in this study showed similar cross-linking activity towards NAM. The similar result was noticeable between NAM solutions for both concentrations (65% and 100% NAM), in which 0.2% OTA exhibited the highest cross-linking activity towards NAM.

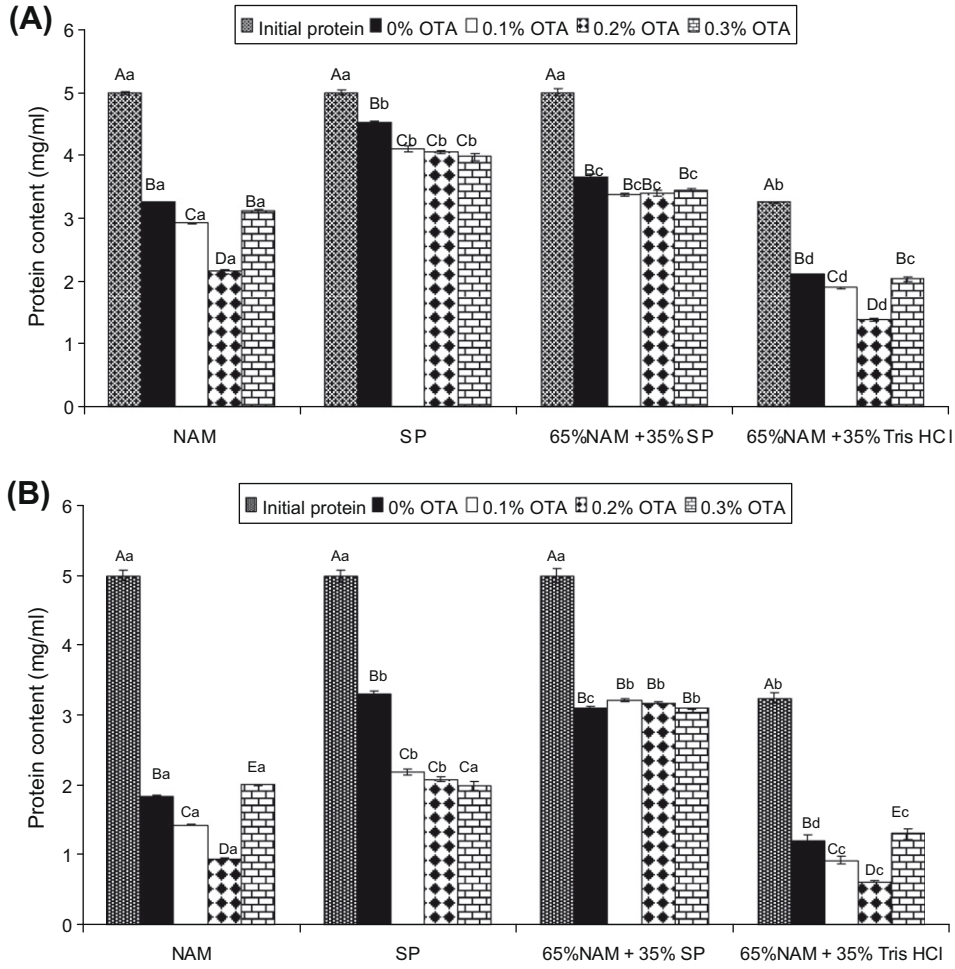

Fig. 2. Solubility of different protein solutions without and with OTA at different concentrations after incubation at room temperature (26–28 °C) (A) or at 40 °C (B) for 30 min. NAM, SP and OTA represent natural actomyosin, sarcoplasmic protein and oxidised tannic acid, respectively. Different capital letters on the bars within the same protein solution indicate the significant differences ($P < 0.05$). The different letters on the bars within the same levels of OTA indicate the significant differences ($P < 0.05$).

3.3. Surface hydrophobicity

Surface hydrophobicity of different protein solutions added without and with OTA at various levels after incubation at room temperature or at 40 °C for 30 min is illustrated in Fig. 3A and B, respectively. At both incubation temperatures, the NAM solution had a higher increase in surface hydrophobicity as the concentration of OTA increased up to 0.2% ($P < 0.05$). The results indicated that OTA likely induced the conformational changes of NAM to some extent, as evidenced by the increase in surface hydrophobicity. ANS, a fluorescence probe, has been found to bind to hydrophobic amino acids containing an aromatic ring, such as phenylalanine and tryptophan, and can be used to indicate the conformational changes occurring in the proteins (Benjakul et al., 1997). The increase in surface hydrophobicity was possibly associated with the exposure of hydrophobic groups of the protein molecules. When OTA attaches to protein molecules, it might cause the alteration of protein conformation to some degree. Nevertheless a slightly lower surface hydrophobicity was found when NAM solution was incorporated with 0.3% OTA and incubated at both temperatures. This was postulated to be due to self-aggregation of OTA at high concentrations, which led to the less efficacy in protein cross-linking. When the NAM solution was heated at 40 °C, a larger amount of reactive groups, including hydrophobic amino acid, became more exposed. In the presence of OTA, the attachment of OTA with NAM could enhance the conformational change as indicated by the higher surface hydrophobicity ($P < 0.05$). Balange and Benjakul (2009c) also found the increase in surface hydrophobicity of bigeye snapper NAM with OTA addition.

SP solution without and with OTA at all levels used had no changes in surface hydrophobicity when incubated at both temperatures (Fig. 3). SP is a water-soluble protein and most of the hydrophobic amino acids might be localised inside the globular molecules, which might require temperatures higher than 40 °C to open up or unfold the molecules.

For the NAM/SP mixture, the addition of OTA had no effect on surface hydrophobicity, regardless of OTA levels ($P > 0.05$). However, higher surface hydrophobicity was noticeable in the solution incubated at higher temperature. OTA might preferably bind with SP, leading to less availability in binding with NAM. As a result, NAM was less attached or bound with OTA and the alteration in NAM conformation became lessened or negligible. For the NAM solution containing lower protein content (65%), the similar result was observed, in comparison with that found in NAM solution at higher protein concentration (100%). This indicated the effect of SP on physicochemical change of NAM, which might be associated with aggregation of NAM.

3.4. Total sulphydryl group and disulphide bond contents

Total sulphydryl group and disulphide bond contents of different protein solutions without and with addition of OTA at different

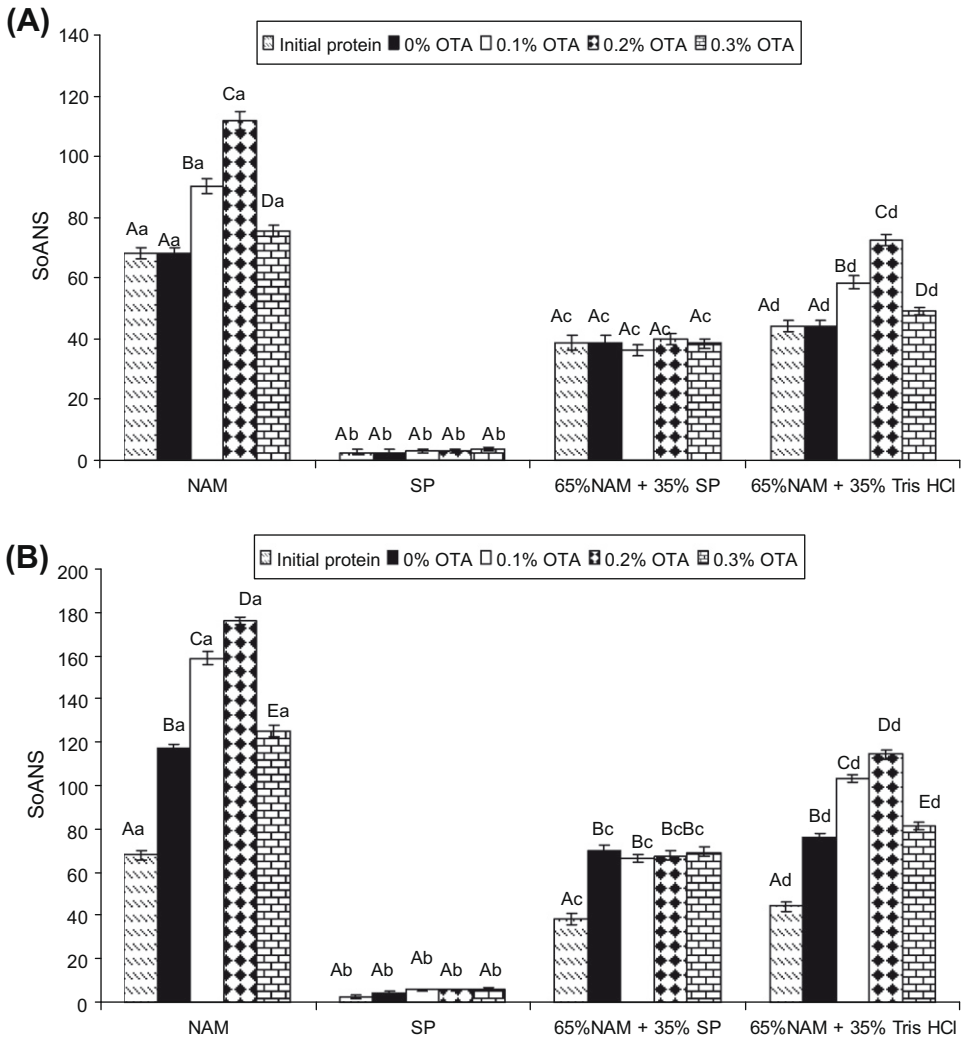

Fig. 3. Surface hydrophobicity of different protein solutions without and with OTA at different concentrations after incubation at room temperature (26–28 °C) (A) or at 40 °C (B) for 30 min. NAM, SP and OTA represent natural actomyosin, sarcoplasmic protein and oxidised tannic acid, respectively. Different capital letters on the bars within the same protein solution indicate the significant differences ($P < 0.05$). The different letters on the bars within the same levels of OTA indicate the significant differences ($P < 0.05$).

levels after incubation at room temperature or at 40 °C for 30 min are shown in Table 1. The total sulphydryl group content of the NAM solution decreased with concomitant increase in disulphide bond formation at both incubation temperatures as the concentration of OTA increased up to 0.2% ($P < 0.05$). However, no differences in total sulphydryl group content and disulphide bond content were found in the NAM solution added with 0.3% OTA, compared with that of the control at both incubation temperatures ($P > 0.05$). At higher temperature, the higher disulphide bond content with the lower sulphydryl groups retained was noticeable in NAM solutions. A decrease in total sulphydryl group content was reported to be due to the formation of disulphide bonds through oxidation of sulphydryl groups or disulphide interchanges (Hayakawa & Nakai, 1985). An inter-molecular disulphide bond is formed by the oxidation of two cysteine molecules on neighbouring protein chains (Lanier, 2000). Elevated temperature most likely resulted in the enhanced oxidation of sulphydryl groups with the accompanied disulphide bond formation. OTA might induce the conformation of NAM, in which sulphydryl groups might be exposed and favoured oxidation process. This was evidenced by the higher disulphide bond contents in NAM added with OTA. Additionally, quinone could interact directly to sulphydryl groups (Strauss & Gibson, 2004). As a result, these sulphydryl groups could be masked by those quinones. At a high concentration of OTA (0.3%), self-aggregation of OTA might lower its ability to interact with sulphydryl groups directly or in induction of sulphydryl group oxidation.

Addition of OTA in SP at all levels had no impact on both total sulphydryl group and disulphide bond contents ($P > 0.05$). Therefore, it can be inferred that OTA was not able to induce the conformational changes of SP molecule in the way, which favoured the oxidation of sulphydryl groups. Furthermore, sulphydryl groups of SP might be present inside molecules and could not readily interact with OTA. It was noted that after incubation at 40 °C, SP had the lowest sulphydryl group content with higher disulphide bond content than those obtained after incubation at room temperature.

No changes in both total sulphydryl group and disulphide bond contents were observed in the NAM/SP mixture when OTA at all levels was added, except for the mixture incorporated with 0.3% OTA and incubated at 40 °C, in which the lowest disulphide bond content was obtained. The results of sulphydryl group and disulphide bond contents correlated well with those of turbidity and solubility (Figs. 1 and 2).

Table 1
Total sulphydryl group and disulphide bond contents of different protein solutions without and with OTA at different concentrations after incubation at room temperature (26–28 °C) or at 40 °C for 30 min.

Protein	% OTA	Incubation at room temperature (26–28 °C)		Incubation at 40 °C	
		Total SH group (mol/10^5 g protein)	SS content (mol/10^5 g protein)	Total SH group (mol/10^5 g protein)	SS content (mol/10^5 g protein)
NAM	0	5.40 ± 0.18Aa*	3.53 ± 0.09Aa	4.20 ± 0.31Aa	3.92 ± 0.35Aa
	0.1	5.20 ± 0.16Aa	3.65 ± 0.25Aa	3.82 ± 0.15Ba	4.51 ± 0.12Ba
	0.2	4.81 ± 0.32Ba	4.20 ± 0.05Ba	3.21 ± 0.21Ca	4.92 ± 0.16Ca
	0.3	5.24 ± 0.18Aa	3.49 ± 0.20Aa	4.10 ± 0.28Aa	3.82 ± 0.30Aa
SP	0	1.14 ± 0.09Ab	0.48 ± 0.03Ab	0.95 ± 0.08Ab	0.68 ± 0.05Ab
	0.1	1.16 ± 0.05Ab	0.51 ± 0.05Ab	0.78 ± 0.05Ab	0.79 ± 0.07Ab
	0.2	1.10 ± 0.11Ab	0.47 ± 0.08Ab	0.75 ± 0.07Ab	0.85 ± 0.06Ab
	0.3	1.22 ± 0.15Ab	0.52 ± 0.04Ab	0.71 ± 0.04Ab	0.92 ± 0.03Ab
65% NAM + 35% SP	0	4.28 ± 0.18Ac	1.61 ± 0.08Ac	3.95 ± 0.38Ac	2.12 ± 0.23Ac
	0.1	4.24 ± 0.21Ac	1.55 ± 0.19Ac	3.84 ± 0.23Ac	2.15 ± 0.35Ac
	0.2	4.35 ± 0.11Ac	1.59 ± 0.21Ac	3.79 ± 0.20Ac	2.21 ± 0.49Ac
	0.3	4.42 ± 0.19Ac	1.42 ± 0.15Ac	3.92 ± 0.26Ac	1.88 ± 0.20Bc
65% NAM + 35% Tris–HCl	0	3.51 ± 0.21Ad	2.29 ± 0.09Ad	2.73 ± 0.08Ad	2.54 ± 0.09Ad
	0.1	3.35 ± 0.31Ad	2.50 ± 0.20Ad	2.48 ± 0.09Bd	2.93 ± 0.20Bd
	0.2	2.67 ± 0.05Bd	2.73 ± 0.08Bd	1.98 ± 0.05Cd	3.19 ± 0.08Cd
	0.3	3.40 ± 0.19Ad	2.27 ± 0.18Ad	2.66 ± 0.11Ad	2.48 ± 0.18Ad

Different capital letters within the same column within the same protein solution indicate the significant differences ($P < 0.05$). The different letters within the same row within the same parameter determined indicate the significant differences ($P < 0.05$).
* Mean ± SD ($n = 3$).

The NAM solution with a lower protein content (65%) showed the similar result of sulphydryl group and disulphide bond contents to that found for NAM with higher protein concentration (100%). Higher disulphide bonds were formed when 0.2% OTA was added, especially when incubated at 40 °C. Therefore, the addition of OTA was associated with the increased formation of disulphide bonds in NAM. The formation of disulphide bonds might contribute to the formation of the NAM aggregate, particularly when NAM was incubated at 40 °C, which is commonly used for the high-temperature setting for surimi or mince gel preparation (Benjakul et al., 2004a, 2004b; Benjakul, Visessanguan, Tueksuban, et al., 2004).

3.5. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of different protein solutions without and with addition of OTA at various concentrations after incubation at room temperature or at 40 °C for 30 min are shown in Fig. 4. No differences in MHC and actin band intensity were noticeable when NAM solutions were incubated at room temperature, regardless of OTA addition (Fig. 4A). However, the lowest MHC and actin band intensity was found in NAM solution added with OTA up to 0.2% when incubated at 40 °C for 30 min. The result suggested that the formation of cross-linking stabilised by non-disulphide covalent bond took place, especially during setting. MHC was most susceptible to cross-linking during setting (Benjakul & Visessanguan, 2003). Benjakul and Visessanguan (2003) reported the decrease in MHC band intensity of surimi gel from bigeye snapper, particularly when the setting at 40 °C was implemented. NAM without OTA incubated at 40 °C showed the slightly lower MHC band intensity compared with those found in NAM incubated at room temperature. This might be governed by remaining endogenous TGase in NAM. At high temperatures (40 °C), MHC might be partially unfolded and OTA could interact with those proteins to a higher extent via non-disulphide covalent bond. This was evidenced by the lowest MHC band intensity found in NAM added with 0.2% OTA and incubated at 40 °C. Conversely, room temperature (26–28 °C) might not be enough for protein unfolding and the reactive groups of NAM might not be available for cross-linking induced by OTA. Ou, Wang, Tang, Huang, and Jackson (2005) reported the polymerisation of soy protein molecules by ferulic acid. Mechanical properties of gelatin films were also improved when ferulic acid and tannic acid were used as cross-linkers (Cao, Fu, & He, 2007). Covalent modification of proteins by phenolic oxidation products generated at alkaline pH was reported extensively (Rawel, Rohn, Kruse, & Kroll, 2002). Thus, the efficacy in protein cross-linking of OTA was maximised when setting at a temperature high enough for conformational alteration of proteins was applied. As a result, the polymerisation of NAM could be enhanced.

SP solution, without and with OTA after incubation at both temperatures, had no marked differences in protein patterns (Fig. 4B). However, the band intensity of all proteins decreased slightly at both incubation temperatures when 0.3% OTA was added. This indicated that non-disulphide covalent bond induced by OTA was negligible. SDS and β-mercaptoethanol used for electrophoresis could destroy all weak bonds and disulphide bond. It was suggested that weak bonds were involved in SP cross-linking, especially hydrophobic interaction or hydrogen bond. The results indicated that OTA, especially at a high concentration, was able to cross-link SP to some degree. Nevertheless, the aggregate might have a small size which did not show a positive response for turbidity or solubility studies. For the NAM/SP mixture, MHC band intensity decreased slightly when incubated at 40 °C for 30 min as compared with that of mixture incubated at room temperature (Fig. 4C). This might be a result of the formation of non-disulphide cross-link induced by endogenous TGase prevalent in SP. The sarcoplasmic fraction from bigeye snapper muscle possessed cross-linking activity towards MHC (Benjakul et al., 2004a, 2004b; Benjakul, Visessanguan, Tueksuban, et al., 2004). Benjakul and Visessanguan (2003) reported the role of TGase in setting of bigeye snapper surimi. However, no differences in band intensity of MHC and other proteins were observed when OTA at all levels was incorporated. The results indicated the interfering effect of SP on NAM cross-linking. OTA might prefer to bind with SP, thus it was not available to cross-link MHC. The result was in accordance with no changes in turbidity, solubility, total sulphydryl groups and disulphide bond contents of NAM/SP mixture (Figs. 1 and 2, and Table 1, respectively).

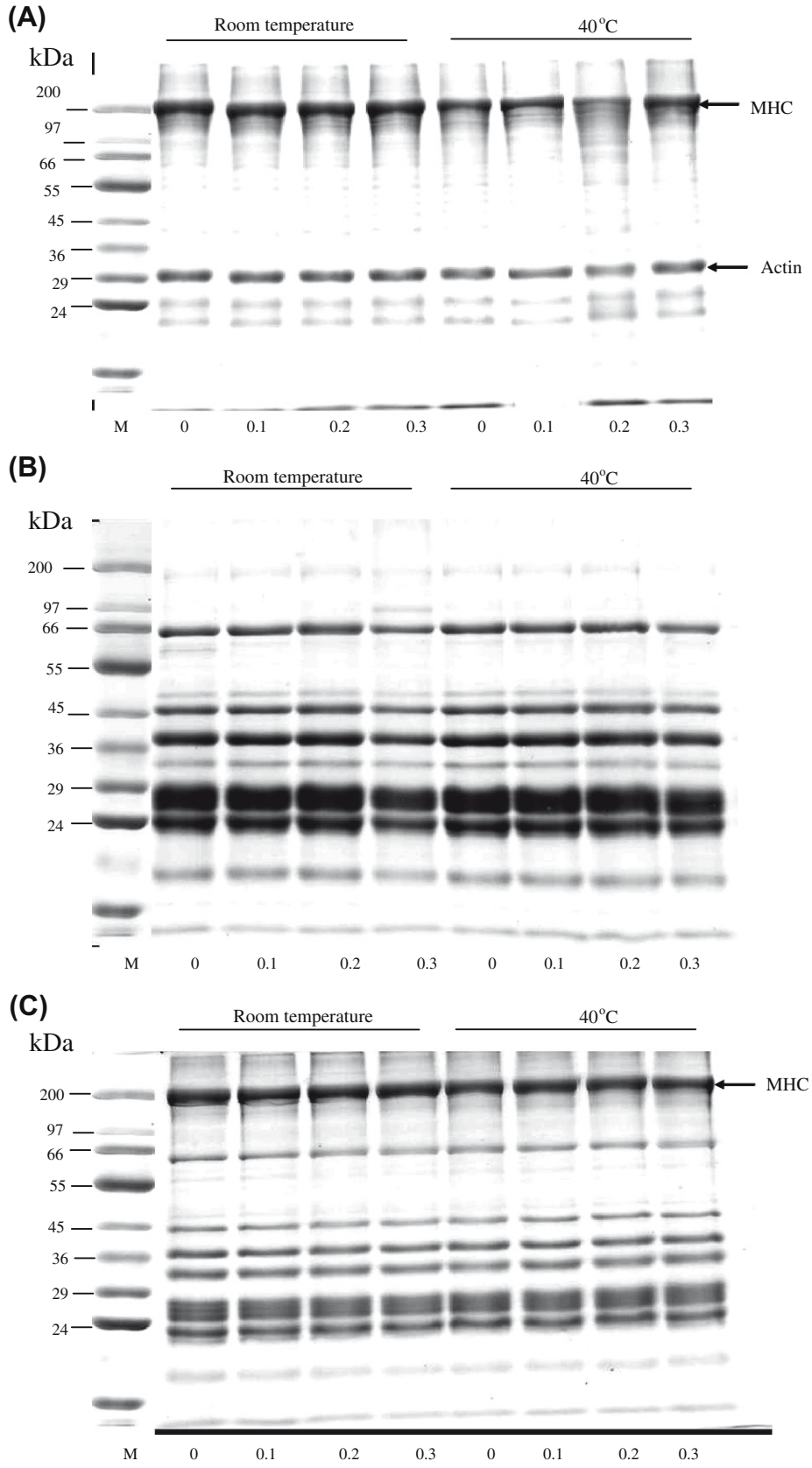

Fig. 4. SDS–PAGE patterns of proteins of NAM (A), SP (B) and NAM/SP (65:35) mixture (C), without and with OTA at different concentrations after incubation at room temperature (26–28 °C) or at 40 °C for 30 min. MHC, myosin heavy chain; M, high molecular weight protein markers; Numbers denote the concentration of OTA added (%).

