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Properties of a protein-based film from round scad (*Decapterus maruadsi*) as affected by muscle types and washing

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Abstract

The effects of muscle types and washing on the properties of a protein-based film from round scad (*Decapterus maruadsi*) mince were investigated. Washing resulted in an increase in the protein content with a coincidental decrease in the fat content of mince, especially from whole muscle and dark muscle. Among all types of muscle (ordinary, dark and whole muscle), the ordinary muscle rendered the film with the highest tensile strength (TS) ($p < 0.05$). TS of films from washed mince was greater than that of films prepared from unwashed mince for the same type of muscle used ($p < 0.05$). Nevertheless, the water vapour permeability (WVP) of films from unwashed mince was higher than that of films prepared from washed mince ($p < 0.05$). Films from washed mince had higher solubility but lower protein solubility than those from unwashed mince ($p < 0.05$). Regardless of washing, films from ordinary muscle showed the highest L^*-value ($p < 0.05$). However, films prepared from dark muscle were more yellowish than those prepared from other muscles, as evidenced by the greater b^*-value. Films from round scad mince and washed mince had excellent barrier properties to UV light at the wavelength of 200–280 nm. Generally, films from round scad mince had a lower preventive effect on visible light transmission than had those from unwashed mince. Among films from all muscles, those prepared from dark muscle exhibited the highest barrier to visible light transmission ($p < 0.05$). Therefore, the properties of films from round scad meat were governed by muscle type as well as by washing.

Keywords: Protein film; Round scad; Muscle; Protein; Washing; Fish

1. Introduction

Nowadays, film and coating from biopolymers have received increasing attention. Synthetic packaging films have led to serious ecological problems due to their non-biodegradability. Therefore, biopolymers can be another source for packaging development, as biodegradable materials. The important functional characteristic of an edible film and coating is to retard the migration of moisture, oxygen, carbon dioxide, microbes or solutes, as well as to prevent collapse of products (Greener & Fennema, 1994). Fish proteins, including myofibrillar and sarcoplasmic proteins, have been used as film-forming material (Iwata, Ishizaki, Handa, & Tanaka, 2000; Paschoalick, Garcia, Sobral, & Habitante, 2003; Shiku, Hamaguchi, & Tanaka, 2003; Shiku, Hamaguchi, Benjakul, Visessanguan, & Tanaka, 2004). Plasticizers are necessarily used in myofibrillar and sarcoplasmic protein films. They reduce brittleness by reducing the intermolecular interactions between adjacent chains of the polymers (Martelli, Moore, Paes, Gandolfo, & Laurindo, 2006).

Dark muscle fish species currently make up 40–50% of the total fish catch in the world (Hultin & Kelleher, 2000). There is great interest in using large quantities of these low-value fatty pelagic fish to produce new products with a higher market value, such as surimi. However, production

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of surimi from the small pelagic species causes difficulty in making high quality surimi (Chen, 2002). Chaijan, Benjakul, Visessanguan, and Faustman (2004) reported that surimi produced from mackerel and sardine exhibited poor gel quality. Washing has been used widely in the surimi industry to remove small molecular-weight proteins and to concentrate myofibrillar proteins, which are most likely essential for gel network formation (Morioka, Nishimura, Obatake, & Shimizu, 1997). However, no information regarding the effect of washing on the properties of fish muscle protein-based films has been reported. To fully utilize those species, the edible/biodegradable films from dark muscle fish species can be an alternative for obtaining new value-added products from those species. Different fish muscles with various constituents may affect the characteristics of resulting films differently. Additionally, washing of muscle can affect the film properties. The objective of this investigation was to study the effect of muscle type and the washing process on the properties of protein-based film from the meat of round scad (*Decapterus maruadsi*), an abundant pelagic species in Thailand.

2. Material and methods

2.1. Chemicals

Sodium chloride (NaCl), sodium dodecylsulfate (SDS), glycerol and β-mercaptoethanol (βME) were purchased from Sigma (St. Louis, MO, USA). Acrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and bis-acrylamide were obtained from Fluka (Buchs, Switzerland).

