CHAPTER 6

EFFECTS OF PLASTICIZERS ON THE PROPERTIES OF EDIBLE FILMS FROM SKIN GELATIN OF BIGEYE SNAPPER AND BROWNSTRIPE RED SNAPPER

6.1 Abstract

The effects of type and concentration of plasticizers on the mechanical properties (tensile strength, TS and elongation at break, EAB), water vapor permeability, light transmission, transparency and color of fish skin gelatin edible films from bigeye snapper (*Priacanthus marcracanthus*) and brownstripe red snapper (*Lutjanus vitta*) were investigated. At the same plasticizer concentration, fish skin gelatin films from both species plasticized with glycerol (Gly) showed the greatest EAB ($P<0.05$), whereas ethylene glycol (EG) plasticized films showed the highest TS ($P<0.05$). Films prepared from brownstripe red snapper skin gelatin exhibited slightly greater TS than those of bigeye snapper skin gelatin ($P<0.05$) when Gly and sorbitol (Sor) were used. EG, polyethylene glycol 200 (PEG 200) and polyethylene glycol 400 (PEG 400) affected the mechanical properties of both films differently. Films generally became more transparent and EAB, water vapor permeability (WVP), as well as light transmission of films increased, but TS and yellowness decreased with increasing plasticizer concentrations.

6.2 Introduction

Edible film is defined as a thin, continuous layer of edible material, which 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 (Torres, 1997; McHugh, 2000). The incorporation of antibiotics or antioxidants to edible films or coating has gained considerable interest owing to the increased potential to retard the deterioration by microorganism or oxidation at the food product surface (Guilbert *et al.*, 1996).

In general, edible films and coating materials are derived from renewable sources such as carbohydrates, lipids and proteins (Lim *et al.*, 1999). Among these materials, proteins are generally superior to polysaccharides in their ability to form films

with greater mechanical and barrier properties (Cuq *et al.*, 1998). However, the stand alone protein-based films are brittle (Tanaka *et al.*, 2001). Therefore, plasticizers are commonly added to polymeric films in order to reduce the protein-protein chains interactions stabilizing the films network, resulting in the increased mobility of protein molecules (Gontard *et al.*, 1992; Banker, 1966). However, plasticizers generally cause the increased gas and water vapor permeability of films (Tanaka *et al.*, 2001). Thus, plasticizers must be added at a certain amount to obtain the films with improved flexibility without significant decrease of barrier properties to mass transfer (Tanaka *et al.*, 2001; Sothornvit and Krochta, 2001). Hydrophilic plasticizers, such as glycerol (Gly), polyethylene glycol (PEG) and sorbitol (Sor) are generally used for protein-based films to improve mechanical properties (Sothornvit and Krochta, 2001). However, the differences in composition, size, structure and shape of plasticizers directly influence their ability to function in the film network (Orliac *et al.*, 2003).

The effect of plasticizers, both types and concentrations, on the properties of protein-based films have been intensively studied on pea proteins (Gueguen *et al.*, 1998), fish water-soluble proteins (Tanaka *et al.*, 2001), β-lactoglobulin (Sothornvit and Krochta, 2001) and sunflower proteins (Orliac *et al.*, 2003). Nevertheless, no information regarding the effect of plasticizers on properties of gelatin-based edible films has been reported. Therefore, the objective of this investigation was to study the effect of various types and concentrations of plasticizers on the mechanical, barrier and physical properties of edible film from the gelatin of bigeye snapper and brownstripe red snapper skins, which are the wastes generated during surimi processing.

6.3 Materials and Methods

Chemicals

Glycerol (Gly), sorbitol (Sor), ethylene glycol (EG), and polyethylene glycol (PEG 200 and PEG 400) were purchased from Wako Pure Chemical Industries, Ltd. (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 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. 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 prior to gelatin extraction.

Extraction of fish skin gelatin

Gelatin was extracted from fish skin according to the method of Sarabia *et al.* (2000) with a slight modification. 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 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 clothes. The resultant filtrate was freeze-dried and the dry matter was referred to as "gelatin powder".

