

## Effect of Emulsifier on Properties and Microstructures of Surimi Protein/Palm Oil Composite Film

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**Abstract:** Effect of different types and concentrations of emulsifier on properties of surimi protein/palm oil composite film was investigated. Different emulsifiers including Hexadecylpyridinium Chloride Monohydrate (HCM), Tween-20, Tween-80 and Span-60 at various concentrations (5, 10 and 15% of palm oil) were incorporated in film from bigeye snapper surimi protein added with palm oil at 75% glycerol substitution. The composite films had decreased Water-Vapor Permeability (WVP), Young's modulus (EM), Tensile Strength (TS) and transparency but increased Elongation at Break (EAB) and yellowness ( $b^*$ -value) as compared to the control surimi protein film ( $p < 0.05$ ), irrespective of emulsifier type and concentration. Increasing concentration of water-soluble emulsifiers (i.e., HCM, Tween-20 and Tween-80) resulted in increased EM and decreased WVP of the films. However, the concentration of oil-soluble emulsifier (i.e., Span-60) had no impact on mechanical properties and WVP of the composite films. At the same level used, films added with different types of water-soluble emulsifiers exhibited similar EM and TS ( $p \geq 0.05$ ). In general, mechanical properties as well as transparency of the composite films stabilized with oil-soluble emulsifier (Span-60) were poorer than those added with water-soluble emulsifiers. From the results, composite films added with Tween-20 at 10% generally had the highest mechanical properties, especially the EAB and water-vapor barrier property ( $p < 0.05$ ). The improved properties of composite film were related with the increased uniform dispersion of oil droplets in the matrix of film as evidenced by SEM morphologies, depending on types and concentrations of emulsifiers used. Therefore, emulsifier types and levels had an influence on properties of surimi protein/palm oil composite film which were governed by different microstructures during film formation.

**Key words:** Composite film, emulsion-based film, surimi protein, emulsifiers, SEM

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### INTRODUCTION

Protein-based films have been of increasing interest as biodegradable packaging or coating. Among a variety of proteins, fish myofibrillar proteins from surimi, has been reported to exhibit good film-forming ability (Paschoalick *et al.*, 2003; Shiku *et al.*, 2004; Chinabhark *et al.*, 2007). Protein-based films from surimi or fish myofibrillar protein generally have good mechanical properties due to the interactions between protein chains via disulfide (S-S) covalent bonding, hydrogen bonding, electrostatic forces and hydrophobic interactions (Paschoalick *et al.*, 2003; Chinabhark *et al.*, 2007). However, protein-based films are highly water and moisture absorptions, owing to hydrophilicity of amino acids in protein molecules. Additionally, hydrophilic plasticizers (such as polyols and mono-, di- and oligosaccharide) necessarily incorporated into the protein

films to improve the film flexibility decrease water vapor barrier properties of the films (Gennadios *et al.*, 1996). To improve water vapor barrier properties of the protein films, hydrophobic substances such as fats and oils could be added. Fat and lipids of different types have been successfully incorporated into protein- and carbohydrate-based films by means of lamination and dispersion or emulsion to form composite films (Morillon *et al.*, 2002; Bertan *et al.*, 2005). The emulsion-based film or composite film based on fish myofibrillar protein from surimi with improved water vapor barrier has been developed for food packaging application (Prodpran *et al.*, 2007).

The homogeneity and stable emulsion of lipids in the film-forming suspension would play an important role in properties of composite or emulsion films. As a result, addition of appropriate type and amount of emulsifier is required in the formulation to prevent colloidal particles from aggregating and to control the stability which in turn

affected to a greater extent the physical properties of the emulsified or composite protein film. However, no information has been reported regarding the influence of emulsifier types on the properties of composite film from fish myofibrillar protein from surimi incorporated with palm oil. Therefore, the objective of this study was to investigate the effect of type and concentration of emulsifier on the properties of the aforementioned film.

## MATERIALS AND METHODS

**Surimi and chemicals:** Frozen surimi (grade A), produced from bigeye snapper (*Priacanthus tayenus*), was purchased from Man A Frozen Food Co. Ltd., Songkhla, Thailand. Surimi was kept at  $-20^{\circ}\text{C}$  until used. Glycerol, Hexadecylpyridinium Chloride Monohydrate (HCM), polyoxyethylene sorbitan monolaurate (Tween-20), polyoxyethylene sorbitan monooleate (Tween-80) and sorbitan monostearate (Span-60) were purchased from Sigma (St. Louis, MO, USA) and were of analytical grade. Figure 1 illustrates chemical structures of different emulsifiers used in this study.

