CHAPTER 7

FATTY ACIDS AND THEIR SUCROSE ESTERS AFFECT THE PROPERTIES OF FISH SKIN GELATIN BASED FILM

7.1 Abstract

The effects of fatty acids (FA) (palmitic acid and stearic acid) and their sucrose esters (FASE) on the mechanical properties, water vapor permeability (WVP), light transmission and color of films from bigeye snapper and brownstripe red snapper skin gelatins were investigated. Tensile strength (TS) of films generally decreased with the addition of FA ($P<0.05$), while gradually increased with increasing FASE amount ($P<0.05$). WVP of films generally decreased with increasing amount of FA or FASE ($P<0.05$). However, films containing FASE exhibited the superior WVP barrier property to those added with FA. Marked increase in elongation at break (EAB) was observed when either FA or FASE at a level of 25% substitution was incorporated. Light transmission of films in both UV (200-280 nm) and visible ranges (350-800 nm) decreased with increasing FA amount. Films added with FASE were generally more transparent than those with FA. Chain length of FA or FASE affected the properties of films differently, depending upon gelatin sources. Therefore, the properties of fish skin gelatin based films, especially water vapor barrier, could be improved by the addition of FA or FASE.

7.2 Introduction

Edible films derived from protein materials have been paid more attention for the use in the food protection and preservation owing to their biodegradable and environmental characteristics (Rayas *et al*., 1997; Tanaka *et al*., 2001a). The films can extend the shelf-life and improve the quality of almost any food system by serving as a barrier to mass transfer or as a mechanical protection (McHugh, 2000). Although edible films prepared from those renewable sources such as proteins and carbohydrates generally have the good mechanical properties, their water vapor barrier properties are usually indigent because of their hydrophilic character (Kester and Fennema, 1986). To retard the water vapor permeability of edible films, lipid materials including edible oil, neutral lipids,

fatty acids, as well as waxes are incorporated into the film (Tanaka *et al.*, 2001a). Bilayer films in which the lipid layer was laminated over a supporting film (Kester and Fennema, 1989a; Donhowe and Fennema, 1993; Lai *et al.*, 1997) and the emulsion films in which the lipid was uniformly dispersed throughout the film (McHugh and Kroachta, 1994; Rhim *et al.*, 1999b; Gallo *et al.*, 2000; Ayranci and Tunc, 2001; Karnnet *et al.*, 2005) have been developed. Although, emulsion films are not the effective barrier as bilayer films, they possess superior mechanical properties (Fairley *et al.*, 1997).

Among all proteins, gelatin has been attracted the attention for the development of edible films due to their abundance (Bigi *et al.*, 2002). Gelatin is generally produced from the land based animal skin or bone, generated during the animal slaughtering and processing. However, the outbreak of bovine spongiform encephalopathy (BSE) and the foot-and-mouth disease crisis has resulted in the anxiety among users of gelatin products from land base animal origin (Helcke, 2000). Recently, the protein based film has been prepared from gelatins of bigeye snapper and brownstripe red snapper skins. However, the films exhibited the poor water vapor barrier property (Jongjareonrak *et al.*, 2005a; Jongjareonrak *et al.*, 2005b). The use of lipid materials incorporated into the gelatin based film would be an effective means to improve the water vapor barrier property. FA have been reported to distribute uniformly and prevent the water vapor permeability of films (Lai *et al.*, 1997; Hagenmaier and Shaw, 1990; Sherwin *et al.*, 1998). Ayranci and Tunc (2001) observed that stearic acid was the most effective fatty acid in decreasing WVP and the CO_2 transmission of cellulose-based films, when compared with palmitic acid and lauric acid. In addition WVP, water vapor transmission rate and water vapor permeance generally decreased with increasing FA content in the film composition (Ayranci and Tunc, 2001). TS and EAB of zein films increased with increasing palmitic acid and stearic acid content plasticized up to 50% of protein, whereas decreased with further increase of the FA content up to 100% of protein (Lai *et al.*, 1997). Nevertheless, a little information regarding the effect of FA, especially FASE, on properties of gelatin-based edible films has been reported. Thus, the objective of this investigation was to study the effect of palmitic acid, stearic acid, and their sucrose esters as well as their amount on the mechanical, barrier and physical properties of films from the skin gelatin of bigeye snapper and brownstripe red snapper.