Preparation of fish skin gelatin films

Gelatin powder was mixed with distilled water to obtain the film-forming solution (FFS) with the protein concentration of 3% (w/v). Gly, Sor, EG, PEG 200 and PEG 400 at various concentrations (25, 50, 75% of protein) were used as plasticizers. To reduce the degradation of bigeye snapper gelatin caused by heat-activated proteinase, 10 mM EDTA was added into FFS. The FFS from both gelatins were incubated at 60°C for

30 min in a water bath with an occasional stirring. The air bubbles in solution were removed by a Hybrid mixer (HM-500; Keyance Co., Tokyo, Japan). De-aerated film forming solution (4±0.01 g) was cast onto a rimmed silicone resin plate (50 mm x 50 mm) and dried with a ventilated oven (Environmental chamber model H110K-30DM; Seiwa Riko Co., Tokyo, Japan) at 25±0.5°C and 50ก5% 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.

Film thickness

Film thickness was measured to the nearest 5 μm with a micrometer (Dial Pipe Gauge, Peacock Co., 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 ColorFles, 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 an Instron (Model TTP-50BXII; Taketomo Electric Co., Ltd. Tokyo, Japan). 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., Australia) according to the method of Fang *et al*. (2003). The transparency of films was calculated by the following equation (Han and Floros, 1997):

$$transparency = -logT600/x$$

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 window, SPSS Inc, Chicago, IL).

6.4 Results and Discussion

Mechanical properties

Fish skin gelatin films from both species showed the different TS and EAB when various types and concentrations of plasticizers were used (Table 14). Generally, TS of bigeye snapper and brownstripe red snapper skin gelatin films decreased with increasing plasticizer concentrations ($P<0.05$). Hydrophilic plasticizer is able to insert between protein

Table 14. Mechanical properties and WVP of fish skin gelatin films as affected by plasticizer types and concentrations

Sources of gelatin	Plasticizer types	Plasticizer conc (%)	TS* (MPA)	EAB* (%)	WVP** (10^{-10} gm^{-1} s^{-1} Pa^{-1})
Bigeye snapper skin	Gly	25	46.87±8.14efgB***	10.87±1.58abcdA	1.35±0.07abA
		50	15.52±2.92abA	42.34±5.38gB	2.58±0.10ijB
		75	11.72±2.35aA	98.14±9.37iC	2.76±0.18cB
	Sor	25	53.52±9.52ghijB	4.88±0.68aA	1.58±0.16bcdA
		50	31.84±2.57cdA	8.69±0.98abcdB	1.73±0.19cdeAB
		75	26.06±3.94bcA	13.58±2.32bcdeC	1.99±0.10efB
	EG	25	67.32±7.90kA	5.12±0.42aA	1.49±0.14bcA
		50	63.18±8.87jkA	5.15±0.66aA	1.59±0.12bcdA
		75	61.41±6.75ijkA	5.44±0.79aA	1.97±0.17efB
	PEG 200	25	ND	ND	ND
		50	41.12±2.55defA	17.75±1.85eA	2.46±0.16hiA
		75	18.31±2.36abB	28.27±3.68fB	3.41±0.18lB
	PEG 400	25	ND	ND	ND
		50	48.54±4.66efghA	7.47±1.93abA	2.71±0.11jA
		75	32.11±5.06cdB	17.50±2.71eB	2.98±0.19kA
Brownstripe red snapper skin	Gly	25	58.10±8.45hijkC	8.20±1.16abcA	1.35±0.10abA
		50	33.58±4.43cdB	39.75±6.09gB	2.16±0.13fgB
		75	18.28±3.10abA	95.04±10.27iC	2.28±0.11ghB
	Sor	25	55.22±7.94ghijB	6.04±1.70aA	1.22±0.09aA
		50	32.41±5.71cdA	15.16±2.51deB	1.57±0.18bB
		75	24.93±5.04bcA	17.63±2.45eB	1.79±0.14deB
	EG	25	53.38±6.65ghijA	5.08±0.97aA	1.38±0.07abA
		50	52.74±8.25ghijA	5.72±0.89aA	1.56±0.10bcdAB
		75	51.07±7.74fghiA	5.94±0.82aA	1.67±0.16cdB
	PEG 200	25	46.70±5.75efgA	6.99±1.14abA	2.00±0.24efA
		50	40.23±4.85defA	14.95±2.94cdeB	2.24±0.12fghAB
		75	39.33±4.39deA	50.86±4.87hC	2.36±0.12lB
	PEG 400	25	48.78±7.18efghB	8.88±1.27abcdA	1.75±0.11cdeA
		50	38.19±5.62efghAB	8.94±1.57abcdA	2.15±0.18fgB
		75	31.42±2.71cdA	10.45±1.32abcdA	2.40±0.11ghiB