### Preparation of film-forming solution/mixture and film:

Film-forming solution without palm oil addition (a control) was prepared according to the method of Prodpran *et al.* (2007). Surimi was added with distilled water to obtain the protein concentration of 2.0 (w/v) and homogenized at 13,000 rpm for 1 min. The solution was then added with glycerol at 50% (w/w) of protein. The mixture was stirred gently for 30 min followed by adjusting the pH to 3 using 1 M HCl. The film forming solution was used for film casting.

To prepare film-forming mixture with palm oil incorporation, palm oil at 75% glycerol substitution was added to the film-forming solution. Prior to oil addition, different emulsifiers, Hexadecylpyridinium Chloride Monohydrate (HCM), Tween-20, Tween-80 and Span-60, at various concentrations (5, 10 and 15% of palm oil) were added and mixed thoroughly with the film-forming solution. Subsequently, the mixture was homogenized at 13,000 rpm for 2 min and allowed to stand for 5 min before film casting.

To prepare the film, the film-forming solution/mixtures (4 g) was cast onto a rimmed silicone resin plate ( $5 \times 5 \text{ cm}^2$ ) and air blown for 12 h at room temperature prior to further drying at  $25^{\circ}\text{C}$  and 50% relative humidity for 24 h in an environmental chamber (NUVE, TK120, Turkey). The resulting films were manually peeled off and used for analyses.

**Determination of film thickness:** The thickness of film was measured by using a digital micrometer (Gotech, Model GT-313-A, Gotech Testing Machines Inc, Tawai). Five random thickness measurements were taken for each film of five films and the average was taken as the result. Determination of mechanical properties:

Prior to testing the mechanical properties, films were conditioned for 48 h at  $25^{\circ}\text{C}$  and  $50 \pm 5\%$  Relative Humidity (RH). Tensile Strength (TS), Elongation at Break (EAB) and Elastic Modulus (EM) of the films were measured according to the ASTM-D882-01 method as described by Iwata using a universal testing machine (Lloyd Instruments, Hampshire, UK). The specimen strip ( $50 \times 20 \text{ mm}$ ) was clamped between the grips with initial

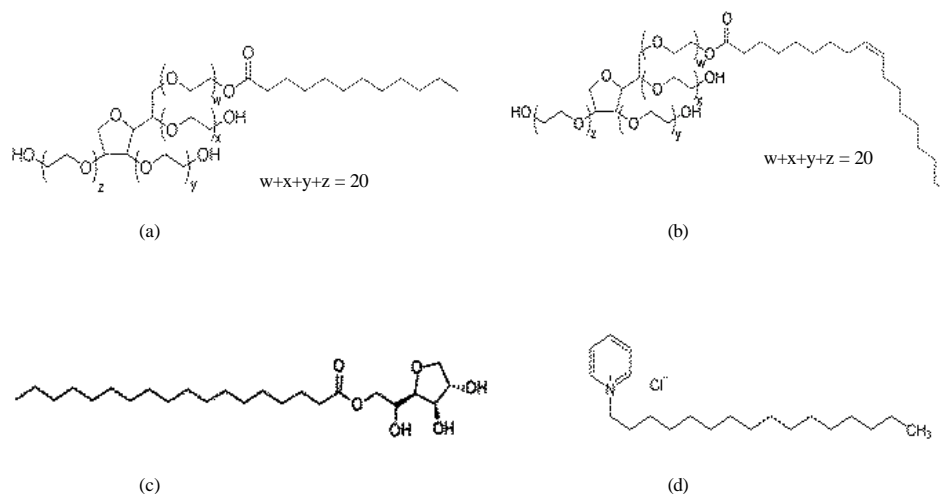


Fig 1: Chemical structure of Tween-20 (a), Tween-80 (b), Span-60 (c) and HCM (d)

separation of 30 mm and then pulled apart at a cross-head speed of 30 mm/min until it was broken. The TS was calculated by dividing the maximum force at break by cross-sectional area of the film. The EAB was calculated by dividing the Length extended ( $\Delta L$ ) by the original Length ( $L_0$ ) of the film. EM was derived from the initial slope of the linear portion of stress-strain curve. Ten specimens were tested for each treatment.