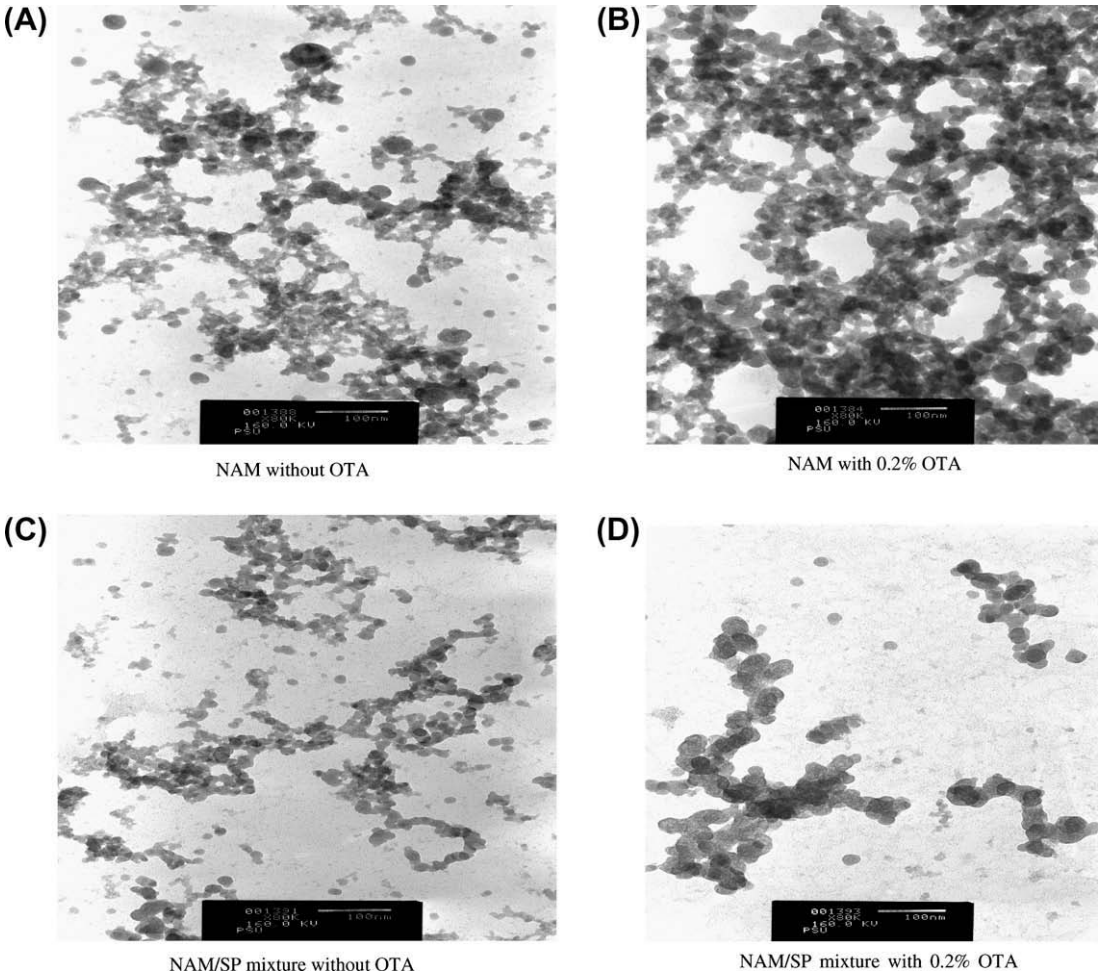

Fig. 5. Transmission electron micrograph of NAM or NAM/SP mixture without and with 0.2% OTA after incubation at 40 °C for 30 min. NAM, SP and OTA represent natural actomyosin, sarcoplasmic protein and oxidised tannic acid, respectively. Magnification: 80,000×.

3.6. Microstructure

Microstructures of NAM or NAM/SP (65:35) mixture incubated at 40 °C for 30 min without and with 0.2% OTA are illustrated in Fig. 5. The NAM aggregate had the larger strand with the denser structure, compared with the aggregate of the NAM/SP mixture. SP with a lower molecular weight might interfere the interaction between NAM molecules, leading to the formation of a looser cluster. A highly interconnected, finer and denser network structure was observed in NAM with 0.2% OTA incubated at 40 °C for 30 min. Gelation is the result of protein denaturation, followed by the aggregation via inter-molecular covalent bonds and non-covalent interactions (Lee & Lanier, 1995). The incubation at 40 °C might provide the sufficient energy for unfolding protein molecules, which allowed OTA to interact easily. This resulted in a highly ordered three dimensional protein network. This was coincidental with the highest turbidity development and disulphide bond formation of NAM added with 0.2% OTA. (Fig. 1B and Table 1). A looser protein network was formed in the NAM/SP mixture incubated at 40 °C for 30 min without OTA (Fig. 5B). In the presence of OTA, the coagulum with a denser structure was formed. A much less interconnected network was observed. Therefore, SP at a level of 35% exhibited the dilution effect on NAM, which had a more fibrous structure (Fig. 5C). Furthermore, OTA most likely preferred to bind SP than NAM, mainly via weak bonds. This resulted in the poor protein network formation.

4. Conclusion

OTA could be used as a cross-linker of NAM when the incubation temperature was high enough to induce the conformational change. Nevertheless, SP exhibited the interfering effect on cross-linking activity of OTA towards NAM, a major contributor for gel formation. OTA might preferably interact with SP instead of NAM, in which a small aggregate or cluster was formed and impede the aggregation of NAM.

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Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidised phenolic compounds

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ABSTRACT

Effects of different oxidised phenolic compounds (ferulic acid, OFA; tannic acid, OTA; catechin, OCT and caffeic acid, OCF) at different levels (0–0.25% of protein content) on the properties of gels from bigeye snapper (*Priacanthus tayenus*) surimi were investigated. Breaking force and deformation of surimi gel varied with types and amounts of oxidised phenolic compounds. Gels added with 0.20% OFA, 0.05% OTA, 0.15% OCF and 0.05% OCT exhibited the marked increases in both breaking force and deformation, compared with the control ($P < 0.05$). Those increases were associated with lower expressible moisture content. No increases in both breaking force and deformation were observed when ferulic acid without oxygenation at alkaline pH was added, regardless of amount added ($P > 0.05$). No changes in the whiteness of gel were found with addition of OFA ($P > 0.05$), but the decreases in whiteness were noticeable as other oxidised phenolics were incorporated ($P < 0.05$). Different microstructures were obtained among gels with different oxidised phenolics. The physicochemical properties of natural actomyosin suggest that oxidised phenolics could induce conformational changes and the cross-linking through amino groups or the induction of disulphide bond formation. Therefore, the addition of oxidised phenolic compounds at the optimum level could increase the gel strength of surimi gel.

1. Introduction

Surimi is minced fish flesh, washed to remove most of lipids, blood, enzymes and sarcoplasmic proteins and stabilised for frozen storage by cryoprotectants. Surimi possesses the functionalities including gelling, binding and emulsifying properties and can be used as a functional protein ingredient in several products (Lanier, 1986). Myofibrillar proteins in surimi are mainly involved in the gel-forming ability (Benjakul, Visessanguan, & Tueksuban, 2003). To increase the gel strength of surimi, various food grade ingredients have been used. However, the addition of these ingredients poses adverse effects on the surimi gel, particularly on off flavour or off colour development (Rawdkuen & Benjakul, 2008). Addition of bovine plasma protein has been prohibited due to the mad cow disease. While addition of egg white is associated with allergy problems.

Polyphenols, which are widely distributed as minor components but are functionally important constituents of plant tissues, occur mainly in rigid tissues, such as the hulls of cereal grains, cell walls of fruits (e.g. grapes, apples), coffee beans, tea leaves, and tubers (e.g. potatoes) (Naczk & Shahidi, 2004; Shahidi & Naczk, 2004). The most common polyphenols are hydroxylated cinnamic acids such as caffeic acid (3, 4-dihydroxycinnamic acid), chlorogenic acid (its quinic acid ester), caftaric acid (its tartaric acid ester), and flavonols such as quercetin and rutin (its rutinoside) (Spanos & Wrolstad, 1992). These compounds have an *ortho*-diphenol (or a 1-hydroxy-2-methoxy) structure (Strauss & Gibson, 2004). The formation of rigid molecular structures by reactions of *ortho*-quinones with proteins is demonstrated by Strauss and Gibson (2004). Diphenol moiety of a phenolic acid or other polyphenol is readily oxidised to an *ortho*-quinone, either enzymatically as in plant tissues, or by molecular oxygen. The quinone forms a dimer in a side reaction, or reacts with amino or sulphydryl side chains of polypeptides to form covalent C–N or C–S bonds with the phenolic ring, with regeneration of hydroquinone. The latter can be reoxidised and bind a second polypeptide, resulting in a cross-link. Alternatively, two quinones, each carrying one chain, can dimerise, also producing a cross-link (Strauss & Gibson, 2004). Plant phenols at pH 8 increased the bloom strength of gelatin gel (Strauss & Gibson, 2004). Addition of phenolic compound in combination with 0.1 M NaCl at pH 8.5 resulted in the improved gel properties of canola protein (Rubino, Arntfield, Nadon, & Bernatsky, 1996). Rawel, Rohn, Kruse, and Kroll (2002) reported that the phenolic compounds react with proteins, resulting in the formation of cross-links. Nevertheless, information regarding the effect of phenolic compounds on the gel property of surimi is very scarce. Thus, the study aimed to investigate the effect of phenolic compounds, including ferulic acid, tannic acid, catechin and caffeic acid on the properties of

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surimi gel from bigeye snapper, a species commonly used as a raw material for surimi production in Thailand.

2. Materials and methods

2.1. Chemicals/surimi

Ferulic acid (FA), tannic acid (TA) and β-mercaptoethanol (βME) were obtained from Sigma (St. Louis, MO, USA). Caffeic acid (CF) and catechin (CT) were purchased from Fluka (Buchs, Switzerland). Sodium dedocylsulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA).

Frozen surimi grade B (breaking force of 300–400 g; deformation of <8 mm), produced from bigeye snapper (*Priacanthus tayenus*) were purchased from Man A Frozen Foods Co., Ltd. (Songkhla, Thailand) and kept at −20 °C not more than two months before use.

2.2. Effect of phenolic compounds on the properties of surimi gel

2.2.1. Preparation of oxidised phenolic solutions

Four phenolic compounds namely ferulic acid, tannic acid, caffeic acid and catechin were dissolved in distilled water as per the method of Strauss and Gibson (2004) with slight modifications. Phenolic solution (100 ml; 1% w/v) was adjusted to pH 8 using 6 M NaOH or 6 M HCl. The prepared solution was placed in a temperature-controlled water bath (40 °C) and subjected to oxygenation for 1 h by bubbling the solution with oxygen to convert the phenolic compounds to quinones. After being oxygenated for 1 h, the solution was then adjusted to pH 7 by using 6 M HCl and was referred to as 'oxidised phenolic compound'. For ferulic acid, another portion (pH 7) was also prepared in the same manner without oxygenation.

2.2.2. Surimi gel preparation

To prepare the gel, frozen surimi was tempered for 30 min in running water (26–28 °C) until the core temperature reached 0–2 °C. The surimi was then cut into small pieces with an approximate thickness of 1 cm. The surimi was placed in a mixer (National Model MK-K77, Tokyo, Japan). The moisture was adjusted to 80% and 2.5% salt was added. Different oxidised phenolic compounds at various concentrations (0%, 0.05%, 0.10%, 0.15%, 0.20% and 0.25% of protein content) were added. The mixture was chopped for 4 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 casing were sealed tightly. Sols were incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Benjakul, Visessanguan, & Chantarasuwan, 2004). All gels were cooled in iced water and stored for overnight at 4 °C prior to analyses.

To study the effect of oxygenation of ferulic acid on the properties of surimi gel, ferulic acid with and without oxygenation at different levels (0%, 0.05%, 0.10%, 0.15%, 0.20% and 0.25% of protein content) was added into surimi sol. Surimi gels were then prepared as previously described.

2.2.3. Texture analysis

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

2.2.4. Determination of expressible moisture content

Expressible moisture content was measured according to the method of Benjakul, Visessanguan, and Srivilai (2001) with a slight modification. Gel samples were cut into a thickness of 5 mm, weighed (X) and placed between 3 pieces of Whatman paper No. 4 at the bottom and 2 pieces on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The sample was then removed from the papers and weighed again (Y). Expressible moisture content was calculated using the following equation

$$\text{Expressible moisture content } (\%) = 100[(X - Y)/X]$$

2.2.5. Determination of whiteness

Colour of surimi gels was determined using a JP7100F colorimeter (Juki Corporation, Tokyo, Japan). L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Lanier, Hart, and Martin (1991) as follows:

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

2.2.6. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of surimi gels were analysed by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85 °C were added to the sample (3 g). The mixture was then homogenised using a homogeniser (IKA Labortechnik, Selangor, Malaysia) for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500 × *g* for 20 min to remove undissolved debris. The samples (20 μg protein) were loaded into the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.2.7. Protein determination

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

2.2.8. Solubility determination

Solubility of protein in surimi gel was determined as described by Benjakul et al. (2001). Finely chopped gel sample (1 g) was solubilised with various solvents including S1 (0.6 M KCl), S2 (20 mM Tris–HCl, pH 8.0), S3 (20 mM Tris–HCl, pH 8.0 containing 1% SDS), S4 (20 mM Tris–HCl, pH 8.0 containing 1% SDS and 8 M urea) and S5 (20 mM Tris–HCl, pH 8.0 containing 1% SDS, 2% β-mercaptoethanol and 8 M urea). The mixture was homogenised for 1 min, boiled for 2 min and stirred for 4 h at room temperature (28–30 °C) using a magnetic stirrer (IKA-Werke, Staufen, Germany). The mixture was centrifuged at 10,000 × *g* for 30 min. Two ml of 50% (w/v) cold trichloroacetic acid (TCA) were added to 10 ml of supernatant. The mixture was kept at 4 °C for 18 h prior to centrifugation at 10,000 × *g* for 20 min. The precipitate was washed with 10% (w/v) TCA, followed by solubilising in 0.5 M NaOH. Protein concentration was determined by the Biuret method (Robinson & Hodgen, 1940). Solubility of protein in surimi samples was expressed as the percentage of total protein in surimi gels solubilised directly in 0.5 M NaOH.

2.2.9. Scanning electron microscopy (SEM)

Microstructure of surimi gels was determined using SEM (JEOL JSM-5800 LV, Tokyo, Japan). The control gel (without oxidised phenolic compound) and those containing oxidised phenolic compounds with a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50%, 70%, 80%, 90% and 100% (v/v). Dried samples were mounted on a bronze stub, and sputter-coated with gold (Sputter coater SPI-Module, PA, USA). The specimens were observed with a scanning electron microscope at an acceleration voltage of 10 kV.

2.3. Effect of phenolic compounds on physico-chemical properties of natural actomyosin from bigeye snapper surimi

2.3.1. Preparation of natural actomyosin (NAM)

Natural actomyosin (NAM) was prepared according to the method of Benjakul, Seymour, Morrissey, and An (1997) with a slight modification. Surimi (10 g) was homogenised in 100 ml of chilled 0.6 M KCl, pH 7.0 for 4 min using a homogeniser (IKA Labortechnik, Selangor, Malaysia). The container with sample was placed in ice. Each 20 sec of homogenisation was followed by a 20 sec rest interval to avoid overheating during extraction. The homogenate was centrifuged at 5,000 × *g* for 30 min at 4 °C. Three volumes of chilled water (0–2 °C) were added to precipitate NAM and NAM was then collected by centrifuging at 5,000 × *g* for 20 min at 4 °C. The pellets were then dissolved by stirring in an equal volume of chilled 0.6 M KCl, pH 7.0 for 30 min at 4 °C.

2.3.2. Incorporation of oxidised phenolic compounds into NAM

NAM was diluted to a concentration of 4 mg/ml. Each oxidised phenolic compound was added into NAM to obtain the final concentration, which was equivalent to that rendering the highest breaking force in surimi gel. The control (without oxidised phenolic compound) was also prepared. The mixture was subjected to heating with two conditions involving (i) 40 °C for 30 min or (ii) 40 °C for 30 min followed by 90 °C for 20 min. Heated samples were cooled rapidly in iced water and subjected to analyses.

2.3.3. Determination of surface hydrophobicity

Surface hydrophobicity was determined as described by Benjakul et al. (1997) using 1-anililonaphthalene-8-sulphonic acid (ANS) as a probe. Different NAM mixtures were diluted to protein contents of 0.1%, 0.2%, 0.3% and 0.5% (w/v) using 10 mM phosphate buffer, pH 6.0 containing 0.6 M NaCl. The diluted protein solution (2 ml) was added with 20 μl of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0. The fluorescence intensity of ANS-conjugates was measured at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. Blanks were prepared using oxidised phenolic compounds at the concentrations equivalent to those found in differently diluted NAM. Net fluorescence intensity of NAM at each concentration was obtained after blank correction. The initial slope of the plot of fluorescence intensity versus NAM concentration was referred to as SoANS.

2.3.4. Determination of total sulphydryl group and disulphide bond contents

Total sulphydryl group content was determined using 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) according to the method of Ellman (1959) as modified by Benjakul et al. (1997). To 1.0 ml NAM solution (4.0 mg/ml), 9 ml of 0.2 M Tris-HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM ethylenediaminetetraacetic acid (EDTA), were added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB was added and incubated at 40 °C for 25 min. Absorbance at 412 nm was then measured. A blank was conducted by replacing the sample with 0.6 M KCl. Sulfhydryl group content was calculated using the extinction coefficient of 13,600 M^{-1} cm^{-1}.

Disulphide bond content was determined by using 2-nitro-5-thiosulphobenzoate (NTSB) assay according to the method of Thannhauser, Konishi, and Scheraga (1987). To 0.5 ml of NAM (4.0 mg/ml), 3.0 ml of freshly prepared NTSB assay solution was added. The mixture was incubated in the dark at room temperature (26–28 °C) for 25 min. Absorbance at 412 nm was measured. Disulphide bond content was calculated using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.3.5. Determination of free amino group contents

Free amino group content was determined according to the method of Benjakul and Morrissey (1997). Diluted samples (125 μl) were mixed thoroughly with 2.0 ml of 0.2 M phosphate buffer, pH 8.2, followed by the addition of 1.0 ml of 0.01% TNBS solution. The mixture was then placed in a water bath at 50 °C for 30 min in dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulphite. The mixture was cooled to room temperature for 15 min. The absorbance was measured at 420 nm and free amino group content were expressed in terms of L-leucine.

2.4. Statistical analysis

The experiments were run in five replicates. Analysis of variance (ANOVA) was performed and the mean comparisons were carried out by Duncan's multiple range tests (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 10.0 for windows: SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Effect of oxidised phenolic compounds on the properties of surimi gel

Gels from bigeye snapper surimi added with different oxidised phenolic compounds at various levels had varying breaking force and deformation (Fig. 1). Gels added with oxidised ferulic acid (OFA) or oxidised caffeic acid (OCF) had the increases in both breaking force and deformation when the levels added increased up to 0.20% and 0.15%, respectively ($P < 0.05$). With the addition of 0.20% OFA, breaking force and deformation of gel increased by 28.98% and 38.06%, respectively, compared with that of the control. The addition of OCF at a level of 0.15% resulted in the increases in breaking force and deformation of gel by 29.78% and 38.63%, respectively, compared with that of the control. For gels added with oxidised tannic acid (OTA) or oxidised catechin (OCT), the highest breaking force and deformation were obtained when both phenolic compounds were added at 0.05% ($P < 0.05$). The continuous decreases in both breaking force and deformation were noticeable as the levels added increased up to 0.25% ($P < 0.05$). OTA at 0.05% resulted in the increase in breaking force and deformation of surimi gel by 39.52% and 38.97%, respectively. The addition of OCT at the same level increased breaking force and deformation of surimi gels by 29.69% and 38.40%, respectively. These results indicate that oxidised phenolic compounds at the optimum concentration showed the enhancing effect on gel formation. Phenolic compounds can interact with proteins in two different ways: via non-covalent (reversible) interactions and via covalent interactions, which in most cases are irreversible (Prigent et al., 2003). Two types of complexation mechanisms can be distinguished: a monodentate and a multidentate mechanism (Haslam, 1989). Both complexation mechanisms lead to aggregation and precipitation of proteins (Haslam, 1989). Lower amounts of TA and CT (0.05%) were

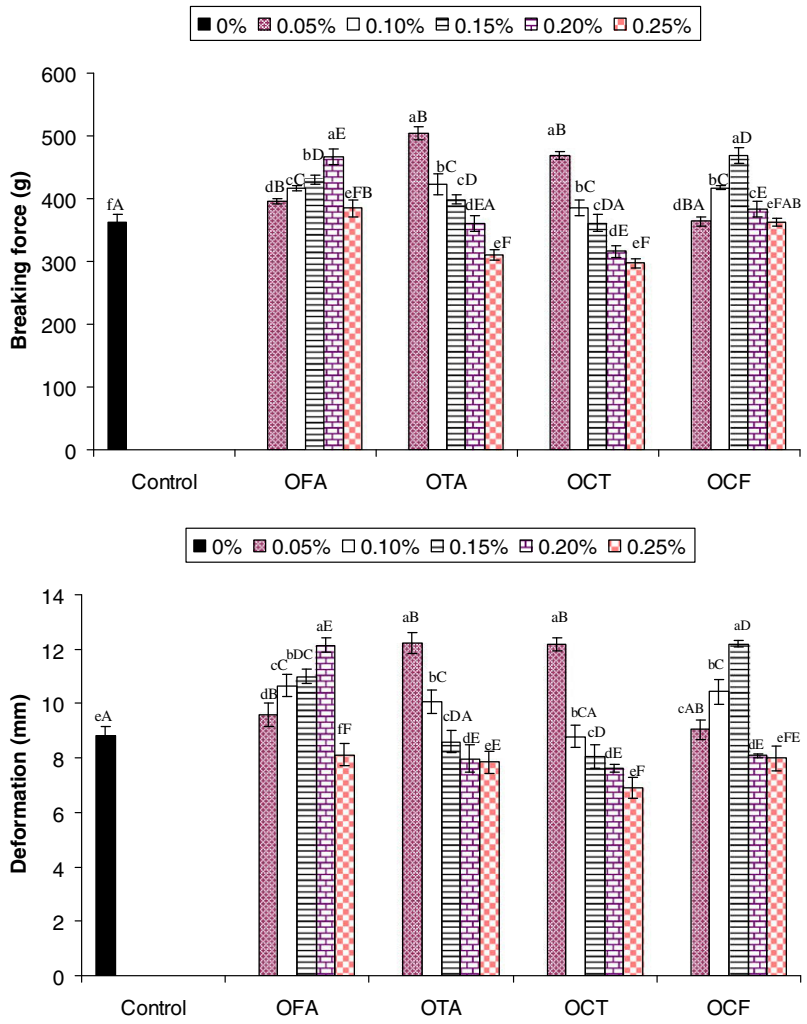

Fig. 1. Breaking force and deformation of gels from bigeye snapper surimi added with oxidised phenolic compounds at different levels. OFA, OTA, OCT and OCF represent oxidised ferulic acid, tannic acid, catechin and caffeic acid, respectively. Bars represent the standard deviation from five determinations. Different letters on the bars within the same oxidised phenolic compounds together with the control indicate the significant differences ($P < 0.05$). The different capital letters on the bars within the same levels of oxidised phenolic compounds indicate the significant differences ($P < 0.05$).

required to increase the breaking force and deformation of surimi gel, compared with FA and CF. The multidentate mechanism generally requires a much lower phenolic compound/protein molar ratio and thus a lower phenolic compound concentration (Haslam, 1989). On the contrary, "Monodentate" means that a phenolic compound interacts with only one protein site at a higher phenolic compound concentration (Haslam, 1989). From the result, both OFA and OCF having 1 and 2 hydroxyl groups, respectively, at higher levels (0.20% and 0.15%) were required to increase breaking force and deformation of surimi gel. The larger the phenolic compound or the more binding sites the phenolic compound possesses, the stronger the association is expected (Hagerman, Rice, & Ritchard, 1998). In the present study, at the lower level (0.05%), higher breaking force and deformation was observed when OTA was added ($P < 0.05$). OTA has a greater number of hydroxyl groups attached to the aromatic benzene ring as compared to others. Nevertheless, the lower solubility of large phenolic compounds at high concentration causes the difficulty to interact with proteins (De Freitas & Mateus, 2001). In addition, the size of the phenolic compound can decrease its conformational flexibility, which is observed to be an important parameter in protein–phenolic compound interactions (Frazier, Papadopoulou, Mueller-Harvey, Kissoon, & Green, 2003). The decreased breaking force and deformation with increasing concentrations of phenolic compounds in the present study might be associated with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking.