7.3 Materials and Methods

Chemicals

Palmitic acid (PA) and stearic acid (SA) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Palmitic acid sucrose ester (PASE) and stearic acid sucrose ester (SASE) were supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan).

Fish skin preparation

Bigeye snapper (*Priacanthus macracanthus*) and brownstripe red snapper (*Lutjanus vitta*) with an average total length of 22-25 cm were caught from Songkhla coast along the Gulf of Thailand, stored in ice and off-loaded after 24-36 h of capture. Upon the arrival to the dock in Songkhla, fish were stored in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Fish were washed with a tap water. Skins were then removed, descaled and cut into small pieces (0.5x0.5 cm). Prepared skins were kept on ice until used for gelatin extraction.

Extraction of fish skin gelatin

Gelatin was extracted from fish skin according to the method of Jongjareonrak *et al.* (2005a). Skins were soaked in 0.2 M NaOH with a skin/solution ratio of 1:10 (w/v) at 4°C with a gentle stirring. The solution was changed every 30 min for 3 times to remove noncollagenous proteins and pigments. Alkaline treated skins were then washed with tap water until neutral or faintly basic pHs (pH 7-7.5) of wash water were obtained. The skins were then soaked in 0.05 M acetic acid with a skin/solution ratio of 1:10 (w/v) for 3 h at room temperature (25°C) with a gentle stirring to swell the collagenous material in fish skin matrix. Acid treated skins were washed as previously described. The swollen fish skins were soaked in distilled water with a skin/water ratio of 1:10 (w/v) at 45°C for 12 h with a continuous stirring to extract gelatin from the skin matter. The mixture was then filtered using two layers of cheese cloth. The resultant filtrate

was freeze-dried using a freeze dryer (Model Dura-Top™ μP/Dura Dry™ μP, FTS® System, Inc., Stone Ridge, New York, USA) and the dry matter was referred to as "gelatin powder".

Preparation of fish skin gelatin films

Gelatin films were prepared according to the method of Jongjareonrak *et al.* (2005a). Gelatin powder was mixed with distilled water to obtain the film-forming solution (FFS) with the protein concentration of 3% (w/v). Glycerol at the concentration of 50% of protein were added into FFS as the plasticizer. To reduce the degradation of bigeye snapper gelatin caused by heat-activated proteinase, 10 mM EDTA was added into FFS. The FFS of skin gelatins from both species were incubated at 70°C for 30 min in a water bath with an occasional stirring for total dissolution. The solution was then homogenized using a homogenizer (Model PT-MR 2100, POLYTRON®, KINEMATICA AG, Littau-Lucerne, Switzerland) at 11,000 rpm for 1 min. Thereafter, FFS (4±0.01 g) was cast onto a rimmed silicone resin plate (50 x 50 mm) and dried with a ventilated oven (Temperature and Humidity Chamber; Model PR-2FT, TABAI ESPEC Corp., Osaka, Japan) at 25±0.5°C and 50±5% relative humidity (RH) for 24 h. Dried films obtained were manually peeled off. The films were conditioned for 48 h at 25±0.5°C and 50±5% RH prior to analyses, however the film thickness was determined without conditioning.

To study the effects of FA (PA or SA) and FASE (PASE or SASE) on film properties, FA or FASE were added into incubated FFS at various amounts (25, 50, 75, and 100% substitution of glycerol). The FFS was then homogenized at a speed of 11,000 rpm for 1 min, cast and dried as previously described. All films without and with the addition of FA or FASE were subjected to analyses.

Film thickness

Film thickness was measured to the nearest 5 μm with a micrometer (Model GT313-A, Dial Micrometer, Gotech testing machines INC., Tokyo, Japan). Nine measurements were taken at random positions. Precision of the thickness measurements was ±5%.

Color

Color of gelatin films was measured in the L*, a*, b* mode of CIE using a colorimeter (Model ColorFlex, HunterLab Reston, VA, USA). Color measurement was carried out in ten replicates for each treatment.

Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of gelatin films were determined using a material testing systems (Model LR30K, LLOYD INSTRUMENTS Ltd., Segensworth Fareham, England). Ten samples were measured for each treatment.

Water vapor permeability

Water vapor permeability (WVP) of films was measured using a modified ASTM method (ASTM, 1989) as described by Shiku *et al.* (2004). Films were sealed onto a glass permeation cup containing silica gel (0% RH) with silicone vacuum grease and an O-ring to hold the film in place. The cups were then placed in a desiccator saturated with water vapor at 30°C. The cups were weighed at 1 h intervals over a 7 h period and WVP of films was calculated as follows (McHugh *et al.*, 1993):

$$WVP = wxA^{-1}t^{-1}(P_2-P_1)^{-1}$$

where w is the weight gain of the cup (g), x is the film thickness (m), A is the area of exposed film (m^2), t is the time of gain (s), and $(P_2-P_1)^{-1}$ is the vapor pressure differential across the film (Pa).

The WVP was expressed as $g.m^{-1}s^{-1}Pa^{-1}$. A total of five samples were determined for each treatment.

Light transmission

The barrier properties of gelatin films against ultraviolet (UV) and visible light were measured at selected wavelengths between 200 and 800 nm, using a UV-Visible Recording spectrophotometer (Model UV-1601, Shimadzu Co., Kyoto, Japan)

according to the method of Fang *et al*. (2002). The transparency of films was calculated by the following equation (Han and Floros, 1997):

$$\text{transparency} = -\log T600/x$$

where T600 is the transmittance at 600 nm and x is the film thickness (mm).

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). Analysis was performed using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL).

7.4 Results and Discussion

Mechanical properties

TS of fish skin gelatin based films from both species without and with addition of FA or FASE at different levels of glycerol substitution is shown in Table 17. Generally, TS of bigeye snapper and brownstripe red snapper skin gelatin based films decreased when PA or SA with 25% substitution was added ($P<0.05$) in comparison with the control (0% substitution). Thereafter, TS of films added with PA or SA with the substitution greater than 50% showed the increase in TS ($P<0.05$), but were still lower than that of the control. FA, both PA or SA, might partially reduce the cross-linking of protein molecules via hydrogen bonds or hydrophobic interactions. FA generally lack the structural integrity of protein films, leading to the decrease in TS (Kroachta, 1992; Gontard *et al*., 1995). A slight increase in TS of films with further increase of both FA amount up to 100% glycerol substitution was possibly associated with the decrease in glycerol added. Glycerol, a polar plasticizer, could distribute uniformly throughout the film matrix. On the other hand, FA, non-polar substances, could not be dissolved and dispersed as did glycerol. At 100% glycerol substitution, no glycerol was used and the cross-linking of protein might be enhanced as evidenced by the greatest increase in TS of this sample.

Table 17. Mechanical properties and WVP of fish skin gelatin films as affected by fatty acid or fatty acid sucrose ester types and concentrations