*Values are given as mean ± SD from ten determinations. **Values are given as mean ± SD from five determinations. ***Different letters in the same column indicate significant differences ($P < 0.05$). Different capital letters under the same plasticizer and gelatin source indicate significant differences ($P < 0.05$). ND : Non-detected (films were too brittle to peel off).

-chains and disrupts the hydrogen bonding, which stabilizes the film network (Gontard *et al.*, 1993). When plasticizer was incorporated in the gelatin film structure, the interactions and the proximity between protein chains were reduced. However, films containing EG at different concentrations had no differences in TS ($P>0.05$). Sunflower protein films

plasticized with EG at 40% and 50% of protein showed the similar mechanical properties (Orliac *et al.*, 2003). Among all plasticizers used, EG resulted in the highest TS of skin gelatin films from both species ($P<0.05$). For both species, gelatin films plasticized with Gly and Sor had the drastic decrease in TS, when plasticizers added increased from 25% to 75% ($P<0.05$). The decreases in TS of gelatin type A films (Lim *et al.*, 1999) and myofibrillar protein films from Atlantic sardine (Cuq *et al.*, 1997a) were also observed with increasing glycerol content. Arvanitoyannis *et al.* (1998b) reported that TS of chitosan/gelatin blended films decreased with increasing Gly and Sor contents.

From the result, TS of brownstripe red snapper skin gelatin films was slightly greater than that of bigeye snapper skin gelatin films ($P<0.05$) when Gly and Sor were used. EG, PEG 200 and PEG 400 affected the mechanical properties of both films differently. Bigeye snapper skin gelatin films plasticized with 25% PEG 200 and PEG 400 were too brittle to peel off. At level of 50%, PEG 200 and PEG 400 addition rendered the films with high TS, suggesting the strong network of protein film. Nevertheless, TS decreased drastically with addition of 75% PEG 200 or PEG 400 ($P<0.05$). In contrast, brownstripe red snapper skin gelatin films plasticized with 25% PEG 200 or PEG 400 were formed and TS slightly decreased with increasing plasticizers concentration up to 75%. Generally, TS of skin gelatin films from both species plasticized with PEG 400 was greater than that of films plasticized with PEG 200 at any concentration used ($P<0.05$). Plasticizers with the smaller size are more efficient in interacting with protein molecules to decrease elastic modulus (EM), TS and to increase EAB of films (Sothornvit and Krochta, 2001). Similar result were observed for β-lactoglobulin films (Sothornvit and Krochta, 2001) and methylcellulose films (Donhowe and Fennema, 1993) plasticized with PEG having various molecular weights.

EAB of gelatin films containing plasticizers with different types and concentrations is shown in Table 14. In general, EAB of films increased with increasing plasticizers concentrations from 25% to 75% of protein ($P<0.05$). The presence of plasticizer causes a reduction of interaction forces between protein chains and also increases the movement of macromolecules (Gontard *et al.*, 1993), leading to the increase in EAB of films. However, EAB of gelatin films was not affected by EG. From the result, EAB of gelatin films from both species plasticized with Gly was greater than those films plasticized by Sor, EG, PEG 200 and PEG 400 at any concentration used ($P<0.05$). This result was in agreement with Sothornvit and Kroachta (2001) who reported that Gly was the most