2.2. Fish samples

Round scad (*D. maruadsi*), with an average weight of 85–90 g/fish, were obtained from Songkhla–Pattani coast along the Gulf of Thailand. The 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 within 2 h. Upon the arrival, fish were immediately washed, filleted and manually excised into ordinary, dark and whole muscles. All muscles were kept on ice until used.

2.3. Preparation of mince and washed mince

To prepare fish mince, fish meat from different muscles was minced to uniformity, using a mincer with a hole diameter of 0.5 cm. Washed mince was prepared according to the method of Benjakul, Leelapongwattana, and Visessanguan (2003). Fish mince was homogenised with 5 vol. of cold 0.05 M NaCl (2–4 °C) at a speed of 13,000 rpm for 2 min, using an IKA Labortechnik homogeniser (Selangor, Malaysia), followed by centrifuging at 9600*g* for 10 min at 4 °C, using a refrigerated centrifuge (Model RC-B Plus centrifuge Newtown, CT, USA). The washing process was repeated twice. Mince, and the washed mince obtained, were stored on ice until used for analysis or for film preparation. Compositional analysis (protein, ash, fat and moisture contents) of mince and washed mince was carried out according to the methods of AOAC (1999).

2.4. Preparation of film-forming solution

The film-forming solution from mince and from washed mince was prepared according to the method of Chinabhark, Benjakul, and Prodpran (2007). The mince or washed mince (200 g) from different muscles was added to 3 vol. of distilled water and homogenised at 13,000 rpm for 1 min, using a homogeniser (IKA Labortechnik, Malaysia). The protein concentration of the film-forming solution was fixed at 2% (w/v). Glycerol, used as a plasticizer, was added at 50% (w/w) of protein. The mixtures were stirred gently for 30 min at room temperature. Subsequently, the pH of the film-forming solution was adjusted to 3, using 1 M HCl. The film-forming solution obtained was filtered through a layer of nylon sheet. The filtrate was used for film casting.

2.5. Film casting and drying

The film-forming solution (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm) and air-blown for 12 h at room temperature prior to further drying at 25 °C and 50% relative humidity (RH) for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and used for analyses.

2.6. Determination of film properties

2.6.1. Film thickness

The thickness of film was measured using a micrometer (Gotech, Model GT-313-A, Gotech testing machines Inc, Tawai). Five random locations around each film of ten film samples were used for thickness determination.

2.6.2. Mechanical properties

Prior to testing the mechanical properties, films were conditioned for 48 h at 50 ± 5% relative humidity (RH) at 25 °C. Tensile strength (TS) and elongation at break (EAB) were determined as described by Iwata et al. (2000) with a slight modification using the Universal Testing Machine (Lloyd Instrument, Hampshire, UK). Five samples (2 × 5 cm) with initial grip length of 3 cm were used for testing. Cross-head speed was set at 30 mm/s.

2.6.3. Water vapour permeability (WVP)

WVP was measured, using a modified ASTM method (American Society for Testing & Materials, 1989) as described by Shiku et al. (2004). The film was sealed on a glass permeation cup containing silica gel (0% RH) with silicone vacuum grease and a rubber band to hold the film in place. The cups were placed in a desiccator containing the distilled water at 30 °C. The cups were weighed at 1 h intervals over a 10 h period. WVP of the film was calculated as follows (McHugh, Avena-Bustillos, & Krochta, 1993):

$$\mathrm{WVP}(\mathrm{g\ m^{-1}\ s^{-1}\ Pa^{-1}}) = wxA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

where w is the weight gain of the cup (g); x is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $(P_2 - P_1)^{-1}$ is the vapour pressure difference across the film (Pa). Three films were used for WVP testing and the measurement was run in duplicate.