3.2. Effect of oxygenation of ferulic acid on the properties of surimi gel

FA, without and with oxygenation under alkaline pH prior to neutralisation at different levels was added in surimi gels. It was noted that no changes in breaking force and deformation were observed with the addition of FA without prior oxygenation (Fig. 2). On the other hand, the continuous increase in breaking force was found when the oxidised FA (OFA) was added up to 0.20%. The highest deformation was also obtained as 0.20% OFA was added ($P < 0.05$). Oxygenation under alkaline conditions induces the deprotonation of phenolic hydroxyl group, leading to quinone formation. Quinones have been indirectly proven to react with amino acids in a peptide chain. This method is used in food industry e.g. to produce ripe olives by treating them with dilute NaOH in order to oxidise caffeic acid and hydroxytyrosol (Garcia, Concepcion, Brenes, & Garrido, 1996). The covalent modification of proteins by phenolic oxidation products generated at alkaline pH has been studied extensively (Rawel et al. 2002). Covalent protein modifica-

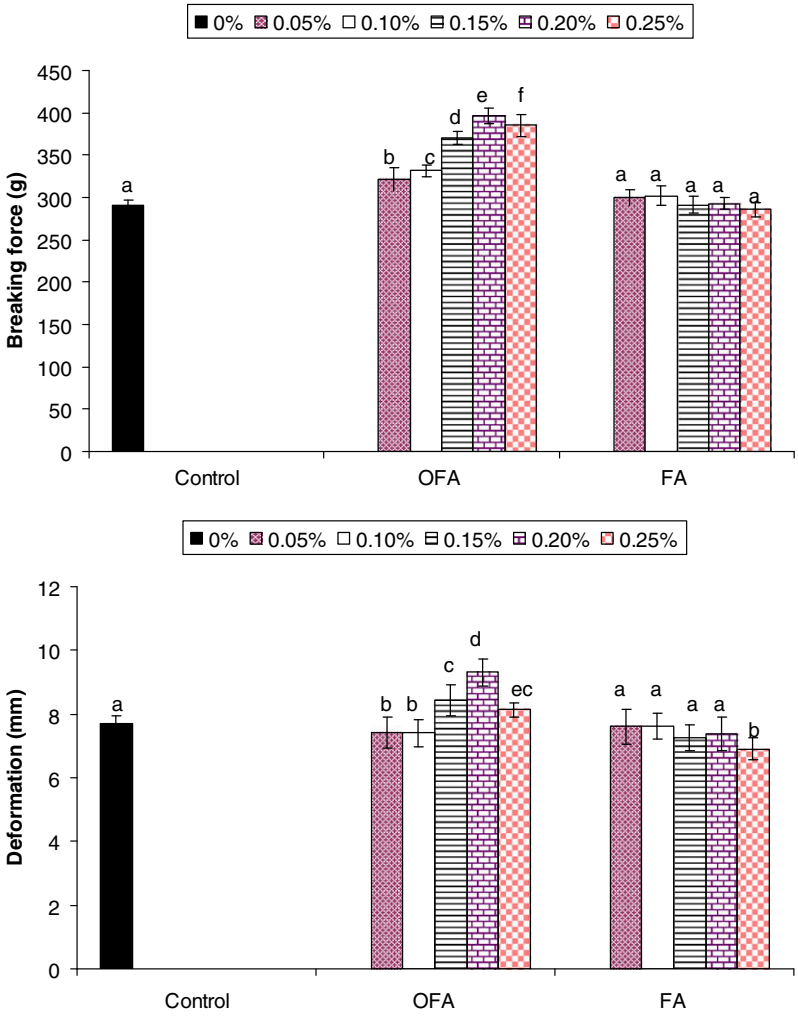

Fig. 2. Breaking force and deformation of gels from bigeye snapper surimi added with ferulic acid with and without oxygenation at different levels. Bars represent the standard deviation from five determinations. Different letters on the bars indicate the significant differences ($P < 0.05$).

tion by phenols oxidised at alkaline pH induced protein cross-linking and a decrease of the isoelectric pH of proteins (Prigent, 2005).

Direct evidence for reaction products with the side-chains of amino acids was demonstrated by the covalent reaction between CQA and cysteine (Richard, Goupy, Nicolas, Lacombe, & Pavia, 1991), caffeic acid and cysteine (Cilliers & Singleton, 1990) and oxidised catechols and histidine (Kerwin et al., 1999). In the present study, OFA at a level of 0.20% increased breaking force and deformation effectively after the oxidation took place. This reveals that the formation of quinone was necessary for the cross-liking of proteins. As a consequence, the protein gel network could be strengthened. The result was in agreement with Strauss and Gibson (2004) who found the increase in bloom strength of gelatin gels incorporated with oxygenated phenolics.

3.3. Effect of oxidised phenolic compounds on expressible moisture of surimi gels

Expressible moisture content of surimi gels added with different oxidised phenolic compounds at various levels is shown in Table 1. Gel added with OFA or OCF had the decreased expressible moisture content as the levels added increased up to 0.20% and 0.15%, respectively. The lowered expressible moisture content was in accordance with the increased breaking force and deformation of resulting surimi gels (Fig. 1). The lowest expressible moisture content was observed in gels added with 0.05% OTA or 0.05% OCT, compared with gels added with higher levels of oxidised phenols. During setting at 40 °C, proteins underwent some denaturation and aligned themselves gradually to form the network, which can imbibe water (Benjakul & Visessanguan, 2003). When the optimal level of oxidised phenolic compound was added, the cross-linking of proteins could be enhanced, resulting in the formation of stronger network with greater water holding capacity.

3.4. Effect of oxidised phenolic compounds on whiteness of surimi gels

Whiteness of all surimi gels added with OTA, OCT and OCF decreased as the concentrations increased ($P < 0.05$) (Table 1). Phenolic compounds were responsible for discoloration in cheese products (O'Connell & Fox, 2001). On the contrary, no changes in whiteness were observed in surimi gels added with different levels of OFA, compared with the control ($P > 0.05$). At alkaline–neutral pH values, FA is inherently unstable and is converted to carbinol base, which is colourless (Asen, Stewart, & Norris, 1979). As a result, no changes in whiteness of surimi gels were noticeable when different levels of OFA were added. Phenolic compounds like tannic acid, catechin and caffeic acids are stable at neutral pH values and thus are suitable colorants (Asen et al., 1979).

Table 1
Expressible moisture content and whiteness of gels from bigeye snapper surimi added with various oxidised phenolic compounds at different levels

Oxidised phenolic compounds	Amount added (%)	Expressible moisture content (%)	Whiteness
Control	-	3.93 ± 0.57a*	73.15 ± 0.28a*
OFA	0.05	2.97 ± 0.75bA**	72.85 ± 0.25aA**
	0.10	2.92 ± 0.72 bA	73.18 ± 0.31aA
	0.15	2.62 ± 0.48cA	72.50 ± 0.24aA
	0.20	2.56 ± 0.51dA	72.53 ± 0.33aA
	0.25	2.62 ± 0.48dA	73.08 ± 0.26aA
OTA	0.05	2.55 ± 0.07bB	65.66 ± 1.68bB
	0.10	2.61 ± 0.43cB	65.30 ± 2.13cbB
	0.15	3.02 ± 0.11dB	62.82 ± 0.43dfB
	0.20	3.05 ± 0.39edB	63.76 ± 1.70edB
	0.25	3.12 ± 0.15fdB	61.89 ± 0.26fB
OCT	0.05	2.62 ± 0.15bC	67.45 ± 0.52bC
	0.10	2.94 ± 0.34caCA	65.83 ± 0.57cB
	0.15	3.48 ± 1.12caC	63.88 ± 0.50dC
	0.20	3.71 ± 1.17caC	63.64 ± 0.43edB
	0.25	3.90 ± 1.09caC	62.64 ± 0.32fC
OCF	0.05	2.81 ± 0.47aD	67.29 ± 0.36bC
	0.10	2.65 ± 0.72aDB	65.70 ± 0.74cB
	0.15	2.06 ± 0.12bD	63.88 ± 0.50dC
	0.20	2.40 ± 0.45cD	63.62 ± 0.44dB
	0.25	2.84 ± 1.43daD	63.32 ± 0.79dD

Values are mean ± standard deviation (n = 3).
* Different letters in the same coloumn within the same oxidised phenolic compound together with the control indicates the significant differences ($P < 0.05$).
** Different capital letters in the same coloumn within the same level of oxidised phenolic compounds used indicates the significant differences ($P < 0.05$).

3.5. Effect of oxidised phenolic compounds on protein patterns of surimi gels

No differences in MHC band intensity were noticeable when oxidised phenolic compounds at the optimum level were used, compared with that of the control (without oxidised phenolic addition) (data not shown). Generally, no changes in actin band intensity were observed. Decrease in MHC band intensity was found in surimi gel, regardless of oxidised phenolic addition, when compared with that observed in sol. This suggests the formation of cross-linking stabilised by non-disulphide covalent bond, especially during setting. MHC was most susceptible to cross-linking during setting (Benjakul & Visessanguan, 2003). Benjakul and Visessanguan (2003) reported the decrease in MHC of surimi gel from bigeye snapper, particularly when the setting was implemented. Since no differences in MHC band intensity among the control gel and those added with oxidised phenolic compounds, non-disulphide covalent bonds induced by oxidised phenolic compounds might be formed at low level. Weak bonds and disulphide bond most likely contributed to the gel strengthening caused by the addition of oxidised phenolic compounds. Nevertheless, covalent modification of proteins by phenolic oxidation products generated at alkaline pH has been reported extensively (Rawel et al., 2002). Thus, bonding involved in protein cross-linking on gel strengthening could be varied with types of phenolics and proteins, etc.

3.6. Effect of oxidised phenolic compounds on solubility of surimi gels

Solubility of surimi gels added with different oxidised phenolic compounds at the optimum level in different solubilising solutions is shown in Fig. 3. Solubility was found to be lower than 10% in all gels with and without oxidised phenolic compounds when solubilised with 0.6 M KCl (S_1) and 20 mM Tris–HCl (pH 8.0) (S_2). The decrease in solubility suggests the formation of protein aggregates during setting and heating. When the gels were solubilised in 20 mM Tris–HCl (pH 8.0) containing 1% SDS (S_3), solubility was increased up to 50% in the control, while gels added with OFA, OTA, OCT and OCF had the increases in solubility by 36%, 34%, 40% and 38%, respectively. SDS is capable of destroying hydrogen and some hydrophobic interactions (Hamada, 1992). Further increases in solubility were observed in S_4, containing urea and SDS, indicating the presence of hydrophobic and hydrogen bonds in surimi gels. Hydrogen bonds might involve in the interactions between hydroxyl groups of phenolic compounds and the nitrogen or oxygen of lysine, arginine, histidine, asparagine, glutamine, serine, threonine, aspartic acid, glutamic acid, tyrosine, cysteine and tryptophan as hydrogen acceptor (Prigent, 2005). Protonation of quinone during oxygenation could take place to some extent after neutralisation. As a consequence, hydroxyl groups could be regenerated partially. Hydrophobic interactions may occur between phenolic compounds and hydrophobic amino acids such as alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine and glycine residues (Prigent, 2005). Surimi gels added with oxidised phenolic compounds had the lower solubility than the control when S_4 was used. This suggests that covalent bonds were formed in gels added with phenolic compounds. When the samples were solubilised in S_5, containing urea, SDS and βME, it was noticeable that a higher increase in solubility was observed, indicating the presence of disulphide bonds in the gel. When quinones attached to protein molecules, the conformational changes of protein possibly occurred in the way that favoured the oxidation of sulphydryl groups, leading to the increases in disulphide bond forma-

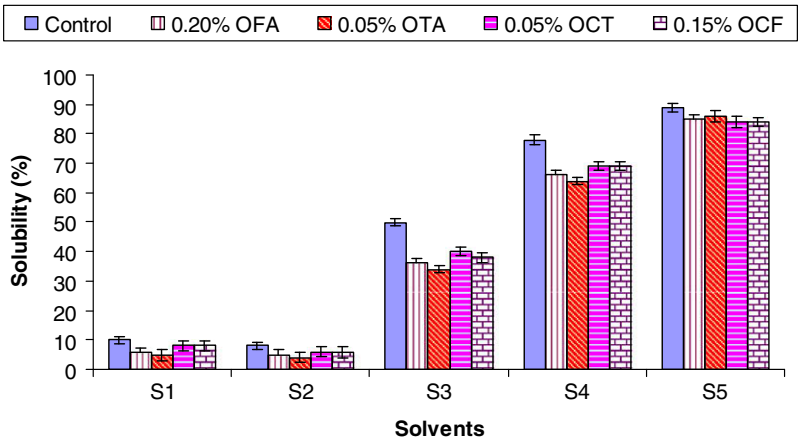

Fig. 3. Solubility of gels added with oxidised phenolic compounds at the selected levels. Samples were solubilised in different solvents and soluble protein was determined by the biuret assay. S1 (0.6 M KCL), S2 (20 mM Tris–HCl pH 8.0), S3 (20 mM Tris–HCl, pH 8.0, containing 1% SDS), S4 (20 mM Tris–HCl, pH 8.0, containing 1% SDS and 8 M urea) and S5 (20 mM Tris–HCl, pH 8.0, containing 1% SDS, 2% β-mercaptoethanol and 8 M urea). Bars represent the standard deviation from triplicate determinations.

tion. Gels added with oxidised phenolic compounds had a slightly lower solubility in S_4, compared with the control gel. Thus, some non-disulphide covalent bonds induced by quinone, oxidised phenolic, could play a partial role in gel strengthening. It was reported that quinone probably attached to amino groups of protein molecules in which the subsequent intermolecular cross-linking could be formed (Rawel et al., 2002; Rubino et al., 1996; Strauss & Gibson, 2004).

3.7. Effect of phenolic compounds on microstructure of surimi gels

Microstructures of gels from bigeye snapper surimi with 0.20% OFA (A), 0.05% OTA (B), 0.05% OCT (C), 0.15% OCF (D) and control (without oxidised phenolic compounds) (E) are shown in Fig. 4. In general, a network with fibrous structure was observed for bigeye snapper surimi gel. However, the surimi gel without oxidised phenolic compound had a larger void, compared with those containing different oxidised phenolic compounds. These observations suggest that the addition of oxidised phenolic compounds resulted in the formation of an ordered structure with finer strands. However, surimi gel added with 0.05% OTA exhibited more compact structure with larger bundles in the matrix as compared to others. For gel added with 0.05% OCT, bead type aggregates were found in the gel matrix. Both OTA and OCT might have a greater number of binding sites and in turn caused a higher aggregation. As a result, different structures of gels were obtained, compared with those added with OFA or OCF.

3.8. Effect of oxidised phenolic compounds on physicochemical properties of natural actomyosin

3.8.1. Surface hydrophobicity

Surface hydrophobicity of NAM extracted from surimi of bigeye snapper added with different oxidised phenolic compounds at the

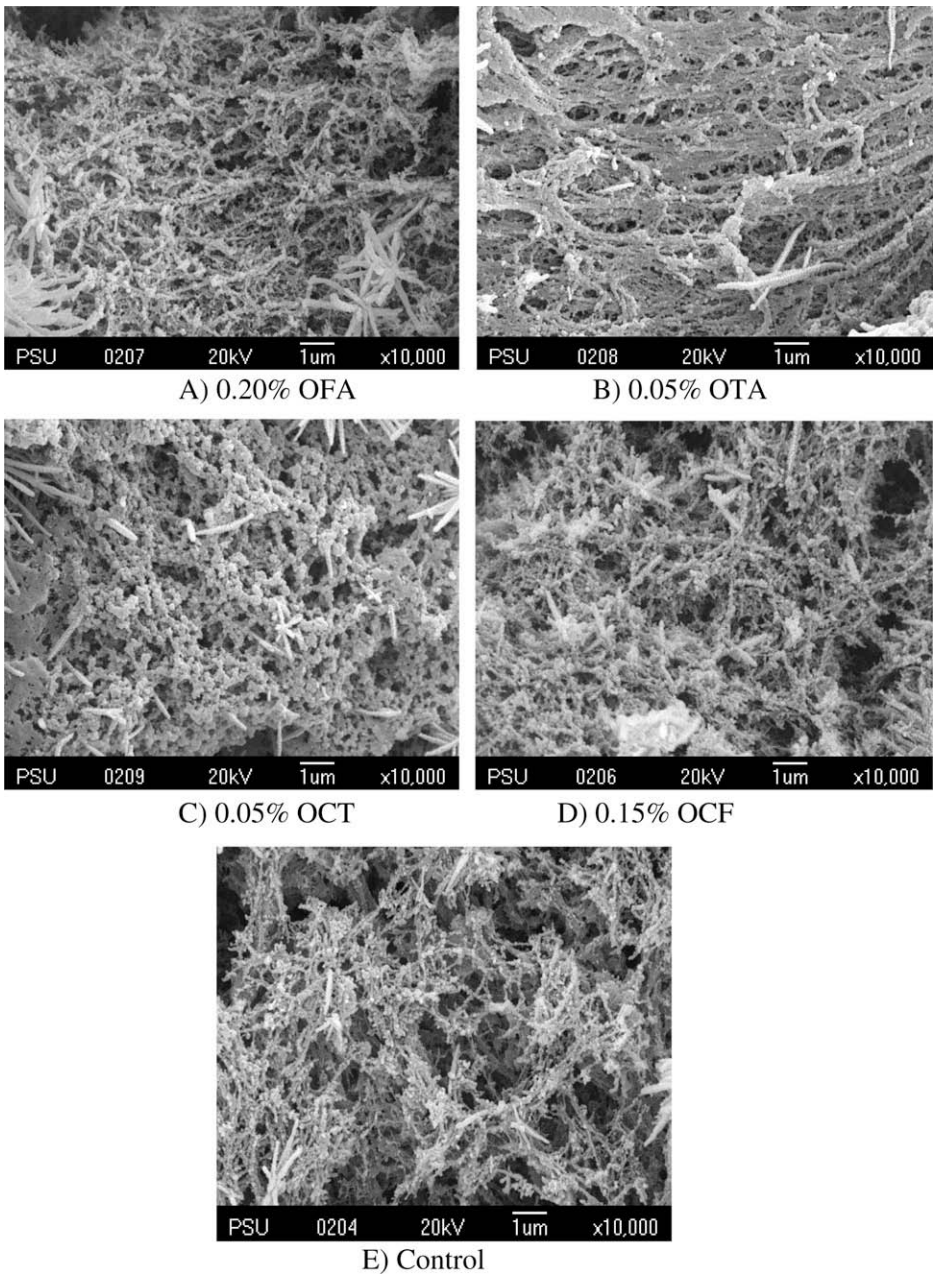

Fig. 4. Electron microscopic images of gels from bigeye snapper surimi added without and with different oxidised phenolic compounds at the selected levels. OFA, oxidised ferulic acid; OTA, oxidised tannic acid; OCT, oxidised catechin; OCF, oxidised caffeic acid. (Magnification: 10,000X).

optimum level and subjected to heating under different conditions: (i) 40 °C for 30 min and (ii) 40 °C for 30 min/90 °C for 20 min, is shown in Fig. 5A. Generally, NAM added with oxidised phenolic compounds had an increase in surface hydrophobicity, compared with the control NAM (without oxidised phenolic compounds) ($P < 0.05$). The results indicate that oxidised phenolic compounds likely induced the conformational changes of NAM to some extent, as evidenced by the increase in surface hydrophobicity. ANS, a fluorescence probe, has been found to bind to hydrophobic amino acids containing an aromatic ring, such as phenylalanine and tryptophan, and can be used to indicate the conformational changes occurring in the proteins (Benjakul et al. 1997). The increase in surface hydrophobicity was possibly caused by the exposure of hydrophobic groups of the protein molecules. Among all oxidised phenolic compounds, OTA even at a lower level caused the greatest increase in surface hydrophobicity. Multifunctional groups of this compound possessed a higher potential to bind or attach to protein molecules, in which the alteration of protein conformation could be more enhanced. Higher surface hydrophobicity of NAM without and with oxidised phenolic compounds was observed when heated at both 40 °C for 30 min and 90 °C for 20 min, compared with that found in NAM heated at 40 °C ($P < 0.05$). Unfolding of protein molecules was due to the instability of hydrogen bonds at higher temperature, exposing greater num-

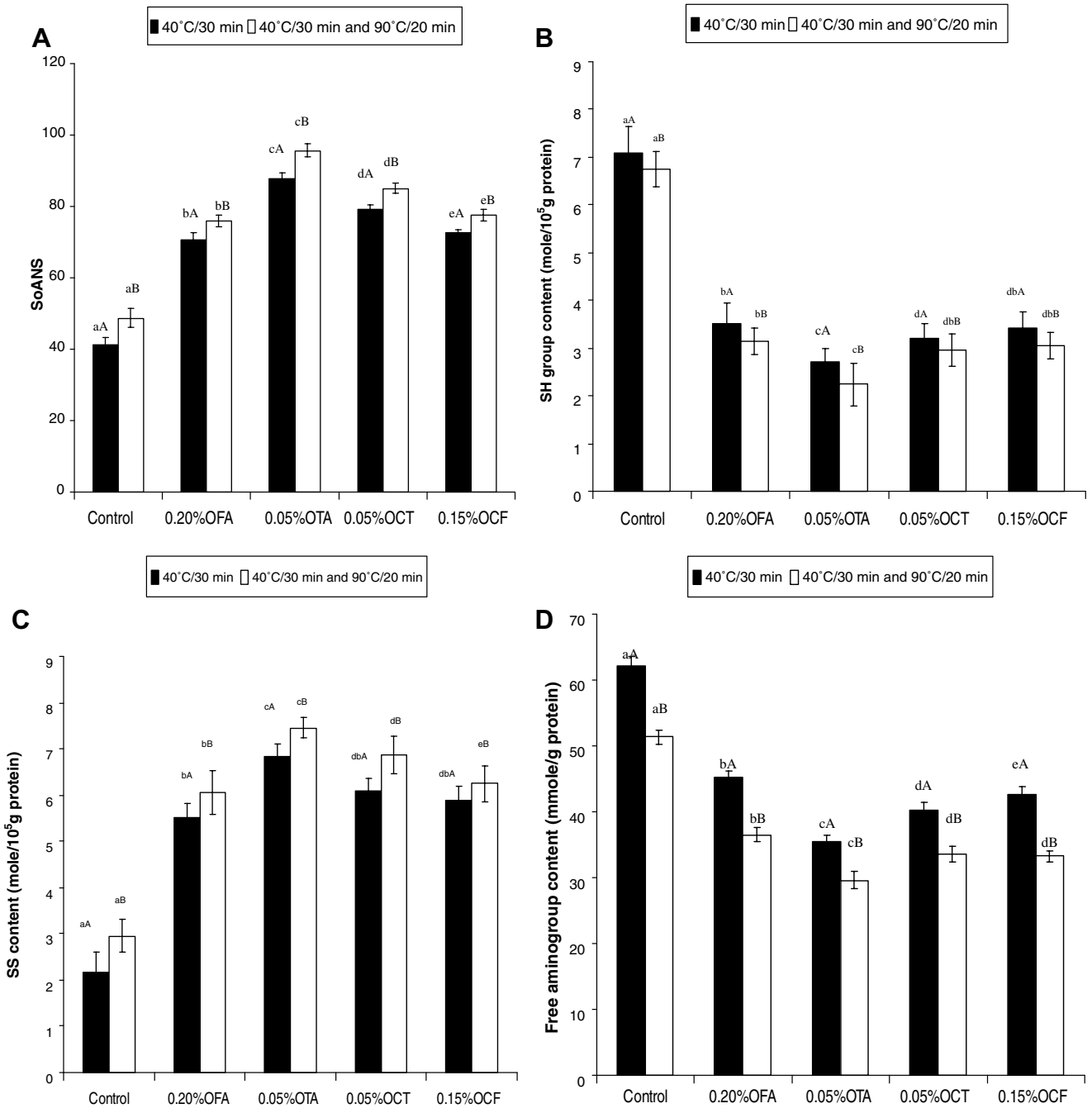

Fig. 5. Effect of oxidised phenolic compounds on surface hydrophobicity, (A); sulphydryl group content, (B); disulphide bond content (C) and free amino group content (D) of natural actomyosin from bigeye snapper surimi heated at two conditions: 1) 40 °C for 30 min and 2) 40 °C for 30 min and 90 °C for 20 min. OFA, oxidised ferulic acid; OTA, oxidised tannic acid; OCT, oxidised catechin; OCF, oxidised caffeic acid. Different letters within the same heating conditions indicates significant difference ($P < 0.05$). Different capital letters within the same treatment of oxidised phenolic compound indicates significant difference ($P < 0.05$).

bers of hydrophobic portions (Niwa, 1992). When NAM was heated at 90 °C, more reactive groups including amino groups became more available. As a consequence, quinone or phenolic could bind more effectively and might induce the conformational change to a greater extent, as indicated by the higher surface hydrophobicity ($P < 0.05$).

