CHAPTER 2

CHARACTERISTICS AND GEL PROPERTIES OF MUSCLES FROM SARDINE (*SARDINELLA GIBBOSA*) AND MACKEREL (*RASTRELLIGER KANAGURTA*) CAUGHT IN THAILAND

2.1 Abstract

Dark and ordinary muscle from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) were characterized. Lipid and myoglobin contents were higher in dark muscle than in ordinary muscle of both species, and higher contents of both constituents were found in sardine muscle than mackerel muscle. The extractable myoglobin contents in sardine dark and ordinary muscle were 14.27 and 2.18 mg/g, while mackerel dark and ordinary muscle contained 4.88 and 1.37 mg myoglobin/g sample, respectively. Alkali-soluble protein and stroma contents were greater in dark muscle than ordinary muscle. Mackerel muscle comprised a higher content of non-protein nitrogenous compounds than sardine muscle. The effect of washing conditions on the myoglobin extractability was investigated. A large amount of myoglobin was removed in the first washing cycle and only a small amount was removed in the second washing cycle. The highest removal of myoglobin from sardine (32.10-46.55%) and from mackerel muscle (103.20-313.66%) was achieved when the mince was washed with 0.2% NaCl and 0.5% NaCl, respectively. Washing media showed the marked effect on the color, expressible drip and textural properties of sardine and mackerel mince gels. The breaking force of directly heated and kamaboko gels from both sardine and mackerel mince washed with NaCl solution was higher than that of unwashed mince and water washed mince. However, no difference in deformation was observed. Washing also resulted in increased whiteness and lowered expressible moisture. In general, sardine surimi showed the superior gel-forming ability and whiteness to mackerel muscle.

2.2 Introduction

Dark muscle fish species currently make up 40-50% of the total fish catch in the world (Hultin and Kelleher, 2000a; 2000b). 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 high content of lipid and myoglobin. Those result in the difficulties in making high-quality surimi from those species (Chen, 2002; Ochiai *et al*., 2001). Due to the limited fish resources for surimi production, dark muscle fish have been paid more attention as a potential alternative raw material (Wu *et al*., 2000; Chen *et al*., 1997; Kelleher *et al*., 1994).

For surimi processing, myoglobin and hemoglobin play an essential role in the whiteness, which is one of factors determining the quality of surimi gel (Chen, 2002). Hemoglobin is lost rather easily during handling and storage, while myoglobin is retained by the muscle intracellular structure (Livingston and Brown, 1981). Therefore, most color changes in meat are due to the reaction of myoglobin with other muscle components, especially myofibrillar proteins (Hanan and Shaklai, 1995). In addition, dark muscle also has a higher proteolytic activity than white muscle (Shimizu *et al*., 1992). This can cause modori, the gel weakening, that occurs if the gel is held for an extended period at a temperature around 50-60°C. Ochiai *et al*. (2001) suggested that high-quality surimi with higher gel strength and better whiteness can be obtained when dark muscle is removed 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 color improvement and gel strengthening of surimi produced from whole muscle. The color of surimi can be improved by increasing the washing cycle, washing time, and water quantity (Kim *et al*., 1996). The color of dark-fleshed fish surimi could be improved by leaching of mince with hydrogen peroxide or sodium percarbonate, or by adding some fat/casein material to mask the color (Chen *et al*., 1997). Ozonized water could improve the color of horse mackerel mince within a short washing period (Chen *et al*., 1997). However, oxidation of the fish oil occurred during ozone treatment. Recently,

Chen (2002) reported that the lowest residual myoglobin content and best color improvement were found in the mince washed with air-flotation washing method. A new approach to obtain the functional protein isolates from dark muscle fish has been developed by Hultin and Kelleher (2000b). An acid or alkaline soluble process potentially overcomes some of the problems caused by the nature of the pelagic species (Undeland *et al*., 2002). The recovered proteins from these processes retain their functionality including their ability to form a gel.

So far, sardine and mackerel have been used for surimi production even to a small portion (Ochiai *et al*., 2001). However, no information regarding compositions as well as washing conditions affecting the color of washed mince from both species caught in Thailand has been reported. Therefore, this study aimed to investigate the chemical compositions and the effect of conventional washing media on removal of myoglobin, color of washed mince and gel properties from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscles.

