ORIGINAL ARTICLE

Properties of composite film based on bigeye snapper surimi protein and lipids

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Abstract

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Lipids were incorporated into bigeye snapper surimi protein films through emulsification using Tween-20 as a surfactant to form protein/lipid composite films. The effects of lipid types (palm oil, butter or shortening) and concentrations (0-100% glycerol substitution) on film properties were investigated. Addition of lipids up to 75% glycerol substitution resulted in the improved water vapor barrier, lowered tensile strength (TS) and increased elongation at break (EAB) of the composite film ($P<0.05$). However, an increase in TS was observed with increasing lipid concentration, plausibly caused by increasing protein aggregation in film matrix. Transparency of films was decreased with increasing lipid concentrations used ($P<0.05$), especially for those added with solid lipids. Generally, the mechanical properties and water resistance of surimi protein films incorporated with palm oil were superior to those modified with butter or shortening. An increase in Tween-20, nonionic surfactant, might be associated with the decrease in non-disulfide covalent cross-links in the film. Scanning electron microscopic study revealed that dispersion of palm oil in the film was more uniform than that of butter and shortening. This might contribute to the varying properties of resulting films.

Key words : surimi, film, bigeye snapper, lipid, composite film

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สมบัติของฟิล์มคอมพอสิทจากโปรตีนซูริมิปลาตาหวานและลิปิด

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ฟิล์มคอมพอสิทจากโปรตีนซูริมิปลาตาหวานและลิปิดเตรียมโดยการเติมลิปิดในสารละลายฟิล์มและทำให้เกิดเป็นอิมัลชันโดยใช้ Tween-20 เป็นสารลดแรงตึงผิวก่อนขึ้นรูปฟิล์ม จากการศึกษาผลของชนิดลิปิด (น้ำมันปาล์ม เนยเหลว หรือเนยขาว) และความเข้มข้นของลิปิดในการทดแทนกลีเซอรอลปริมาณ 0 ถึง 100% ต่อสมบัติของฟิล์ม พบว่าการเติมลิปิดเพื่อทดแทนกลีเซอรอลจนถึงระดับ 75% ในฟิล์มโปรตีนซูริมิ มีผลในการปรับปรุงความสามารถในการป้องกันการซึมผ่านของไอน้ำ ลดค่าการทนต่อแรงดึงสูงสุด และเพิ่มค่าระยะยืดดึงเมื่อขาดของแผ่นฟิล์ม ($P<0.05$) อย่างไรก็ตาม การทนต่อแรงดึงสูงสุดของฟิล์มมีค่าเพิ่มขึ้น เมื่อระดับความเข้มข้นของลิปิดที่ใช้ทดแทนกลีเซอรอลเพิ่มขึ้น ซึ่งอาจเป็นผลจากการรวมตัวของโปรตีนในโครงข่ายฟิล์มที่เพิ่มขึ้น การเติมลิปิดโดยเฉพาะลิปิดที่มีสถานะเป็นของแข็งในระดับที่เพิ่มขึ้นมีผลทำให้ความโปร่งใสของฟิล์มลดลง ($P<0.05$) โดยทั่วไปฟิล์มคอมพอสิทโปรตีนซูริมิที่เติมน้ำมันปาล์มมีสมบัติเชิงกลและการทนน้ำดีกว่าฟิล์มคอมพอสิทที่เติมเนยเหลวหรือเนยขาว การใช้ Tween-20 ซึ่งเป็นสารลดแรงตึงผิวชนิดไม่มีประจุในปริมาณสูงขึ้นอาจทำให้การเชื่อมประสานของโปรตีนโดยพันธะโควาเลนท์ที่ไม่ใช่พันธะไดซัลไฟด์ในแผ่นฟิล์มลดลง จากการศึกษาโดยใช้กล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด (SEM) พบว่าน้ำมันปาล์มสามารถกระจายตัวในแผ่นฟิล์มได้สม่ำเสมอดีกว่าเนยเหลวและเนยขาว ซึ่งอาจมีผลต่อสมบัติที่แตกต่างกันของฟิล์มที่ได้

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Protein-based films have been of increasing interest as biodegradable packaging or coating. Both plant and animal proteins can be used as protein based films with varying properties (Park and Chinnan, 1995; Vannin *et al*., 2005). Among a variety of proteins, surimi, the stabilized myofibrillar fish proteins, has been reported to exhibit the film-forming ability (Shiku *et al*., 2003; Cuq *et al*., 1995; Paschoalick *et al*., 2003). However, the film properties were governed by many factors including pH, plasticizers, etc. Recently, the transparent and flexible edible/biodegradable films were made from frozen threadfin bream surimi (Prodpran and Benjakul, 2005). The properties of films from surimi produced from tropical fish were affected by the pH used to solubilize the proteins, which directly influenced the proteolysis of muscle proteins (Prodpran and Benjakul, 2005; Chinabhark *et al*., 2005).