Source of gelatin	Plasicizer type	Platicizer amount (%)	TS[1]	EAB[1]	WVP[2]
Bigeye snapper skin	Gly	0	15.41±1.12^{d*}	24.50±2.32^{f}	2.61±0.09^{c}
	PA	25	12.36±2.21^{ef}	69.99±3.62^{b}	2.45±0.13^{cd}
		50	7.81±1.05^{g}	36.33±3.45^{e}	3.01±0.09^{b}
		75	10.23±2.94^{efg}	4.31±0.68^{i}	2.61±0.07^{c}
		100	17.33±3.57^{d}	3.46±0.50^{i}	2.44±0.14^{cd}
	SA	25	9.75±1.53^{fg}	42.96±5.46^{d}	2.87±0.16^{b}
		50	11.29±1.86^{ef}	19.65±3.94^{g}	3.46±0.15^{a}
		75	16.68±2.88^{d}	5.38±0.46^{i}	2.23±0.18^{ef}
		100	20.45±5.76^{c}	4.08±0.73^{i}	2.17±0.06^{ef}
	PASE	25	18.89±2.37^{d}	81.51±5.13^{a}	2.05±0.02^{fg}
		50	25.36±1.55^{b}	25.39±4.83^{f}	1.70±0.07^{hi}
		75	45.48±2.22^{a}	14.68±2.80^{h}	1.68±0.09^{hi}
		100	47.80±6.35^{a}	13.88±1.92^{h}	1.56±0.07^{i}
	SASE	25	12.80±1.78^{ef}	48.43±4.29^{c}	2.12±0.04^{ef}
		50	23.61±2.05^{b}	14.68±2.44^{h}	1.86±0.06^{gh}
		75	43.06±1.86^{a}	7.79±0.82^{i}	1.01±0.08^{j}
		100	ND	ND	ND
Brownstripe red snapper skin	Gly	0	33.54±2.09^{e}	39.82±6.10^{e}	2.16±0.10^{a}
	PA	25	20.80±4.09^{h}	59.32±4.31^{c}	2.30±0.22^{a}
		50	20.11±2.13^{h}	21.89±1.71^{g}	1.85±0.19^{bc}
		75	29.01±3.20^{g}	19.18±2.10^{gh}	1.25±0.12^{de}
		100	31.65±3.01^{fg}	14.67±1.04^{ij}	1.17±0.23^{ef}
	SA	25	16.11±2.28^{i}	63.11±5.69^{c}	1.68±0.22^{c}
		50	22.41±2.93^{h}	35.46±3.55^{f}	1.63±0.23^{c}
		75	29.04±2.76^{g}	14.06±2.97^{ijk}	1.11±0.29^{ef}
		100	31.07±1.72^{fg}	10.51±1.45^{jkl}	1.06±0.15^{f}
	PASE	25	39.27±4.34^{d}	95.55±5.62^{a}	2.04±0.04^{b}
		50	36.89±3.87^{d}	47.56±7.76^{d}	1.71±0.06^{c}
		75	46.92±4.84^{c}	13.61±1.64^{ijk}	1.24±0.12^{def}
		100	53.75±4.84^{b}	8.71±0.81^{l}	1.14±0.04^{ef}
	SASE	25	36.49±1.33^{de}	73.54±4.85^{b}	2.31±0.06^{a}
		50	44.41±2.94^{c}	46.58±3.99^{d}	1.73±0.04^{c}
		75	54.34±2.00^{b}	15.47±1.52^{hi}	1.29±0.05^{de}
		100	60.73±3.10^{a}	10.17±1.08^{kl}	1.02±0.06^{f}

ND: Non-detected (films were too brittle to peel off) [1]Values are given as mean ± SD from 10 determinations. [2]Values are given as mean ± SD from five determinations. *Different superscripts in the same column under the same species indicate significant differences ($P<0.05$)

For films added with FASE, TS of films from both fish skin gelatins gradually increased with increasing amount of PASE and SASE ($P<0.05$). FASE, an emulsifier, contained both hydrophilic and hydrophobic character in the molecules (Soultani *et al.*, 2003). When the FASE was incorporated into FFS, the intermolecular interaction between proteins and FASE possibly occurred via the hydrophilic head of FASE. As a

result, the structural integrity between gelatin and sucrose ester molecules was obtained in the resulting film. Furthermore, the decreasing glycerol content with the concomitant increased amount of FASE as glycerol substitute might enhance the formation of hydrogen bonds or other inter-chain interactions between gelatins and FASE, resulting in the stronger film matrix as shown by the increase in TS of films. From the result, the greater increase in TS of films was observed with the film added with SA or SASE, compared with those containing PA or PASE (Table 17). The result revealed that the chain length of FA or FASE somehow determined the mechanical property of resulting film. FA or FASE with the longer chain dispersed in FFS and might interact with gelatin in the fashion which strengthened the film network, probably via hydrophobic interaction. The result was in agreement with that reported on soy protein-fatty acid composite films (Rhim *et al.*, 1999b).

EAB of gelatin films without and with FA or FASE at different levels is shown in Table 17. In general, EAB of films increased when either FA or FASE were added at a level of 25% glycerol substitution ($P<0.05$). However, a drastic decrease in EAB was observed with increasing FA and FASE amount up to 100% glycerol substitution ($P<0.05$). The presence of hydrophobic plasticizer causes a reduction of direct interaction forces between protein chains via hydrogen bonds or hydrophobic interactions and also increases the movement of macromolecules (Gontard *et al.*, 1993). This led to the increase in EAB of films. For films added with FA, the increasing FA amount with concomitant decreasing glycerol content might lessen the integrity of films network structure. This was possibly due to the incompatibility of FA in gelatin films matrix. Additionally, FA might impede the cross-linking of gelatin molecules by disturbance or alteration in gelatin structure (Karnnet *et al.*, 2005).