2.3 Materials and Methods

Chemicals

Sodium dodecyl sulfate (SDS), dithiothreitol (DTT), β-mercaptoethanol (βME) were purchased from Sigma (St. Louise, MO, USA). Trichloroacetic acid was obtained from Merck (Darmstadt, Germany). Sodium dithionite was purchased from Riedel (Seeize, Germany). All chemicals were of analytical grade.

Fish samples

Sardine (*Sardinella gibbosa*) with an average weight of 55-60 g and mackerel (*Rastrelliger kanagurta*) with an average weight of 85-90 g were caught from Songkhla-Pattani 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 Department of Food Technology, Prince of Songkla University, Hat Yai within 2 h. The fish were immediately washed, filleted and manually excised into ordinary and dark muscles. The muscles were kept on

ice during preparation and analysis. The pH values of sardine and mackerel muscle were 6.53-6.58 and 6.24-6.27, respectively.

Compositional analysis

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

Electrophoresis

Protein patterns of protein fractions were analyzed on SDS-PAGE according to the method of Laemelli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution were added to the sample (3 g). The mixture was homogenized for 1 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The sample was centrifuged at 8,500×*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 and Hodgen, 1940), using bovine serum albumin as a standard. Protein samples were applied into the gel. After separation, the proteins were stained in 0.125% (w/v) Coomassie brilliant blue R-250 and destained in 25% (v/v) ethanol and 10% (v/v) acetic acid.

Myoglobin analysis

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 sec, followed by centrifuging at 3,000×*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 and Regenstein, 1992). The myoglobin content was expressed as mg/g sample.

Effect of washing conditions on myoglobin content and color of mince

To prepare fish mince, fish fillets were minced to uniformity using a mincer (a diameter of 4 mm). The mince was subjected to 1 or 2 washing cycles using different washing media (0, 0.2, 0.5, and 1.0 % NaCl (w/v) at 4°C with a solution/mince ratio of 3:1 (v/w)). The mixtures with the pH ranges of 5.43-5.50 were stirred gently for 5 min in a walk-in cold room (4° C) and centrifuged at 1,000×*g* for 5 min. Wash water was measured for myoglobin content. Redness index (*a**/*b**) of washed mince was determined according to the method of Chen *et al*. (1997).

Effect of washing conditions on gel forming abilities of sardine and mackerel mince

Fish mince prepared as mentioned above was washed with cold distilled water (5°C) or cold NaCl solution (0.2% NaCl (w/v) for sardine and 0.5% NaCl (w/v) for mackerel) using a washing media/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 for three times. Finally, the washed mince was centrifuged at 700 *Xg* for 15 min using a basket centrifuge (Model CE 21K, 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.

To prepare the gels, the frozen samples were thawed at 4°C, cut into small pieces and the moisture content was adjusted to 80%. The samples were added with 2.5% 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. Directly heated gels were prepared by heating the sol at 90°C for 20 min. Kamaboko gels were prepared by incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. The gels were cooled in iced water and stored for 24 h at 4°C prior to analysis.

Texture analysis

Texture analysis of the gels was performed using a TA-XT2i texture analyzer (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 analyzer equipped with a cylindrical plunger (diameter 5 mm; depression speed 60 $mm.min^{-1}$).

Determination of whiteness

Surimi gel color 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:

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

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 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.

Statistical analysis

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

2.4. Results and Discussion

Chemical compositions and myoglobin in sardine and mackerel muscles

Dark and ordinary muscles from sardine and mackerel were similar in composition (Table 1). As a major constituent in fish muscle, protein accounted for approximately 13.74-17.54%. Crude protein content in the muscle was usually in the broad range

of 11-24% (wet weight), depending on the species and variety, the state of nutrition, and the reproductive cycle of the animals, as well as the parts of the organisms (Sikorski, 1994; Spinelli and Dassow, 1982). Lipid content was generally higher in dark muscle. Sardine dark muscle contained 4.8 times greater lipid content than mackerel muscle. Dark muscles were especially rich in chromoproteins and contained about two to five times more lipids than the ordinary muscles (Sikorski *et al*., 1990). The dark muscle is normally concentrated along the lateral line of the body and may represent 15-30% of the total muscle in migratory fish such as mackerel and 2-12% of less active fish (Haard, 1992). Moisture content in ordinary muscle of both species was generally higher than that in dark muscle. Nevertheless, similar ash content was observed among all muscles from both species.