Proteins are thermoplastic heteropolymers containing both polar and non-polar amino acids, which are able to form numerous intermolecular linkages. Generally, globular proteins must be denatured by heat, acid, base and/or solvent to form more extended structures that are required for film formation (Krochta, 1997). Protein films are quite stiff and brittle due to the extensive interactions between protein chains through hydrogen bonding, electrostatic forces and hydrophobic interaction (Krochta, 2002). Normally, low-molecular-weight plasticizers must be added in order to improve film flexibility by reducing those interactions. Plasticizers commonly used in protein films include mono-, di- and oligosaccharides, polyols and lipids (Avena-Bustillos and Krochta, 1993; Shellhammer and Krochta, 1997). Although protein films are excellent oxygen and carbondioxide barriers, they are generally poor barriers to moisture, due to the high content of hydrophilic components (Kim and Ustunol, 2001). In contrast, films produced from hydrophobic substances such as lipid and lipid emulsion possess high moisture barrier properties,

but are opaque and have rather poor mechanical properties (slightly flexible and brittle) (Guilbert, 1986; Bertan *et al*., 2005). Therefore, several attempts have been made to produce the composite or emulsion films by incorporating lipids into hydrophilic films to improve their moisture barrier properties (Morillon *et al*., 2002; Yang and Paulson, 2000; Bertan *et al*., 2005). This can be accomplished by laminating a hydrophilic film with a lipid layer (Debeaufort *et al*., 1993; Park *et al*., 1994), by dispersing both hydrophilic and hydrophobic components in a solvent and then drying (Shellhammer and Krochta, 1997; Gontard *et al*., 1994; Pommet *et al*., 2003), or by emulsifying the lipid within the hydrophilic phase (Avena-Bustillos and Krochta, 1993; McHugh and Krochta. 1994). The homogineity and stable emulsion of lipids in the film-forming solution would play an important role in properties of composite or emulsion films. The effectiveness of emulsified lipids in reducing the WVP of whey protein films has been reported (Avena-Bustillos and Krochta, 1993; McHugh and Krochta, 1994). Moreover, the physical state of the lipid, liquid or solid (crystalline), has a strong influence on barrier efficiency of the film. Crystalline lipids generally provide a better barrier to moisture transport than do liquid lipids (Kamper and Fennema, 1984; Martin-Polo *et al*., 1992). However, there is no report regarding the influence of lipid on the film from muscle protein, especially surimi. Therefore, the objective of this study was to investigate the effect of lipid types at different concentrations on the properties of protein-based film produced from frozen bigeye snapper surimi.

Materials and methods

1. Frozen surimi

Frozen surimi (grade A), produced from bigeye snapper (*Priacanthus tayenus*), was purchased from Man A Frozen Foods Co. Ltd., Muang, Songkhla. Surimi was kept at -20ºC until used.

2. Preparation of film-forming solution

Frozen surimi was thawed using a running water (26-28ºC) until the core temperature reached 0ºC. To prepare the film-forming solutions, surimi was added with the distilled water and homogenized at 13,000 rpm for 1 min using a homogenizer (IKA Labortechnik, Malaysia). The protein concentration of the film-forming solution was fixed at 2% (w/v) (Prodpran and Benjakul, 2005). Film-forming solution was then added with the glycerol at 50% (w/w) of protein and was used as the control. The mixtures were stirred gently for 30 min at room temperature. The pH of the film-forming solution was then adjusted to 3 using 1 M HCl. The solution was subsequently filtered through two layers of nylon cloth.

To study the effect of various lipids on film properties, lipids (palm oil, butter or shortening) at different levels (25, 50, 75 and 100% glycerol substitution) were added to the solution. Prior to lipid addition, Tween-20 (polysorbate-20) at a level of 10% (w/w) of lipid amount used was mixed thoroughly with the film forming solution. Subsequently, the mixture was homogenized at 13,000 rpm for 2 min and allowed to stand for 2 min before film casting.

3. Film casting and drying

The film-forming solution (4 g) was cast onto a rimmed silicone resin plate (50x50 mm) and dried overnight using an electric fan prior to drying in a ventilated oven at 25ºC and 50% relative humidity (RH) for 48h (Environmental chamber; WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and used for analyses.

4. Determination of film properties

4.1 Film thickness

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

4.2 Mechanical properties

The films were conditioned for 48 h at 25ºC and 50% RH prior to testing. 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 Instruments, Hampshire, UK). Eight samples (2x5 cm) with initial grip length of 3 cm were used for testing. Cross-head speed was 0.5 mm/s.