3.8.2. Total sulphydryl group and disulphide bond contents

Total sulphydryl group content of NAM extracted from bigeye snapper surimi and added with different oxidised phenolic compounds at the optimum level and subjected to heating under two different conditions is shown in Fig. 5B. NAM added with different oxidised phenolic compounds had lower sulphydryl group content than that of the control ($P < 0.05$). Phenolic compound might induce the conformation, in which oxidation of sulphydryl group could occur more easily as evidenced by the lowered sulphydryl group content. Additionally, quinone could interact directly to sulphydryl group (Strauss & Gibson, 2004). As a result, these sulphydryl groups could be masked by those quinones. A decrease in total sulphydryl group content was reported to be due to the formation of disulphide bonds through oxidation of sulphydryl groups or disulphide interchanges (Hayakawa & Nakai, 1985). Sulphydryl group content of NAM without and with phenolic compounds was lower ($P < 0.05$) when NAM mixtures were heated at 40 °C for 30 min and 90 °C for 20 min in comparison with those heated at 40 °C for 30 min ($P < 0.05$). Elevated temperature during heating most likely resulted in further oxidation of sulphydryl groups with the accompanied disulphide bond formation. The decrease in sulphydryl group content was in accordance with the increase in disulphide bond formation in all treatments and heating conditions used (Fig. 5C). NAM had lower disulphide bond content than those added with all oxidised phenolic compounds ($P < 0.05$) (Fig. 5C). Therefore, the addition of oxidised phenolic compounds was associated with the increased formation of disulphide bonds. An intermolecular disulphide bond is formed by the oxidation of two cysteine molecules on neighbouring protein chains (Lanier, 2000). The formation of disulphide bonds might contribute to the improved gel property of surimi added with the oxidised phenolic compounds at the optimum level (Fig. 1). Among all oxidised phenolic compounds used, OTA at a level of 0.05% caused the greater decrease in sulphydryl group content with the concomitant increase in disulphide bond. This was coincidental with the highest increase in both breaking force and deformation of gel added with 0.05% OTA (Fig. 1).

3.8.3. Free amino group content

Free amino group contents of NAM from surimi of bigeye snapper added without and with different oxidised phenolic compounds at optimum level and subjected to heating under different conditions are shown in Fig. 5D. NAM of surimi added with all oxidised phenolic compounds had lower free amino group content than that of control ($P < 0.05$). This indicates that the amino groups might undergo cross-linking via quinone. The rate of loss in free amino group was generally higher in NAM added with 0.05% OTA ($P < 0.05$). This was associated with the more number of hydroxyl groups present in the tannic acid compared with others. The hydroxyl groups were presumably converted to quinone, which functioned as cross-linkers. Additionally, remaining hydroxyl groups might interact with amino groups. The interactions between hydroxyl groups of phenolic compounds and the nitrogen or oxygen of some amino acids were reported by Prigent (2005). Hydrophobic interactions may occur between phenolic compounds and amino acids (Prigent, 2005). Free amino group content of NAM without and with oxidised phenolic compounds decreased to a higher degree when heated at 40 °C for 30 min and 90 °C for 20 min, compared with that of NAM heated at 40 °C for 30 min ($P < 0.05$). Unfolding and exposing of free amino groups at elevated temperature allowed the oxidised phenolic compounds to react with amino groups more effectively, thereby lowering free amino group contents. The lower free amino group content in NAM without oxidised phenolic compound indicates that cross-linking of protein via remaining TGase in NAM could occur, particularly during heating at 40 °C. TGase from bigeye snapper exhibited the optimal temperature at 40 °C (Benjakul & Visessanguan, 2003).

4. Conclusions

Type and concentration of oxidised phenolic compounds had varying influence on surimi gels. OTA exhibited superior gel strengthening effect to others. However, addition of OTA, OCT and OCF caused the decreased whiteness, especially with increasing amount. Addition of OFA had no adverse effect on whiteness of resulting gel. Therefore, the use of oxidised phenolic compounds can be a suitable option to improve the gel strength of surimi manufactured from dark flesh fishes which may not have a negative effect on the whiteness of resulting surimi gel.

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Gel Strengthening Effect of Wood Extract on Surimi Produced from Mackerel Stored in Ice

A.K. BALANGE, S. BENJAKUL, AND S. MAQSOOD

ABSTRACT: The effect of ethanolic kiam wood extract (EKWE) and commercial tannin (CT) on the gel properties of surimi produced from mackerel (*Rastrelliger kanagurta*) stored in ice for different times (0 to 12 d) was studied. During 12 d of iced storage, pH, total volatile base (TVB), trimethylamine (TMA), and trichloroacetic acid (TCA)-soluble peptide contents as well as thiobarbituric acid-reactive substances (TBARS) of mackerel mince increased while myosin heavy chain (MHC) band intensity decreased continuously ($P < 0.05$). The result suggested that deterioration, protein degradation, and lipid oxidation proceeded with increasing storage time. For corresponding surimi, TVB and TMA were almost removed and TBARS and TCA soluble peptide contents were decreased. Conversely, MHC became more concentrated. Decreases in gel-forming ability of surimi were observed when fish used as raw material were stored in ice for a longer time, regardless of EKWE or CT addition. Whiteness of surimi gel decreased and expressible moisture increased especially when the storage time increased. However, superior breaking force and deformation of surimi gel with 0.15% EKWE or 0.30% CT added, compared to those of the control gel were observed during the first 6 d of the storage. Thereafter, EKWE and CT had no gel enhancing effect on surimi. Therefore, freshness was a crucial factor determining gel enhancing ability of EKWE or CT toward mackerel surimi.

Keywords: degradation, denaturation, gelation, iced storage, kiam wood, mackerel, muscle, pH, surimi

Introduction

Freshness of fish is generally considered to be the most important factor determining the gel-forming ability of surimi. Time and temperature of postmortem storage of fish can affect the final surimi quality (Park and Morrissey 2000). The rate of deterioration is associated with many factors such as fish species, size, lipid content, state at the moment of capture, microbial load, and storage temperature. Fresh fish are extremely perishable and should be handled with a great care. Low-temperature storage, especially iced storage, is one of the primary methods to maintain fish freshness (Benjakul and others 2002). However, microbiological, chemical, and physical changes of fish muscle still occur during iced storage (Benjakul and others 2003; Riebroy and others 2007). Lower gel quality is generally associated with extended storage times in ice and the rate of loss of gel-forming ability appears to vary among different species (Benjakul and others 2002, 2003). The gel strength of kamaboko made from lizardfish kept in ice for 3 d was 50% of that made from fresh fish (Kurokawa 1979). Northern squawfish surimi could be made from fish stored for up to 9 d (Lin and Morrissey 1995). Generally, the prolonged holding times and elevated temperatures can cause severe proteolysis of myofibrillar proteins, which directly results in an inferior gel quality (Suzuki 1981). During handling, leakage of digestive enzymes into the muscle contributes to the subsequent hydrolysis of muscle proteins. Degradation of myosin heavy chain (MHC) also occurred in Pacific whiting muscle during iced storage (Benjakul and others 1997).

Mackerel (*Rastrelliger kanagurta*) is a pelagic fish species containing a high amount of dark muscle, fat, and myoglobin. This makes it less appealing for human acceptance and is considered to be an underutilized species (Chaijan and others 2004). When dark fleshed fish are used for surimi production, the dark color and low gel-forming ability of surimi still limit its manufacturing and marketing. To improve surimi gel properties, several approaches such as the addition of protein additives (Benjakul and others 2004a; Julavittayanukul and others 2006), an appropriate setting (Benjakul and others 2004b), and the use of microbial transglutaminase (Benjakul and others 2008) have been implemented. Also, the washing process was shown to enhance the gel strength of surimi (Park and Morrissey 2000).

Phenolic compounds are natural additives and can be derived from different parts of the plants (Shahidi and Naczk 2004). Recently, phenolic–protein interaction has been used extensively for improving food properties (Kroll and others 2003). Balange and Benjakul (2009a, 2009b, 2009c) used the phenolic compounds in oxidized form to improve the gel strength of mackerel surimi. Since the oxidized phenolic compounds can induce the cross-linking of proteins, they might be able to cross-link the degraded proteins found in surimi with poor quality. Therefore, the gel-forming ability might be recovered in surimi produced from low-quality fish associated with the degradation during extended iced storage. Recently, kiam wood extract containing tannin was reported to improve the gel strength of mackerel surimi (Balange and Benjakul 2009d). The objective of this study was to investigate the changes in chemical composition of mackerel during iced storage and to study the impact of oxidized kiam wood extract (EKWE) and oxidized tannic acid on gel properties of surimi prepared from mackerel kept in ice for different times.

Materials and Methods

Chemicals

Tannic acid (TA), sodium hydroxide (NaOH), hydrochloric acid, sodium carbonate, $CuSO_4 \cdot 5H_2O$, bovine serum albumin, Folin–Ciocalteu reagent, and β-mercaptoethanol (βME) were obtained from Sigma (St. Louis, Mo., U.S.A.). Sodium dedocyl sulfate

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(SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED), and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, Calif., U.S.A.). Sodium chloride and ethanol were obtained from Merck (Darmstadt, Germany).

Preparation of kiam wood extracts

Collection of kiam wood. Kiam wood was obtained from a forest of the Phattalung province in southern Thailand. The tree was about 15 to 20 y old and harvested in June 2008. The tree was cut by using a sawing machine; the leaves and branches were separated manually by cutting, and the trunk was sun-dried for 3 mo. The pieces of wood with an average thickness of 1.5 cm were dried in an oven at 70 °C for 8 h. Prepared wood was then subjected to a portable grinding machine (Spong-90, Leeds, U.K.) with a sieve size of 6 mm. This coarse form was further blended using a blender (Natl. Model MK-K77, Tokyo, Japan) and finally sieved using a stainless steel sieve of 80 mesh size. The kiam wood powder obtained was subjected to extraction.

Extraction of phenolic compounds from kiam wood using ethanol. Ethanolic extract from kiam wood powder was prepared according to the method of Santoso and others (2004) with slight modifications. Wood powder (10 g) was mixed with 150 mL of absolute ethanol. The mixture was homogenized for 2 min at 15000 rpm using a homogenizer (IKA Labortechnik, Selangor, Malaysia) and stirred at room temperature using a magnetic stirrer (IKA-Werke, Staufen, Germany) at room temperature (28 to 30 °C) for 3 h. The homogenate was then centrifuged at 5000 × *g* for 10 min at 25 °C using a Beckman centrifuge (Beckman Coulter Inc., Avanti –J-E Centrifuge, Fullerton, Calif., U.S.A.). The supernatant was filtered using Whatman filter paper nr. 1 (Whatman Schleicher & Schuell, Maidstone, U.K.). The filtrate was then evaporated at 40 °C using a rotary evaporator model N-1000 (Eyela, Tokyo Rikakikai, Co. Ltd, Tokyo, Japan) until ethanol was almost removed. The extract was then dried in a forced-air oven at 70 °C for 4 h. Dried extract was powdered using a mortar and pestle. Extract powder referred to as "ethanolic kiam wood extract, EKWE" was kept in a desiccator at 28 to 30 °C until used.

Preparation and iced storage of fish samples. Mackerel (*Rastrelliger kanagurta*) with an average weight of 85 to 90 g were caught from Songkhla coast along the Gulf of Thailand during February–March 2009. Fish were stored in ice and off-loaded approximately 36 h after capture. Upon the arrival to the dock in Songkhla, fish were iced with a fish/ice ratio of 1 : 2 (w/w) and transported to the Dept. of Food Technology, Prince of Songkla Univ., Hat Yai, within 1 h. The fish were immediately washed and kept in a styrene foam box containing crushed ice. The fish were placed and distributed uniformly between the layers of ice using a fish/ice ratio of 1 : 2 (w/w). The box was kept at room temperature (27 to 30 °C) for up to 12 d. To maintain the ice content, the molten ice was removed and replaced with an equal amount of ice every 2 d. At the time designated (0, 3, 6, 9, and 12 d), fish were washed and filleted manually. The flesh was minced to uniformity using a mincer with a hole diameter of 5 mm. The mince was subjected to chemical analyses and surimi production.

Preparation of surimi. Surimi was prepared according to the method of Shimizu (1965) with a slight modification. Mince was suspended in 4 volumes of cold (5 °C) diluted alkaline salt solution (0.15% NaCl in 0.2% $NaHCO_3$ of which the ionic strength was 0.05 and the final pH was 7 to 7.1). The mixture was stirred gently for 20 min and the washed mince was filtered with a layer of nylon screen (Butterfly brand, Lao Hah Seng Lee Co., Ltd., Bangkok, Thailand). The washing process was repeated twice. Finally, the washed mince was subjected to centrifugation at a speed of 700 × *g* at 4 °C for 10 min using a Model CE 21K basket centrifuge (Grandiumpiant, Belluno, Italy). Washed mince or raw surimi (without 4% sucrose and 4% sorbitol) was subjected for chemical analyses.

To the washed mince, 4% sucrose and 4% sorbitol were added and mixed well. A portion of mixture (500 g) was packaged in a polyethylene bag and frozen using an air-blast freezer at −20 °C (Patkol, Patanakolkarn Co. Ltd., Bangkok, Thailand). Frozen surimi was stored at −20 °C for no longer than 2 wk and used for gel preparation.

Chemical analyses

Determination of pH. The pH was determined according to the method of Benjakul and others (1997). Sample was mixed with 10 volumes of deionized water (w/v) and homogenized at 11000 rpm for 60 s using an IKA homogenizer (Model T25, Selangor, Malaysia). The pH of homogenate was measured using a pH meter (Sartorious, PB 10, Goettingen, Germany).

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 (1987). A sample (2 g) was added to 8 mL of 4% trichloroacetic acid (TCA) (w/v) and homogenized with an IKA homogenizer at a speed of 11000 rpm for 2 min. The homogenate was centrifuged at 3000 × *g* for 15 min using a Beckman centrifuge at room temperature. The supernatant referred to as "sample extract" (1 mL) was placed in the outer ring of the Conway apparatus. The inner ring solution (1% boric acid containing the Conway indicator) was then pipetted into the inner ring. To initiate the reaction, K_2CO_3 (1 mL) was mixed with the sample extract. The Conway unit was closed and incubated at 37 °C for 60 min. The inner ring solution was then titrated with 0.02 N HCl until the green color turned to pink. Determination of TMA content was done in the same manner except that 1 mL of 10% formaldehyde was added to the sample extract to fix ammonia present in the sample prior to the assay.

Determination of thiobarbituric acid-reactive substances (TBARS). TBARS value was determined according to the method of Buege and Aust (1978). A sample (5 g) was homogenized with 25 mL of TBARS solution (0.375% TBA, 15% TCA, and 0.25 N HCl) at a speed of 11000 rpm for 1 min using an IKA homogenizer. The homogenate was heated for 10 min in boiling water (95 to 100 °C) to develop a pink color. Then the mixture was cooled with running water and centrifuged at 5500 × *g* for 25 min at 25 °C using a Beckman centrifuge. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). The TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg malonaldehyde/kg sample.

Determination of TCA-soluble peptides. TCA-soluble peptide content was determined according to the method of Green and Babbitt (1990). A sample (3 g) was homogenized with 27 mL of 15% TCA at a speed of 11000 rpm for 1 min using an IKA homogenizer. The homogenate was kept in ice for 1 h and centrifuged at 12000 × *g* for 5 min at 4 °C using a Beckman centrifuge. The soluble peptides in the supernatant were measured by the method of Lowry and others (1951) and expressed as μmol tyrosine/g sample.

Effect of oxidized EKWE and oxidized commercial tannin (CT) on gel properties of surimi prepared from ice-stored mackerel

Preparation of oxidized EKWE and oxidized commercial tannin. EKWE and CT were oxidized according to the method of Strauss and Gibson (2004) with slight modifications. The extract solutions (100 mL; 1% [w/v]) were adjusted to pH 8 using 6M NaOH

or 6M HCl. The prepared solutions were placed in a temperature-controlled water bath (Memmert, Schwabach, Germany) at 40 °C and subjected to oxygenation for 1 h by bubbling the solution with oxygen with a purity of 99.5% to 100% (TTS Gas Agency, Hat Yai, Songkhla, Thailand) to convert phenolic compound to quinone. After being oxygenated for 1 h, the solutions were then adjusted to pH 7 by using 6M HCl. Both oxidized EKWE and CT were used as additives in the surimi gels.

Surimi gel preparation. Frozen surimi was tempered for 30 min in running water (26 to 28 °C) until the core temperature reached 0 to 2 °C. The surimi was then cut into small pieces with an approximate thickness of 1 cm and placed in a mixer (Natl. Model MK-K77, Tokyo, Japan). The moisture was adjusted to 80% and 2.5% salt was added. Oxidized EKWE and CT solutions were added into the sols at levels of 0.30% and 0.15% (based on protein content), respectively (Balange and Benjakul 2009d). The mixtures were chopped for 4 min at 4 °C to obtain the homogeneous sols. The sols were then stuffed into polyvinylidine casings with a diameter of 2.5 cm and both ends of the casings were sealed tightly. Sols were incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min (Benjakul and Visessanguan 2003). All gels were cooled in iced water and stored overnight at 4 °C prior to analyses.

Measurement of surimi gel properties

Texture analysis. Texture analysis of gels was performed using a texture analyzer Model TA-XT2 (Stable Micro Systems, Surrey, U.K.). Gels were equilibrated and tested at room temperature. Five cylinder-shaped samples of 2.5 cm in length were prepared. The breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyzer equipped with a spherical plunger (5 mm dia). The probe was pressed into the cut surface of a gel specimen perpendicularly at a constant plunger speed (60 mm/min) until the puncture occurred. The force in gram (g) required to puncture into the gel (breaking force) and the distance (mm) at which the spherical probe punctured into the gel (deformation) were recorded.

Determination of expressible moisture content. Expressible moisture content was measured according to the method of Benjakul and others (2001) with a slight modification. Gel samples were cut into a thickness of 5 mm, weighed (X), and placed between 3 pieces of Whatman paper nr 4 at the bottom and 2 pieces on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The samples were then removed from the papers and weighed again (Y). Expressible moisture content was calculated using the following equation:

$$\text{Expressible moisture content (\%)} = 100[(X - Y)/X]$$

Determination of whiteness. Color of gels was determined using a JP7100F colorimeter (Juki Corp., Tokyo, Japan). The colorimeter was standardized for whiteness by using black plate, followed by white plate. L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Lanier and others (1991) as follows:

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

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Protein patterns of samples were analyzed under a reducing condition by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 mL of 5% (w/v) SDS solution heated to 85 °C were added to the sample (3 g). The mixture was then homogenized at a speed of 11000 rpm for 2 min using an IKA homogenizer. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500 × g for 20 min to remove undissolved debris. Protein concentration was determined by the Biuret method (Robinson and Hodgen 1940) using bovine serum albumin as a standard. The sample was then mixed with sample buffer (4 mL of 10% SDS, 2 mL of glycerol, 1 mL of β-mercaptoethanol, 2.5 mL of 0.5 M Tris-HCl (pH 6.8), and 0.03 g Bromophenol blue) at a 1 : 1 ratio (v/v). The samples (20 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, Calif., U.S.A.). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

Statistical analysis

All experiments were run in triplicate. For each run, chemical analyses were performed in triplicate. For physical analyses, for example, expressible moisture, whiteness, and textural properties, 5 determinations were conducted. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range tests (Steel and Torrie 1980). t-test was used for pair comparison. Analysis was performed using a SPSS package (SPSS 10.0 for Windows, SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Changes in pH

Changes in pH of mackerel mince and the resulting surimi during iced storage of 12 d are shown in Figure 1. Mackerel mince had a pH of 6.2 at day 0 of storage. Generally, dark muscle fish has low pH values due to the postmortem glycolytic reaction, in which lactic acid is accumulated (Hultin and Kelleher 2000). For corresponding surimi, a higher pH (6.9) was obtained. During washing, the acidic compounds, particularly lactic acid might be leached out, which may have led to the increased pH in surimi. Additionally, the neutralization of acids by sodium bicarbonate in the washing medium might contribute to the raised pH. During iced storage, the continuous increase in the pH of fish mince was observed up to 12 d ($P < 0.05$). Nevertheless, no differences in pH of surimi were found within the first 6 d of the storage ($P > 0.05$). Thereafter a slight increase in pH was found ($P < 0.05$). The increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes (Benjakul and others 2002, 2003; Riebroy and others 2007). Decomposition of nitrogenous compounds causes an increase in pH in fish flesh (Sikorski and others 1990). The changes in pH also depend on the liberation of inorganic phosphate and ammonia due to the enzymatic degradation of ATP (Sikorski and others 1990).