Table 1. Proximate compositions of sardine and mackerel muscles

Compositions (%wet wt.)	Sardine		Mackerel	
	Dark	Ordinary	Dark	Ordinary
Protein	14.64±1.26[a]	13.74±1.15	17.14±0.68	17.54±1.27
Lipid	4.77±0.38	0.52±0.20	0.99±0.43	0.27±0.22
Moisture	78.88±0.24	81.57±0.05	78.93±0.40	79.37±0.32
Ash	1.65±0.16	1.35±0.01	1.34±0.07	1.32±0.01
Myoglobin[b]	14.27±0.47	2.18±0.28	4.88±0.08	1.37±0.46

[a]Values are given as mean ± SD from triplicate determinations.

[b]mg/g sample.

Different extractable myoglobin content was observed between different muscle types and species (Table 1). Sardine muscle contained a larger amount of extractable myoglobin, especially in dark muscle, when compared to mackerel muscle. The results were in agreement with Spinelli and Dassow (1982) and Hashimoto *et al*. (1979) who reported that myoglobin was dominant in dark muscle. Dark muscle, both superficial and deep-seated, contained more

hemoglobin, myoglobin and cytochrome *c* than the ordinary muscles (Sikorski *et al*., 1990). Muscle of yellowfin tuna contained myoglobin ranging from 37 to 128 mg/100 g in light muscle and 530 to 2,440 mg/100 g in dark muscle (Brown, 1961). Sardine dark muscle comprised myoglobin 2.9 times greater than mackerel dark muscle. For ordinary muscle, sardine contained myoglobin 1.6 times higher than mackerel. Myoglobin contributed to the redness of muscle, which is associated with the lowered whiteness of surimi gel (Chen, 2002; Hultin and Kelleher, 2000a; 2000b).

Nitrogenous compositions in sardine and mackerel muscles

Proteins in dark and ordinary muscles from both species were classified into five fractions based on solubility (Table 2). Myofibrillar protein was found as a major protein component for both muscle types and species (41.6-69.9%). Myofibrillar proteins were the dominant proteins, which are involved in muscle contraction (Sikorski, 1994). Electrophoretic patterns indicated that myofibrillar fraction consisted of several protein bands corresponding to myosin heavy chain (MHC), actin, troponin and tropomyosin (data not shown). MHC was a major protein in myofibrillar fraction. The result was in agreement with Hashimoto *et al*. (1979) who found that MHC showed the highest band intensity in the myofibrillar fraction from sardine (*Sardinops melanosticta*) and mackerel (*Pneumatophorus japonicus japonicus*) muscle. Similar results were found in muscle from two species of bigeye snapper (Benjakul *et al*., 2002). For the same type of muscle, both species contained a similar amount of sarcoplasmic proteins. However, dark muscle consisted of a greater content of sarcoplasmic protein, compared to ordinary muscle.

Table 2. Nitrogenous constituents in sardine and mackerel muscles

Compositions	Sardine		Mackerel	
(mgN/g muscle)	Dark	Ordinary	Dark	Ordinary
Non-protein nitrogen	4.84±0.76[a]	5.33±0.58	7.10±0.76	7.85±0.00

Sarcoplasmic protein	8.16±0.37	6.79±0.56	8.39±0.69	7.98±0.35
Myofibrillar protein	16.69±2.39	17.60±1.86	24.40±2.99	25.89±2.30
Alkali soluble protein	13.15±0.45	10.88±0.82	6.60±0.19	2.77±0.25
Stromal protein	2.09±0.05	0.95±0.16	1.05±0.03	0.37±0.04

[a]Values are given as mean ± SD from triplicate determinations.