4.3 Water vapor permeability (WVP)

WVP of films was determined using a modified ASTM method as described by Shiku *et al*. (2003). The film was sealed on a glass permeation cup containing silica gel (0% RH) with silicone vacuum grease and a rubber band. The cups were placed at 30ºC in a desiccator containing the distilled water. The cups were weighed at 1 h intervals over a 7-h period. WVP of the film was calculated as follows (McHugh *et al*., 1993):

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

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

4.4 Color and film transparency

Color of the film was determined as L*, a* and b* using CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). The films were also subjected to the measurement of transparency. The transmittance of films were determined at 600 nm using the UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) as described by Han and Floros (1997). The transparency of the films was calculated by the following equation:

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

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

4.5 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein patterns of the films were analyzed by SDS-PAGE according to the method of Laemmli (1970) using a 10% running gel and 4% stacking gel. To prepare the film samples for analysis, the samples were mixed with a solubilizing solution containing 1% SDS and 8M urea. The mixtures were homogenized with a homogenizer at 13,000 rpm for 1 min using an IKA homogenizer. The homogenate was stirred continuously for 12 h at room temperature, followed by centrifugation at 3,000xg for 5 min. The supernatants obtained were subjected to SDS-PAGE analysis in the presence of β-mercaptoethanol (βME). Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8 containing 4% SDS, 20% glycerol and 10% βME) and boiled for 3 min. 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 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% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

4.6 Measurement of film solubility and protein solubility

Film solubility was determined as described by Shiku *et al*. (2003). Films dried in dessicator containing the silica gels over 3 weeks (2 g) were placed in 10 ml of distilled water comprising 0.1% (w/v) sodium azide. The mixture was stirred continuously at room temperature for 24 h. Undissolved matter was removed by centrifuging the mixture at 3,000xg for 20 min. The pellet was dried in an oven at 105ºC for 24 h. The solubility was then calculated based on the weight differences between the initial dry film and undissolved matter.

The supernatant obtained was then subjected to protein determination according to the Lowry method (Lowry *et al*., 1951). To obtain the total protein content in the dry film, the film was solubilized in 1 M NaOH. Protein solubility was expressed as percentage of soluble protein content relative to total protein content.

4.7 Scanning electron microscopy

Surface morphology of selected films was examined by scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan). Film samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, PA, USA). The surface was observed at an acceleration voltage of 10 kV.

5. Statistical analysis

The experiments were run in duplicate. Different two lots of films were prepared. Data obtained was subjected to analysis of variance (ANOVA) and mean comparisons were run by Duncan's multiple range test (Steel and Torrie, 1980). Analysis was performed using the SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL).

Results and Discussion

1. Effect of lipid types and concentrations on the mechanical properties of surimi protein/ lipid composite film

Films with different types and amounts of lipids had the similar thickness (Table 1). TS of bigeye snapper surimi film added with different lipids at various concentrations is shown in Table 1. In general, the addition of lipids (25-75% glycerol substitution) to surimi protein-based films reduced their TS ($P<0.05$) due to the partial replacement of protein polymer by lipids in the film matrix. The interactions between non-polar lipid molecules and between polar polymer and non-polar lipid molecules are believed to be much lower than those between the polar polymer molecules (Yang and Paulson, 2000). With 25% glycerol substitution,

Table 1. Tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP) and thickness of bigeye snapper surimi protein-based film as affected by types and amounts of lipids

Films	Lipid conc. (%)	TS[#] (MPa)	EAB[#] (%)	WVP[##] (x 10^{-10}gm^{-1}s^{-1}Pa^{-1})	Thickness[#] (mm)
Control	0	3.55±0.77^{b*}	73.73±3.48^{cd}	1.15±0.08^{a}	0.030±0.001^{d}
Palm oil	25	0.72±0.05^{fg}	77.94±1.39^{c}	0.95±0.07^{b}	0.031±0.001^{cd}
	50	1.02±0.25^{ef}	83.13±4.96^{b}	0.93±0.12^{bc}	0.032±0.002^{bcd}
	75	1.34±0.19^{e}	102.50±10.89^{a}	0.80±0.12^{d}	0.032±0.001^{bcd}
	100	3.39±0.66^{b}	2.47±0.44^{g}	0.78±0.06^{d}	0.032±0.001^{bc}
Butter	25	0.51±0.03^{g}	73.23±1.54^{cd}	0.93±0.15^{bc}	0.031±0.001^{cd}
	50	0.63±0.09^{fg}	66.75±3.02^{e}	0.93±0.07^{bc}	0.032±0.002^{bcd}
	75	1.90±0.31^{d}	60.07±7.96^{f}	0.81±0.06^{cd}	0.031±0.001^{cd}
	100	2.54±0.56^{c}	1.26±0.78^{g}	0.78±0.08^{d}	0.033±0.001^{ab}
Shortening	25	0.99±0.14^{ef}	71.46±3.17^{d}	1.10±0.08^{a}	0.032±0.001^{bcd}
	50	0.77±0.13^{fg}	63.38±3.44^{ef}	1.08±0.08^{a}	0.032±0.001^{bcd}
	75	1.79±0.36^{d}	63.04±4.30^{ef}	0.88±0.22^{bcd}	0.033±0.001^{ab}
	100	4.10±0.75^{a}	1.92±1.20^{g}	0.87±0.09^{bcd}	0.034±0.001^{a}