Changes in TVB and TMA contents

TVB and TMA contents of mackerel mince and the corresponding surimi were monitored during iced storage of 12 d as depicted in Figure 2A and 2B, respectively. At day 0 of storage, the initial TVB content of mince was 4.17 mg N/100 g (Figure 2A), whereas a negligible content of TMA was obtained. Surimi produced from fresh mackerel mince had TVB and TMA of 0.883 and 0.431 mg N/100 g sample, respectively. When the storage time increased, both TVB and TMA contents in mince increased ($P < 0.05$). For surimi, both TVB and TMA contents remained constant throughout 12 d

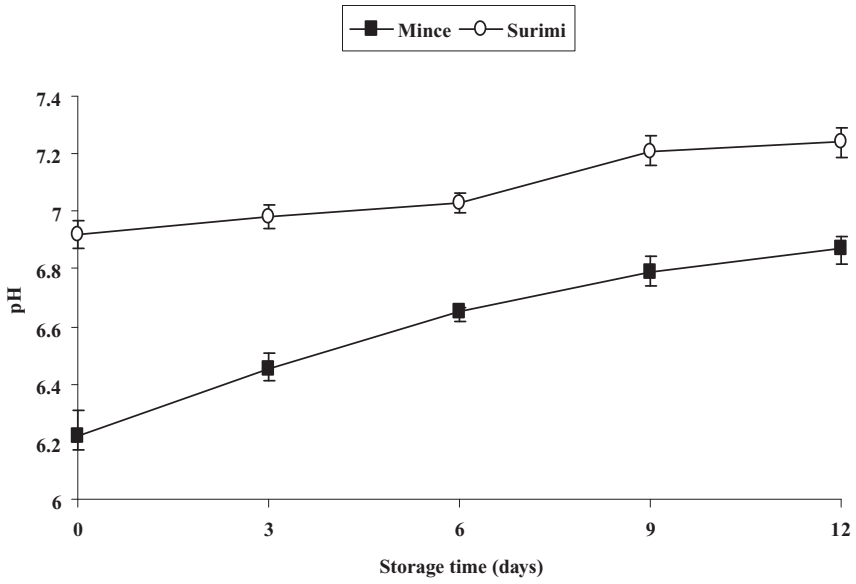


Figure 1—Changes in pH of mince and corresponding surimi from mackerel stored in ice for different times. Bars represent standard deviation from 3 determinations (n = 3).

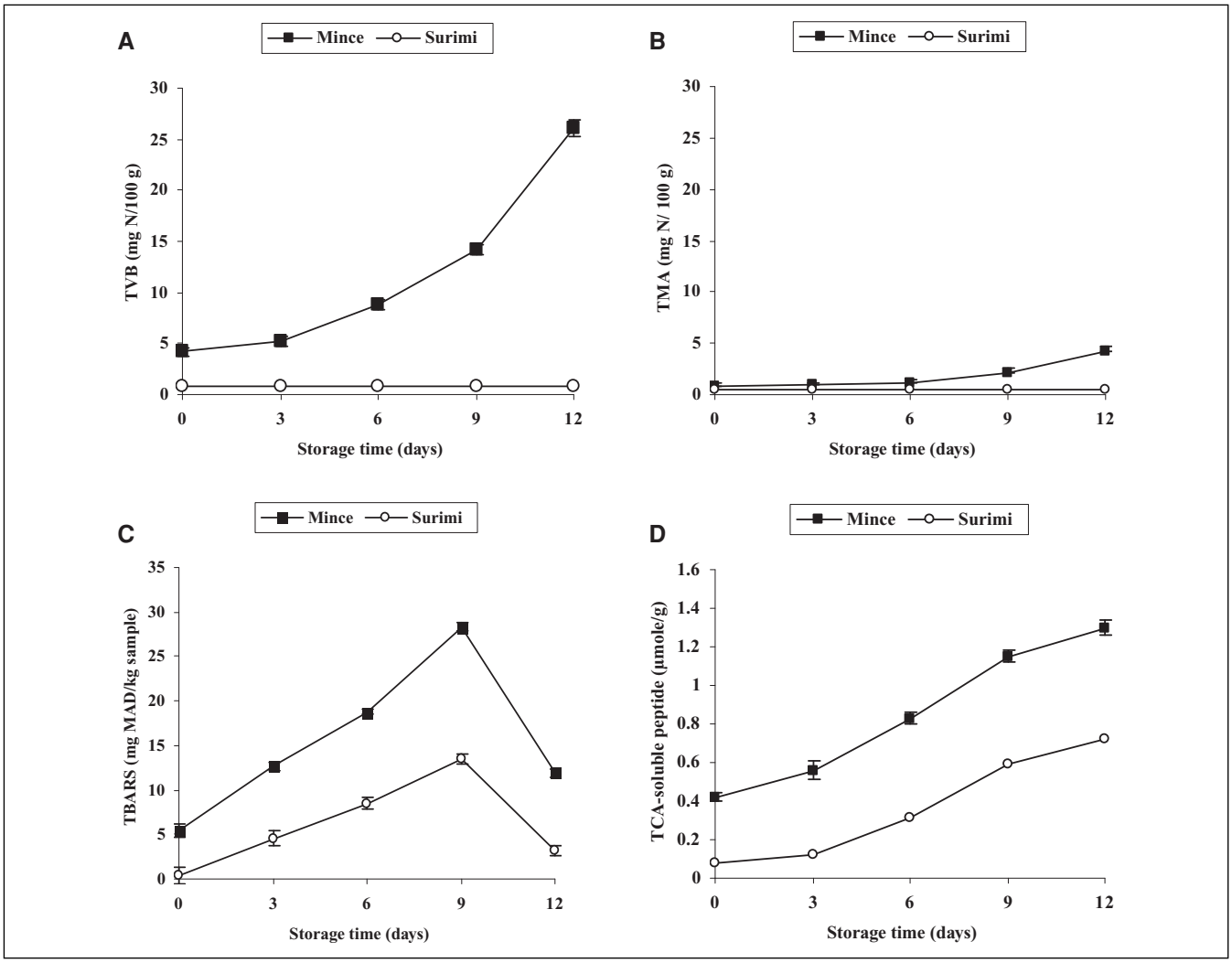


Figure 2—Changes in TVB (A), TMA (B), TBARS (C), and TCA-soluble peptide contents (D) in mince and corresponding surimi from mackerel stored in ice for different times. Bars represent standard deviation (n = 3).

of storage ($P > 0.05$) and the contents were much lower than those found in mince ($P < 0.05$). These results indicated that TVB and TMA contents could be reduced markedly by the alkaline saline washing process. The increases in TVB and TMA contents in mince indicated that the decomposition of nitrogenous compounds became more pronounced with increasing storage time. The increases in both TVB and TMA contents in mince were in accordance with the increase in pH, especially when the storage time increased (Figure 1). The formation of TVB is generally associated with the growth of microorganisms and can be used as an indicator of spoilage (Benjakul and others 2002). Generally, TVB consists mainly of TMA and ammonia, which are produced by both microbial and endogenous enzymes. A number of specific spoilage bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Vibrionaceae*, and so on typically use TMAO as an electron acceptor in anaerobic respiration, resulting in off-odor and off-flavor due to the formation of TMA (Gram and Huss 1996). Since fish samples were kept in ice, the formation of TVB and TMA was probably mediated by psychrotropic bacteria (Sasajima 1974). TVB content of 30 mg N/100 g is generally regarded as the fish acceptability limit (Sikorski and others 1990). For up to 12 d of iced storage, TVB content of mackerel mince was lower than the limiting level (26.12 mg N/100 g). The washing process used for surimi production could remove volatile base compounds including TMA effectively. This could reduce offensive odor, particularly fishy odor, in resulting surimi.

Changes in TBARS

Changes in TBARS value of mackerel mince and the corresponding surimi during iced storage are shown in Figure 2C. Mince contained TBARS of 5.51 mg MDA/kg sample at day 0, indicating that lipid oxidation took place during handling after capture. However, corresponding surimi had the negligible TBARS. TBARS of mince and surimi increased continuously during storage up to 9 d ($P < 0.05$), indicating that lipid oxidation occurred intensively during the extended iced storage. Fish muscle typically has a high content of polyunsaturated fatty acids and is consequently prone to oxidative reaction (Stamman and others 1990). TBARS has been widely used to indicate lipid oxidation in meat and meat products (Jo and Anh 2000). The lipid oxidation can be initiated and accelerated by various mechanisms including the production of singlet oxygen, enzymatic and nonenzymatic generation of free radicals and active oxygen (Kubow 1992). In general, surimi possessed a lower TBARS than did mince at all storage times used ($P < 0.05$). This was probably due to the removal of some lipids, prooxidants, particularly heme protein, as well as oxidation products from mince during washing. At day 12 of storage, a sharp decrease in TBARS was noticeable ($P < 0.05$). This might be caused by the loss of volatile lipid oxidation products at the extended time of storage. Eymard and others (2009) reported that 2,4-heptadienal in horse mackerel mince increased rapidly within the first 24 h of storage at 5 °C, followed by the slight decrease with the extended storage time up to 96 h.

Changes in TCA-soluble peptide content

TCA-soluble peptide content in mackerel mince and the resulting surimi during iced storage is illustrated in Figure 2D. At day 0, TCA-soluble peptide content in mince and surimi were 0.42 and 0.08 μmol tyrosine/g sample, respectively. TCA-soluble peptides detected in mince at day 0 indicated the indigenous oligopeptides and free amino acids as well as degradation products accumulated during postharvest handling (Benjakul and others 1997). During the extended storage, TCA-soluble peptide content in both mince and surimi increased continuously up to 12 d of storage ($P < 0.05$). This suggested that proteolysis caused by either indigenous or microbial proteases, took place during the storage of mackerel. Cathepsins play a role in autolysis of fish myofibrillar protein during the postmortem storage (An and others 1994; Riebroy and others 2007). It was noted that TCA-soluble peptide content in surimi produced from ice-stored fish was lower than that of mince at the same storage time. Washing might remove the small peptides, resulting in the lowered TCA-soluble peptides in surimi. Protease from *Pseudomonas marinoglutinosa* was reported to hydrolyze actomyosin at 0 to 2 °C and the optimal pH was above 7 (Venugopal and others 1983). The result suggested that the washing process could remove peptides with low molecular weight, leading to the concentration of the larger MW proteins or peptides, which are generally involved in gelation.

Changes in protein patterns

Protein patterns of mince and surimi prepared from mackerel stored in ice for different times are shown in Figure 3. The lower band intensity of myosin heavy chain (MHC) and actin was found in mince, in comparison with corresponding surimi. This might be attributed to the effective removal of low molecular weight components, especially sarcoplasmic proteins, which can exhibit the interfering effect on gel-forming ability by disrupting the aggregation of the myofibrillar proteins, the major contributors for gelation. Sarcoplasmic proteins are soluble in water and salt solutions of low ionic strength of 0.05 (Govindan 1985). Solubility of sarcoplasmic proteins of dark fleshed species is increased in "alkaline saline leaching" solution (Shimizu 1965). The greater removal of sarcoplasmic proteins from the mince by alkaline-saline washing resulted in the higher concentration of myofibrillar proteins including MHC and actin in the surimi.

MHC band intensity of both mince and surimi prepared from mackerel stored in ice decreased continuously with the increasing storage period up to 12 d. Nevertheless, no marked changes were noticeable in the actin band intensity of both mince and surimi prepared from ice-stored mackerel, irrespective of the storage period. SDS–PAGE patterns revealed that MHC was much more susceptible to hydrolysis than was actin. This result was in agreement with Benjakul and others (1997) who reported that MHC was more prone to proteolytic degradation than other muscle proteins, for example, actin, troponin, and tropomyosin.

Breaking force and deformation of mackerel surimi gel

Breaking force and deformation of surimi gels prepared from mackerel stored in ice for different times without and with 0.15% EKWE or 0.30% CT are shown in Figure 4. At day 0 of storage, surimi gels with EKWE or CT added had increased breaking force by 112% or 130% and deformation by 34% or 50%, respectively, compared with those of the control ($P < 0.05$). This might be attributed to the cross-linking activity of oxidized tannin, which could induce the formation of both covalent and noncovalent bonds of gel matrix (Prigent and others 2003). EKWE has been reported to contain a high amount of tannin (Balange and Benjakul 2009d). The result was in accordance with that of Balange and Benjakul (2009a) who reported the increases in breaking force and deformation of bigeye snapper surimi with the addition of oxidized tannin. The addition of EKWE and CT increased the breaking force to a higher degree, compared with the deformation. Quinone in oxidized EKWE and CT acted as the protein cross-linker via the strong covalent bonds. Moreover, the oxidation of phenolic compounds resulted in the lowering OH– groups, which were mainly responsible

for protein cross-linking by noncovalent bonds, mainly hydrogen bonds, in the gel matrix. As a consequence, the gels were more rigid but less elastic.

Decreased breaking force and deformation of surimi were observed with increasing storage time of mackerel ($P < 0.05$). Decreases were concomitant with the increase in TCA-soluble peptide content (Figure 2D) and the decrease in MHC band intensity of surimi (Figure 3). Myosin integrity is of paramount importance for gelation (An and others 1996). The degradation of MHC resulted in an inferior gel network formation, causing a lower elasticity with poor water holding capacity in the gel matrix. Yean (1993) also found a decrease in gel strength of surimi produced from threadfin

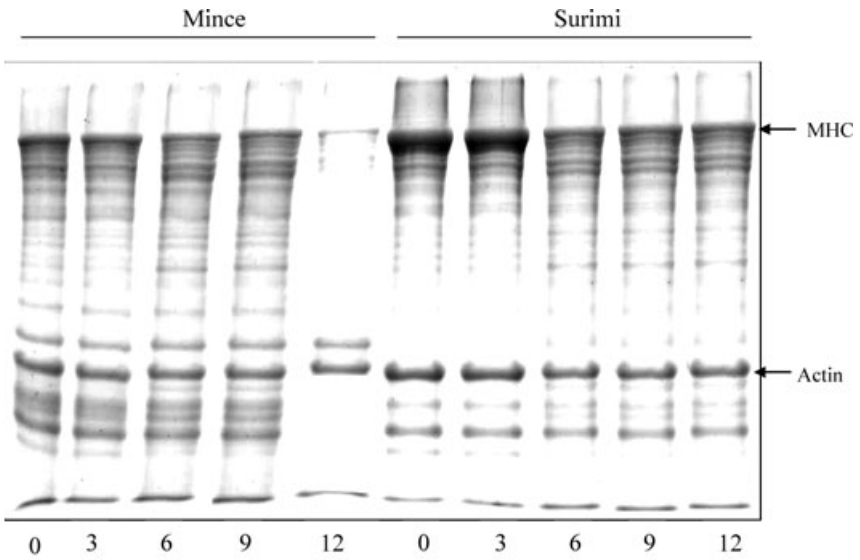
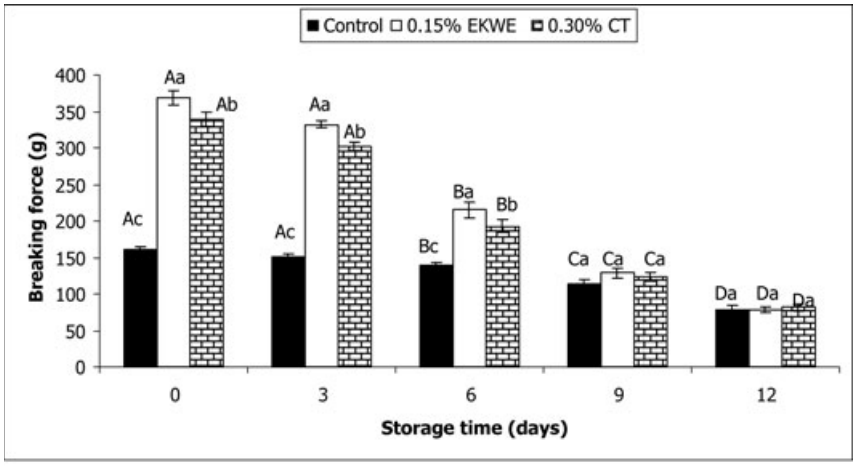


Figure 3—SDS–PAGE patterns of mince and corresponding surimi from mackerel stored in ice for different times. Numbers designate storage time (days). MHC = myosin heavy chain.



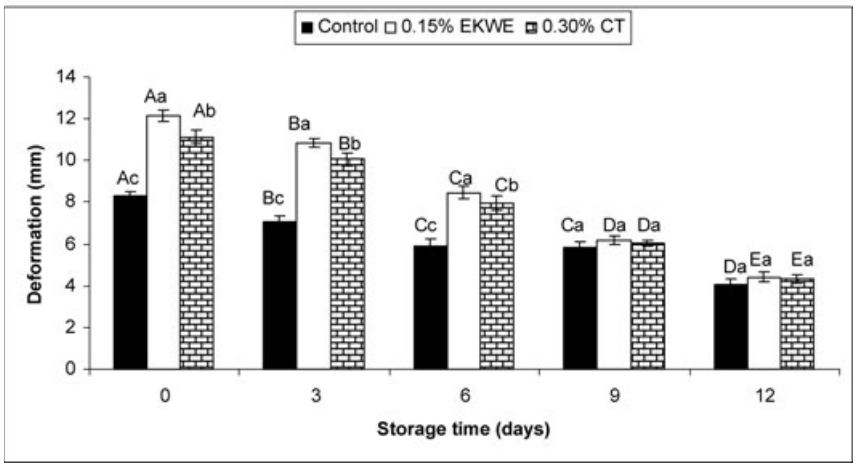


Figure 4—Breaking force and deformation of gels of surimi from mackerel stored in ice for different times without and with 0.15% ethanolic kiam wood extract (EKWE) or 0.30% commercial tannin (CT). Bars represent the standard deviation ($n = 3$). The different small letters on the bars within the same storage time indicate significant differences between treatments ($P < 0.05$). Different capital letters on the bars within the same treatment indicate significant differences over time ($P < 0.05$).

bream stored in ice for more than 2 d. Benjakul and others (2003) reported a decrease in gel strength of surimi from lizardfish and bigeye snapper stored in ice with the extended time.

During 0 to 6 d of storage, gel with EKWE added showed a higher breaking force and deformation, compared with gel with 0.30% CT added ($P < 0.05$). This might be attributed to the presence of lignin along with tannin in the extracts. Lignin, also being a polyphenolic compound with 2 hydroxyl groups and 2 benzene rings, might function as a synergist to tannin in improving the gel strength of mackerel surimi. The major chemical constituents in the bark and wood from different trees were reported to be tannin with small amounts of lignin (Yazaki and Collins 1994; Fradinho and others 2002). However, no differences in breaking force and deformation of surimi gels without and with EKWE or CT were noticeable when the storage time was greater than 6 d ($P > 0.05$). With increasing storage time, the degradation was more pronounced. Partially hydrolyzed proteins with lower molecular weight were retained after washing, while very low molecular weight peptides were leached out. Those smaller peptides might be preferably cross-linked with the multiple binding sites of quinones in oxidized EKWE or CT. As a consequence, quinones became less available for cross-linking of high molecular weight proteins or peptides. Additionally, the short chain peptides could not form the junction zones, in which the interconnection could be formed, even in the presence of oxidized EKWE or CT.

Expressible moisture content of mackerel surimi gel

When 015% EKWE or 0.30% CT was added into the gel of surimi prepared from fresh mackerel mince (day 0), the expressible moisture content was decreased, compared with that of the control gel ($P < 0.05$; Table 1). The decreases in expressible moisture contents were in accordance with the increased breaking force and deformation of resulting surimi gels (Figure 4). During setting at 40 °C, proteins underwent some denaturation and aligned themselves gradually to form the network, which could imbibe water (Benjakul and Visessanguan 2003). In the presence of oxidized EKWE or CT, quinones of tannin and other components could interact with unfolded muscle proteins, in which the network capable of holding water could be formed. The continuous increases in the expressible moisture content of surimi gels without and with 0.15% EKWE or 0.30% CT were noticeable throughout the storage of 12 d ($P < 0.05$). During the first 6 d of storage, the expressible moisture content of surimi gels with 0.15% EKWE was lowest, followed by gel with 0.30% CT added and the control gel, respectively. The lowered expressible moisture content was in a good agreement with the increased breaking force and deformation during the first 6 d of storage (Figure 4). This might be due to the formation of an ordered network stabilized by strong bondings, which could possibly imbibe the water effectively than the control gel. Nevertheless, no differences in the expressible moisture content were observed in all gels when surimi was prepared from mackrel stored in ice for 9 to 12 d ($P > 0.05$). This reconfirmed the role of protein integrity in gel formation, regardless of cross-linkers addition. Surimi with the pronounced degradation or denaturation of myofibrillar proteins could not yield a good gel, even when oxidized EKWE or TA was incorporated.

Whiteness of mackerel surimi gel

Whiteness of surimi gels prepared from ice-stored mackerel without or with addition of EKWE or CT decreased continuously as the storage time increased up to day 12 ($P < 0.05$) (Table 1). At day 0, the lower whiteness was observed in surimi gels with the addition of 0.30% CT, compared with the control gel and gel with 0.15% EKWE added ($P < 0.05$). This might be associated with the higher level used (0.30%) of CT, compared with EKWE. These results are in agreement with O'Connell and Fox (2001) who reported that phenolic compounds were responsible for discoloration in the cheese products. During iced storage, the oxidation of pigments in fish muscle, particularly myoglobin and hemoglobin, occurred. These oxidized products possibly bound tightly with muscle proteins and could not be removed by washing. As a consequence, surimi gel produced from fish kept for a longer time had a lower whiteness. During extended storage, blood and liquid from internal organs in whole samples could penetrate through the muscle, especially when autolysis proceeded and caused a looser muscle structure. Therefore, storage time directly affected the whiteness of surimi gels from mackerel and EKWE could be used as gel enhancer without the adverse effect on whiteness of resulting gel.

Protein patterns of mackerel surimi gels

Protein patterns of gels of surimi prepared from mackerel stored in ice for 0, 6, and 12 d without and with the addition of 0.15% EKWE

Table 1 — Expressible moisture content and whiteness of gels of surimi from mackerel stored in ice for different times without and with 0.15% ethanolic kiam wood extract (EKWE) or 0.30% commercial tannin (CT).

