CHAPTER 5

CHARACTERIZATION OF EDIBLE FILMS FROM SKIN GELATIN OF BROWNSTRIPE RED SNAPPER AND BIGEYE SNAPPER

5.1 Abstract

Edible films were successfully prepared from fish skin gelatin of brownstripe red snapper (*Lutjanus vitta*) and bigeye snapper (*Priacanthus marcracanthus*). Films with greater protein content had higher thickness and tensile strength (TS) and elongation at break (EAB) but lower water vapor permeability (WVP) than those with lower protein content. Films without glycerol were mostly brittle, and became more flexible in the presence of glycerol. TS generally decreased with increasing glycerol concentration from 25 to 75%. Films prepared from bigeye snapper skin gelatin exhibited the lower mechanical properties than those of brownstripe red snapper at any glycerol concentrations used. Marked decreases of transition temperature and transition enthalpy of the films were observed with increasing glycerol content. Films from bigeye snapper skin gelatin contained the lower content of high-molecular-weight cross-links with the concomitant increased degradation peptides, compared with gelatin powder and films from brownstripe red snapper skin gelatin. The addition EDTA and soybean trypsin inhibitor into the film forming solution (FFS) of bigeye snapper skin gelatin totally inhibited and mostly retarded the degradation of gelatin components, respectively. This suggested that metallo- and serine-proteinase/collagenase actively involved in protein hydrolysis in bigeye snapper skin gelatin, especially during FFS preparation. Addition of EDTA also increased TS and EAB of film from bigeye snapper skin gelatin.

5.2 Introduction

Edible films and coating materials are potentially used to extend the shelf-life and improve the quality of almost any food system by serving as mass transfer barriers to moisture, oxygen, carbon dioxide, lipid, flavor and aroma between food components and the surrounding atmosphere (McHugh, 2000). Coating materials have been reported to

improve the appearance of some foods such as candies by reducing their stickiness (Donhowe and Fenema, 1994). The composite edible films or edible films containing some preservatives, antibiotics or antioxidants, which are concentrated at the product surface where the protection is needed, have been paid more attention (Guilbert *et al.*, 1996). Furthermore, the increasing concern in health, nutrition, food safety and environmental problems leads to the interest in edible and biodegradable films or materials.

Generally, edible films is defined as a thin, continuous layer of edible material (Torres, 1997). The materials used are mainly the renewable sources such as proteins, carbohydrates and lipids. Among these materials, proteins have been extensively selected for the development of edible films owing to their abundance and the uniqueness in film-forming ability and properties of films obtained (Irissin-Mangata *et al.*, 2001; Ou *et al.*, 2004). Protein-based films are generally superior to polysaccharide-based films in their mechanical and barrier properties (Cuq *et al.*, 1998). This is because proteins have a specific structure (based on 20 different monomers) which provides a wider range of potential functionalities. The stronger intermolecular binding potential via covalent bonds was found in protein-based films, not in the film from homopolymer polysaccharides (Cuq *et al.*, 1995).

Among all proteins, gelatin has been attracted the attention for the development of edible films due to its abundance and biodegradability (Bigi *et al.*, 2002). Collagen and gelatin films have been used for sausage casing (Johnston-Banks, 1990), production of hard and soft capsules, wound dressing and adsorbent pad in the pharmaceutical industry (Digenis *et al.*, 1994). Gelatin edible films, with high puncture strength, low puncture deformation and high water vapor permeability, prepared from bovine and porcine skin have been reported (Sobral *et al.*, 2001b). Edible wrappings based on blends of gelatin with other constituents have been marketed (Torres, 1994). Gelatin is normally manufactured from the waste generated during the animal slaughtering and processing, such as skin and bone (Patil *et al.*, 2000). So far, the main sources of gelatin are limited to those of land animal origin such as bovine or porcine skin and bone. However, the outbreak of mad cow disease and the foot-and-mouth disease crisis has resulted in the anxiety among users of collagen and gelatin products from land-based animal (Helcke, 2000). Additionally, gelatin obtained from porcine skins or bones cannot be applied for some foods due to esthetic and religious objections (Sadowska *et al.*, 2003). Therefore, alternative sources, especially fish processing wastes including skin, bone or

scale have been paid increasing attention for gelatin extraction. So far, a little information regarding the characteristics of edible films from fish skin gelatin, especially from commercially important species involving those used for surimi production, has been reported. Therefore, the aim of this investigation was to prepare and characterize the edible films from the skin gelatin of brownstripe red snapper (*Lutjanus vitta*) and bigeye snapper (*Priacanthus marcracanthus*) which are the prevalent species used for surimi production in Thailand.