2.6.4. Colour, light transmission and film transparency

Colour of the film was determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA) and expressed as L^*, a^* and b^*. The ultraviolet (UV) and visible light barrier properties of the films were measured at selected wavelengths between 200 and 800 nm, using the UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan) as described by Fang, Tung, Britt, Yada, and Dalgleish (2002). The transparency of film was calculated by the following equation (Han & Floros, 1997):

$$\mathrm{Transparency} = -\log T_{600}/x$$

where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm).

2.6.5. Film solubility and protein solubility

Film solubility was determined according to the method of Gennadios, Handa, Froning, Weller, and Hanna (1998). The conditioned film samples (2 × 5 cm) were weighed and placed in a 50 ml centrifuge tube containing 10 ml of distilled water with 0.1% (w/v) sodium azide. The mixture was shaken at a speed of 250 rpm using a shaker (Heidolth Inkubator 10000, Schwabach, Germany) at 30 °C for 24 h. Undissolved debris was removed by centrifugation at 3000*g* for 20 min. The pellet was dried at 105 °C for 24 h and weighed. The weight of solubilised dry matter was calculated by substracting the weight of unsolubilised dry matter from the initial weight of dry matter and expressed as a percentage of the total weight.

To determine the protein solubility, the protein concentration in the supernatant was determined using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Protein solubility was expressed as the percentage of total protein in the film, which was solubilised with 0.5 M NaOH at 30 °C for 24 h.

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

Protein patterns of different muscles and their films were determined by SDS–PAGE, using 4% stacking gel and 10% running gel according to the method of Laemmli (1970). Muscles (3 g) were solubilised in 27 ml of 5% SDS. The mixture was homogenised for 1 min at a speed of 13,000 rpm, using an IKA homogenizer and incubating at 85 °C for 1 h to dissolve total proteins. To solubilise the films prior to SDS–PAGE analysis, films were mixed with 20 mM Tris–HCl (pH 8.8) containing 2% SDS and 8 M urea in the presence and the absence of 2% βME. The mixture was homogenised at 13,000 rpm for 1 min. The homogenate was stirred continuously for 24 h at room temperature (28–30 °C). Then, the sample was centrifuged at 7500*g* for 10 min at room temperature, using a Biofuge primo Centrifuge (Sorvall, Hanau, Germany). The solubilised muscle samples were mixed with the sample buffer containing βME, while the solubilised film samples were mixed with sample buffer without βME. Proteins (15 μg) determined by the Biuret method (Robinson & Hodgen, 1940) were loaded onto the gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protean 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% (v/v) methanol and 7.5% (v/v) acetic acid for 12 min, followed by 5% (v/v) methanol and 7.5% (v/v) acetic acid for 3 h.

2.7. Statistical analysis

Analysis of variance (ANOVA) was performed and mean comparison was carried out by Duncan's multiple range test (Steel & Torrie, 1980). Analysis was performed using the SPSS package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Chemical composition of mackerel mince and washed mince

Mackerel mince from different muscles, ordinary, dark and whole muscles, had moisture contents in the range of 75.30–76.63% and protein contents ranging from 16.2% to 19.0% (Table 1). Among all muscles tested, dark muscle had a lower protein content, but higher fat content, than had ordinary and whole muscles ($p < 0.05$). However, no differences in ash content were noticeable among all muscles ($p > 0.05$). Crude protein content in fish flesh varies, depending on the species, the nutritional condition, the state of nutrition, the productive cycle, as well as the parts of fish (Sikorski, Kolakowska, & Pan, 1990; Sikorski, 1994). Lipid content was generally higher in dark muscles, which contained about 2–5 times more lipids than did the ordinary muscle (Sikorski et al., 1990). After washing, the washed mince of all muscles generally had an increased moisture content, leading to a dilution of other constituents. For dark muscle and whole muscle, a marked increase in protein content with a coincidental decrease in lipid content was observed after washing. During washing, sarcoplasmic proteins and other components, including fat or inorganic substances, could be removed (Hultin & Kelleher, 2000). Since a large amount of fat was leached out from dark and whole muscles, the protein content (dry basis) was concomitantly increased from 65.6% to 71.1% for dark muscle and from 78.2% to 82.6% for whole muscle. Nevertheless, no difference in protein content (dry