[#] Mean±SD from eight determinations.
[##] Mean±SD from four determinations.
*** The different superscripts in the same column indicate significant differences ($P<0.05$).**

the lowest TS was observed in all films added with any type of lipids. Strength reduction of edible composite films with lipid incorporation has been typically reported (Debeaufort and Voilley, 1995; Shellhammer and Krochta, 1997; Yang and Paulson, 2000; Morillon *et al*., 2002; Bertan *et al*., 2005). However, TS of the composite film generally increased as the lipid levels used to substitute the glycerol increased up to 100% ($P<0.05$). The highest TS was observed with 100% glycerol substitution. The result suggested that the addition of a greater amount of lipid into film-forming solution resulted in the less incorporation of lipids in dispersed two-phase system even though Tween-20, a non-ionic surfactant, was used to stabilize the emulsion of the system. Prior to film casting, surimi proteins were solubilized by adjusting the pH to the acidic range. Charge repulsion contributes to the protein solubility, which is prerequisite for film formation. Unfolding of protein molecules at low or high pH values occurs owing to a decrease in electrostatic bonds (Vojdani, 1996). As a consequence, the lipids added could not be compatibly incorporated with the charged protein molecules in the solution. Without the aid of Tween-20, the added lipids were totally separated from the protein-rich phase and located on the top of the film. Additionally, in the presence of the less amount of glycerol, a hydrophilic plasticizer, with the concomitant increasing amount of lipids, the higher aggregation of protein molecules in the protein-rich phase of the film was presumed. This led to the increased TS, especially film without glycerol. However, film containing 100% palm oil as the plasticizer (100% glycerol substitution) had the similar TS to the control (100% glycerol). For the sample added with solid lipids at 100% glycerol substitution, TS was found to be varied with lipid types. It was noted that film containing 100% shortening had the greater TS than that consisting of 100% butter. Butter has the discontinuous structure of fat globules and a crystalline fat matrix, while shortening posesses the plate-like three dimensional crystalline network with crystal bridge (Juriaanse and Heertje, 1988). Fat globules remaining in the butter structure could partially contribute as the plasticizer of film as evidenced by the lower TS (Table 1). This observation was inconsistent with some previous reports concerning whey protein/lipid composite films reported by Gontard et al. (1994) and Shellhammer and Krochta (1997), and gelatin/lipid composite films reported by Bertan *et al*. (2005). The differences were most likely due to the differences in protein structure and protein solubilizing process used for the preparation of film-forming solution. The results suggested that the strength of surimi protein/lipid composite films was governed not only by the nature of lipid phase but also by molecular interactions (aggregation) in the protein-rich phase.

When EAB of films added with different lipids at varying levels of glycerol substitution was determined (Table 1), it was noted that EAB of films added with lipids at 25% glycerol substitution showed the similar EAB to the control. However, EAB increased gradually when the palm oil was added up to 75% glycerol substitution. For the samples added with butter or shortening, the slight decrease in EAB was noticeable when the lipids were added up to 75% glycerol substitution. The differences in changing pattern of EAB between films added with liquid or solid lipids were most likely due to the differences in their molecular flexibility and phase solidity. The liquid lipid could be dispersed in the protein-rich phase more effectively than the solid lipids and possibly exhibited more potentially plasticizing effect. Similar results have been reported for whey protein films (Anker *et al*., 2002), zein films (Lai and Padua, 1998) and egg albumen films (Gennadios *et al*., 1996). Conversely, the solid lipid became solidified and larger phase separation occurred upon film formation. The non-uniform system might be associated with the lack of film structural integrity. Martin-Polo (1992) observed that film incorporated with solid lipid exhibited heterogeneous and porous structure while film incorporated with liquid lipid was denser in structure. The lower continuity and coherence (cohesiveness) of protein network in the presence of lipid globules might result in the decrease in EAB as pointed out by Anker *et al*. (2002) and Peroval *et al*. (2002). Lower water

content of film containing lipids may also cause the decreased elongation (Gallo *et al*., 2000).

At level of 100% glycerol substitution, EAB of all samples sharply decreased and reached the values ranging from 1.26 to 2.47%. The excessive protein aggregation occurred in the absence of water soluble plasticizer resulted in the complete loss in plasticity. This was in agreement with the increased TS in the samples added with 100% lipid (100% glycerol substitution). In the absence of glycerol, no differences in EAB were observed among all films added with 100% lipid regardless of lipid types. In general, low-molecular-weight plasticizers are added to protein film in order to improve film flexibility by reducing protein-protein interaction (Krochta, 1997).