For films added with FASE, the interactions between gelatin and FASE were possibly formed via the hydrophilic head of the molecule. Hydrophobic portion of FASE might expose to protein-riched phase and functioned as a plasticizer by reducing the protein interaction. In addition, films added with SA or SASE exhibited the lower EAB than those films added with PA or PASE ($P<0.05$). SA possessing the longer hydrocarbon chain has the lower chain mobility, compared with PA (Ayranci and Tunc, 2001). Thus, the formation of film network structure with less movement of the macromolecules was obtained in film incorporated with SA.

From the results, similar patterns of TS and EAB as affected by the addition of FA or FASE were observed between bigeye snapper and brownstripe red snapper skin gelatin films. However, TS and EAB of brownstripe red snapper skin gelatin films generally greater than those of bigeye snapper skin gelatin film under the same condition tested. This was possibly due to the different compositions, particularly in term of amino acid composition and size of protein chains between both gelatins (Jongjareonrak *et al.*, 2005a; Paschoalick *et al.*, 2003; Muyonga *et al.*, 2004b). Differences in hydrophobic and hydrophilic amino acids between both gelatins might cause the differences in the interaction between gelatin molecules with FA or FASE. This possibly contributed to the differences in the mechanical property of resulting films.

Water Vapor Permeability

WVP of fish skin gelatin films of both species added with different types and amounts of FA or FASE is depicted in Table 17. WVP of films decreased when FA or FASE amounts increased ($P<0.05$), except the film from bigeye snapper skin gelatin added with PA or SA at 50% glycerol substitution, which had the increased WVP ($P<0.05$). The hydrophobicity of FA or FASE presented in the film composition contributed to the reduced WVP of the film (Ayranci and Tunc, 2001). For bigeye snapper skin gelatin film, the incorporation of PA and SA at 50% glycerol substitution might cause the incompatibility of FA and gelatin in FFS. This might result in the discontinuous protein matrix and some cracks at the interphase might be formed. As a consequence, WVP was increased ($P<0.05$). However, WVP of films gradually decreased with further increase in FA amount up to 100% glycerol substitution ($P<0.05$). Increasing hydrophobicity with increasing FA at 75-100% glycerol substitution led to the decrease in WVP of the film. From the result, WVP of films added with FASE were generally lower than those of film added with FA at the same amount used. FASE have both hydrophilic and hydrophobic characters and could be easily dispersed as well as interacted with protein chains via the formation of hydrogen bonds with the hydrophilic head of the molecule. As a result, continuous hydrophobicity mainly from FASE domain could be increased throughout the film. Nevertheless, no marked differences in WVP were observed between film from brownstripe red snapper skin gelatin added with all lipids tested at levels above 50% glycerol substitution. The differences found between gelatin films from two species were possibly governed by the varying protein

composition as well as the emulsifying properties of gelatin. This might contribute to the differences in compatibility of FA in gelatin between both species, which was associated with film property.

From the result, WVP of films added with SA or SASE was generally lower than those added with PA or PASE. The increase in hydrophobicity of FA with increasing chain length in the order of PA (C_{16}) and SA (C_{18}) was reported by Ayranci & Tunc (2001). The similar result was also reported on cellulose-based films added with lauric acid, PA, and SA (Ayranci and Tunc, 2001). However, at 25 and 50% glycerol substitution, the greater WVP was noticeable in films from bigeye snapper skin gelatin added with PA, compared with those incorporated with SA.