Table 1
Proximate compositions of round scad mince and washed mince of different muscle types

Samples	Muscle types	Compositions (% wet wt. basis)[A]			
		Moisture	Protein	Fat	Ash
Mince	Ordinary	76.63 ± 0.33^{c}	19.0 ± 0.29^{a}	1.32 ± 0.06^{d}	1.29 ± 0.07^{a}
	Dark	75.30 ± 0.69^{d}	16.2 ± 0.10^{b}	5.23 ± 0.50^{a}	1.20 ± 0.02^{a}
	Whole	76.34 ± 0.69^{c}	18.5 ± 0.25^{a}	2.20 ± 0.10^{c}	1.28 ± 0.09^{a}
Washed mince	Ordinary	84.97 ± 0.07^{a}	12.3 ± 0.12^{c}	0.50 ± 0.07^{e}	0.42 ± 0.07^{b}
	Dark	83.91 ± 0.73^{b}	11.4 ± 0.18^{d}	3.40 ± 0.22^{b}	0.44 ± 0.06^{b}
	Whole	84.42 ± 0.54^{ab}	12.9 ± 0.79^{c}	0.60 ± 0.32^{e}	0.36 ± 0.04^{b}

The same superscript in the same column indicates non-significant difference ($p > 0.05$).
[A] Means ± SD from triplicate determinations.

basis) was observed between mince (81.3%) and washed mince (81.7%) from ordinary muscle.

3.2. Mechanical properties of protein-based film

Mechanical and physical properties of films from different types of muscles without and with washing are shown in Table 2. All films had similar thicknesses (0.036–0.040 mm). Tensile strength (TS) of protein-based films varied with types of muscle (ordinary, dark and whole muscle). Among all muscles, the ordinary muscle rendered the film with the highest TS ($p < 0.05$). From the result, films from whole and dark muscles showed no differences in TS ($p > 0.05$). Different protein constituents, as well as other components, in different muscles, might govern the film formation. Slight differences in protein patterns (shown in SDS–PAGE) of film-forming solutions from different muscles were observed (Fig. 1). Myosin heavy chain (MHC) and actin were found to be the major proteins in all muscles used. In general, MHC is the dominant protein in fish muscle (Shahidi, 1994). From the result, protein molecules in ordinary muscle possibly underwent aggregation to a greater extent and stronger bonds formed. As a result, a stronger film network was obtained as indicated by higher TS. For elongation at break (EAB), no differences were observed among films from different types of muscles ($p > 0.05$).

When comparing the mechanical properties of films prepared from mince and washed mince, it was noted that TS of films from washed mince, from all types of muscles, was much higher than that of films prepared from unwashed mince ($p < 0.05$). After washing, films from ordinary, dark and whole muscles showed increases in TS of 86.5%, 130.5% and 163.7%. The increase in TS of films prepared from washed mince indicated that washing contributed to the removal of sarcoplasmic proteins as well as other constituents, such as lipids (Table 1). This led to the concentrated salt soluble proteins, which are involved in the

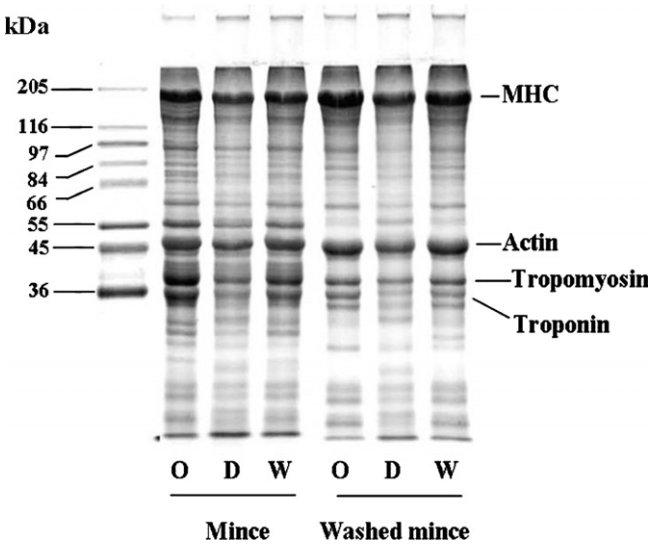

Fig. 1. Protein patterns of film-forming solution from round scad mince and washed mince of different muscle types: O, ordinary muscle; D, dark muscle; W, whole muscle.