effective plasticizer among all plasticizers tested (Sor, Sucrose, PEG 200 and PEG 400) for β-lactoglobulin films. Gly is the smallest straight chain molecule and is the most hygroscopic among all plasticizers tested (Sothornvit and Krochta, 2001). Thus, Gly could be easily inserted between protein chains and attracted more water to the protein films structure, resulting in the increase in its effectiveness as a plasticizer (Sothornvit and Krochta, 2001; Gontard *et al.*, 1993). When the effect of Sor and PEG 200 on EAB was compared, PEG 200 showed higher plasticizer efficiency as evidenced by the greater EAB, compared with Sor ($P<0.05$) (Table 14). Sor and PEG 200 are similar in molecular weight, shape and number of oxygen atoms in the molecules (Sothornvit and Krochta, 2001). The differences in plasticizing effect between both plasticizers were possibly due to the different availability of oxygen atoms for hydrogen bonding (Sothornvit and Krochta, 2001). The differences in TS and EAB of films plasticized with different plasticizers suggested that the appropriate plasticizer for an individual protein film system depends on the nature, size and the structure of plasticizer as well as the compatibility between protein molecules and plasticizers.

From the results, the differences in TS and EAB between bigeye snapper and brownstripe red snapper skin gelatin films plasticized with various types and concentrations of plasticizers could possibly due to the different compositions, particularly in term of amino acid compostion and size of protein chains between both gelatins (Muyonga *et al.*, 2004b; Paschoalick *et al.*, 2003). This might result in the different film formation.

Water Vapor Permeability

WVP of fish skin gelatin films of both species plasticized with different types and concentrations of plasticizers are shown in Table 14. In general, WVP of the films increased when the plasticizer concentration increased ($P<0.05$). With increasing Gly concentration from 25% to 50%, WVP of gelatin films from both species increased ($P<0.05$). However, WVP of films slightly increased with further increase of plasticizers concentration up to 75% of protein ($P<0.05$). For other plasticizers, the constant increases were found in the range of 25-75%. The increase in plasticizer concentration resulted in the increase of WVP of films from gluten (Gontard *et al.*, 1993), sunflower proteins (Orliac *et al.*, 2003), chitosan/gelatin blended (Arvanitoyannis *et al.*, 1998b), muscle

proteins (Paschoalick *et al*., 2003), fish water soluble proteins (Tanaka *et al*., 2001), gelatin (Sobral *et al*., 2001b) and methyl cellulose/soluble starch blends (Arvanitoyannis and Biliaderis, 1999). The insertion of plasticizers between chains of macromolecules increases the free volume of the system and favors the mobility of polymeric chains. Consequently, films network structure became less dense and more permeable (Gontard *et al*., 1993). Since all plasticizers used are hydrophilic, an increase in their concentrations enhanced the absorption of more water to the network and increased water vapor transfer (Orliac *et al*., 2003). However, the different rate of water vapor transmission was observed among gelatin films plasticized with different types and levels of plasticizers. This might be due to the differences in the hygroscopic nature of the plasticizers used, which caused the different ability to attract water to the film network system (Leung, 1986).

Light transmission

Transmission of UV and visible light at selected wavelength of 200-800 nm to fish skin gelatin films for both species is shown in Table 15. Generally, films prepared from the skin gelatin of both fish species exhibited the low transmission to light in the UV ranges (200-280 nm). At the same level of plasticizer used, films plasticized with PEG 200 and PEG 400 showed the lower light transmission at 280 nm, compared with those plasticized with Gly, Sor and EG. The differences in light transmission at 280 nm of films plasticized with various types of plasticizers might be due to the different molecular weight, composition, size, nature and some properties of plasticizers used that might interfere with the light transmission properties of the films (Orliac *et al*., 2003). From the result, the addition of plasticizers at the higher content resulted in the increase in light transmission of films for both UV and visible ranges. Plasticizers used are the transparent compound (Paschoalick *et al*., 2003), which could increase the light transmission of the film. This result was in accordance with Pascoalick *et al*. (2003) who observed that the muscle protein films from Nile tilapia became more transparent with the increase in plasticizer contents.