Light transmission and film transparency

Light transmission for both UV and visible ranges at selected wavelength of 200-800 nm of fish skin gelatin films of both species is shown in Table 18. In general, the skin gelatin films of both fish species exhibited the low transmission to light in the UV ranges (200-280 nm). At the same level of FA or FASE used, films added with PA and SA showed the lower light transmission at 280 nm, compared with those added with PASE and SASE as well as the control film ($P<0.05$). The droplet of dispersed FA in the gelatin film might prevent the light transmission, whereas FASE could be dissolved and had lower barrier property to light transmission. PA added films exhibited the lower light transmission than SA, while those added with PASE showed slightly greater light transmission than those with SASE at all wavelength tested. The differences in light transmission of films added with various types of FA or FASE might be due to the different molecular weight, composition, size, nature and some properties of those lipid materials that might interfere with the light transmission properties of the films (Orliac *et al.*, 2003). From the result, the amounts of lipids used were found to affect the light transmission of the films. Films added with FA at the higher amount resulted in the decrease in light transmission of films for both UV and visible ranges. Gallo *et al.* (2000) reported that the methylcellulose emulsion based films became more opaque with increasing solid fat contents.

Table 18. Light transmission (% T) and transparency of fish skin gelatin films as affected by fatty acid or fatty acid sucrose ester types and concentrations

Source of gelatin	Plasicizer type	Platicizer amount (%)	Wavelength (nm)								Transparency[1]
			200	280	350	400	500	600	700	800	
Bigeye snapper skin	Gly	0	0.15	31.90	73.05	76.40	78.85	79.60	80.00	80.15	2.22±0.08^{j*}
	PA	25	0.20	8.65	22.80	25.20	28.50	30.75	32.65	34.35	10.11±0.14^{f}
		50	0.05	3.65	10.65	12.25	14.45	15.95	17.10	18.10	14.97±0.10^{b}
		75	0.00	1.60	5.55	8.10	9.40	11.50	12.75	14.55	14.40±0.70^{c}
		100	0.00	0.50	1.90	2.40	3.50	4.55	5.60	6.65	20.26±0.07^{a}
	SA	25	0.20	8.10	18.70	19.75	20.95	21.70	22.20	22.65	10.42±0.15^{f}
		50	0.25	4.70	9.60	10.30	11.20	11.75	12.20	12.45	11.92±0.14^{d}
		75	0.15	4.60	10.45	11.20	12.30	12.95	13.60	14.30	11.50±0.17^{e}
		100	0.00	1.65	3.45	4.25	4.70	5.95	6.60	7.40	15.22±0.15^{b}
	PASE	25	0.00	18.35	63.55	72.10	79.25	82.35	84.20	85.50	1.58±0.09^{k}
		50	0.10	8.00	43.40	53.95	65.35	71.20	75.00	77.70	2.70±0.04^{i}
		75	0.00	6.65	34.50	43.70	54.50	60.65	64.85	68.05	3.60±0.10^{gh}
		100	0.10	4.40	31.20	41.05	52.50	59.00	61.20	66.40	3.77±0.11^{g}
	SASE	25	0.00	8.85	44.05	52.80	61.15	65.50	68.50	70.85	3.42±0.07^{h}
		50	0.10	8.25	43.80	57.55	66.70	67.80	69.60	71.65	2.87±0.09^{i}
		75	0.00	4.50	36.05	47.25	59.50	66.15	70.45	73.55	2.79±0.05^{i}
		100	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brownstripe red snapper skin	Gly	0	0.15	18.35	63.20	73.45	81.15	83.45	84.40	84.95	1.82±0.03^{i}
	PA	25	0.05	4.20	15.15	18.00	20.75	21.95	22.80	23.35	12.62±0.32^{g}
		50	0.10	1.60	6.75	8.35	9.95	10.75	11.30	11.65	15.52±0.16^{e}
		75	0.15	1.35	4.60	5.50	6.55	7.15	7.65	7.95	17.88±0.24^{d}
		100	0.05	0.90	3.40	4.00	4.85	5.35	5.65	5.90	19.36±0.19^{b}
	SA	25	0.05	6.00	22.60	27.35	29.75	34.55	36.05	37.25	8.56±0.34^{h}
		50	0.15	2.65	10.20	12.50	15.05	16.40	17.35	18.15	14.85±0.35^{f}
		75	0.15	1.25	6.10	7.70	9.70	10.90	11.85	12.60	18.52±0.08^{c}
		100	0.15	1.20	4.75	6.05	7.65	8.60	9.35	10.00	19.76±0.37^{a}
	PASE	25	0.20	14.80	56.95	68.50	77.65	80.40	81.35	82.05	2.08±0.06^{i}
		50	0.00	15.85	64.30	76.50	86.10	88.40	89.10	89.45	1.03±0.03^{k}
		75	0.00	17.90	66.15	78.25	86.80	88.95	89.70	90.00	1.05±0.01^{k}
		100	0.15	15.35	65.70	78.50	86.85	89.00	89.75	90.15	0.93±0.01^{k}
	SASE	25	0.10	13.75	60.35	71.20	76.40	78.45	79.70	81.00	2.11±0.11^{i}
		50	0.15	13.15	58.50	69.25	78.20	81.35	82.70	83.55	1.90±0.08^{i}
		75	0.15	15.50	65.30	75.20	82.30	84.40	85.10	85.45	1.47±0.00^{j}
		100	0.10	13.00	61.65	72.20	80.40	83.40	84.75	85.65	1.47±0.01^{j}