Sources of phenolic compounds	Amount added (%)	Storage period (d)	Expressible moisture content (%)	Whiteness
Control	0	0	8.18 ± 0.49aA	67.46 ± 0.51aA
		3	12.32 ± 0.61bA	65.35 ± 0.81bA
		6	17.41 ± 0.52cA	63.71 ± 0.66cA
		9	21.23 ± 0.41dA	61.83 ± 0.32dA
		12	28.14 ± 1.08eA	59.81 ± 0.89eA
EKWE	0.15	0	3.50 ± 0.53aB	66.92 ± 0.57aA
		3	7.12 ± 0.62bB	65.81 ± 0.42bA
		6	11.89 ± 0.71cB	63.20 ± 0.81cA
		9	19.92 ± 0.81dA	61.21 ± 0.91dA
		12	27.89 ± 0.95eA	59.12 ± 0.39eA
CT	0.30	0	4.85 ± 0.39aC	65.34 ± 0.33aB
		3	7.22 ± 0.47bC	63.48 ± 0.45bB
		6	12.35 ± 0.62cC	61.81 ± 0.35cB
		9	20.45 ± 0.71dA	59.63 ± 0.81dB
		12	27.97 ± 0.92edA	57.85 ± 0.52eB

Different small letters in the same column within the same source of phenolic compounds indicate significant differences over time ($P < 0.05$).
Different capital letters in the same column within the same storage period indicate significant differences between treatments ($P < 0.05$).
Values are mean ± standard deviation ($n = 3$).
CT = commercial tannin; EKWE = ethanolic kiam wood extract.

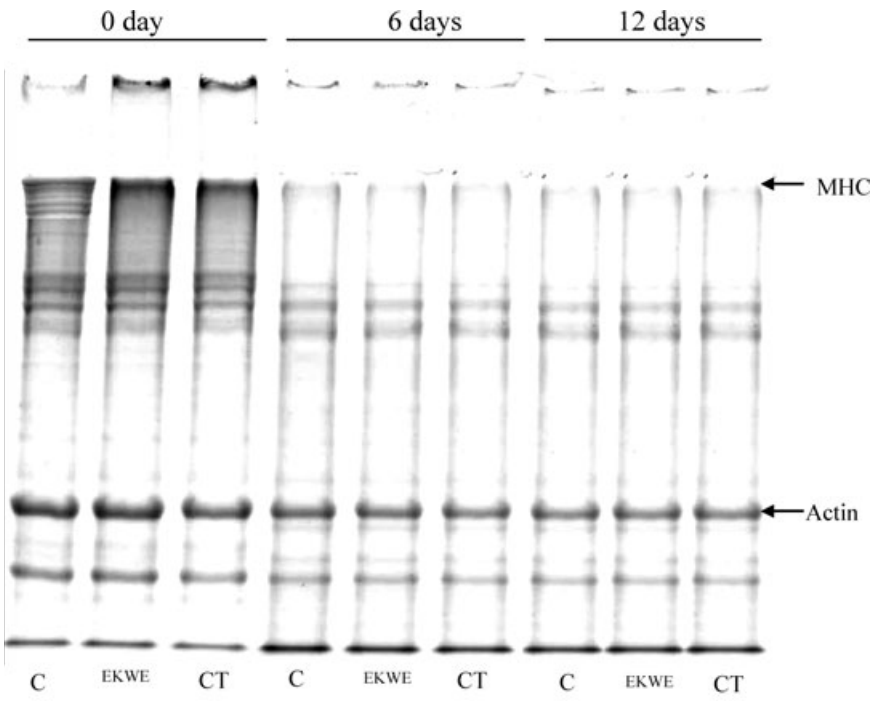


Figure 5—SDS–PAGE patterns of protein in gels from surimi from mackerel stored in ice for 0, 6, and 12 d without and with 0.15% ethanolic kiam wood extract (EKWE) or 0.30% commercial tannin (CT). MHC = myosin heavy chain; C = the control gel.

or 0.30% CT are illustrated in Figure 5. Decrease in MHC band intensity was found in the control gel (without addition of EKWE or CT), compared with that observed in surimi sol at day 0 (Figure 3). The result suggested that the formation of cross-linking stabilized by nondisulfide covalent bonds mediated by indigenous transglutaminase took place, especially during setting. MHC was most susceptible to cross-linking during setting (Benjakul and Visessanguan 2003). Benjakul and Visessanguan (2003) reported the decrease in MHC of surimi gel from bigeye snapper, particularly when the setting was implemented. For gels of surimi prepared from mackerel stored in ice at day 0 and with 0.15% EKWE or 0.30% CT added, MHC band intensity decreased markedly with the concomitant formation of cross-links with high molecular weight found in the stacking gel. The results suggested that MHC underwent cross-linking induced by oxidized phenolic compounds in EKWE or CT via nondisulfide covalent bonds. The results reconfirmed that the enhanced cross-linking of proteins in surimi was most likely due to the cross-linking activity of quinones in oxidized EKWE or CT. The quinone, a reactive electrophilic intermediate, can readily undergo attack by nucleophiles such as lysine, methionine, cysteine, and tryptophan residues in a protein chain, forming strong covalent bonds (Hurrell and Finot 1984). Nevertheless, no MHC band was observed in surimi gels prepared from mackerel stored in ice for 6 or 12 d, regardless of the addition of EKWE or CT. The disappearance of MHC in gel of surimi prepared from mackerel stored in ice for 6 or 12 d might be a result of heat-activated autolysis or cross-linking of MHC. As a consequence, no MHC was retained in all gels from surimi containing initially low MHC content. Actin was the dominant protein remaining in gel samples. At the same storage time, corresponding surimi gels had a similar actin band intensity, irrespective of EKWE or CT addition. Actin was less susceptible to proteolysis and to cross-linking induced by indigenous transglutaminase (An and others 1994). Similar protein patterns between different gels correlated well with similar gel properties of surimi produced from mackerel stored in ice for 6 to 12 d, regardless of EKWE or CT addition.

Conclusions

Mackerel mince rapidly underwent deterioration and physicochemical changes especially proteolytic degradation and lipid oxidation during iced storage, leading to the loss in gel-forming ability. The addition of 0.15% EKWE or 0.3% CT effectively improved surimi gel quality, when mackerel kept in ice up to 6 d were used for surimi preparation. Both additives showed no gel strengthening effect toward surimi produced from poor-quality fish.

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Thermal properties and heat-induced aggregation of natural actomyosin extracted from goatfish (*Mulloidichthys martinicus*) muscle as influenced by iced storage

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ABSTRACT

Thermal properties and aggregation of natural actomyosin (NAM) extracted from fresh and 9 days ice-stored goatfish were comparatively studied. Myosin of fresh goatfish had the higher thermal stability than that of ice-stored counterpart as indicated by the higher maximum transition temperature (T_{max}). Additionally, the thermal inactivation rate constant (K_D) of Ca^{2+}-ATPase in NAM from fresh goatfish was lower than that of ice-stored counterpart when incubated at the temperature range of 20–40 °C, indicating the lower thermal stability of the latter NAM. NAM from fresh goatfish exhibited the higher turbidity, surface hydrophobicity and disulfide bond formation than did NAM of iced-stored sample when heated at temperature from 35 to 75 °C, suggesting the higher extent of aggregation of the former. However, goatfish NAM showed the lower extent of heat-induced aggregation than did bigeye snapper NAM. As visualized by transmission electron microscopy, network strands of aggregates from bigeye snapper NAM were finer and more uniform than those from goatfish. NAM from goatfish stored in ice showed the lower extent of heat-induced aggregation than did NAM from fresh counterpart. Therefore, heat-induced aggregation of goatfish muscle proteins was governed by freshness.

1. Introduction

Fish muscle proteins typically undergo heat-induced gelation, which has the impact on quality of surimi seafood products. Among fish muscle proteins, myofibrillar proteins, especially myosin or actomyosin, are the major proteins contributing to the functional properties including gelation (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001; Ko, Yu, & Hsu, 2007). Gelation of food protein generally involves denaturation and aggregation (Ko et al., 2007). Denaturation is a process, in which proteins undergo conformational changes, primarily unfolding, without alteration of the amino acid sequence. Then, protein–protein interactions, known as association, aggregation and polymerization, take place and a three-dimensional network can be formed (Ko et al., 2007). The change of actomyosin conformation correlates with the exposure of functional groups such as sulfhydryl groups and hydrophobic groups (Benjakul et al., 2001). Subsequently, those groups most likely undergo disulfide bond formation and hydrophobic interaction, respectively. Additionally, gelation is dependent upon temperature (Sano, Ohno, Otsuka-Fuchino, Matsumoto, & Tsuchiya, 1994), heating rate (Yongsawatdigul & Park, 1999), pH and type of actomyosin (Lefevre, Fauconneau, Thompson, & Gill, 2007) used. Chan, Gill, and Paulson (1992a) reported that herring, cod and silver hake aggregated in different fashions, accounting for the differences in gel elasticity between species. When the rate of protein aggregation is slow, heat-denatured proteins are allowed to align in an ordered fashion to form a fine gel network, resulting in more elastic gels (Hermansson, 1979).

Functional properties of muscle protein are closely associated with the integrity of proteins. Denaturation and degradation mainly contribute to the loss of those functionalities. Freshness is generally considered as the most crucial factor determining the final gel quality of surimi (Benjakul, Visessanguan, & Tueksuban, 2003). Superior surimi gel quality cannot be produced from denatured or degraded myosin (Benjakul, Seymour, & Morrissey, 1997). Proteolysis and denaturation of fish muscle proteins are related with the quality loss of fish flesh as well as poor gel forming ability of fish stored for the extended holding times, especially at elevated temperatures (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002).

Nowadays, surimi is served as a potential raw material for a variety of seafood products, which become more increasingly popular due to their unique textural properties as well as high nutritional value. However, raw materials for surimi production

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such as threadfin bream (*Nemipterus* spp.), bigeye snapper (*Priacanthus* spp.) and lizardfish (*Saurida* spp.) have been decreasing. Goatfish is an abundant species in the Gulf of Thailand and has low commercial values. Recently, it has become more economically important as a raw material for surimi production. However, poor gel forming ability of goatfish muscle is generally recognized and limits the utilization of this species. Inferior gelation of this species might be associated with the poor gel quality. Additionally, inappropriate handling or storage of this species, commonly treated as by-catch, may cause the loss in gel forming ability. However, the information regarding heat-induced gelation of goatfish muscle proteins is very limited. Therefore, the objective of this study was to investigate the thermal properties and heat-induced aggregation of natural actomyosin from fresh and ice-stored goatfish, in comparison with that of bigeye snapper.

2. Materials and methods

2.1. Chemical reagents

Adenosine-5′-triphosphate (ATP), 8-anilino-1-naphthalenesulphonic acid (ANS), guanidine thiocyanate, sodium hydrogen sulfite, β-mercaptoethanol (β-ME) and Tris-maleate were procured from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Potassium chloride, sodium chloride, calcium chloride, trichloroacetic acid, potassium dihydrogen phosphate and ammonium molybdate were purchased from Merck (Darmstadt, Germany). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Bovine serum albumin (BSA) was purchased from Fluka (Buchs, Switzerland).

2.2. Materials

Goatfish (*Mulloidichthys martinicus*) with a weight of 50–100 g/fish and bigeye snapper (*Priacanthus tayenus*) with a weight of 100–130 g/fish were caught off the Songkhla–Pattani Coast in the Gulf of Thailand during January and February, 2008. Fish were immediately stored in ice after capture and off-loaded within 24 h. From a dock in Songkhla, Thailand, fish were transported in ice with the fish/ice ratio of 1:2 (w/w) to the Department of Food Technology, Prince of Songkla University, Songkhla, Thailand within 1–2 h. Upon arrival, goatfish were separated into 2 groups. The first group was headed, gutted and washed with water. The flesh was separated manually from skin and bone and kept on ice not longer than 2 h for natural actomyosin (NAM) preparation. For another portion, fish were stored in ice with fish/ice ratio of 1:2 (w/w) for 9 days. To maintain the ice ratio, the molten ice was removed every day and the same amount of ice was replaced. The temperature of fish was 0–2 °C throughout the storage of 9 days. The flesh of fresh bigeye snapper was also separated in the same manner and used for NAM preparation.

2.3. Preparation of natural actomyosin (NAM)

NAM was prepared according to the method of Benjakul et al. (2001) with a slight modification. Goatfish muscle, both fresh and 9 days ice-stored, or 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 over heating, the sample was placed in ice and homogenized for 20 s, followed by a 20 s rest interval for a total extraction time of 4 min. The extract was centrifuged at 5000×g for 30 min at 4 °C using a Sorvall Model RC-5B plus (Mandel Scientific, Newton, CT, USA). 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 dissolved in chilled 50 mM potassium phosphate buffer, pH 7.0 containing 0.6 M KCl and then centrifuged at 5000×g for 20 min at 4 °C. The supernatant was collected and used as NAM. NAM from fresh and 9 days ice-stored goatfish was referred to as 'G_0' and 'G_9', respectively. NAM from fresh bigeye snapper was also prepared in the same manner and referred to as 'B'. All NAM were kept in ice until use or analysis.

2.4. Thermal denaturation and stability of NAM

2.4.1. Differential scanning calorimetry

Thermal transition of NAM from goatfish, G_0 and G_9, was studied using the differential scanning calorimetry (DSC) (Perkin-Elmer, Model DSCM, Norwalk, CT, USA). NAM samples (15–20 mg wet weight) were placed in the DSC hermetic pans, assuring a good contact between the sample and the pan bottom. An empty hermetic pan was used as a reference. The samples were scanned at 10 °C/min over the range of 20–100 °C. The maximum transition temperature (T_{max}) was measured and enthalpy change (ΔH) was estimated by measuring the area under the DSC transition curve. The system was calibrated using indium.

2.4.2. Determination of inactivation rate constant of Ca^{2+}-ATPase of NAM

Three milliliters of NAM solution (3–5 mg/ml), both G_0 and G_9, were incubated at different temperatures (0, 10, 20, 30, 40, 50 and 60 °C). At definite times (0, 5, 10, 30 and 60 min), a sample solution was immediately cooled in iced water. The sample was then equilibrated at 25 °C prior to determination of Ca^{2+}-ATPase activity. The inactivation rate constant (K_D) of NAM was calculated according to Arai, Kawamura, and Gayashi (1973) as follows:

$$K_D = \frac{\ln C_0 - \ln C_t}{t}$$

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).

2.5. Preparation of NAM heated to different temperatures

NAM extracted from goatfish, both G_0 and G_9, and NAM from bigeye snapper (B) were diluted to 1 mg/ml with chilled 50 mM potassium phosphate buffer containing 0.6 M KCl (pH 7.0). The solutions were heated at heating rate of 0.65 °C/min from 20 to 75 °C using a digital thermoregulator (TH5/150, Ratek, Boronia, Victoria, Australia). The samples were taken every 5 °C of temperature increment. At temperature designated, the samples were cooled immediately with iced water. The samples obtained were subjected to analyses.

2.5.1. Determination of turbidity

Heated NAM solutions were placed in cuvette (light path length of 1 cm). Degree of protein aggregation was estimated by measuring the turbidity at 660 nm (Benjakul et al., 2001) using a UV–visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan).

2.5.2. Determination of surface hydrophobicity

Surface hydrophobicity was measured according to the method of Benjakul et al. (2001) using 8-anilo-1-naphthalenesulfonic acid (ANS) as a probe. Heated NAM solutions were diluted to 0.125, 0.25, 0.5 and 1 mg/ml using the same buffer. To 2.0 ml of diluted NAM solution, 10 μl of 10 mM ANS dissolved in 50 mM potassium phosphate buffer (pH 7.0) was added and the mixtures were mixed thoroughly. Sample blanks of each protein concentration were

prepared in the same manner, except the same volume of 50 mM potassium phosphate buffer (pH 7.0) was used instead of ANS solution. Fluorescence intensity was measured using a RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan) at the excitation and emission wavelength of 374 and 485 nm, respectively. Surface hydrophobicity was calculated from initial slope of plot of fluorescence intensity against protein concentration using a linear regression analysis. The initial slope was referred to as S_0ANS.

2.5.3. Determination of total sulfhydryl group and disulfide bond contents

Total sulfhydryl group content was measured according to the method of Ellman (1959) as modified by Benjakul et al. (2001). To 0.25 ml aliquot of heated NAM solutions, 3 ml of 0.2 M Tris–HCl buffer containing 8 M urea, 2% SDS and 10 mM EDTA (pH 6.8) and 0.25 ml of 0.1% DTNB in 0.2 M Tris–HCl buffer (pH 6.8) were added. The mixture was incubated at 40 °C for 40 min and the absorbance at 412 nm was read using a UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan). Reagent blank was prepared by replacing the sample with 50 mM potassium phosphate buffer containing 0.6 M KCl (pH 7.0). For sample blank, the reaction was run in the same manner except 0.2 M Tris–HCl (pH 6.8) was used instead of DTNB solution. Sulfhydryl group content was calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}. Disulfide bond content was determined by using 2-nitro-5-thiosulfobenzoate (NTSB) assay as described by Thannhauser, Konishi, and Scheraga (1987). To 0.5 ml of NAM solutions, 3.0 ml of freshly prepared NTSB assay solution were added. The mixture was incubated in the dark at room temperature (25 °C) for 25 min. Absorbance was then measured at 412 nm. Disulfide bond content was calculated from the absorbance using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.6. Transmission electron microscopy

NAM solutions (B, G_0 and G_9) heated to reach the final temperature of 75 °C, followed by cooling, were diluted to 0.2 mg/ml with 50 mM potassium phosphate buffer containing 0.6 M KCl (pH 7). A drop of sample was fixed for 5 min on a carbon-coated grid, negatively stained with 4% uranyl acetate for 5 min and washed with distilled water until the grid was cleaned. The specimens were visualized using a JEOL JEM-2010 transmission electron microscope (JEOL Ltd., Tokyo, Japan) (80,000×) at an accelerating voltage of 160 kV.

2.7. Protein determination

Protein content was determined by the Biuret method (Robinson & Hodgen, 1940) using bovine serum albumin as the standard.

2.8. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range tests (Steel & Torrie, 1980). Analysis was performed using a SPSS package (SPSS 11.0 for Windows, SPSS Inc, Chicago, IL, USA).

3. Results and discussion

3.1. Thermal denaturation of NAM from fresh and ice-stored goatfish

Thermal transition of goatfish NAM extracted from fresh (G_0) and 9 days ice-stored (G_9) goatfish determined by DSC is shown in Table 1. G_0 exhibited two major peaks with the maximum transition temperatures (T_{max}) of myosin and actin of 47.4 and 63.5 °C, respectively. After stored in ice for 9 days (G_9), T_{max} of myosin shifted to a lower level (45.5 °C) with a coincidental decrease in enthalpy change (ΔH) ($p < 0.05$). Lower T_{max} and ΔH indicated that myosin from ice-stored goatfish had lower thermal stability as compared with that from fresh counterpart. However, no differences in T_{max} and ΔH of actin were found between G_0 and G_9 ($p > 0.05$). Rattanasatheirn, Benjakul, Visessanguan, and Kijroongrojana (2008) found that both T_{max} and ΔH of myosin peak of white shrimp shifted from 50.1 to 49.8 °C and from 1.65 to 0.76 J/g, respectively, after kept in ice for 7 days. T_{max} of red claw fish for myosin head (50.2 °C) and actin (72.6 °C) showed a significant decrease after 7 days of iced storage to 46.3 and 69.7 °C and dropped to 39.4 and 60.3 °C after 14 days of iced storage (Tseng, Xiong, Webster, Thompson, & Muzinic, 2002). Goatfish myosin was susceptible to denaturation during extended iced storage. During storage, autolysis might take place, leading to the loss in protein integrity. Endogenous proteolytic enzymes including calpain and lysosomal proteinases might partially degrade MHC, leading to the ease of denaturation at lower temperature with less energy input. Moreover, the protein conformation changes during iced storage were not only caused by proteolysis but also chemical reaction, such as lipid oxidation (Tseng et al., 2002). The lipid oxidation products could interact with muscle proteins, resulting in the destabilized proteins (Tseng et al., 2002). Thus, the iced storage had the influence on thermal property of muscle proteins from goatfish.

Table 1
T_{max} and enthalpy change (ΔH) of natural actomyosin from fresh goatfish and goatfish stored in ice for 9 days.

Samples	Peak I		Peak II	
	T_{max}(°C)	ΔH (J/g protein)	T_{max}(°C)	ΔH (J/g protein)
G_0	47.4 ± 0.3a[a]	1.19 ± 0.04a	63.5 ± 0.5a	0.27 ± 0.04a
G_9	45.5 ± 0.2b	0.31 ± 0.02b	62.9 ± 0.3a	0.26 ± 0.03a

Different alphabets in the same column indicate significant differences ($p < 0.05$). The protein content of NAM samples was 21.2–22.0% as determined by the biuret assay.
[a] Values are given as mean ± SD from triplicate determinations.

3.2. Thermal stability of NAM from fresh and ice-stored goatfish

The inactivation rate constants (K_D value) of Ca^{2+}-ATPase of NAM from G_0 and G_9 at different temperatures are shown in Table 2. No changes in K_D were observed for G_0 and G_9 when heated up to 30 and 20 °C, respectively. A significant increase in K_D value was observed for G_0 and G_9 at temperatures of 40 and 30 °C, respectively. However, no K_D of both samples was detected between 50

Table 2
Thermal inactivation rate constant of natural actomyosin extracted from fresh goatfish and goatfish stored in ice for 9 days.

Temperature (°C)	$K_D \times 10^5$ (S^{-1})	
	G_0	G_9
0	0.66 ± 0.44bA[a]	0.93 ± 0.22cA
10	0.87 ± 0.46bA	1.03 ± 0.45cA
20	1.23 ± 0.09bB	2.36 ± 0.39cA
30	5.04 ± 0.61bB	9.70 ± 0.85bA
40	81.60 ± 7.24aB	120.03 ± 5.75aA
50	ND[b]	ND
60	ND	ND

Different alphabets in the same column indicate significant differences ($p < 0.05$). Different capital alphabets in the same row indicate significant differences ($p < 0.05$).
[a] Values are given as mean ± SD from triplicate determinations.
[b] ND: Not detectable.

and 60 °C. At the same heating temperature, the higher K_D value was found in G_9 than was G_0. The higher K_D generally indicated the greater loss in Ca^{2+}-ATPase. Ca^{2+}-ATPase is used as a good indicator of the integrity of myosin molecule (Benjakul et al., 1997). This result indicated that myosin from G_9 was unstable and underwent partial denaturation during handling and storage in ice. The result was in agreement with the DSC analysis (Table 1), which revealed that the lower ΔH of protein denaturation of G_9 was obtained in comparison with G_0. The changes were probably associated with proteolysis caused by muscle bound proteinases or microbial proteinases. Hydrolysis might result in the ease of conformational changes of proteins, particularly the head portion of MHC. Benjakul et al. (1997) found that the changes in troponin–tropomyosin complex of Pacific whiting muscle during ice storage was caused by proteolysis. Visessanguan and An (2000) reported that myosin from arrowtooth flounder in the presence of 2.5 mU papain exhibited the lower enthalpy required to induce myosin denaturation (1.9 J/g) than in the absence of papain (5.2 J/g). It was inferred that the longer storage time, the greater denaturation of MHC occurred. This might affect gelation of muscle proteins from goatfish stored in ice.