Haard *et al*. (1994) suggested that the sarcoplasmic proteins from fish included myoglobin, enzymes and other albumins. The content of sarcoplasmic protein was generally high in pelagic fish, such as sardine and mackerel (Haard *et al*., 1994; Hashimoto *et al*., 1979). For both species, dark muscles had a larger amount of alkali soluble protein and stroma than ordinary muscle. Greater stroma content in dark muscle could be related to the high mechanical strength of this muscle (Hultin and Kelleher, 2000a; 2000b). Mackerel muscle was composed of a higher content of non-protein nitrogenous compounds than sardine muscle. This might be due to a higher content of amino acids, dipeptide, nucleotide, trimethylamine, urea, and the products of postmortem changes in this species (Foegeding *et al*., 1996; Sikorski, 1994). For the same species, dark muscle contained lower amount of non-protein nitrogenous compounds than ordinary muscle.

Effect of washing conditions on myoglobin removal and color of mince

Myoglobin was removed to a great extent with the first washing cycle and only a small amount was removed with the second washing cycle (Figure 6). The highest amount of myoglobin from sardine was removed when the mince was washed with 0.2% NaCl (P<0.05). For mackerel muscle, 0.5% NaCl was shown to be the most effective washing media for myoglobin removal (P<0.05). The difference in NaCl concentration needed between two species might be due to the different interaction or bonding between myoglobin and muscle. NaCl could weaken those bonds, leading to the release of myoglobin from the muscle. As Na^+ and Cl^- are bound to acidic and basic amino acid residues, intermolecular ionic bonds among protein molecules are broken (Lee, 1992). As a result, more myoglobin was removed from the muscle. From the result, washing with 0.2% NaCl removed 32.10 and 46.55% of myoglobin from sardine dark and

ordinary muscle, whereas 0.5% NaCl removed 103.20 and 313.66% of myoglobin from mackerel dark and ordinary muscle. From the result, more than 100% of total extractable myoglobin was removed from the mackerel ordinary muscle, especially with 0.2% and 0.5% NaCl solution. Since mackerel ordinary muscle contained a low content of lipid, myoglobin might bind with muscle proteins more tightly by ionic bonds. Those bonds were more weakened by 0.2% or 0.5% NaCl solution, compared with phosphate buffer used for total myoglobin determination. As a consequence, the greater amount of myoglobin was extracted from mackerel ordinary muscle as evidenced by more than 100% extractable myoglobin. However, a decrease in total myoglobin removed was observed when 1.0% NaCl was used. This might be due to the denaturation of myoglobin at high salt concentration. Denatured myoglobin possibly interacted with muscle

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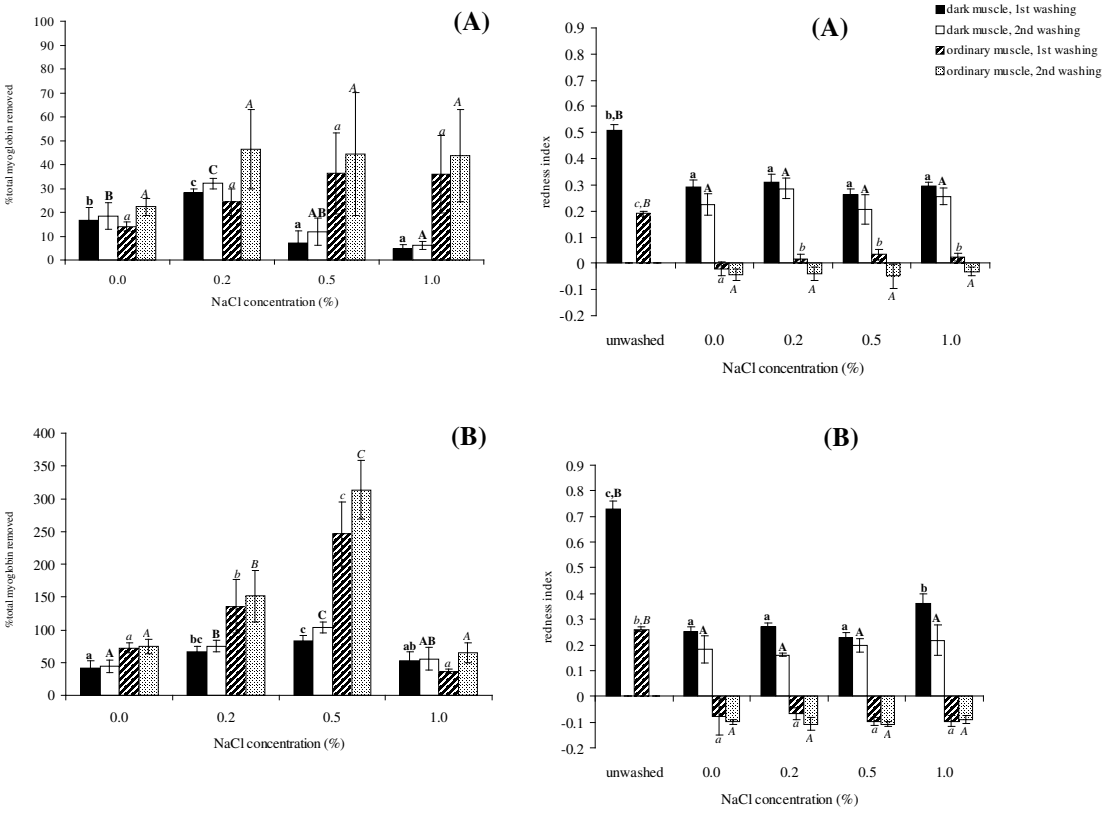