ND: Non-detected (films were too brittle to peel off). [1]Values are given as mean ± SD from triplicate determinations. *Different superscripts in the same column under the same species indicate significant differences ($P<0.05$).

The transparency of fish skin gelatin films added with FA or FASE is shown in Table 18. Films added with PASE and SASE were more transparent, compared with those added with PA and SA at the same amount used ($P<0.05$). The different characteristics of plasticizers used might cause the differences in film transparency (Orliac *et al.*, 2003). For skin gelatin films added with PA and SA, films become less transparent (greater value) with further increase in FA amount ($P<0.05$). The similar behavior was also reported in the emulsified films based on methylcellulose and alkanes or triglycerides mixtures at different solid fat content (Gallo *et al.*, 2000). Conversely, films added with

PASE or SASE were more transparent when a greater amount was added ($P<0.05$). Generally, PASE added films were slightly more transparent, compared with SASE added films. FASE with different chain length might align in the film matrix in different fashion, leading to the different characteristics of resulting film in term of film transmission (Table 18). However, bigeye snapper skin gelatin films added with PASE became less transparent with increasing PASE amount.

Color

Color of fish skin gelatin films from both species added with different types and amounts of FA or FASE is shown in Table 19. Marked increase in b* values (-b* = blueness, +b* = yellowness) of films was observed with increasing FA amount from 25% to 100% glycerol substitution ($P<0.05$), whereas L* value (lightness) slightly increased ($P<0.05$). However, L* value of bigeye snapper skin gelatin films added with SA was lower than that of the control films (without FA). In addition, films added with PA generally had higher b* value than those added with SA at the same amount used. An increase in fat solid content might enhance the reflection of light at the film surface, leading to the increased L* value of film. For films added with FASE, L* value slightly decreased with increasing PASE and SASE amount except brownstripe red snapper skin gelatin film added with SASE. From the result, b* value of bigeye snapper skin gelatin films slightly increased with the addition of FASE amount, but that of brownstripe red snapper skin gelatin films tended to decrease. For a* value (-a* = greenness, +a* = redness) of bigeye snapper skin gelatin films, no changes in a*-value were observed with the addition of FA or FASE ($P>0.05$). Nevertheless, the a*-value of brownstripe red snapper skin gelatin film decreased as all FA and FASE were added, except when SASE was used. The difference in color values of the films added with various type and amount of FA or FASE could be associated with the different characteristics, properties, nature of lipids and the compatibility between the lipid and gelatin molecules in the film matrix (Orliac *et al.*, 2003). Thus, FA or FASE not only influenced the mechanical property and WVP, but also affected the color as well as light transmission of gelatin film.