**Determination of Water Vapor Permeability (WVP):**

WVP was determined using a modified ASTM E-96-01 method similar to that reported by Shiku *et al.* (2004). The preconditioned film was sealed on an aluminum permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket 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:

$$WVP (gm^{-1} s^{-1} Pa^{-1}) = w l A^{-1} t^{-1} (P_2 - P_1)^{-1}$$

Where:

- w = The weight gain of the cup (g)
- l = The film thickness (m)
- A = The exposed area of film (m<sup>2</sup>)
- t = The time of gain (s)
- $P_2 - P_1$  = The vapor pressure difference across the film (Pa)

**Determination of color:** Color of the film was determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA).  $D_{65}$  (day light) and a measure cell with opening of 30 mm were used. The color of the films was expressed as  $L^*$ ,  $a^*$  and  $b^*$  values.

**Determination of transparency value:** The transmission of visible light at 600 nm of films was measured using UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Then, the transparency value of the films was calculated by the following equation, as described by Han and Floros:

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

Where:

- $T_{600}$  = The fractional value of transmittance at 600 nm
- x = The film thickness (mm)

According to this equation, higher transparency value indicates lower degree of film transparency.

**Scanning Electron Microscopy (SEM):** Morphology of the film samples was visualized using a Scanning

Electron Microscope (SEM) (Quanta 400, FEI, Eindhoven, Netherlands). The samples were mounted on bronze stub and sputtered with gold (Sputter coater SPI-Module, PA, USA) in order to make the sample conductive. The photographs were taken at an acceleration voltage of 15 kV.

**Statistical analysis:** Experiments were run in triplicate. Data were subjected to Analysis of Variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test. Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

**Thickness and mechanical properties:** Thickness and mechanical properties of surimi protein/palm oil composite films added with different emulsifiers at various concentrations are shown in Table 1. Thickness of the films was slightly different which was in the range of 0.042-0.049 mm. All composite films had decreased Young's modulus (EM) and TS but increased EAB as compared with the control film without oil incorporation ( $p < 0.05$ ), regardless of type and concentration of emulsifier used. The dispersed oil droplets might impede protein-protein interactions in the film matrix, resulting in decreased stiffness and strength of the films (Bertan *et al.*, 2005; Prodpran *et al.*, 2007). Among surimi protein/palm oil composite films, those added with water-soluble emulsifiers (i.e., HCM, Tween-20 and Tween-80) showed gradually increased EM and slightly differed TS with increase in emulsifier concentration, irrespective of type of emulsifier used. This result suggested that the addition of greater amount of water-soluble emulsifiers resulted in stiffer surimi protein film incorporated with palm oil. However, concentration of Span-60, an oil-soluble emulsifier, did not significantly affect EM and TS of the composite film ( $p \geq 0.05$ ). For EAB of the composite films added with water-soluble emulsifiers, the EAB seemed to increase as the emulsifier concentration increased, regardless of type of emulsifier. However, films added with Span-60 at different concentrations studied showed similar EAB ( $p \geq 0.05$ ).

At the same emulsifier concentration, films added with different water-soluble emulsifiers tested in this study exhibited similar TS ( $p \geq 0.05$ ). Similar results were also observed for EM value, except that added with HCM which showed lower EM. From the results, films with Tween-20 possessed higher EAB than those with Tween-80 and HCM. Varying mechanical properties of composite films obtained would be most likely due to the differences in structure, molecular weight and hydrophilicity of different emulsifiers used.

Table 1: Thickness, mechanical properties and WVP of surimi protein film and surimi protein/palm oil composite films added with different emulsifiers at various concentrations