2. Effect of lipid types and concentrations on the water vapor barrier property of surimi protein/lipid composite film

Protein-based films have typically high WVP due to hydrophilic nature of protein. Transmission of water vapor through protein-based film is also facilitated by the presence of glycerol, a hydrophilic plasticizer which favorably absorbs the water or moisture (Cuq *et al*., 1995). Additionally, cryo-protectants including sucrose and sorbitol in surimi also provided the polar groups in the film. Those polar groups provide for hydrogen bonding with water (Prodpran and Benjakul, 2005). As a result, the surimi-based film could absorb the water from the surrounding air or from the food product. To prevent the moisture migration into the protein-based film, hydrophobic substance such as lipids are commonly used (Morillon *et al*., 2002).

Table 1 shows WVP of bigeye snapper surimi protein-based film as affected by different types and amounts of lipids added. In general, WVP of film added with palm oil or butter decreased when the added lipid increased ($P<0.05$). Among all samples, the control containing 100% glycerol (without lipid addition) possessed the greatest WVP. From the result, the increasing amount of lipids increased the barrier efficiency against moisture transfer of film by increasing the hydrophobic substance, while lowering the hydrophilic glycerol amount (Martin-Polo *et al*., 1992; Morillon *et al*., 2002; Yang and Paulson, 2000; Bertan *et al*., 2005). Yang and Paulson (2000) reported that both beeswax and the blend of stearic and palmitic acids effectively reduced WVP of gellan film. The solid or liquid state of lipids strongly influenced the barrier efficiency of the film. It has been reported that the increase of the solid fat content allowed for the improvement of the barrier efficiency (Martin-Polo *et al*., 1992; Yang and Paulson, 2000; Bertan *et al*., 2005). This is because CH_2 groups of liquid aliphatic chains have a greater volume than when they are crystallized (Morillon *et al*., 2002).

However, WVP of films added with shortening at 25 and 50% glycerol substitution was not different from that of the control ($P>0.05$). Though the shortening was incorporated as the hydrophobic substance, it might cause the formation of fat crystal and the micro-cracks or the disconnection of interstitial zone could occur. These structural defects within the film might result in some leakage of film. Sapru and Labuza (1994) reported that the moisture migration was favored when the formation of large crystals of stearic acid was formed, allowing interstitial zones free of lipid. Therefore, the difference in WVP between film added with two solid lipids, butter and shortening, was possibly owing to the differences in lipid nature, particularly their crystallinity as well as fatty acid composition. Water vapor permeability of film was highly dependent on fatty acid chain length and degree of saturation (Morillon *et al*., 2002; Pommet *et al*., 2003).

3. Effect of lipid types and concentrations on the color and transparency of surimi protein/lipid composite film

Bigeye snapper surimi film became lighter as evidenced by the increase in L*-values when the amount of lipid incorporated in the film increased ($P<0.05$). The decrease in a*-value was noticeable as the lipids was incorporated into the films ($P<0.05$). However, no differences were observed among all samples added with varying types and amounts of lipids ($P>0.05$). For b*-value, films added with palm oil or shortening had the lowered

value, compared with that of the control. On the other hand, b*-value of film added with butter increased as the amount of butter added increased ($P<0.05$). This indicated the increasing yellowness of film, possibly due to the greater amount of pigments naturally found or added in the butter.

Lower transparency of surimi film as indicated by the greater values was found when the greater amount of lipids was incorporated ($P< 0.05$) (Table 2). At the same level of lipid used, films with palm oil addition showed less opacity or more transparency than those added with butter or shortening. Among all samples, film added with shortening was the most opaque. Increasing opacity of the protein film by addition of hydrophobic substances has been reported (Bertan *et al*., 2005; Pommet *et al*., 2003; Yang and Paulson, 2000; Gontard *et al*., 1994). The increase in opacity was possibly caused by the light scattering from the lipid droplets distributed throughout the protein network. The surface of solid lipid droplet most likely exhibited more light scattering effect than that of liquid lipid. The difference in crystallinity and surface of both solid lipids might contribute to the different light scattering of resulting film. Yang and Paulson (2000) reported that the differences in opacity of film were determined by the optical properties of lipids incorporated. Solid fat was also reported to increase the opacity of polysaccharide-based film (Gallo *et al*., 2000).

4. Effect of lipid types and concentrations on the film solubility and protein solubility of surimi protein/lipid composite film

Table 3 shows water solubility of the bigeye snapper surimi protein-based films with and without lipid modification. For the control, solubility of 67.69% was noted. The loss in film solubility of the control was owing to the aggregation of proteins in the film network. Film solubility markedly decreased when the lipids were incorporated. The loss in solubility was pronounced when greater levels of lipids were incorporated. The solubility decreased to a greater degree as the higher lipid amount was added. Water solubility is an indicative of the film hydrophilicity. Addition of lipid thus increased hydrophobicity of the protein-based film. Similar results were reported by Kim and Ustunol (2001) on lipid-whey protein emulsion films. From the results, the higher lipid content

Table 2. Color parameters and transparency of bigeye snapper surimi protein-based film as affected by types and amounts of lipids