Table 2
Mechanical and physical properties of protein-based film from round scad mince and washed mince of different muscle types

Samples	Muscle types	TS[A] (MPa)	EAB[A] (%)	WVP[B] ($\times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1})	Thickness[A] (mm)
Mince	Ordinary	4.21 ± 0.81^{c}	159.43 ± 7.56^{a}	1.19 ± 0.05^{b}	0.038 ± 0.001^{a}
	Dark	2.00 ± 0.49^{d}	154.13 ± 10.15^{a}	1.22 ± 0.04^{b}	0.037 ± 0.002^{a}
	Whole	2.51 ± 0.30^{d}	151.67 ± 4.32^{a}	1.20 ± 0.03^{b}	0.039 ± 0.005^{a}
Washed mince	Ordinary	7.85 ± 0.93^{a}	159.34 ± 2.46^{a}	0.89 ± 0.08^{a}	0.036 ± 0.001^{a}
	Dark	4.61 ± 0.40^{c}	138.49 ± 4.57^{b}	0.90 ± 0.08^{a}	0.040 ± 0.001^{a}
	Whole	6.62 ± 0.96^{b}	151.38 ± 1.52^{a}	0.90 ± 0.11^{a}	0.038 ± 0.001^{a}

The same superscript in the same column indicates non-significant difference ($p > 0.05$).
[A] Means ± SD from five determinations.
[B] Means ± SD from triplicate determinations.

strong film network formation. With appropriate washing, sarcoplasmic proteins were removed, resulting in an increased concentration of myofibrillar proteins, which play an essential role in protein–protein interaction (Chaijan et al., 2004). Okada (1964) concluded that washing during surimi processing was necessary to obtain a higher concentration of myosin by removing sarcoplasmic proteins. The characteristics of protein-based films are determined by the nature of protein–protein interactions (Perez-Gargo & Krochta, 2001). The distribution and concentration of inter- and intra-molecular interactions most likely determine the mechanical properties of myofibrillar protein-based films (Cuq, 2002). For EAB, no differences were found between films prepared from washed and unwashed mince of both ordinary and whole muscles ($p > 0.05$). Nevertheless, EAB of film from washed mince of dark muscle was lower than that of film from unwashed mince ($p < 0.05$). Dark muscle contained a high amount of lipid as well as water-soluble proteins such as haemoglobin and myoglobin (Chaijan, Benjakul, Visessanguan, & Faustman, 2006). The removal of lipids might increase the interaction between protein molecules, resulting in increased rigidity of the film. This was associated with the reduced EAB of resulting films.

3.3. Water vapour permeability of protein-based film

Water vapour permeability (WVP) of films produced from mince and washed mince of different types of muscles is shown in Table 2. No differences in WVP were observed among films prepared from different types of muscles ($p > 0.05$). Similar amino acid compositions among all types of muscles might be associated with the similar WVP of all films tested. After washing, mince from all types of muscles rendered films with lowered WVP ($p < 0.05$). This result revealed that polar proteins or amino acids, especially from the sarcoplasmic fraction were leached out, leading to a higher amount of non-polar components. Blue marlin myofibrillar protein comprises a large amount of ionised polar amino acids (approximately 33%) (Shiku et al., 2003). Nile tilapia myofibrillar protein has a high content of polar ionic amino acids, such as aspartic acid, glutamic acid, arginine and lysine (Paschoalick et al., 2003). Constituents with lowered polarity could absorb less water from the surrounding atmosphere. Water vapour permeation through a hydrophilic film depends on both diffusivity and solubility of moisture in the film matrix. Glycerol, used as a plasticizer, also increased the hydrophilicity of resulting films. An increase in the inter chain spacing due to inclusion of glycerol molecules between the polymer chains may promote water vapour diffusion through the film (Gontard, Guilbert, & Cuq, 1993). Additionally, more pronounced protein cross-linking of the film with more rigidity and denser structure might retard diffusion of water through the films. From this result, washing could be an effective means to lower WVP of fish muscle protein-based film.