Figure 6. %Myoglobin removed and redness index of washed mince from sardine (A) and mackerel (B) prepared with different washing conditions. The myoglobin in the muscle samples was extracted with 40 mM phosphate buffer, pH 6.8 and myoglobin content was then determined by direct spectrophotometric method. *Bars indicate standard deviation from triplicate determinations. **Different letters and capital letters among

dark muscle with 1st washing and 2nd washing, respectively, indicate significant differences (P<0.05). Different italic letters and capital italic letters among ordinary muscle with 1st washing and 2nd washing, respectively, indicate significant differences (P<0.05).

Hultin and Keheller (2000a; 2000b) reported that some sarcoplasmic proteins, although soluble in a solvent, may not be extractable from muscle tissue. Those proteins may bind to muscle subcellular structures or they may be located in cellular compartments, which can not be reached by the solvent, such as the mitrochondrial matrix (Hultin and Keheller, 2000a; 2000b). Dark muscle fibers have greater quantities of mitochondria, myoglobin, fats, glycogen and cytochromes and have a more abundant vascular supply (Kisia, 1996). Myoglobin may be localized in close proximity to mitochondria and had a greater affinity for mitochondrial, structural protein, than did cytochrome *c* (Gidding, 1974). This could contribute to the difficulties in myoglobin removal from the muscle. Although myoglobin content in sardine dark muscle was greater than that in mackerel dark muscle, the redness index of unwashed mince in the former was lower than that of the latter. This might be due to the higher oxidation of myoglobin in sardine mince, especially during mincing process, resulting in the higher formation of metmyoglobin. Additionally, sardine dark muscle contained the higher fat content than mackerel dark muscle. Sardine and mackerel lipids are rich in polyunsaturated fatty acids, which are highly susceptible to oxidation (Ohshima *et al*., 1988). Therefore, the higher oxidation might occur in sardine muscle. Lipid oxidation products were prooxidative towards oxymyoglobin (Faustman *et al*., 1999). Free radicals generated during lipid oxidation also initiate oxymyoglobin oxidation (Yin and Faustman, 1994). Thus, oxidation of myoglobin was postulated to occur, leading to the decreased redness index in sardine dark muscle.