Films	Lipid conc. (%)	$L^{*\#}$	$a^{*\#}$	$b^{*\#}$	Transparency$^{\#}$
Control	0	90.18±0.13^{g*}	-1.33±0.06^{a}	3.39±0.05^{c}	5.83±0.02^{l}
Palm oil	25	90.65±0.01^{f}	-1.58±0.04^{b}	2.80±0.03^{fg}	9.86±0.00^{k}
	50	91.43±0.02^{e}	-1.64±0.04^{bcd}	2.65±0.05^{h}	11.72±0.15^{j}
	75	92.06±0.05^{c}	-1.60±0.05^{bc}	2.81±0.01^{fg}	19.35±0.01^{f}
	100	92.50±0.02^{a}	-1.70±0.03^{cd}	2.76±0.04^{g}	21.00±0.01^{d}
Butter	25	91.46±0.02^{e}	-1.60±0.02^{bc}	2.76±0.03^{g}	13.93±0.01^{i}
	50	91.64±0.02^{d}	-1.66±0.04^{bcd}	2.97±0.03^{e}	16.31±0.05^{h}
	75	92.02±0.02^{c}	-1.65±0.10^{bcd}	4.34±0.05^{b}	22.99±0.05^{c}
	100	92.23±0.05^{b}	-1.91±0.07^{c}	4.83±0.04^{a}	30.97±0.17^{b}
Shortening	25	90.66±0.03^{f}	-1.71±0.06^{cd}	2.62±0.03^{h}	17.11±0.06^{g}
	50	91.43±0.02^{e}	-1.76±0.08^{d}	3.37±0.05^{c}	20.72±0.02^{e}
	75	91.64±0.06^{d}	-1.67±0.08^{bcd}	3.14±0.05^{d}	22.87±0.06^{c}
	100	92.30±0.15^{b}	-1.66±0.10^{bcd}	2.85±0.03^{f}	36.07±0.04

$^{\#}$ Mean±SD from three determinations.
*** The different superscripts in the same column indicate significant differences ($P<0.05$).**

Table 3. Film solubility and protein solubility of bigeye snapper surimi protein-based film as affected by types and amounts of lipids

Films	Lipid conc. (%)	Film solubility# (%)	Protein solubility# (%)
Control	0	67.69±0.21^{a*}	26.78±2.64^{a}
Palm oil	25	34.65±1.21^{ef}	17.61±1.16^{bc}
	50	32.13±0.38^{g}	16.83±1.26^{bc}
	75	30.36±0.98^{h}	14.31±0.22^{cd}
	100	27.04±0.45^{i}	10.40±0.59^{d}
Butter	25	36.33±1.03^{d}	20.79±4.90^{b}
	50	35.60±0.45^{de}	16.32±2.99^{bc}
	75	34.30±0.38^{ef}	14.27±2.98^{cd}
	100	33.97±0.64^{f}	13.94±3.59^{cd}
Shortening	25	42.88±0.95^{b}	17.11±3.62^{bc}
	50	42.66±0.57^{b}	17.01±2.45^{bc}
	75	40.99±1.13^{c}	14.99±1.26^{cd}
	100	40.96±0.58^{c}	14.72±1.51^{cd}

 # Mean±SD from three determinations.
*** The different superscripts in the same column indicate significant differences (P<0.05).**

used was concomitant with the lower glycerol content in the film. As a consequence, the glycerol dissolved in extracting water was lowered when the percent lipid substitution was increased. Also, the lowered preventive effect of glycerol towards the protein aggregation was presumed. As previously discussed, the hydrophobic lipids were rarely dispersed throughout the very polar protein-rich phase. Without the sufficient glycerol, a hydrophilic plasticizer, the more aggregation took place, leading to a great loss in film solubility. At the same level of lipid used, film added with palm oil showed the lowest solubility, and the greater solubility's were observed for the films with butter and shortening additions.

Similar results were observed with protein solubility (Table 3). Surimi-based film was formed by the cross-links stabilized by various bonding including hydrogen bond, hydrophobic interaction as well as disulfide bond (Shiku *et al*., 2003). The cross-liked proteins in the surimi-based film generally tend to loss their solubility. However, the loss in protein solubility was more pronounced as the lipid used increased, suggesting the greater aggregation of proteins in the film. This was in accordance with the increase in TS and the decrease in EAB of the film added with a greater amount of lipids (Table 1).