5.3 Materials and Methods

Chemicals

α-Chymotrypsin, glycerol, sodium dodecyl sulfate and Coomassie Brilliant Blue R-250 were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). β-mercaptoethanol (βME) and high- and low-molecular-weight protein markers were obtained from Sigma Chemical Co. (St. Louis, Mo., USA).

Fish skin preparation

Brownstripe red snapper (*Lutjanus vitta*) and bigeye snapper (*Priacanthus macracanthus*) 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). Skins were kept on ice prior to gelatin extraction.

Extraction of 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 was 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 with tap water until neutral or faintly acidic pHs of wash water was obtained. 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 gelatin films

Gelatin powder was mixed with distilled water to obtain the film-forming solution (FFS) with the protein concentration of 1, 2, 3 and 4% (w/v). Glycerol was used as a plasticizer at the concentration of 25% of protein. The solution was 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 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 50±5% relative humidity (RH) for 24 h. Dried films obtained were manually peeled off.

To study the effect of plasticizer concentrations on fish gelatin film, glycerol at various concentrations was used (25, 50, 75% of protein). The FFS was prepared, cast and dried as previously described.

To investigate the effect of proteinase inhibitors on the degradation of gelatin in FFS solution, soybean trypsin inhibitor (0.01 and 0.1 mM) and EDTA (10 and 20 mM) were added into FFS and the mixtures were then incubated at 60°C for 30 min. The solutions without inhibitors incubated at 60°C for 30 min and 100°C for 10 min were used as the positive and negative controls, respectively. All samples were subjected to SDS-PAGE analysis.

Analyses

The films were conditioned for 48 h at 25±0.5°C and 50±5% RH prior to analyses. The film thickness was determined without conditioning.

Film thickness

Film thickness was measured to the nearest 5 μm with a hand-held micrometer (Dial Pipe Gauge, Peacock Co., Tokyo, Japan). Nine measurements were taken at random positions. Precision of the thickness measurements was ±5%.

Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of gelatin films were determined using a Tensipressor (Model TTP-50BXII; Taketomo Electric Co., Ltd. Tokyo, Japan). A total of ten samples were measured for each film.

Water vapor permeability

Water vapor permeability (WVP) of films was measured using a modified ASTM method (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 film.

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-160, Shimadzu Co., Kyoto, Japan) according to the method described by Fang *et al.* (2002).

Digestibility by protease

Protein digestibility of films was determined according to the method described by Shiku *et al.* (2003) and Ou *et al.* (2004) with a slight modification. Ground film sample (15 mg) was suspended in 10 ml of α-chymotrypsin solution (0.2 mg/ml in 40 mM Tris-HCl buffer, pH 7.6). The suspension was then incubated at 37°C for 2 h. The reaction was stopped by addition of 5 ml of cold 50% trichloroacetic acid (TCA). The mixture was allowed to stand for 30 min at 4°C and was then centrifuged at 9,500 x *g* for 30 min using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The resultant precipitate was dissolved in 1.0 M NaOH and protein concentration was measured using the method of Lowry, Rosebrough, Farr and Randall (1951). The degree of hydrolysis of protein film sample was expressed as % digestibility using the following equation:

Protein digestibility (%) = (A - B) / A x 100

where A is total protein amount (mg) in film sample. B is total protein amount (mg) in TCA precipitate.

Electrophoretic analysis

Protein patterns of gelatin film was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Film samples (200 mg) were dissolved in 10 ml of 1% (w/v) SDS. The mixture was stirred continuously at room temperature for 12 h. Supernatants were collected after centrifuging at 3,000 x *g* for 3 min. For FFS, the solution was mixed with 2 % (w/w) SDS at a ratio of 1:1 (v/v). The mixture was stirred and supernatant was obtained in the same manner. The supernatants were then mixed with sample buffer (0.5 M Tris-HCl, pH 6.8 containing 4% (w/v) SDS, 20% (v/v) glycerol with 10% (v/v) βME at

the ratio of 1:1 (v/v). Samples were loaded on the PAGEL® -Compact precast gel (Atto Co., Tokyo, Japan) and subjected to electrophoresis at a constant current of 20 mA per gel using a Compact-PAGE apparatus (Atto Co., Tokyo, Japan). After electrophoresis, gel was stained with 0.05% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. High- and low-molecular-weight markers were used to estimate the molecular weight of proteins.