The transparency of fish skin gelatin films was in the range of 1.03 – 2.40 (Table 15), indicating that the films were fairly transparent. Films plasticized with PEG 200 and PEG 400 were more transparent, compared with those plasticized with Gly, Sor and EG at the same concentration used. The different characteristics of plasticizers used

might cause the differences in film transparency (Orliac *et al*., 2003). It was noticeable that films became more transparent with further increase of plasticizer concentrations. The similar result was reported in edible films from Nile tilapia muscle protein plasticized with increasing glycerol concentration (Paschoalick *et al*., 2003). The transparency of fish skin gelatin films obtained in this study was greater than those of pea protein isolate films (Choi and Han, 2002), myofibrillar protein films (Shiku *et al*., 2003), fish water soluble protein films (Hamaguchi *et al*., 2003) and surimi films (Shiku *et al*., 2004).

Table 15. Light transmission (%T) and transparency of fish skin gelatin films as affected by plasticizer types and concentrations

Sources of gelatin	Plasticizer types	Plasticizer conc (%)	Wavelength (nm) 200	280	350	400	500	600	800	Trans-parency
Bigeye snapper skin	Gly	25	0.3	29.4	69.3	76.8	80.6	82.3	83.9	2.40
		50	0.3	29.4	69.4	76.2	79.6	81.7	82.5	1.82
		75	0.3	29.5	69.5	75.3	79.0	80.8	82.7	1.72
	Sor	25	0.3	24.6	69.9	77.6	81.2	82.4	83.7	1.76
		50	0.3	28.5	70.3	78.6	82.1	83.2	84.1	1.66
		75	0.3	29.9	72.6	79.2	82.6	83.8	84.8	1.46
	EG	25	0.3	19.0	70.5	79.3	83.2	84.5	85.8	1.63
		50	0.3	26.7	73.2	80.6	84.0	85.3	86.0	1.53
		75	0.3	31.0	74.4	81.4	84.4	85.6	86.9	1.33
	PEG 200	25	0.3	ND	ND	ND	ND	ND	ND	ND
		50	0.3	16.2	70.1	78.6	81.8	83.7	84.6	1.56
		75	0.3	17.5	71.1	79.5	82.2	83.8	84.9	1.29
	PEG 400	25	0.3	ND	ND	ND	ND	ND	ND	ND
		50	0.3	19.2	72.8	80.5	82.1	84.5	85.7	1.40
		75	0.3	23.9	73.2	81.1	83.2	85.8	86.8	1.21
Brownstripe red snapper skin	Gly	25	0.3	29.0	73.2	78.5	82.6	83.7	84.4	2.08
		50	0.3	27.5	72.5	78.7	82.5	83.6	84.4	1.63
		75	0.3	27.8	72.3	78.9	82.8	84.3	84.9	1.35
	Sor	25	0.3	19.1	66.7	76.4	81.4	83.1	84.2	1.72
		50	0.3	23.3	71.6	79.0	83.3	84.6	85.2	1.53
		75	0.3	26.7	75.3	81.0	84.4	85.6	86.0	1.33
	EG	25	0.3	15.0	63.6	74.8	80.6	82.5	83.6	2.11
		50	0.3	20.4	69.2	78.2	83.0	84.4	84.9	1.93
		75	0.3	27.3	73.8	79.5	83.0	84.6	85.4	1.80
	PEG 200	25	0.3	16.6	60.7	71.3	76.7	78.8	80.3	2.16
		50	0.3	16.6	66.5	77.0	82.6	84.3	85.4	1.38
		75	0.3	24.2	76.6	83.0	86.8	88.0	88.8	1.03
	PEG 400	25	0.3	15.7	65.6	76.3	82.0	84.0	85.5	1.82
		50	0.3	19.3	66.2	76.7	82.7	84.7	85.6	1.37
		75	0.3	20.8	70.1	78.4	84.0	85.9	86.9	1.15

ND: Non-detected (films were too brittle to peel off)

Table 16. Color of fish skin gelatin films as affected by plasticizer types and concentrations