5. Effect of lipid types and concentrations on the protein pattern of surimi protein/lipid composite film

SDS-PAGE protein patterns of surimi films added with different types and amounts of lipids are depicted in Figure 1. It was found that the control film contained both myosin heavy chain (MHC) and actin as the major constituents. In general, MHC band intensity increased when the levels of lipids incorporated increased. The MHC band intensity of sample added with lipid at 100% glycerol substitution was similar to that found in the surimi (data not shown). The result suggested that non-disulfide covalent bond was formed in the control film (without lipid addition) to some extent. Reducing sugar in the film forming solution might undergo Maillard reaction with the amino

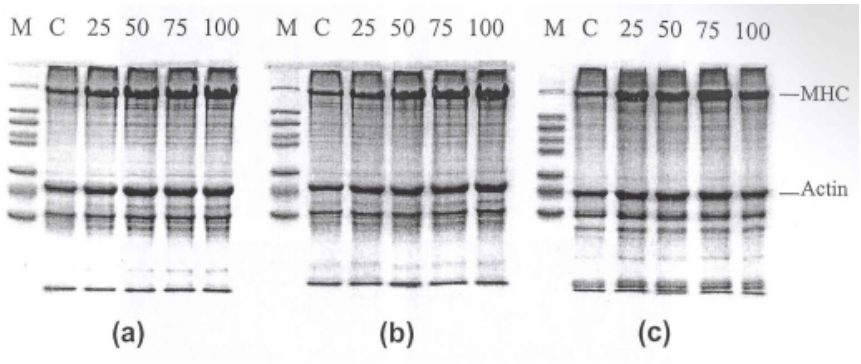
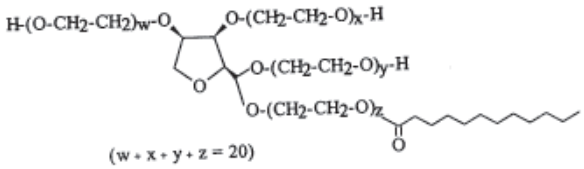


Figure 1. Protein patterns of bigeye anapper surimi protein-based films incorporated with palm oil (a), butter (b) and shortening (c). M: high-molecular-weight protein marker; C: control film (without lipid). Numbers denote lipid levels as % glycerol substitution.

groups of protein, leading to the covalent cross-linking of proteins. The reaction might be enhanced during the drying process of films Chinabhark *et al*. (2005) reported that the reducing sugar was produced in acidic film forming solution of frozen surimi from bigeye snapper. Polymerization of protein was induced by Maillard glycation (Chevalier *et al*., 2002).

As the lipid was added to a great extent, this bonding was much reduced as evidenced by the greater MHC band intensity retained. Tween-20 might play a role in impeding the non-disulfide covalent bond formation in a concentration dependent manner. Tween-20, a nonionic surfactant [I], was added proportionally to the amount of lipids used.



Tween-20 [I]

It localized at the interface by exposing the hydrophobic portion to lipid phase. The hydrophilic portion was imposed in the protein-rich phase. With increasing Tween-20 at the interface, the reducing sugars might migrate to hydrophilic shield of interface surrounded by Tween-20. The diffusion of sugar through colloidal dispersions has been reported in the literature (Basaran and McClements, 1999; Matsumoto *et al*., 2000). As a consequence, the lower amount of reducing sugar was retained in the protein-rich phase and Maillard reaction was decreased. As a result, the non-disulfide cross-links via Maillard glycation was reduced. Although the degradation of MHC in edible films based on sardine myofibrillar proteins, especially in the acidic pH ranges, due to the cathepsins was reported (Cuq *et al*., 1995), negligible degradation was noted in the acidic film forming solution of bigeye snapper surimi (Chinabhark *et al*., 2005). Furthermore, Tween-20 showed no inhibitory effect towards proteolysis (data not shown). From the result, similar protein patterns were obtained among films added with different lipids. This confirmed that lipid types had no pronounced effect on protein pattern of the surimi protein/lipid composite film.

6. Effect of lipid types and concentrations on the surface characteristics of surimi protein/lipid composite film

The SEM micrographs of surimi film incorporated with palm oil at different levels as the substitute of glycerol are shown in Figure 2. The control film (without lipids) had the smooth and continuous surface without grainy and porous structure. This indicated that the film with ordered network was formed without the air bubbles. With addition of palm oil, the surface of surimi film had