Differential scanning calorimetry

Prior to analysis, films were conditioned in a desiccator containing silica gel for 3 weeks at room temperature to obtain the most dehydrated possible films (Sobral *et al.*, 2001b). Thermal properties of films were determined using Perkin Elmer Differential Scanning Calorimetry (DSC) (Model DSC-7, Norwalk, CT, USA). Temperature calibration was performed using the Indium thermogram. The film samples (5-10 mg) were accurately weighed into aluminum pans, sealed, and scanned over the range of 20 to 150°C with a heating rate of 5°C/min. The ice water was used as a cooling medium and the system was equilibrated at 20°C for 5 min prior to the scan. The empty aluminum pan was used as a reference. The maximum transition temperature was estimated from the maximum endothermic peak of DSC thermogram and referred to as transition temperature and the peak area was defined as transition enthalpy.

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 8.0 for window, SPSS Inc, Chicago, IL).

5.4 Results and discussion

Effect of protein concentration on the properties of fish skin gelatin films

Mechanical properties

Skin gelatin films from both species showed the different TS and EAB when different protein concentrations were used (Table 8). Film prepared from film-forming solution (FFS) with 1% protein content was too thin to peel off. On the contrary, the viscosity of FFS with 4% protein content was too high and could not be used for film casting. TS of skin gelatin films of both species increased with increasing protein concentration from 2% to 3% ($P<0.05$). At the same protein concentration used, film from bigeye snapper skin gelatin exhibited the lower TS than that from brownstripe red snapper, suggesting the difference in film forming ability of gelatin between two species. The marked increase in EAB of films from bigeye snapper skin gelatin was observed when the protein content increased from 2 to 3% ($P<0.05$). However, no change in EAB of films from brownstripe red snapper skin gelatin was obtained as the protein concentration increased ($P>0.05$). Therefore, the higher protein content in FFS of bigeye snapper skin gelatin might result in the higher aggregation of protein to form the film with improved mechanical properties. The result was in accordance with those reported on fish water soluble protein (Iwata *et al.*, 2000) and casein films (Brault *et al.*, 1997), in which the TS and EAB increased with increasing protein concentration.

Table 8. Mechanical properties, WVP and thickness of fish skin gelatin films as affected by protein concentration

Source of gelatin	Protein conc. (%)	TS* (MPa)	EAB* (%)	WVP** (10^{-10} g m^{-1} s^{-1} Pa^{-1})	Thickness (μm)
Bigeye snapper skin	1	ND	ND	ND	ND
	2	$28.28\pm6.76^{a***}$	2.68 ± 0.64^{a}	1.22 ± 0.11^{a}	23.30 ± 2.97^{a}
	3	44.28 ± 10.06^{ab}	7.00 ± 1.85^{b}	1.37 ± 0.07^{a}	35.32 ± 1.83^{b}
Brownstripe red snapper skin	1	ND	ND	ND	ND
	2	41.09 ± 9.81^{ab}	7.02 ± 1.46^{b}	1.33 ± 0.04^{a}	24.10 ± 1.87^{a}
	3	58.10 ± 8.45^{b}	8.20 ± 1.16^{b}	1.35 ± 0.10^{a}	37.18 ± 1.56^{b}

ND: Non-detected (films were too weak to handle). *Values are given as mean ± SD from ten determinations. **Values are given as mean ± SD from five determinations.
***Different superscripts in the same column indicate significant differences ($P<0.05$).

Water vapor permeability

For WVP, it was noted that similar WVP was observed between two fish skin gelatin films at the same protein concentration used (Table 8). Nevertheless, higher WVP was found with gelatin film containing a greater protein content and higher thickness.

Since gelatin contained a wide range of hydrophilic amino acids (Cho *et al.*, 2004), films with higher amount of protein and thickness could adsorb more water from the environment (McHugh, 1993). Thus, the film with higher protein content was most likely hygroscopic, compared with that containing the lower protein content.

Light transmission

Transmission of UV and visible light to fish skin gelatin films from both species was affected by protein concentration (Table 9). Generally, light transmission for both UV and visible ranges (200-800 nm) decreased with increasing protein concentration from 2 to 3%. Gelatin films with 3% protein content with the greater thickness (Table 8) tended to have the lower transmission of light at both UV and visible ranges. Thus, films with greater protein content and thickness would absorb the light more effectively than those with lower protein content and thickness. At the same protein level used, film from bigeye snapper skin gelatin showed the lower transmission than that from brownstripe red snapper gelatin. Collagen, a native form of gelatin, from both fish species had some differences in amino acid content and compositions (Jongjareonrak *et al.*, 2005a; Jongjareonrak *et al.*, 2005b). The difference in light transmission between the films from both species might be due to the differences in composition, density and the aggregation or alignment of gelatin molecules in the film between two species.