Emulsifier type	Emulsifier conc.	Thickness (mm)	EM (MPa)	TS (MPa)	EAB (%)	WVP ( $\times 10^{11}$ g.m/m <sup>2</sup> .s.Pa)
HCM	5%	0.048 $\pm$ 0.001 <sup>ab</sup>	122.48 $\pm$ 4.50 <sup>f</sup>	4.38 $\pm$ 0.69 <sup>cd</sup>	87.84 $\pm$ 5.74 <sup>de</sup>	2.65 $\pm$ 0.13 <sup>b</sup>
	10%	0.048 $\pm$ 0.001 <sup>ab</sup>	165.48 $\pm$ 3.95 <sup>de</sup>	4.96 $\pm$ 0.40 <sup>cd</sup>	82.57 $\pm$ 4.82 <sup>e</sup>	2.03 $\pm$ 0.10 <sup>e</sup>
	15%	0.049 $\pm$ 0.001 <sup>a</sup>	178.35 $\pm$ 5.66 <sup>d</sup>	4.98 $\pm$ 0.58 <sup>cd</sup>	72.50 $\pm$ 9.16 <sup>ef</sup>	3.52 $\pm$ 0.32 <sup>a</sup>
Tween-20	5%	0.048 $\pm$ 0.001 <sup>abc</sup>	168.56 $\pm$ 5.71 <sup>de</sup>	5.03 $\pm$ 0.32 <sup>cd</sup>	99.52 $\pm$ 5.86 <sup>bc</sup>	2.33 $\pm$ 0.20 <sup>bc</sup>
	10%	0.049 $\pm$ 0.001 <sup>ab</sup>	202.17 $\pm$ 8.20 <sup>f</sup>	5.42 $\pm$ 0.69 <sup>bc</sup>	125.10 $\pm$ 9.76 <sup>a</sup>	1.74 $\pm$ 0.05 <sup>de</sup>
	15%	0.046 $\pm$ 0.001 <sup>cd</sup>	214.88 $\pm$ 2.26 <sup>b</sup>	6.16 $\pm$ 0.51 <sup>ab</sup>	119.95 $\pm$ 7.95 <sup>a</sup>	1.88 $\pm$ 0.21 <sup>cd</sup>
Tween-80	5%	0.049 $\pm$ 0.001 <sup>ab</sup>	182.35 $\pm$ 9.05 <sup>d</sup>	4.16 $\pm$ 0.60 <sup>de</sup>	92.24 $\pm$ 5.36 <sup>cd</sup>	2.54 $\pm$ 0.27 <sup>bc</sup>
	10%	0.049 $\pm$ 0.002 <sup>ab</sup>	202.93 $\pm$ 6.53 <sup>d</sup>	5.25 $\pm$ 0.42 <sup>bc</sup>	93.93 $\pm$ 6.67 <sup>cd</sup>	1.58 $\pm$ 0.16 <sup>ef</sup>
	15%	0.049 $\pm$ 0.001 <sup>ab</sup>	223.73 $\pm$ 6.41 <sup>b</sup>	6.06 $\pm$ 0.73 <sup>ab</sup>	114.42 $\pm$ 9.90 <sup>ab</sup>	1.76 $\pm$ 0.28 <sup>de</sup>
Span-60	5%	0.046 $\pm$ 0.001 <sup>d</sup>	225.41 $\pm$ 4.55 <sup>b</sup>	3.99 $\pm$ 0.45 <sup>ef</sup>	54.01 $\pm$ 7.21 <sup>e</sup>	1.88 $\pm$ 0.30 <sup>de</sup>
	10%	0.048 $\pm$ 0.003 <sup>bc</sup>	220.94 $\pm$ 7.25 <sup>b</sup>	3.23 $\pm$ 0.49 <sup>f</sup>	59.97 $\pm$ 3.22 <sup>e</sup>	1.81 $\pm$ 0.12 <sup>de</sup>
	15%	0.047 $\pm$ 0.001 <sup>cd</sup>	220.68 $\pm$ 5.60 <sup>b</sup>	3.37 $\pm$ 0.39 <sup>f</sup>	62.48 $\pm$ 9.49 <sup>e</sup>	1.63 $\pm$ 0.29 <sup>ef</sup>
Control	-	0.042 $\pm$ 0.003 <sup>a</sup>	230.10 $\pm$ 8.45 <sup>a</sup>	6.98 $\pm$ 0.25 <sup>a</sup>	52.31 $\pm$ 8.02 <sup>e</sup>	3.69 $\pm$ 0.26 <sup>a</sup>

Table 2: Color parameters and transparency value of surimi protein film and surimi protein/palm oil films added with different emulsifiers at various concentrations