3.3. Turbidity of NAM during heating

Turbidity development of solutions (1 mg/ml) of G_0, G_9 and B heated from 20 to 75 °C was monitored (Fig. 1). All NAM solution became more turbid as temperature increased, suggesting an increased formation of protein aggregates. Each myosin molecule is bound to the actin filament at its head portion with its tail portion sticking out (Sano, Noguchi, Tsuchiya, & Matsumoto, 1988). NAM molecules tended to interact each other and form protein aggregates upon heating. A distinct increase in turbidity of G_0 and B was observed at 35 °C, while G_9 showed the increased turbidity at 40 °C. Bigeye snapper NAM (B) exhibited the higher rate in turbidity development than did goatfish NAM, both G_0 and G_9, at temperatures above 40 °C. The differences in turbidity were most probably due to the varying size and/or rate of protein aggregation (Chan & Gill, 1994). Muscle protein molecules can associate with one another through hydrophobic, electrostatic and hydrogen bonds as well as disulfide bond (Chawla, Venugopal, & Nair, 1996; Xiong, 1997). The result suggested that NAM from bigeye snapper could undergo aggregation at a higher extent, compared to that from goatfish. Bigeye snapper has been used as potential raw material for surimi production owing to its excellent gel formability of this species (Benjakul et al., 2002). On the other hand, goatfish yields the surimi with poor gel (Yarnpakdee, 2008). Benjakul et al. (2001) reported that the difference in aggregation of NAM from bigeye snapper of two species was due to the differences in amount of hydrophobic domain and in intermolecular disulfide bonds formed during heating process. When comparing NAM from goatfish, G_0 and G_9, the greater turbidity was observed in the former than was the latter. The result suggested that G_0 underwent higher extent of aggregation than did G_9. When proteins underwent denaturation or degradation during iced storage, they might lose their ability in aggregation, possibly due to the hindrance of reactive groups or the shorter chain length caused by autolysis. Formation of large aggregates is presumably a prerequisite to formation of a good elastic gel (Chan et al., 1992a). Chan et al. (1992a) reported that the poorer aggregating ability of herring actomyosin reflected the inferior gelling properties of surimi. The differences in aggregation behavior of NAM were postulated to result in the different gelling properties between species.

3.4. Surface hydrophobicity of NAM during heating

Changes in surface hydrophobicity (S_0ANS) of proteins in NAM solutions, G_0, G_9 and B, are depicted in Fig. 2. S_0ANS increased continuously after heating at the temperature above 35 °C up to 60 °C. Thereafter, slight decreases in S_0ANS were observed at temperature above 65 °C for G_0 and G_9, and above 70 °C for B sample. The increase in S_0ANS indicated the structural changes of NAM during heating. Upon heating, the aromatic hydrophobic amino acid residues, i.e. phenylalanine and tryptophan, could be exposed to a greater extent (Visessanguan, Ogawa, Nakai, & An, 2000). ANS, an effective fluorescent probe, has been found to bind at non-polar regions of protein (Wicker, Lanier, Hamann, & Akahane, 1986). The increase in ANS binding could be due to either the presence of exposed hydrophobic sites on unfolded, not aggregated proteins, or unfolded and aggregated proteins. A slight decrease in S_0ANS at temperatures above 65 or 70 °C suggested that the hydrophobic interaction between proteins molecules most likely took place. Higher S_0ANS in bigeye snapper NAM (B) was found, compared with goatfish NAM (G_0 and G_9), possibly caused by the differences in amino acid composition, especially hydrophobic amino acids. The exposure of hydrophobic domains has been suggested as a prerequisite for formation of large myosin aggregates via hydrophobic interaction (Chan, Gill, & Paulson, 1992b). For goatfish NAM, S_0ANS of G_9 was higher than that of G_0 at a temperature in the range of 20–35 °C. Nevertheless, G_0 exhibited the higher S_0ANS than did G_9 when heated at temperatures above 40 °C. With the sufficient heat, G_0 could undergo conformational changes or the exposure of interior domain where hydrophobic amino acids were located, to the greater extent than G_9. The rate of

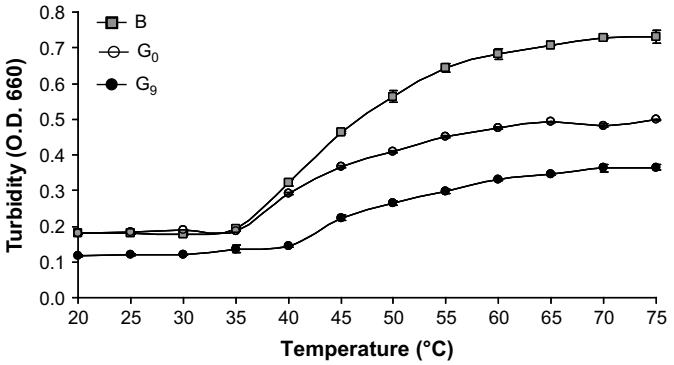

Fig. 1. Turbidity of different NAM solutions (1 mg/ml) during heating from 20 to 75 °C at 0.65 °C/min. B, G_0 and G_9 represent NAM solutions extracted from bigeye snapper, fresh goatfish and goatfish stored in ice for 9 days, respectively. Bars represent standard deviation from triplicate determinations.

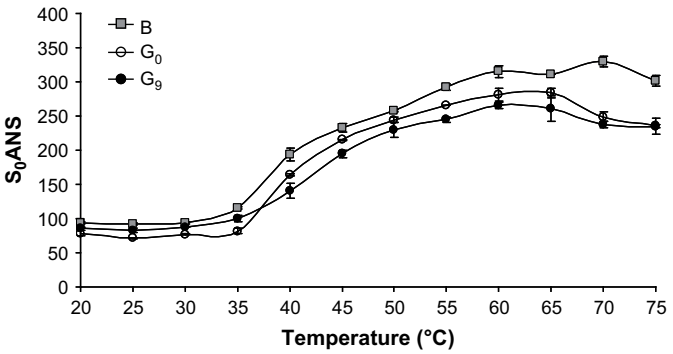

Fig. 2. Surface hydrophobicity (S_0ANS) of proteins in different NAM solutions (1 mg/ml) during heating from 20 to 75 °C at 0.65 °C/min. B, G_0 and G_9 represent NAM solutions extracted from bigeye snapper, fresh goatfish and goatfish stored in ice for 9 days, respectively. Bars represent standard deviation from triplicate determinations.

the increase in S_0ANS in G_0 was higher than G_9 for further heating at temperature above 40 °C. This result suggested that hydrophobic portion of G_9 might be less exposed during heating. This might be caused by the prior denaturation during iced storage.

3.5. Total sulfhydryl group content and disulfide bond formation of NAM during heating

Total sulfhydryl group content (total SH) and disulfide bond formation of proteins in different NAM solutions after heating from 20 to 75 °C are shown in Fig. 3. The continuous decrease in total SH content was observed in all samples when heated at temperatures higher than 30 °C (Fig. 3A). Decreases in total SH content were concomitant with the increased disulfide bond formation of all NAM samples. During heating, the formation of disulfide bond might be induced and involved in aggregation (Fig. 3B). This indicated that thermally exposed sulfhydryl groups could be oxidized to disulfide bond, especially at temperature above 40 °C. A decrease in total SH groups was reported to be due to the formation of disulfide bond through oxidation of SH group or disulfide interchanges (Hayakawa & Nakai, 1985). Chan, Gill, Thompson, and Singer (1995) reported that myosin contained 42 SH groups. Two type of SH groups on the myosin head portion, named SH_1 and SH_2, have been reported to be involved in ATPase activity of myosin (Benjakul et al., 1997; Yamaguchi & Sekine, 1966). Another SH group (SH_a) localized in the light meromyosin contributes to oxidation (Sompongse, Itoh, & Obatake, 1996). Niwa (1992) reported that the disulfide bond formation of fish actomyosin and myosin occurred at 40 and 45 °C, respectively. Yongsawatdigul and Park (2003) reported that the disulfide bond formation in tilapia actomyosin required high temperature (above 50 °C). Disulfide bond content was higher in NAM from bigeye snapper, in comparison with goatfish NAM at all temperatures tested. This was in accordance with the higher extent of aggregation of the former as indicated by the turbidity data (Fig. 1). This was possibly due to the differences in amino acid composition as well as the structural rigidity. Disulfide bond formation of G_9 was lower than was G_0, especially at temperature higher than 40 °C. This result indicated that G_9 possibly underwent oxidation during iced storage. As a result, during heating, the intermolecular disulfide bond formation could occur to a lower content, leading to the less aggregation. Benjakul et al. (2001) reported that the hydrophobic interaction and disulfide bond formation determined the aggregation behavior of protein as well as gel properties of bigeye snapper.

3.6. Transmission electron micrograph of heated NAM

Microstructure of G_0, G_9 and B samples after heating to 75 °C revealed that the aggregation of NAM occurred after heat treatment differently as depicted in Fig. 4. Heated NAM exhibited the networks with the fibrous strand. Network of G_0 had the larger

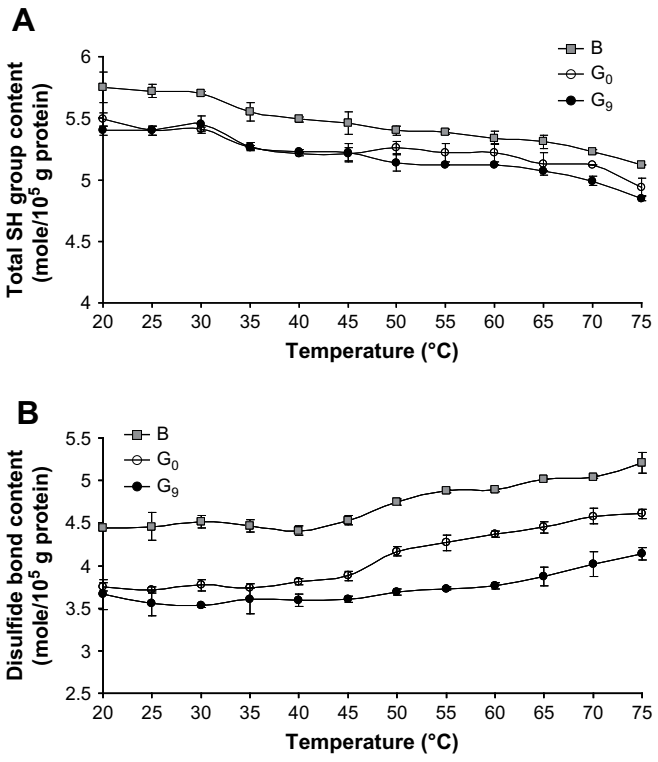

Fig. 3. Total sulfhydryl group content (A) and disulfide bond content (B) of proteins in different NAM solutions (1 mg/ml) during heating from 20 to 75 °C at 0.65 °C/min. B, G_0 and G_9 represent NAM solution extracted from bigeye snapper, fresh goatfish and goatfish stored in ice for 9 days. Bars represent standard deviation from triplicate determinations.

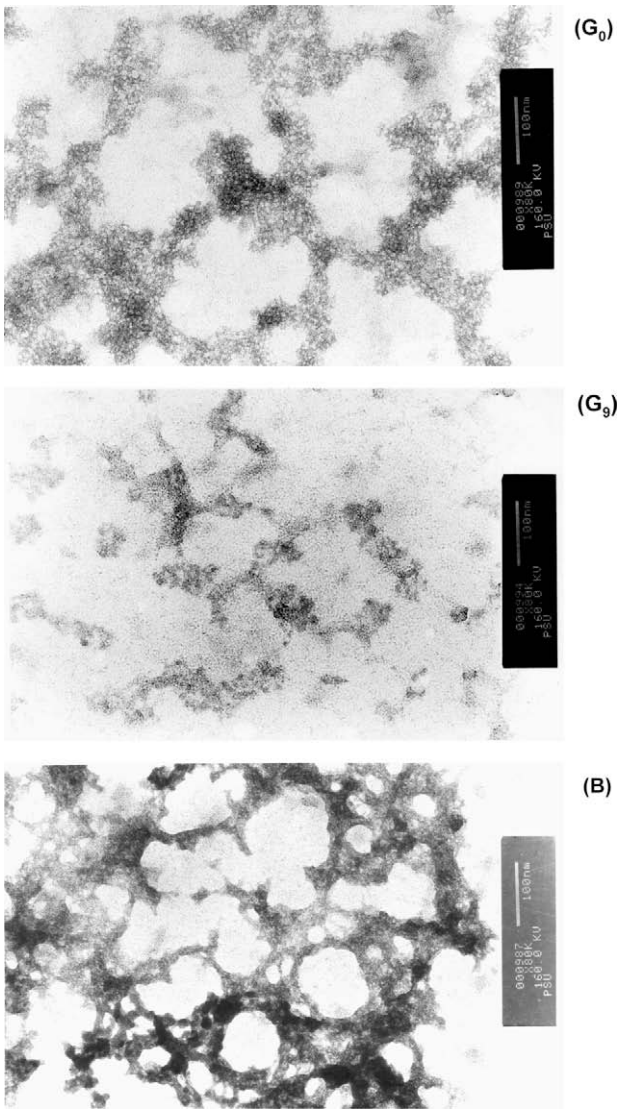

Fig. 4. Transmission electron micrograph on aggregation of NAM from bigeye snapper (B), fresh goatfish (G_0) and goatfish stored in ice for 9 days (G_9) after heating at 0.65 °C/min to 75 °C. Heated NAM was cooled rapidly in iced water prior to TEM analysis. Magnification: 80,000×.

strand with the coarser structure (Fig. 4 G_0), compared to that of B sample (Fig. 4B). Gelation is the result of protein denaturation, followed by the aggregation via intermolecular covalent bonds and noncovalent interactions (Lee & Lanier, 1995). The thermal treatment produces enough protein denaturation to cause the interactions and the formation of a network structure (Totosaus, Montejano, Salazar, & Guerrero, 2002). Large aggregates were formed by various bonds including hydrophobic interaction and disulfide bonds (Figs. 2 and 3). The native actomyosin was found as an arrowhead structure on the filaments and became shortened after heat treatments (Ko et al., 2007). Looser protein network was formed in G_9 (Fig. 4 G_9). This was coincidental with the lower turbidity development (Fig. 1). From the result, the alignment of myofibrillar proteins as well as aggregation pattern induced by heat treatment was different between species. Moreover, NAM from goatfish with lower freshness exhibited the inferior gel network to NAM from fresh counterpart. Benjakul et al. (2001) noted that the differences in gelation of NAM from two species of bigeye snapper were caused by different intrinsic properties of muscle proteins, mainly myosin and actin.

4. Conclusion

Heat-induced aggregation of NAM from goatfish was governed by its freshness. However, goatfish NAM exhibited the lower extent of aggregation with the lower extent of hydrophobic interaction and disulfide bond during heating in the temperature range of 20–75 °C than did bigeye snapper NAM. This suggests the importance of freshness of fish used as raw material on the quality of surimi gels. Additionally, the gel forming ability most likely varies with fish species.

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COMBINATION EFFECTS OF WHEY PROTEIN CONCENTRATE AND CALCIUM CHLORIDE ON THE PROPERTIES OF GOATFISH SURIMI GEL

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ABSTRACT

*Effect of whey protein concentrate (WPC) in combination with calcium chloride (50 mmol/kg) on the gel properties of surimi produced from goatfish (*Mulloidichthys martinicus*) was investigated. Breaking force and deformation of both kamaboko (40/90C) and modori (60/90C) gel increased with increasing WPC (0–3%) (*$P < 0.05$*). Myosin heavy chain (MHC) band intensity of all gels was more retained as WPC concentration increased. The coincidental increase in water-holding capacity was obtained, however, the whiteness was slightly decreased. At all levels of WPC added, kamaboko gels added with $CaCl_2$ exhibited the superior properties to those without $CaCl_2$ (*$P < 0.05$*). On the other hand, $CaCl_2$ addition resulted in the poorer gel properties of modori gel, regardless of WPC addition. The microstructure of surimi gels added with 3% WPC generally became denser and more ordered, compared with the control gel, irrespective of $CaCl_2$ addition.*

PRACTICAL APPLICATIONS

Goatfish is the new raw material for surimi production; however, it yields the gel with poor quality. The addition of WPC and $CaCl_2$ at the appropriate levels can be a promising means to improve the gel quality via the inhibition

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of endogenous proteinases and the enhancement of setting phenomenon, respectively. As a consequence, surimi industry is able to produce surimi from goatfish with the quality equivalent to that of surimi from commonly used species. Thus, the greater benefit can be gained from goatfish, especially for the surimi industry.

KEYWORDS

Autolysis, $CaCl_2$, gel, goatfish, setting, surimi, whey protein concentrate

INTRODUCTION

Surimi possesses the functional properties including gelling, binding and emulsifying properties and can be used as a functional protein ingredient in several products (Lanier 1986). The thermal aggregation of myosin molecule is a crucial process for developing the elastic gels that largely affect consumer preference and palatability of surimi-based products (Sano *et al.* 1988). However, surimi gels from some fish species undergo thermal degradation, which is associated with the weakening of the resulting gel. The presence of endogenous proteinases brings about the gel softening of surimi (Morrissey *et al.* 1993). Alkaline proteinases are considered to be responsible for weakening of minced fish (Makinodan *et al.* 1985). However, the active proteinases in fish muscle, which soften the surimi gel, vary with fish speices. Cathepsin L, a cysteine proteinase, has been reported to hydrolyze muscle proteins of Pacific whiting and arrowtooth flounder at the elevated temperature (55–65C) (Seymour *et al.* 1994; Visessanguan and An 2000). Myofibril-associated proteinases in lizardfish were cysteine and serine proteinases, whereas serine proteinase was the major proteinase in muscle of bigeye snapper (Benjakul *et al.* 2003a,b). To alleviate the gel weakening caused by endogenous proteinases, some protein additives such as bovine plasma proteins, porcine plasma proteins, chicken plasma proteins, egg white, potato extract, etc., have been widely used to enhance the gel strength via inhibition of proteolysis (Wasson *et al.* 1992; Reppond and Babbitt 1993; Visessanguan *et al.* 2000; Rawdkuen *et al.* 2004; Benjakul *et al.* 2004a). However, the applications of BPP and EW in surimi have recently been limited by the outbreak of mad cow disease and allergy problem, respectively. The addition of blood plasma is associated with off-color and off-flavor of resulting surimi gel (Rawdkuen *et al.* 2004). Recently, Rawdkuen and Benjakul (2008) reported that whey protein concentrate (WPC) addition increased the breaking force and deformation of surimi produced from some tropical fish without the adverse effect

of whiteness. Setting phenomenon plays a role in strengthening the surimi gel and is mediated by endogenous transglutaminase (TGase) (Benjakul and Visessanguan 2003). In general, fish TGase requires the sufficient amount of calcium ion for full activation (Araki and Seki 1993). The addition of calcium ion into surimi resulted in the increases in mechanical properties of the gel (Benjakul and Visessanguan 2003; Rawdkuen *et al.* 2005) and the concentration required varies with fish species (Julavittayanukul *et al.* 2006)

Goatfish has become an important alternative raw material for surimi production because of its abundance and low price. Nevertheless, the poor gel properties limit the uses of this species. The inhibition of thermal degradation by acceptable food grade proteinase inhibition together with the maximized setting could be a promising approach to obtain the better gel properties. The objective of this study was to investigate the effects of WPC in combination with calcium chloride on the gel properties of goatfish surimi.

MATERIALS AND METHODS

Chemicals

Sodium chloride was obtained from Fisher Scientific (Pittsburgh, PA). Folin-Ciocalteu's phenol reagent and trichloroacetic acid (TCA) were purchased from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) was obtained from Fluka Chemika (Buchs, Switzerland). β-mercaptoethanol (β-ME) and L-tyrosine were procured from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate (SDS), *N,N,N′,N′*-tetramethyl ethylene diamine (TEMED) and Coomassie Brilliant Blue R-250 were obtained from Bio-Rad Laboratories (Hercules, CA). Whey protein concentrate (WPC; Proliant 8600) was obtained from I.P.S. International, Co., Ltd. (Bangkok, Thailand).

Goatfish Collection and Preparation

Goatfish with the size of 15–20 fishes/kg were obtained from a dock in Songkhla, Thailand. Fish, off-loaded approximately 36–40 h after capture, were stored in ice with fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Songkhla, Thailand within 1 h. Upon arrival, fishes were washed, beheaded, eviscerated and the flesh was separated manually. The flesh was minced using a mincer with a 4-mm diameter hole. The mince obtained was kept in ice before use.

Surimi Preparation

Surimi was prepared according to the method of Benjakul and Visessanguan (2003). Goatfish mince was washed with cold water (5C) 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 subjected to centrifugation using a model CE 21K basket centrifuge (Grandiumpiant, Belluno, Italy) with a speed of 700× g for 15 min. The washed mince was mixed with cryoprotectant (4% sorbitol and 4% sugar) prior to freezing at −18C and referred to as "surimi."

Effect of Whey Protein Concentrate in Combination with Calcium Chloride on Gel Properties of Surimi Gel

Surimi Gel Preparation. To prepare the gel, frozen surimi was tempered in running water (25C) until the core temperature reached 0–2C. The surimi was then cut into small pieces with an approximate thickness of 1 cm. The surimi was placed in a mixer (National Model MKK77, Tokyo, Japan). The moisture was adjusted to 80% and 2.5% NaCl was added. WPC was added to obtain the final concentrations of 0, 1, 2 and 3% in the presence and in the absence of $CaCl_2$ (50 mmol/kg). The mixture was chopped for 4 min at 4C to obtain a homogenous sol. The sol was stuffed into a polyvinylidine chloride casing with a diameter of 2.5 cm and both ends were sealed tightly. Kamaboko gels were prepared by incubating the sol at 40C for 30 min, followed by heating at 90C for 20 min. To prepare modori gels, the sol was incubated at 60C for 30 min, followed by heating at 90C for 20 min. All gels were cooled in iced water and stored for 24 h at 4C before analyses.

Texture Analysis. Texture analysis of all gels was carried out using a model TA-XT2 texture analyzer (Stable Micro System, Surrey, UK). Gels were equilibrated at room temperature (25–30C) for 2 h before analysis. Five cylindrical samples (2.5 cm in length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyzer equipped with a spherical plunger (5-mm diameter) with a cross-head speed of 60 mm/min and 60% compression.

Determination of Whiteness. All gels were subjected to whiteness measurement using a HunterLab (ColorFlex, Hunter Associates Laboratory, Reston, VA). Illuminant C was used as the light source of measurement. *Commission Internationale d'Eclairage L**, *a** and *b** values were measured. Whiteness was calculated using the following equation (Park 1994):

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

Determination of Expressible Moisture Content. Expressible moisture was measured according to the method of Ng (1987). Cylindrical gel samples were cut to a thickness of 5 mm, weighed (X) and placed between two pieces of Whatman paper (No. 1) (Whatman International Ltd., Maidstone, England) at the bottom and one piece of paper on the top. A standard weight (5 kg) was placed on the top of the sample for 2 min, and then the sample was removed from the papers and weighed again (Y). Expressible moisture content was calculated and expressed as percentage of sample weight as follows:

$$\text{Expressible moisture content } (\%) = [(X - Y)/X] \times 100$$

Determination of Autolysis of Surimi Gel. To 3 g of finely chopped gel samples, 27 mL of 5% TCA were added and homogenized for 1 min using an IKA Labortechnik homogenizer (Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 4C for 1 h and centrifuged at 8,000× g for 5 min, using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). TCA-soluble peptide content in the supernatant were measured according to the Lowry method (Lowry *et al.* 1951) and expressed as μmol tyrosine/g sample.