Washed mince had the lowered redness index than unwashed mince. Generally, no marked differences in redness index were observed among the samples washed with different NaCl concentrations. With 1.0% NaCl, mackerel dark muscle had the higher redness than those washed with the lower NaCl concentrations. This was probably due to the insolubilization of myoglobin at high NaCl concentration, leading to the tight association of myoglobin with the muscle proteins. This was coincidental with the lower removed myoglobin content at this NaCl

concentration. Chen *et al*. (1998) showed that myoglobin extracting efficiency was strongly dependent on the pH values of washing media used. The pH value of sardine muscle homogenate washed with 0.2% NaCl solution was in the range of 6.51-6.54, while the mackerel muscle homogenate washed with 0.5% NaCl solution had the pH of 6.17. Mackerel and sardine myoglobins had the isoelectric point (p*I*) of 5.8-5.9 (Yamaguchi *et al*., 1979). Since the pH of washing media was quite close to p*I* of myoglobin, the solubility of myoglobin at pHs used in this study might be lower than that at acidic or alkaline pHs. Therefore, acidic or alkaline solublization process (Undeland *et al*., 2002) would be an effective means to decrease the redness of fish mince caused by myoglobin, a main pigment in the dark-fleshed fish.

Effect of washing conditions on gel forming abilities of sardine and mackerel mince

The breaking force and deformation of sardine and mackerel mince and washed mince gels prepared with two different heating conditions are depicted in Figure 7. With washing process used, the yield of 30-32% was obtained. Generally, breaking force of directly heated gels and kamaboko gels from mince washed with NaCl solution was higher than that of gels from mince washed with water ($P<0.05$). However, deformation was not different ($P>0.05$). From the result, sardine mince gels showed a greater breaking force than mackerel mince gels. With the appropriate washing, sarcoplasmic proteins could be removed, resulting in the concentrated myofibrillar proteins, which play an essential role in gel formation. Small quantities of sarcoplasmic proteins can have an adverse effect on the strength and deformability of myofibril protein gels (Hultin and Kelleher, 2000a; 2000b; Haard *et al*., 1994). These proteins may interfere with myosin cross-linking during gel matrix formation because they do not form gels and have poorer water holding capacity according to Sikorski (1994). The presence of sarcoplasmic proteins may also change the rheological properties of the fish gels. Some sarcoplasmic proteins may be bound to the myofibrils during the heat treatment, thus decreasing the strength of the gel (Sikorski, 1994). When comparing the properties of gels, it was noted that mince and washed

mince from sardine had a higher breaking force. However, no difference in deformation of gels between these two species was observed. With direct heating, breaking force of sardine mince and washed mince was 41.89-55.28% higher than that of mackerel. For kamaboko gel, those from sardine mince and washed mince were 43.25-70.05% higher in breaking force than those from mackerel. The differences in gel forming ability might result from the differences in protein integrity and bonding formed during thermal process (Benjakul *et al*., 2001). The gel-forming ability of dark muscle has been known to be lower than that of ordinary muscle. This apparently resulted from the differences in the unfolding abilities and thermal stability of myosin between those two muscles (Lo *et al*., 1991). Difference in heat-activated proteolysis possibly contributed to the different gel forming ability between two species. Alvarez *et al*. (1999) reported that thermal gel degradation (modori) in sardine (*Sardina pilchardus*) surimi gels occurred when incubated at 50 and 60°C.