Emulsifier type	Emulsifier conc.	L*	a*	b*	Transparency value
HCM	5%	89.43 $\pm$ 0.50 <sup>abc</sup>	-2.57 $\pm$ 0.02 <sup>cd</sup>	3.34 $\pm$ 0.17 <sup>bc</sup>	7.15 $\pm$ 0.05 <sup>g</sup>
	10%	89.37 $\pm$ 0.22 <sup>bc</sup>	-2.54 $\pm$ 0.02 <sup>cd</sup>	3.33 $\pm$ 0.08 <sup>bc</sup>	8.59 $\pm$ 0.02 <sup>f</sup>
	15%	89.32 $\pm$ 0.07 <sup>bc</sup>	-2.42 $\pm$ 0.01 <sup>abcd</sup>	3.39 $\pm$ 0.21 <sup>ab</sup>	11.02 $\pm$ 0.07 <sup>ab</sup>
Tween-20	5%	89.98 $\pm$ 0.31 <sup>ab</sup>	-2.25 $\pm$ 0.11 <sup>ab</sup>	3.47 $\pm$ 0.03 <sup>ab</sup>	10.70 $\pm$ 0.03 <sup>a</sup>
	10%	89.73 $\pm$ 0.46 <sup>abc</sup>	-2.40 $\pm$ 0.11 <sup>abc</sup>	3.07 $\pm$ 0.12 <sup>d</sup>	11.24 $\pm$ 0.07 <sup>d</sup>
	15%	89.76 $\pm$ 0.34 <sup>abc</sup>	-2.68 $\pm$ 0.20 <sup>d</sup>	3.09 $\pm$ 0.31 <sup>d</sup>	13.38 $\pm$ 0.34 <sup>e</sup>
Tween-80	5%	89.58 $\pm$ 0.10 <sup>abc</sup>	-2.56 $\pm$ 0.13 <sup>cd</sup>	3.59 $\pm$ 0.44 <sup>ab</sup>	10.31 $\pm$ 0.05 <sup>e</sup>
	10%	90.24 $\pm$ 0.18 <sup>a</sup>	-2.50 $\pm$ 0.07 <sup>bcd</sup>	3.11 $\pm$ 0.26 <sup>d</sup>	10.86 $\pm$ 0.04 <sup>e</sup>
	15%	89.61 $\pm$ 0.13 <sup>abc</sup>	-2.66 $\pm$ 0.12 <sup>cd</sup>	3.62 $\pm$ 0.08 <sup>ab</sup>	13.36 $\pm$ 0.09 <sup>e</sup>
Span-60	5%	89.66 $\pm$ 0.13 <sup>abc</sup>	-2.43 $\pm$ 0.21 <sup>abcd</sup>	3.43 $\pm$ 0.62 <sup>ab</sup>	15.31 $\pm$ 0.03 <sup>b</sup>
	10%	90.05 $\pm$ 0.10 <sup>ab</sup>	-2.21 $\pm$ 0.11 <sup>a</sup>	3.01 $\pm$ 0.20 <sup>d</sup>	15.86 $\pm$ 0.04 <sup>b</sup>
	15%	89.15 $\pm$ 0.31 <sup>c</sup>	-2.17 $\pm$ 0.04 <sup>a</sup>	3.42 $\pm$ 0.22 <sup>ab</sup>	18.38 $\pm$ 0.03 <sup>a</sup>
Control	-	89.80 $\pm$ 0.77 <sup>abc</sup>	-2.42 $\pm$ 0.08 <sup>abcd</sup>	2.35 $\pm$ 0.06 <sup>e</sup>	3.81 $\pm$ 0.12 <sup>h</sup>

\*Mean $\pm$ SD (n = 3); Different super script letters in the same column indicate significant differences (p<0.05)

**Water vapor permeability (WVP):** WVP of surimi protein/palm oil composite films added with different emulsifiers at various concentrations is shown in Table 1. Control surimi protein film without palm oil addition had the highest WVP (p<0.05). Protein films possess typically high WVP due to hydrophilic nature of protein. Transmission of water vapor through protein film is also facilitated by the presence of glycerol, a hydrophilic plasticizer which favorably absorbs the water or moisture (Cuq *et al.*, 1995). Additionally, cryoprotectants including sucrose and sorbitol in surimi also provided the polar groups in the film. Those polar groups provide for hydrogen bonding with water. WVP of surimi protein/palm oil composite films had lower WVP than did the control film. Irrespective of type of emulsifier used, WVP of composite films added with water-soluble emulsifiers generally decreased when emulsifier concentration increased from 5-10%. Composite films with Tween-20 and Tween-80 had similar WVP (p $\geq$ 0.05). Among water-soluble emulsifiers tested, films incorporated with HCM, a cationic emulsifier, had higher WVP than those added with Tween-20 and Tween-80 which are non-ionic emulsifiers. The charged emulsifier used might result in higher hydrophilicity of the film as compared to the non-ionic counterpart. Composite film

emulsified with HCM at 15% showed similar WVP (p $\geq$ 0.05), compared to the control film. Composite films stabilized with Span-60 at 5-15% had similar WVP (p $\geq$ 0.05).