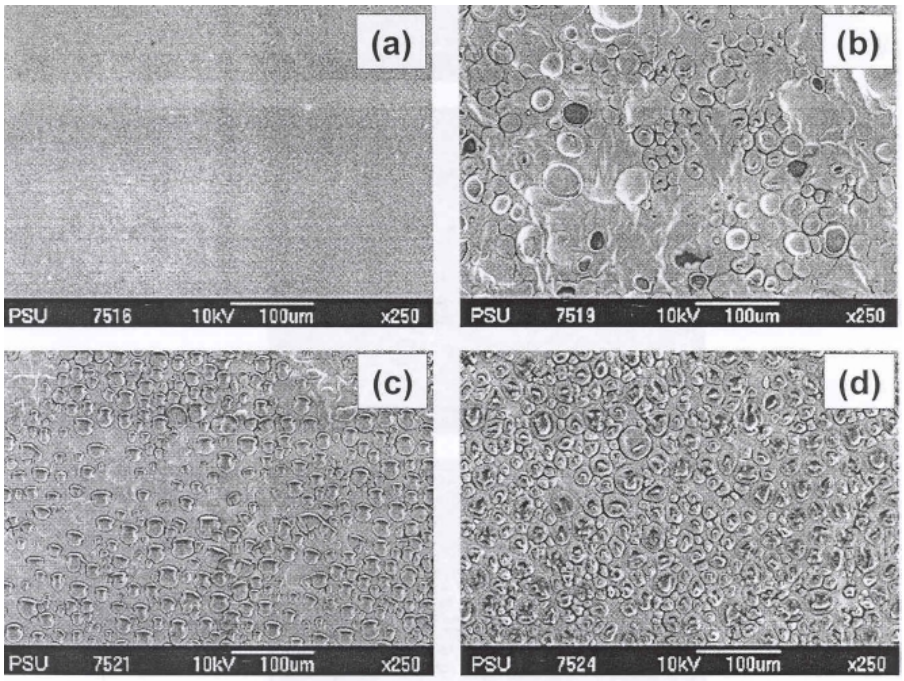


Figure 2. SEM micrographs of bigeye snapper surimi protein-based films incorporated without and with palm oils at different levels. Control film (without lipid) (a) and films incorporated with palm oil at 25% (b), 75% (c) and 100% (d) glycerol substitution.

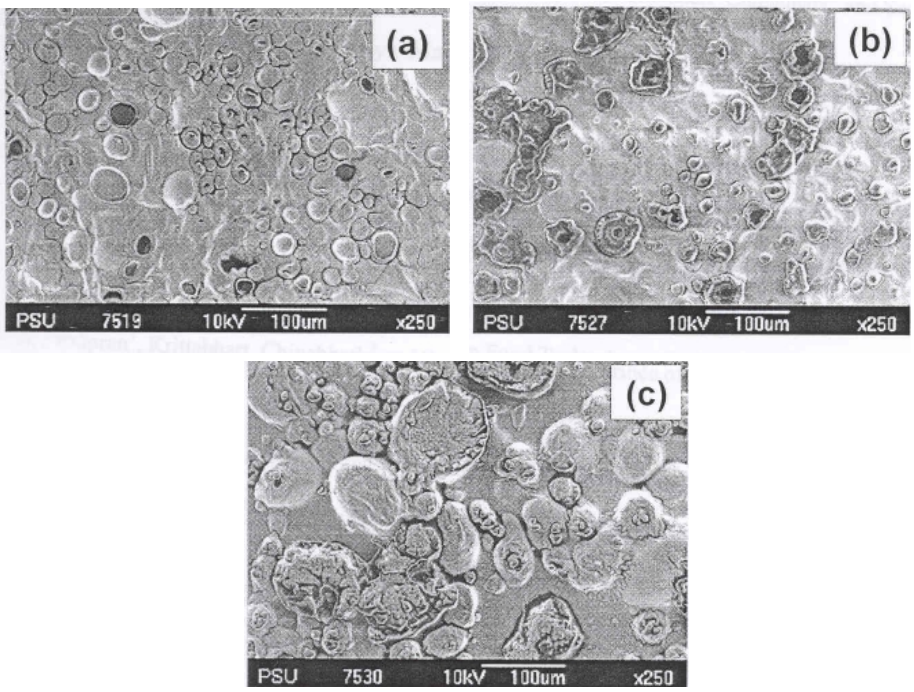


Figure 3. SEM micrographs of bigeye anapper surimi protein-based films incorporated with palm oil (a), butter (b) and shortening (c) at 75% glycerol substitution.

the irregular surface with the distribution of oil droplets. The oil droplets were more intense on the surface as the amount of oil incorporated increased. The continuously spread oil droplets on the film surface might be associated with the reduced WVP of film with a greater amount of oil (Table 1).

When the surface characteristics of films added with different lipids, palm oil, butter and shortening, at 75% glycerol substitution were compared (Figure 3), different surface was observed. The greatest irregularity was noticeable with the film containing shortening. The large and irregular shaped-droplets were found throughout the film. For the butter containing film, smaller droplet of butter was found to distribute in the film, compared with that obtained in shortening containing film. The differences in surface among all films tested might be caused by the different crystallinity of lipids used. This might result in the varying distribution and solidity of lipids in films. The surface characteristics of gellan film were determined by varying lipid types and the differences in surface structure might contribute to some extent to the differences in WVP of the films (Yang and Paulson, 2000).

Conclusion

Incorporation of lipids directly affected the properties of surimi protein/lipid composite film by decreasing water vapor permeability and water solubility, owing to the hydrophobic nature of lipids. Compared to solid lipid, surimi protein films modified by liquid lipid generally exhibited better mechanical properties, due to the regularity of dispersed lipid phase and structural integrity of the films. Hydrophilic plasticizer was still required in concert with lipids to maintain the plasticity or extensibility of the surimi protein films.

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