Table 3
Film solubility and protein solubility of protein-based film from round scad mince and washed mince of different muscle types

Samples	Muscle types	Film solubility[A] (%)	Protein solubility[A] (%)
Mince	Ordinary	46.34 ± 1.20^{cd}	26.9 ± 0.71^{c}
	Dark	45.09 ± 0.51^{d}	39.4 ± 1.91^{d}
	Whole	47.78 ± 0.59^{c}	38.7 ± 1.40^{d}
Washed mince	Ordinary	54.78 ± 0.02^{b}	13.2 ± 0.12^{ab}
	Dark	64.47 ± 1.51^{a}	14.6 ± 0.75^{b}
	Whole	63.45 ± 0.16^{a}	11.9 ± 0.35^{a}

The same superscript in the same column indicates non-significant difference ($p > 0.05$).
[A] Means ± SD from triplicate determinations.

3.4. Film solubility and protein solubility

Film solubility and protein solubility of protein-based film from different types of muscles are shown in Table 3. For the films from unwashed mince, those from ordinary muscle showed similar film solubility to those from dark muscle and from whole muscle ($p > 0.05$). However, the solubility of film prepared from whole muscle was higher than that of film from dark muscle ($p < 0.05$). For protein solubility, a greater protein solubility was found in films prepared from dark and whole muscle than in films from ordinary muscle ($p < 0.05$). The lower protein solubility observed in film from ordinary muscle suggested that the proteins in ordinary muscle underwent more aggregation, leading to more cross-linking and larger molecular weight. This was associated with lower solubility.

When washed mince from different types of muscles was used to prepare the film, a greater film solubility but lower protein solubility were noticeable for all types of muscle, compared with all films from unwashed mince ($p < 0.05$). This result suggested that a greater amount of cross-linked proteins was formed inter- and/or intra-molecularly between protein chains in the films from washed mince as evidenced by the lessened protein solubility. Also, sarcoplasmic proteins had been mostly removed during the washing process and the remaining myofibrils were mainly soluble in salt solution. As the cross-linking became intensive, glycerol was imbibed to a lesser extent in the film network. This resulted in a higher solubility of the resulting film. The cross-linked proteins in film network were insoluble, whereas almost all of the glycerol was released (Orliac, Rouilly, Silvestre, & Rigal, 2002). The greater protein solubility observed in films from unwashed mince was also probably due to the ease of sarcoplasmic proteins being leached from the film. Among all films from washed mince, it was noted that films from washed mince of ordinary muscle showed the lowest film solubility (compared with those from dark and whole muscle ($p < 0.05$)). Nevertheless, no differences in protein solubility were obtained between films prepared from washed mince of whole and ordinary muscles ($p > 0.05$).

Table 4
Colour of protein-based film from round scad mince and washed mince of different muscle types

Samples	Muscle types	L^{*A}	a^{*A}	b^{*A}
Mince	Ordinary	87.28 ± 0.04^{a}	-1.84 ± 0.04^{a}	4.69 ± 0.19^{a}
	Dark	83.53 ± 0.39^{c}	-2.46 ± 0.04^{c}	20.65 ± 0.88^{c}
	Whole	85.96 ± 0.63^{b}	-2.26 ± 0.06^{b}	9.87 ± 0.17^{b}
Washed mince	Ordinary	87.02 ± 0.27^{a}	-1.85 ± 0.09^{a}	4.88 ± 0.20^{a}
	Dark	82.38 ± 0.56^{d}	-2.95 ± 0.08^{d}	22.87 ± 0.94^{d}
	Whole	85.73 ± 0.37^{b}	-2.43 ± 0.07^{c}	10.23 ± 0.35^{b}

The same superscript in the same column indicates non-significant difference ($p > 0.05$).
[A] Means ± SD from five determinations.