**Color and transparency of films:** Table 2 shows color parameters and transparency value of surimi protein film and surimi protein/palm oil composite films incorporated with different types and concentrations of emulsifiers. L\* and a\* values were slightly different among all films which were in the range of 89.15-90.24 and -2.68 to -2.21, respectively. All composite films almost had similar b\* value which was higher than the control film, more likely due to the color of pigments presented in incorporated palm oil.

Transparency value of composite films seemed to increase with increasing emulsifier concentration, regardless of type of emulsifier used (Table 2). The control film without palm oil addition had the lowest transparency value (p<0.05). It was noted that according to the equation used, the higher transparency value represents the lower degree of film transparency. Films added with water-soluble emulsifiers were more transparent (lower transparency value) than those added with oil-soluble emulsifier (Span-60) (p<0.05).

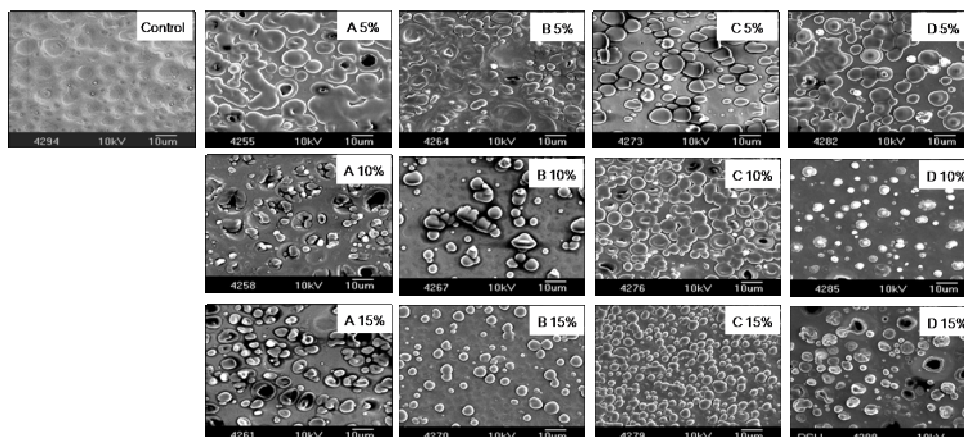


Fig 2: SEM micrographs (magnification: 1000×) of surimi protein film (control) and surimi protein/palm oil films added with different emulsifiers at various concentrations. A: HCM, B: Tween-20, C: Tween-80, D: Span-60

Transparency of films seemed to decrease when emulsifiers were added at increasing concentration, regardless of type of emulsifier.

**SEM morphology:** Figure 2 shows SEM microstructures of surimi protein/palm oil composite films added with different emulsifiers at various concentrations. The control film (without palm oil) had smoother internal microstructure. With addition of palm oil, the SEM showed the irregular microstructure with the distribution of oil droplets.

In general, the size of oil droplets decreased and uniformity of the oil droplet distribution in the film matrix seemed to increase when emulsifier with increasing amount was incorporated, especially from 5-10%. The distribution of oil droplets in the films using Tween-20 and Tween-80 was (water-soluble, uncharged emulsifiers) generally more uniform with smaller droplet size as compared to those using other emulsifiers. The continuously spread oil droplets in the film matrix might be associated with the more improvement in flexibility, extensibility and water-vapor barrier of those films (Table 1). For films with Span-60 (oil-soluble emulsifier), their SEM images showed larger and greater irregularity of oil droplet distribution. Some white crystals were also noticed in films using Span-60, since this emulsifier was difficult to totally dissolve during preparation of film-forming mixture. Typically, oil-soluble emulsifiers including Span-60 possessed lower emulsifying ability in oil-in-water emulsion system. This might contribute to the lower mechanical properties and transparency observed in composite films added with Span-60 (Table 1 and 2).

## CONCLUSION

Physical properties of surimi protein/palm oil composite films were generally affected by type and concentration of emulsifier incorporated. In general, use of water-soluble emulsifier was more effective in emulsifying ability than oil-soluble emulsifier, providing more uniform oil droplet distribution. This significantly contributed to better mechanical properties of the resulting composite films. However, addition of water-soluble emulsifier at excessive amount might have negative effect on the water-vapor barrier as well as the appearance of the resulting films. Among emulsifiers used, Tween-20 at 10% was the most appropriate emulsifier for surimi protein/palm oil composite films, which yielded the films with the most improved mechanical and water-vapor barrier properties.

## ACKNOWLEDGEMENTS

Researcher would like to thank Prince of Songkla University for the financial support. The TRF Distinguished Research Professor Grant was also acknowledged.

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