From the study, film with 3% protein content showed the greater TS and EAB than that containing 2% protein content. Therefore, gelatin film with 3% protein was used for further study.

Table 9. Light transmission (%T) of fish skin gelatin films as affected by protein concentration

Source of gelatin	Protein conc (%)	Wavelength (nm)						
		200	280	350	400	500	600	800
Bigeye snapper	2	0.3	35.9	72.4	78.8	82.7	83.6	84.8
Skin	3	0.3	29.4	69.3	76.8	80.6	82.3	83.9
Brownstripe	2	0.3	36.7	73.6	80.0	83.5	84.5	85.1
Red snapper skin	3	0.3	29.0	73.2	78.5	82.6	83.7	84.4

Effect of plasticizer levels on the properties of fish skin gelatin films

Mechanical properties

TS of films prepared from skin gelatin of both species plasticized with glycerol at different concentrations is shown in Table 10. In general, TS of films decreased with increasing glycerol concentrations ($P<0.05$). TS of brownstripe red snapper and bigeye snapper skin gelatin films decreased from 67.78 to 18.28 MPa and from 57.34 to 7.97 MPa, respectively, with increasing glycerol content from 25 to 75%. Glycerol has the relatively small molecule with hydrophilic characteristic which could be easily inserted between protein chains and establish hydrogen bonds with amide group and amino acid side chains of proteins (Gontard *et al.*, 1993). When glycerol was incorporated in the gelatin film network, direct interactions and the proximity between protein chains were reduced. This result was in agreement with Lim *et al.* (1999) who reported the decrease in TS of gelatin type A films with increasing glycerol content. The reduction of puncture force of myofibrillar protein film from Atlantic sardine was observed with the addition of glycerol as a plasticizer at the concentration between 0 and 40% of protein (Cuq *et al.*, 1997b). Sobral *et al.* (2001b) also reported that puncture force of bovine hide and pig skin gelatin films decreased with the addition of sorbitol at the high content (45 and 55 g sorbitol/100 g gelatin).

Table 10. Mechanical properties and WVP of fish skin gelatin films as affected by glycerol concentration

Source of gelatin	Glycerol conc (%)	TS* (MPa)	EAB* (%)	WVP** (10^{-10} g m^{-1} s^{-1} Pa^{-1})
Bigeye snapper skin	0	$57.34 \pm 15.08^{e***}$	3.40 ± 1.14^{a}	1.31 ± 0.08^{a}
	25	44.28 ± 10.06^{d}	7.00 ± 1.85^{a}	1.37 ± 0.07^{a}
	50	15.41 ± 2.93^{ab}	24.42 ± 6.68^{b}	2.61 ± 0.15^{c}
	75	7.97 ± 1.19^{a}	50.30 ± 5.72^{d}	2.73 ± 0.22^{c}
Brownstripe red snapper skin	0	67.78 ± 14.67^{f}	5.24 ± 1.56^{a}	1.32 ± 0.07^{a}
	25	58.10 ± 8.45^{e}	8.20 ± 1.16^{a}	1.35 ± 0.10^{a}
	50	33.58 ± 4.43^{c}	39.75 ± 6.09^{c}	2.16 ± 0.13^{b}
	75	18.28 ± 3.10^{b}	95.04 ± 10.27^{e}	2.28 ± 0.11^{b}

*Values are given as mean ± SD from ten determinations. **Values are given as mean ± SD from five determinations. ***Different superscripts in the same column indicate significant differences ($P<0.05$).

EAB of gelatin films containing different glycerol levels is shown in Table 10. EAB of films increased with increasing glycerol from 25 to 75% of protein ($P<0.05$). EAB increased from 5.24 to 95.04% and from 3.40 to 50.30% for the gelatin film from brownstripe red snapper and bigeye snapper skin, respectively. From the result, EAB of film from brownstripe red snapper skin gelatin was greater than that of film from bigeye snapper skin gelatin, especially at the high level of glycerol added. The result was in accordance with Gontard *et al.* (1993) who observed that puncture force of gluten films increased from 6 to 20% with increasing glycerol concentration from 16 to 33 g glycerol/100 g dry matter. EAB of gluten films increased from 4 to 22% when glycerol content increased from 10 to 20% (Irissin-Mangata *et al.*, 2001). The presence of plasticizer causes a reduction of intermolecular interaction and also increases the mobility of macromolecules (Gontard *et al.*, 1993), leading to the increase in EAB of films. Additionally, the increase in the plasticizer concentration increases the moisture content of films because of its high hygroscopic character, which contributes to the reduction of the forces between the adjacent macromolecules (Sobral *et al.*, 1999). At the same glycerol concentration used, the differences in TS and EAB between two fish skin gelatin films were possibly caused by the different compositions, particularly in term of amino acid composition and size of protein chains (Muyonga *et al.*, 2004b; Paschoalick *et al.*, 2003), resulting in the different film formation.