3.5. Colour of protein-based film

Colour of films from different types of muscles expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values is shown in Table 4. Among all films from different muscles, films from ordinary muscle showed the highest L^*- and the lowest b^*-values ($p < 0.05$). Conversely, films prepared from dark muscle were more yellowish than those prepared from other muscles, as evidenced by the greater b^*-values ($p < 0.05$). Dark muscle was reported to contain a higher amount of myoglobin than ordinary muscle (Chaijan et al., 2004). Those pigments most likely contributed to the darker and yellowish colour of films from dark muscle, compared with films from other muscles.

When the films were prepared from washed mince, only films from dark muscle showed decreases in L^*- and a^*-values ($p < 0.05$), suggesting a decrease in redness of the films. However, a slight increase in b^*-value was noticeable ($p < 0.05$). The removal of water-soluble pigments, especially myoglobin and haemoglobin, might result in a decrease in redness of resulting films. Heme protein in sarcoplasmic fraction affects the colour of muscle tissue (Chaijan et al., 2004, 2006). Chaijan et al. (2004) reported that washing of sardine and mackerel muscles resulted in a decrease of myoglobin content. Therefore, washing mainly affected the colour of films from round scad mince, especially from dark muscle.

3.6. Light transmission and transparency of protein-based film

Light transmission at selected wavelengths, for the films from round scad mince of different muscle types, is shown in Table 5. Films prepared from all muscle types had low transmission in the UV range (200–280 nm). This result suggested that films could prevent the lipid oxidation induced by UV light in a food system. The result was in agreement with Shiku et al. (2004) who found low transmission in the UV range of fish muscle protein-based film. Among all films from different muscle types, the films from dark muscle showed the lowest transmission in the visible range (250–800 nm), followed by those from whole muscle and ordinary muscle, respectively. Therefore, films from dark muscle could prevent absorption of visible light more effectively than could other films. The pigments in dark muscle might contribute to absorb some visible light, as evidenced by the lowered transmission. When comparing the transmission of films prepared from mince and washed mince, it was found that transmission in the visible range of film from mince was lower than that of washed mince films. Among all films from washed mince, those of dark muscle also showed the lowest transmission ($p < 0.05$). Washing could leach the pigments from all muscles, especially those which are water-soluble. This might result in a lower efficacy in preventing the transmission of visible light of the resulting film.

The transparency of film from round scad mince of different muscles is shown in Table 5. Films prepared from ordinary and whole muscles were more transparent than were those from dark muscle, as indicated by the lower transparency value. With the high value, film was less transparent. Therefore, heme pigments in the muscle might affect the transparency of film. The films from washed mince of ordinary and whole muscles showed similar transparencies to those from unwashed mince ($p > 0.05$). However, films from dark muscle were more transparent when washed mince was used for preparation ($p < 0.05$). Sarcoplasmic proteins, including myoglobin, were removed from dark muscle to a great extent during washing. As a consequence, more transparent films were obtained. Myofibrillar protein films are transparent and clear enough for use as

Table 5
Light transmission and transparency of protein-based film from round scad mince and washed mince of different muscle types

Samples	Muscle types	Wavelength (nm)								Transparency[A]
		200	280	350	400	500	600	700	800	
Mince	Ordinary	0	0	40.88	70.35	77.40	79.74	80.74	80.99	1.50 ± 0.01^{a}
	Dark	0	0	1.95	27.11	45.22	51.81	54.87	56.41	1.66 ± 0.03^{c}
	Whole	0	0	16.45	48.04	60.23	63.98	67.52	67.97	1.55 ± 0.01^{a}
Washed mince	Ordinary	0	0.10	51.89	68.76	77.49	80.04	81.00	81.25	1.51 ± 0.01^{a}
	Dark	0	0	6.66	33.57	61.58	70.29	73.73	75.39	1.55 ± 0.01^{b}
	Whole	0	0	31.45	58.20	73.28	77.41	78.97	79.43	1.52 ± 0.01^{a}

The same superscript in the same column indicates non-significant difference ($p > 0.05$).
[A] Means ± SD from five determinations.