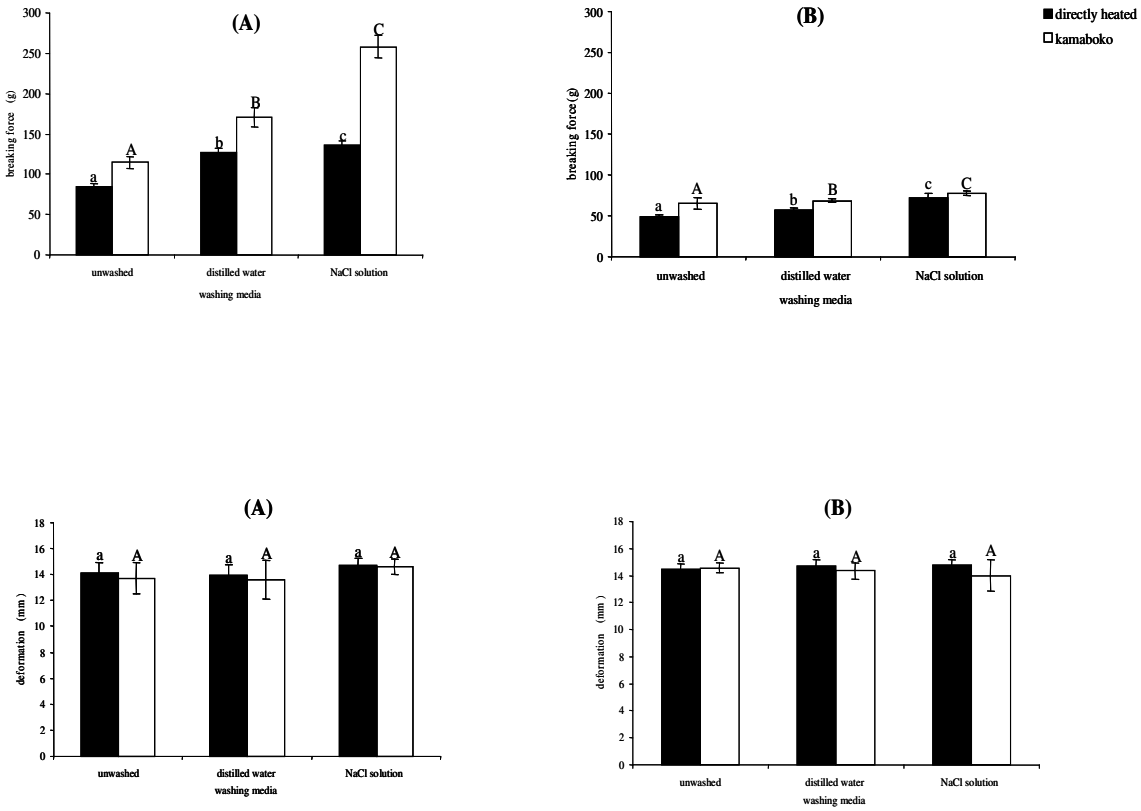

Figure 7. Breaking force and deformation of gels from sardine (A) and mackerel (B) mince and mince washed with different washing media and heating conditions. *Bars indicated standard deviation from seven determinations. Kamaboko gels were prepared by

incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. Directly heated gels were prepared by heating the sol at 90°C for 20 min. **Different letters under the same heating condition indicate significant differences (P<0.05).

When comparing the breaking force of directly heated and kamaboko gels from mince and mince washed with water or NaCl solution, it was found that the former showed a lower breaking force than the latter. The result indicated that protein-protein interactions in kamaboko gels were established during heating, which strengthened the network previously formed by setting at 40°C. Alvarez and Tejada (1997) reported that sardine (*Sardina pilchardus*) kamaboko gels (35°C for 30 min, followed by heating at 90°C for 30 min) had much higher gel strength than the corresponding suwari gels (35°C for 30 min). Additionally, endogenous transglutaminase might play a role in protein cross-linking during setting, resulting in the enhanced gel strength (Benjakul and Visessanguan, 2003). Benjakul *et al*. (2003) suggested that setting at different temperatures might lead to different gel characteristics, especially with different fish species. Gel strength of surimi can be increased by subjecting surimi sol to setting below 40°C prior to cooking (An *et al*., 1996). Gelation of fish paste during setting has been reported to have a close relationship to the formation of cross-linking between myosin heavy chains induced by endogenous transglutaminase (Benjakul and Visessanguan, 2003; Kumazawa *et al*., 1995). Setting occured to a higher extent in sardine mince and washed mince, compared to those from mackerel as evidenced by the much higher increase in breaking force of sardine kamaboko gels with prior setting. It was postulated that sardine might contain a higher endogenous transglutaminase than mackerel. Also, more unfolding and rearrangement of sardine proteins during setting probably resulted in the better setting of sardine muscle.