Table 19. Color of fish skin gelatin films as affected by fatty acid or fatty acid sucrose ester types and concentrations

Source of gelatin	Plasicizer type	Platicizer amount (%)	L^{*1}	a^{*1}	b^{*1}
Bigeye snapper skin	Gly	0	89.32±0.13^{b**}	−1.23±0.06^{bc}	1.95±0.10^{h}
	PA	25	89.88±0.18^{b}	−1.38±0.10^{bc}	2.67±0.08^{efg}
		50	89.90±0.21^{b}	−1.42±0.06^{bc}	3.28±0.12^{cd}
		75	89.95±0.28^{b}	−1.46±0.10^{bc}	3.32±0.23^{cd}
		100	91.01±0.21^{a}	−1.51±0.06^{c}	3.42±0.23^{c}
	SA	25	85.66±0.14^{g}	−1.29±0.07^{bc}	2.41±0.14^{g}
		50	86.44±0.12^{f}	−1.37±0.08^{bc}	2.72±0.15^{efg}
		75	86.67±0.13^{f}	−1.39±0.07^{bc}	2.80±0.10^{efg}
		100	87.83±0.11^{cde}	−1.39±0.12^{bc}	2.96±0.11^{def}
	PASE	25	88.38±0.49^{c}	−1.37±0.13^{bc}	2.59±0.42^{fg}
		50	88.31±0.60^{cd}	−1.44±0.04^{bc}	3.30±0.30^{cd}
		75	87.69±0.52^{de}	−1.47±0.08^{bc}	4.38±0.22^{b}
		100	87.28±0.41^{e}	−1.47±0.10^{bc}	4.40±0.21^{b}
	SASE	25	88.41±0.51^{c}	−1.12±0.23^{a}	2.48±0.29^{g}
		50	88.32±0.59^{cd}	−1.12±0.10^{ab}	3.01±0.12^{de}
		75	88.28±0.39^{cd}	−1.24±0.10^{bc}	4.75±0.21^{a}
		100	ND	ND	ND
Brownstripe red snapper skin	Gly	0	89.40±0.16^{cde}	−1.73±0.09^{abc}	5.40±0.08^{hi}
	PA	25	89.25±0.16^{def}	−1.73±0.09^{abc}	8.87±0.14^{e}
		50	89.28±0.13^{def}	−1.84±0.06^{cd}	11.14±0.10^{b}
		75	89.98±0.17^{a}	−1.96±0.05^{de}	11.65±0.10^{a}
		100	90.04±0.14^{a}	−2.01±0.08^{ef}	11.76±0.12^{a}
	SA	25	89.53±0.13^{bcd}	−1.61±0.08^{ab}	7.60±0.21^{f}
		50	89.30±0.12^{def}	−1.76±0.06^{bc}	9.46±0.12^{d}
		75	89.72±0.11^{abc}	−1.84±0.07^{cd}	10.59±0.05^{c}
		100	89.80±0.11^{ab}	−1.98±0.05^{def}	10.75±0.16^{bc}
	PASE	25	89.39±0.45^{cde}	−2.13±0.09^{f}	6.27±0.62^{g}
		50	89.29±0.33^{def}	−2.04±0.11^{ef}	6.20±0.41^{g}
		75	89.05±0.11^{efg}	−2.04±0.08^{ef}	5.73±0.36^{h}
		100	88.84±0.34^{gh}	−1.98±0.06^{def}	4.58±0.18^{k}
	SASE	25	88.35±0.44^{i}	−1.66±0.06^{ab}	5.78±0.06^{h}
		50	88.64±0.21^{hi}	−1.64±0.12^{ab}	5.14±0.25^{ij}
		75	88.92±0.15^{fgh}	−1.63±0.13^{ab}	4.84±0.10^{jk}
		100	88.95±0.13^{fgh}	−1.59±0.09^{a}	4.51±0.22^{k}

ND: Non-detected (films were too brittle to peel off). [1]Values are given as mean ± SD from 10 determinations. **Different superscripts in the same column under the same species indicate significant differences ($P<0.05$).

7.5 Conclusion

Incorporation of FASE generally increased TS and reduced EAB as well as WVP of both fish skin gelatin films more effectively than FA. TS of films added with FA or FASE with the greater chain length was generally higher than that with a lower chain length. On the other hand, EAB and WVP of films were lowered with the addition of FA or FASE posessing a longer chain length. Films added with FASE were more transparent than those added with FA. Yellowness of films generally increased with the addition of FA and

FASE, but that of brownstripe red snapper skin gelatin films slightly decreased with increasing FASE amount added. Therefore, chain length of FA or FASE affected the properties of lipid/gelatin composite films.