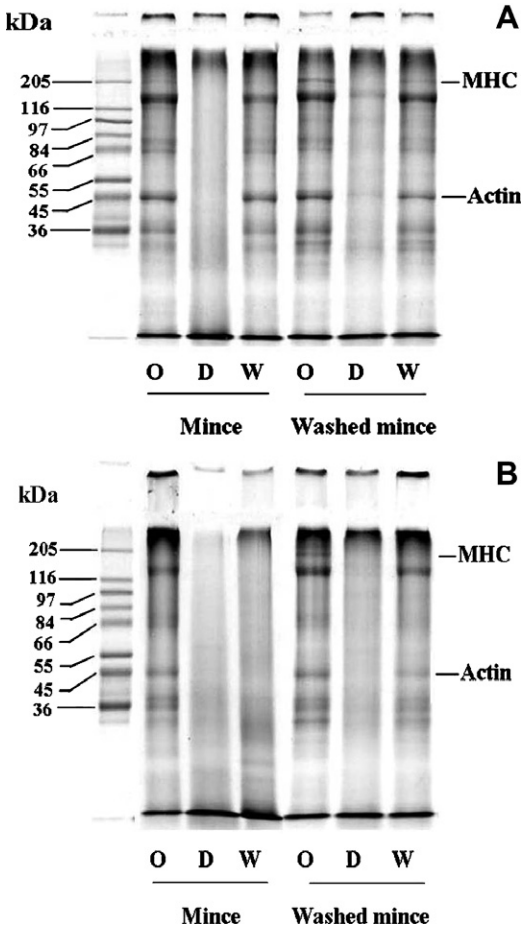

Fig. 2. Protein patterns of film from round scad mince and washed mince of different muscle types in the presence (A) and the absence (B) of βME: O, ordinary muscle; D, dark muscle; W, whole muscle.

seen by packaging (Shiku et al., 2003, 2004). Generally, films from washed mince had a lower preventive effect on visible light transmission than had those from unwashed mince. Nevertheless, washing could make the films from dark muscle more transparent.

3.7. Protein pattern of protein-based film

Protein patterns of film from round scad mince and washed mince of different muscle types are shown in Fig. 2. Similar protein patterns were noticeable between films from ordinary and whole muscle, regardless of washing. Both MHC and actin band intensity, in all types of muscles, were much decreased, compared with that found in the film-forming solution (Fig. 1). The decreases in band intensity of MHC and actin in all films were concomitant with the formation of high-molecular-weight cross-links as appeared on the top of the SDS–PAGE. From this result, some differences in protein pattern were observed between those run under reducing (Fig. 2A) and non-reducing (Fig. 2B) conditions. This suggested the role of disulfide bonds in stabilizing the film matrix. It was possible that sulfhydryl groups in muscle proteins formed disulphide bonds to yield the film structure upon casting and drying of the film-forming solution (Shiku et al., 2003). Among all muscles, dark muscle proteins underwent polymerisation to a greater extent, than did other muscles. From this result, films from dark muscle showed lower TS than did other films, regardless of washing (Table 2). Therefore, it appears that bonding, which stabilised the protein cross-links of dark muscle films, might be weaker than that found in the other films.

4. Conclusion

Mechanical and physical properties of round scad muscle protein-based film were affected by types of muscle and by the washing process. Films from washed mince showed a greater TS, but lower WVP, than did films from unwashed muscle. Regardless of washing, films from ordinary muscle showed greater lightness and less yellowness than did those from other muscles. However, films from dark muscle could prevent the transmission of UV and showed superior visible light barrier properties to those from other muscles.

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