SDS-Polyacrylamide Gel Electrophoresis. Protein patterns of goatfish surimi or gels were analyzed by SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (1970). To solubilize the protein, surimi and gel (3 g) were added with 27 mL of 5% SDS solution (85C). The mixture was then homogenized using a homogenizer (IKA Labortechnik, Selangor, Malaysia) at speed of 11,000 rpm for 2 min. The homogenate was incubated at 85C for 1 h to dissolve total proteins. The samples were centrifuged at 7,500 × g for 20 min to remove undissolved debris. Protein concentration in the supernatants was determined as per the method of Lowry *et al.* (1951) using bovine serum albumin as standard. The samples (15 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

Scanning Electron Microscopy. Microstructure of surimi gels was determined using scanning electron microscopy (SEM). The control

kamaboko and modori gels (without WPC) and those containing WPC at the level rendering the hightest breaking force and deformation without and with the addition of 50 mmol $CaCl_2$/kg (thickness of 2–3 mm) were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 20 kV.

Statistical Analysis. The experiments were run in triplicate. Each analysis was carried out in triplicate. Data were subjected to analysis of variance. Comparison of means was carried out by Duncan's multiple range test (Steel and Torrie 1980). Analysis was performed using a SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL, USA).

RESULTS AND DISSCUSSION

Effect of WPC in Combination with Calcium Chloride on the Properties of Goatfish Surimi Gel

Textural Properties of Surimi Gel. Breaking force and deformation of both kamaboko and modori gels containing WPC at different levels in the absence and in the presence of $CaCl_2$ at 50 mmoles/kg are shown in Fig. 1. $CaCl_2$ at 50 mmoles/kg was shown to yield the highest breaking force and deformation of goatfish surimi, in comparison with $CaCl_2$ at other concentrations (data not shown). The control gel (without WPC) had the lower breaking force and deformation when compared with samples added with WPC, regardless $CaCl_2$ addition ($P < 0.05$). Breaking force and deformation increased with increasing WPC levels ($P < 0.05$). Addition of 3% WPC resulted in the increase in breaking force by 49.9 and 115.8% for kamaboko and modori gel containing $CaCl_2$, respectively, and by 45.1 and 157.3% for kamaboko and modori gel without $CaCl_2$, respectively. The result revealed that the strengthening of surimi gel could be achieved by the addition of WPC. WPC has been reported to enhance gel properties of some tropical fish by inhibiting the proteolysis or acting as the filler in the gel matrix (Rawdkuen and Benjakul 2008). Akazawa *et al.* (1993) reported that the addition of WPC resulted in an improvement in heat-set gel texture and an apparent inhibition of proteolysis during gel formation caused by indigenous proteinases.

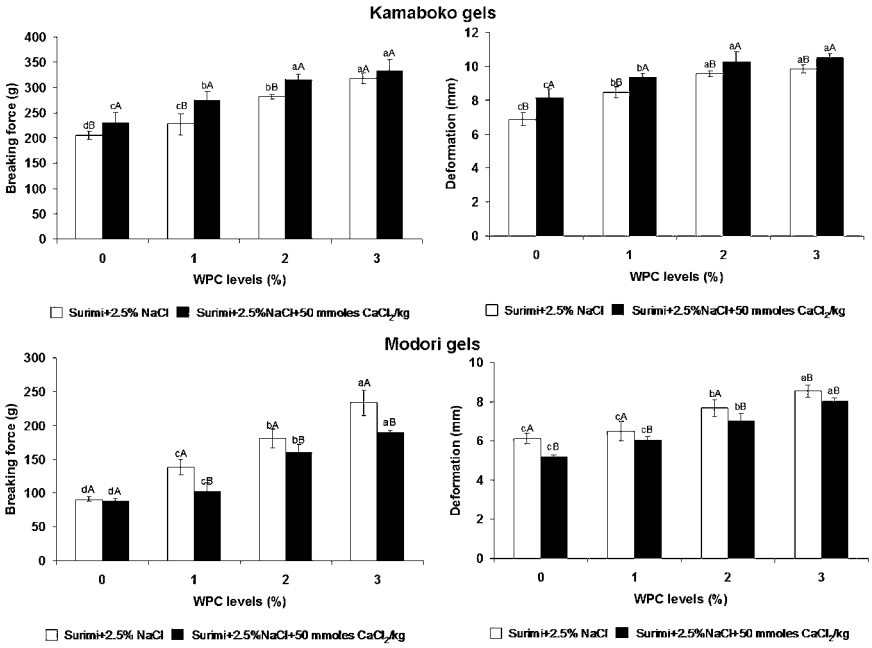

FIG. 1. BREAKING FORCE AND DEFORMATION OF KAMABOKO AND MODORI GELS ADDED WITH WPC AT DIFFERENT LEVELS IN THE ABSENCE AND IN THE PRESENCE OF 50 mmol $CaCl_2$/kg

Bars represent the standard deviation from triplicate determinations. Different letters on the bars within the same $CaCl_2$ level indicate the significant differences ($P < 0.05$). Different capital letters on the bars within the same level of WPC indicate the significant differences ($P < 0.05$).
WPC, whey protein concentrate.

Lower breaking force and deformation were found in modori gel, compared with kamaboko gel. Genrally, modori gel with softened texture is ascribed to the degradation of myosin by endogenous proteinases (An *et al.* 1996; Jiang *et al.* 1996). Gel structure disintegration reaction was observed at 50–60C (An *et al.* 1996). Benjakul *et al.* (2004c) found that the lower breaking force and deformation were observed with modori gel (60/90C) compared with kamaboko gel (40/90C) from lizard fish surimi. Addition of $CaCl_2$ affected breaking force and deformation of kamaboko and modori gels differently. For kamaboko gel, at all levels of WPC, the addition of 50 mmol $CaCl_2$/kg resulted in the superior gel quality as evidenced by the increases in breaking force and deformation obtained ($P < 0.05$). This indicated that $CaCl_2$ played the essential role in setting, in which endogenous TGase was activated. Fish TGase has been found to be Ca^{2+}-dependent, however the requirement varies among fish species (Nozawa *et al.* 1997). Endogenous

TGase has been reported to induce the formation of ε-(γ-glutamyl) lysine linkage during setting (Tsukamasa *et al.* 2002). Calcium compounds are commonly added in surimi or fish mince as a gel enhancer (Yamamoto *et al.* 1991). Julavittayanukul *et al.* (2006) found that breaking force and defomation of bigeye snapper surimi increased with increasing $CaCl_2$ concentrations (0, 25 and 50 mmol/kg). $CaCl_2$ in combination with WPC contributed to gel strengthening via enhancing myosin cross-linking and inhibiting proteolysis. Furthermore, cross-linked proteins formed during setting were more likely resistant to proteolysis. Therefore, the combination effect between cross-linking activity and proteolysis inhibition contributed to the improved gel strength of goatfish surimi.

At the same level of WPC added, modori gels containing $CaCl_2$ showed the lower breaking force and deformation than did those without $CaCl_2$ ($P < 0.05$). This result indicated that $CaCl_2$ addition caused the adverse effect on modori gel. Some proteinases such as calpain, a calcium activated neutral proteinase, might be activated by calcium ion. Park *et al.* (2003) reported that $CaCl_2$ addition in squid paste resulted in the lower breaking force and deformation of resulting gel, compared with control (no addition of $CaCl_2$). The superior gel strength was obtained when the combination of $CaCl_2$ with calpain inhibitor was used (Park *et al.* 2003). However, the breaking force and deformation of modori gel was increased with the addition of WPC. This result confirmed that the strength of kamaboko and modori gels could be improved by the addition of WPC.

Whiteness of Surimi Gel. Whiteness of kamaboko and modori gel added with WPC at different levels in the presence and in the absence of $CaCl_2$ (50 mmol/kg) is shown in Table 1. The slight decrease in whiteness was noticeable in all gels, except kamaboko gel without $CaCl_2$, as WPC levels increased ($P < 0.05$). Protein additives generally have the different impact on surimi gel color, depending on the initial color of each protein additives (Rawdkuen *et al.* 2004; Benjakul *et al.* 2004c). WPC is predominantly light-cream-colored in nature. It might reduce the whiteness of surimi gel to some extent, especially when a higher amount was used. Ramirez *et al.* (2007) reported that the color attributes of strip mullet restructure fish product changed from a yellow hue to a slightly more reddish hue after adding of WPC. Regardless of heating processes, whiteness of both kamaboko and modori gels in the presence of 50 mmol $CaCl_2$/kg was greater than that of gels without $CaCl_2$ ($P < 0.05$). $CaCl_2$ might form complex with some anion in the muscle, resulting in the formation of insoluble particles, leading to the light scattering in resulting gels (Benjakul *et al.* 2004b). Julavittayanukul *et al.* (2006) reported that whiteness of kamaboko gel of bigeye snapper surimi was increased with increasing $CaCl_2$ concentrations.

TABLE 1.
WHITENESS AND EXPRESSIBLE MOISTURE CONTENTS OF KAMABOKO AND MODORI GELS ADDED WITH WHEY PROTEIN CONCENTRATE (WPC) AT DIFFERENT LEVELS IN THE PRESENCE AND IN THE ABSENCE OF 50 mmol $CaCl_2$/kg

Samples	Treatment	WPC levels (%)	Whiteness	Expressible moisture content (%)
Kamaboko gel	Surimi + 2.5% NaCl	0	$73.4 \pm 0.7^{aB*,**,\dagger}$	4.5 ± 0.2^{aA}
		1	73.1 ± 0.1^{aB}	4.0 ± 0.1^{bA}
		2	73.0 ± 0.5^{aB}	3.8 ± 0.1^{bcA}
		3	73.1 ± 0.4^{aB}	3.6 ± 0.3^{cA}
	Surimi + 2.5%	0	77.2 ± 0.5^{aA}	3.6 ± 0.2^{aB}
	NaCl + 50 mmol $CaCl_2$/kg	1	76.1 ± 0.2^{bA}	3.4 ± 0.1^{aB}
		2	75.8 ± 0.2^{cA}	3.2 ± 0.1^{bB}
		3	75.6 ± 0.4^{cA}	2.9 ± 0.2^{cB}
Modori gel	Surimi + 2.5% NaCl	0	74.2 ± 0.4^{aB}	8.5 ± 0.3^{aA}
		1	73.3 ± 0.4^{aB}	4.8 ± 0.1^{bB}
		2	72.7 ± 0.2^{bB}	4.4 ± 0.3^{bcB}
		3	72.4 ± 0.4^{cB}	4.1 ± 0.1^{cB}
	Surimi+2.5%	0	76.4 ± 0.4^{abA}	9.0 ± 0.6^{aA}
	NaCl + 50 mmol $CaCl_2$/kg	1	76.8 ± 0.4^{aA}	6.3 ± 0.2^{bA}
		2	76.1 ± 0.3^{bA}	5.9 ± 0.4^{bA}
		3	76.2 ± 0.2^{bA}	5.8 ± 0.4^{bA}

* Different letters within the same $CaCl_2$ levels of the same gel indicate the significant differences ($P < 0.05$). ** Different capital letters within the same WPC levels of the same gel indicate significant differences ($P < 0.05$).

† Values are given as means ± standard deviation ($n = 3$).

Expressible Moisture Content of Surimi Gel. Expressible moisture content of kamaboko and modori gels added with WPC at different levels in the presence and in the absence of $CaCl_2$ is shown in Table 1. Decreasing expressible moisture contents were obtained in all gel samples as WPC levels increased ($P < 0.05$). Expressible moisture contents of kamaboko and modori gels decreased by 18.9 and 35.9% with the addition of 3% WPC and $CaCl_2$. Expressible moisture content decreased by 18.8 and 51.4% for kamaboko and modori gels added with only 3% WPC. WPC might be able to bind water, resulting in a greater amount of water retained in the gel matrix. Thus, addition of WPC increased water-holding capacity of gel matrix.

In general, the greater expressible moisture content of modori gel was observed, compared with kamaboko gel, irrespective of WPC addition. This indicated the poor gel matrix with low water-holding capacity as mediated by indigenous proteinases. For the kamaboko gel, higher water-holding capacity was obtained when $CaCl_2$ was present at all levels of WPC used as evidenced by lower expressible moisture content ($P < 0.05$). During setting, $CaCl_2$ most

likely improved gel forming ability via the formation of non-disulfide covalent bonds. As a consequence, a stronger gel network formed could imbibe more water. This was in agreement with higher breaking force and deformation of gels in the presence of $CaCl_2$ (Fig. 1). At the same level of WPC, the expressible moisture content of modori gel containing $CaCl_2$ was higher than that of gels without $CaCl_2$ ($P < 0.05$). Ca^{2+} might activate endogenous proteinases, particularly calpain. As a result, the network formed could be destroyed to some extent, leading to the lower ability to imbibe water in gel network. Rawdkuen and Benjakul (2008) also found that the addition of WPC (0–3%) resulted in the increase water-holding capacity of surimi gel from some tropical fish.

TCA-Soluble Peptide Content of Surimi Gel. TCA-soluble peptide content in kamaboko and modori gels containing WPC at different levels with and without $CaCl_2$ is present in Fig. 2. Generally, TCA-soluble peptide content in surimi gels has been used as an indicator of autolytic degradation of fish muscle protein (Thammatinna *et al.* 2007). The highest TCA-soluble peptide content was obtained in both kamaboko and modori gels without WPC addition ($P < 0.05$). TCA-soluble peptide content decreased as WPC levels increased ($P < 0.05$). This result suggested that WPC exhibited the inhibitory activity toward degradation of muscle protein. The degradation occurred during heat-induced gelation is considered to result from the action of endogenous proteinases (An *et al.* 1996), especially heat activated proteinases (Benjakul *et al.* 2003a). Heat activated alkaline proteinase in bigeye snapper classified as a serine proteinase contributed to degradation of gel texture at 60C (Benjakul *et al.* 2003a). The enzymes induced modori gel of lizardfish was characterized to be heat activated alkaline cysteine proteinase (Benjakul *et al.* 2003b). High molecular weight protein (101,000 daltons) of WPC was reported as the effective inhibitor toward papain and trypsin (Weerasinghe *et al.* 1996). For the kamaboko gel, the lower TCA-soluble peptide content was obtained in gels added with $CaCl_2$, compared with those without $CaCl_2$ ($P < 0.05$). With addition of 3% WPC, TCA-soluble peptide content of gel without and with $CaCl_2$ decreased by 54.5% and 68.6%, respectively, compared with that of the control gel. This suggested that $CaCl_2$ and WPC could function synergistically to inhibit protein degradation. $CaCl_2$ more likely induced protein cross-linking mediated by TGase, resulting in the resistance to proteolysis. Additionally, Ca^{2+} could bind protein substrates and caused the conformation changes, in which proteinases could not hydrolyze easily. Nevertheless, TCA-soluble peptide content was higher in modori gels containing $CaCl_2$ than those without $CaCl_2$ ($P < 0.05$). This reconfirmed that $CaCl_2$ might involve in gel softening mediated by proteinases. The addition of WPC, especially at higher levels, could lower protein degradation. The result was in

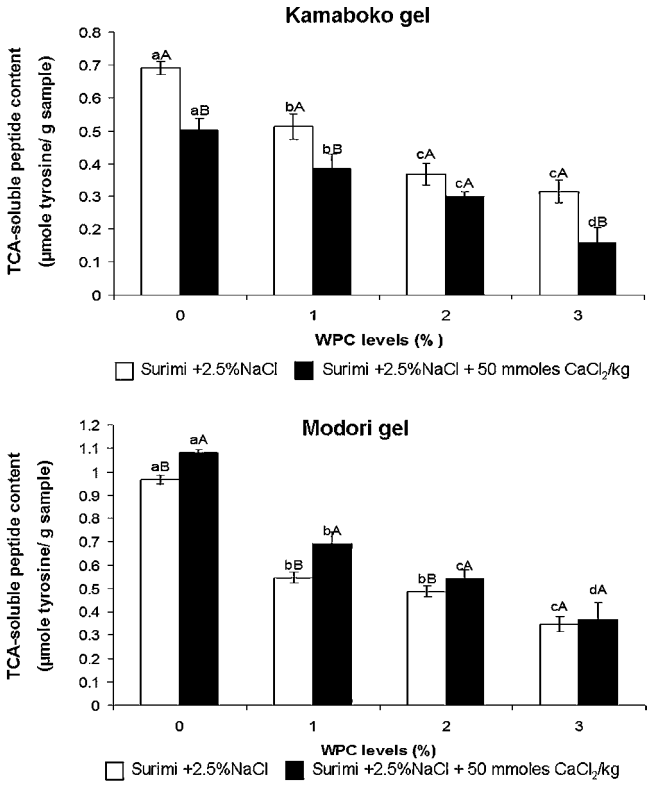

FIG. 2. TCA-SOLUBLE PEPTIDE CONTENT OF KAMABOKO AND MODORI GELS ADDED WITH WPC AT DIFFERENT LEVELS IN THE ABSENCE AND IN THE PRESENCE OF 50 mmol $CaCl_2$/kg

Bars represent the standard deviation from determinations. Different letters on the bars within the same $CaCl_2$ level indicate the significant differences ($P < 0.05$). Different capital letters on the bars within the same level of WPC indicate the significant differences ($P < 0.05$).
WPC, whey protein concentrate.

accordance with the increases in breaking force and deformation of the gels added with 3% WPC, irrespective of $CaCl_2$ addition (Fig. 1). The results reconfirmed that the improved gel strength of surimi was partially associated with lowering of proteolysis.

Protein Patterns of Surimi Gel. Protein patterns of all surimi gels are shown in Fig. 3. The intensive degradation of myosin heavy chain (MHC) was observed in the sample without the addition of WPC as indicated by the lowest MHC band intensity retained in both kamaboko and modori gels, regardless of $CaCl_2$ addition. However, no changes in actin were observed in all treatments.

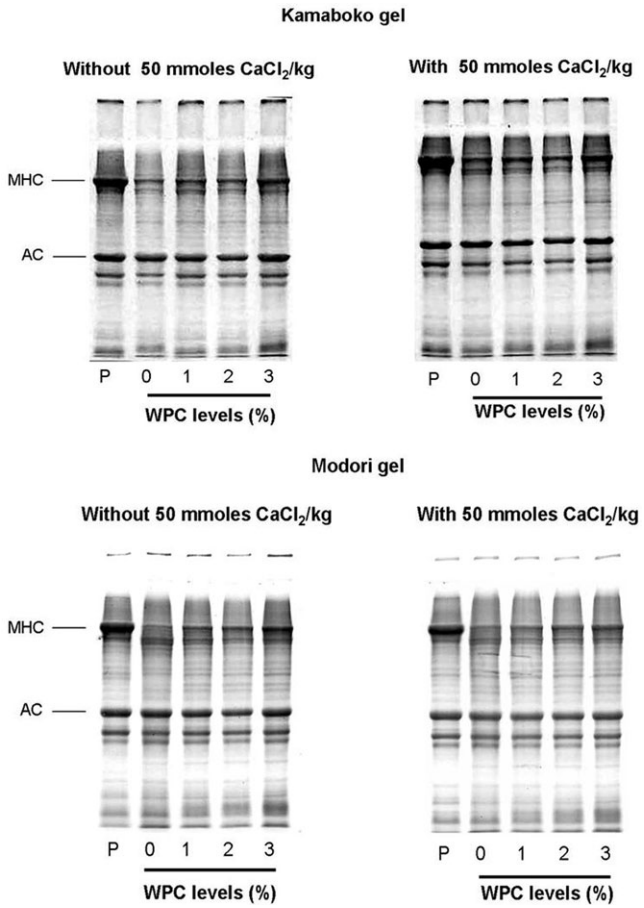

FIG. 3. PROTEIN PATTERN OF KAMABOKO AND MODORI GELS ADDED WITH WPC AT DIFFERENT LEVELS IN THE ABSENCE AND IN THE PRESENCE OF 50 mmol $CaCl_2$/kg
Numbers designate the amounts of WPC added (%).
P, surimi paste; MHC, myosin heavy chain; AC, actin; WPC, whey protein concentrate.

MHC band intensity became increased with increasing levels of WPC, indicating that WPC could inhibit the degradation of MHC to some extent. At a level of 3% WPC, which rendered the highest breaking force and deformation, the highest MHC intensity was observed. This suggested that the proteolysis of goatfish gel could be minimized when 3% WPC was used. For the control gels, MHC band intensity of kamaboko gel was slightly higher than that of modori gel. Degrading products with molecular weight lower than 200 kDa were found in the latter, indicating the greater protein degradation.

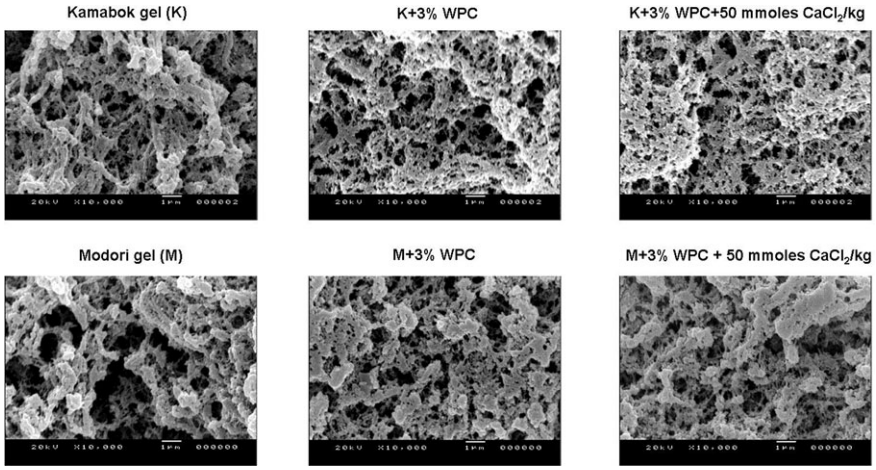

FIG. 4. MICROSTRUCTURES OF KAMABOKO AND MODORI GELS FROM GOATFISH SURIMI ADDED WITHOUT AND WITH 3% WPC IN THE ABSENCE AND IN THE PRESENCE OF 50 mmol $CaCl_2$/kg
Magnification ×10,000.
WPC, whey protein concentrate.

When comparing protein patterns between gels with and without $CaCl_2$ addition, no obvious differences were found at the same level of WPC added. Occurrence of higher molecular weight protein cross-links were observed in kamaboko gel, regardless of $CaCl_2$ addition. The formation of cross-linked proteins coincided with higher breaking force and deformation of surimi gel (Fig. 1). Conversely, lower protein polymerization took place in modori gels. Disappearance of MHC band in modori gel most likely resulted mainly from proteolysis. Additionally, TGase might be destabilized at 60C, an incubation temperature for modori gel preparation. The changes in MHC, either polymerization or degradation, which were found in kamaboko and modori gels, were mirrored by the increases and decreases in gel strength, respectively. Thus, the addition of WPC could prevent the degradation of proteins in goatfish surimi, allowing a stronger three-dimensional gel network to be formed.

Microstructure of Surimi Gel. The selected micrographs of different kamaboko and modori gels from goatfish surimi: (1) control gel; (2) gel added with 3% WPC; and (3) gel added with 3% WPC and 50 mmol $CaCl_2$/kg, visualized by SEM are shown in Fig. 4. The microstructure of kamaboko gel without WPC showed the higher interconnected three-dimensional protein network, compared with the modori gel. When 3% WPC was added, more compact structure with smaller voids was obtained, in comparison with the