Source of gelatin	Plasticizer type	Plasticizer conc (%)	L*[1]	a*[1]	b*[1]
Bigeye snapper skin	Gly	25	95.39±0.13abcdA**	−0.35±0.03klA	1.63±0.16fgB
		50	95.45±0.14abcdefA	−0.34±0.03klmA	1.42±0.19efB
		75	96.10±0.18jkB	−0.27±0.03mnB	0.80±0.15aA
	Sor	25	95.48±0.19abcdefgA	−0.30±0.02klmnA	1.34±0.19defB
		50	95.88±0.09hijB	−0.28±0.02lmnAB	0.96±0.08abcA
		75	96.10±0.16jkB	−0.25±0.03nB	0.91±0.12abA
	EG	25	95.20±0.08aA	−0.36±0.03kA	1.42±0.13efB
		50	95.73±0.11fghijB	−0.31±0.02klmnA	1.17±0.08bcdeA
		75	95.95±0.21ijkB	−0.31±0.04klmnA	1.05±0.06abcdA
	PEG 200	25	ND	ND	ND
		50	95.57±0.43cdefgA	−0.49±0.02jA	2.00±0.19hujB
		75	96.18±0.21kB	−0.32±0.04klmB	1.65±0.15efA
	PEG 400	25	ND	ND	ND
		50	95.93±0.14ijkA	−0.35±0.02klA	1.45±0.20efA
		75	95.96±0.19hijkA	−0.34±0.04klmA	1.25±0.15cdeA
Brownstripe red snapper skin	Gly	25	95.56±0.04cdefgA	−0.61±0.04hiA	2.18±0.22ijB
		50	95.71±0.17efghiA	−0.55±0.04ijAB	1.97±0.16hijAB
		75	95.77±0.16ghiA	−0.49±0.02jB	1.80±0.07ghA
	Sor	25	95.21±0.08aA	−0.73±0.02efA	2.92±0.12iB
		50	95.24±0.09aA	−0.52±0.03jB	1.96±0.16hijA
		75	95.43±0.11abcdeB	−0.51±0.04jB	1.96±0.18hijA
	EG	25	95.26±0.10abA	−0.90±0.04bA	4.04±0.16nC
		50	95.46±0.07abcdefB	−0.64±0.04ghB	2.60±0.20kB
		75	95.54±0.07bcdefgB	−0.49±0.02jC	1.87±0.10ghiA
	PEG 200	25	95.32±0.10abcA	−0.84±0.05bcA	3.49±0.17mB
		50	95.42±0.11abcdeA	−0.81±0.06cdA	3.21±0.21lB
		75	95.66±0.07defghB	−0.62±0.04ghB	2.04±0.16nhijA
	PEG 400	25	95.18±0.15aA	−0.99±0.07aA	3.77±0.14nC
		50	95.39±0.11abcdAB	−0.77±0.05deB	2.87±0.24klB
		75	95.48±0.05abcdefgB	−0.68±0.05fgB	2.22±0.19jA

[1]Values are given as mean ± SD from ten determinations. **Different letters in the same column indicate significant differences (P<0.05). Different capital letters under the same plasticizer and gelatin source indicate significant differences (P<0.05). ND: Non-detected (films were too brittle to peel off).

Color

Color of fish skin gelatin films from both species plasticized with different types and concentrations of plasticizers is presented in Table 16. From the result, b* values (-b* = blueness, +b* = yellowness) of films decreased with increasing plasticizer

concentrations from 25% to 75% of protein ($P<0.05$), whereas L* (lightness) and a* values (-a* = greenness, +a* = redness) slightly increased ($P<0.05$). The decrease in b* value of film plasticized with greater concentration of plasticizers might be owing to the dilution of the proteins by colorless plasticizers (Paschoalick *et al*., 2003). The result was in accordance with Paschoalick *et al*. (2003) who reported that the color values of muscle protein films from Nile tilapia decreased linearly with increasing plasticizer concentration. Additionally, the increase in plasticizer content might enhance the reflection of light at the film surface, leading to the increased L* value of film added with some plasticizer, especially Gly.

6.5 Conclusion

Transparent edible films were successfully prepared from fish skin gelatin of brownstripe red snapper and bigeye snapper. The properties of fish skin gelatin films from both fish species were affected by types and concentrations of plasticizers used. Films plasticized with Gly showed the highest EAB among all plasticizers at the same concentration used, whereas EG plasticized films showed the highest TS. EAB, WVP and light transmission increased but TS and yellowness decreased with increasing plasticizers concentration. The properties of gelatin films from both species were affected by plasticizers differently.