Breaking force of the kamaboko gel from sardine mince washed with NaCl solution was 47.17% greater than that of directly heated gels. With prior setting, a lower increase in breaking force was found in unwashed mince and distilled water washed mince, compared with NaCl washed mince. The same trend was observed in mackerel samples. However, only 5.73% increase in breaking force was obtained in NaCl washed mackerel mince with two-step heating (kamaboko gel), compared to that with one-step heating (directly heated gel). Washing with NaCl

solution could improve the gel strength of cod and flounder muscles, as indicated by the increase in fold test scores. However no influence of NaCl solution on gel strength of red hake muscle was observed (Hennigar *et al*., 1988). The endogenous transglutaminase in sardine muscle might associate with muscle more strongly than that in mackerel. Furthermore, NaCl probably weakened the enzyme binding in mackerel muscle more effectively than in sardine. As a consequence, lower setting phenomenon was observed in NaCl washed mince from mackerel, compared with unwashed mince or water washed mince.

Whiteness and expressible moisture of the gel from sardine and mackerel mince and washed mince are shown in Table 3. The higher whiteness was found in gels from washed mince, compared with that of unwashed mince. Gels from sardine generally showed a higher whiteness than those from mackerel. For both directly heated gels and kamaboko gels from sardine, those prepared from water washed mince showed a greater whiteness than NaCl solution washed mince. However, no difference in whiteness between gels of water washed mince and NaCl solution washed mince was found in mackerel gels. NaCl might exhibit prooxidative activity with the following mechanisms: alterations in the reactivity of iron by the chloride ion (Osinchak *et al*., 1992) or the displacement of iron from macromolecules by the sodium ion (Kanner *et al*., 1991). Due to the higher content of myoglobin and lipid in sardine muscle, the greater oxidation enhanced by NaCl might take place in this species, compared with mackerel muscle. Metmyoglobin formation, caused by oxidation process, possibly resulted in the darker color in sardine mince washed with NaCl solution.

Table 3. Whiteness and expressible drip of sardine and mackerel surimi gels prepared with different washing media and heating conditions

Species	Heating conditions	Washing media	Whiteness[a]	Expressible drip (%)[a]
Sardine	Directly heated	Unwashed	59.68±0.06a[b]	23.83±1.33b
		Distilled water	63.32±0.01c	8.60±1.72a
		NaCl solution	62.88±0.10b	7.53±3.57a
	Kamaboko	Unwashed	60.17±0.01a	17.46±1.33b

		Distilled water	63.47±0.02c	6.20±1.31a
		NaCl solution	62.25±0.05b	4.00±0.76a
Mackerel	Directly heated	Unwashed	57.58±0.27a	19.51±4.53b
		Distilled water	59.45±0.43b	12.59±3.92ab
		NaCl solution	59.85±0.07b	5.47±2.02a
	Kamaboko	Unwashed	57.80±0.10a	9.59±1.64b
		Distilled water	59.94±0.50b	10.70±1.51b
		NaCl solution	60.38±0.23b	5.52±1.25a

[a]Values are given as mean ± SD from triplicate determinations. Kamaboko gels were prepared by incubating the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. Directly heated gels were prepared by heating the sol at 90°C for 20 min.

[b]Different letters in the same column under the same species and heating condition indicate significant differences ($P<0.05$).

Expressible moisture was lower in gels from washed mince, compared with those of unwashed mince. Among all samples, NaCl washed mince had the lowest expressible moisture. Marked decrease in expressible moisture was found in kamaboko gel from NaCl washed mince. Nevertheless, no differences in expressible moisture were found between directly heated and kamaboko gels of NaCl washed mince from mackerel. In general, the lower expressible moisture was coincidental with the increased breaking force. Different expressible moisture suggested the difference in water holding capacity of gel network. Directly heated gels showed a higher expressible moisture than that the kamaboko gels, indicating that protein network of the former was lower in water binding properties (Niwa, 1992). 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). Therefore, washing water containing an appropriate amount of NaCl could be used to improve gel-forming ability and whiteness of sardine and mackerel mince. However, these conventional washing methods were not completely improved the gel properties from both species. Hence, an alkaline solubilization process as described by Hultin and Kelleher (2000a; 2000b) is used in our laboratory to improve the color and gel-forming ability of sardine and mackerel surimi.

2.5 Conclusion

The compositions of dark and ordinary muscles from sardine and mackerel were different. Myoglobin was higher in dark muscle, however, it could be removed with washing, particularly with increasing NaCl concentration and washing cycle. In general, sardine washed mince or surimi gel showed a better color and textural properties than that of mackerel.