Water Vapor Permeability

WVP of fish skin gelatin films containing the different glycerol levels is shown in Table 10. Generally, WVP of films from the skin gelatin of both fish species increased with increasing glycerol concentration ($P<0.05$). With increasing glycerol content from 25 % to 50 %, WVP increased from 1.31×10^{-10} to 2.61×10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ and from 1.32×10^{-10} to 2.16×10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ for films prepared from the skin gelatin of bigeye snapper and brownstripe red snapper, respectively. However, WVP of films remained constant with further increase in glycerol content up to 75% of protein. The increase in glycerol content resulted in the increased WVP of films from muscle proteins (Paschoalick *et al.*, 2003), fish water-soluble proteins (Tanaka *et al.*, 2001b), gelatin (Lim *et al.*, 1999; Sobral *et al.*, 2001b) and wheat gluten (Gontard *et al.*, 1993). Glycerol, a small straight chain hydrophilic plasticizer consisting of 3 hydroxyl groups (-OH), is able to

attract water to the plasticized protein system due to its high hygroscopic character (Sobral *et al*., 1999; Sothornvit and Krochta, 2001). The increase in water vapor transmission rate of films was possibly caused by an increase in free volume of system which enhanced the mobility of the polymeric chains, due to the insertion of glycerol between protein molecules. Consequently, the network structure of films became less dense and more permeable (Gontard *et al*., 1993). This led to the increase in the rate of water diffusion of the film matrix (Cuq *et al*., 1997b).

At the same glycerol level used, films produced from bigeye snapper skin gelatin had generally greater WVP than those obtained from brownstripe red snapper skin gelatin. WVP of gelatin films from the skin of both species incorporated with 50% glycerol was higher than those of fish water soluble protein and muscle protein films plasticized with glycerol at the same level as reported by Iwata *et al*. (2000) and Paschoalick *et al*. (2003), respectively. The resistance of fish skin gelatin based film to water vapor transmission was lower than that of muscle protein film, possibly due to the higher inherent hydrophilic properties of gelatin.

Light transmission

Transmission of UV and visible light at selected wavelength in the range of 200-800 nm to fish skin gelatin films from both species is presented in Table 11. Transmission in visible range (350-800 nm) of gelatin films was from 69.3 to 86.8 %. The transmission of UV light at 280 nm was in the range of 24.9-29.5%. Very low transmission (0.3%) was found at 200 nm. Therefore, gelatin film effectively prevented the UV light. On the other hand, those synthetic films did not prevent the transmission of UV light above 200 nm except for polyester film. These results suggested the potential preventive effect of gelatin films on the retardation of lipid oxidation induced by UV light. The similar results were also observed for fish myofibrillar protein film (Shiku *et al*., 2003), surimi film (Shiku *et al*., 2004), fish water soluble protein film (Hamaguchi *et al*., 2003) and whey protein film (Fang *et al*., 2002). However, skin gelatin films prepared from both species had the greater UV transmissible at 280 nm than those of other fish protein previously reported (Hamaguchi *et al*., 2003; Shiku *et al*., 2003, 2004). The collagen obtained from both fish skins (native form of gelatin) has lower content of aromatic amino acids (tyrosine and phenylalanine) (Jongjareonrak *et al*., 2005a; 2005b)

than those of fish muscle protein (Paschoalick *et al.*, 2003). In general, tyrosine and phenylalanine are well known to be sensitive chromophores, which absorb the light at the wavelength below 300 nm (Li *et al.*, 2004). The aromatic amino acid content of protein material might play an important role in UV barrier properties of protein films.

From the result, glycerol levels (25-75%) did not affect the light transmission of fish skin gelatin film as evidenced by the similar transmission in both UV and visible ranges. The addition of glycerol resulted in the slight decrease in transmission at 400-800 nm, while led to the increase in transmission at 280 nm. Therefore, the glycerol used as the plasticizer affected the light transmission property of fish skin gelatin film to some extent.

Table 11. Light transmission (%T) of fish skin gelatin films as affected by glycerol concentration

Source of gelatin	Glycerol conc (%)	Wavelength (nm)						
		200	280	350	400	500	600	800
Bigeye	0	0.3	24.9	68.1	77.2	82.2	84.5	86.7
snapper skin	25	0.3	29.4	69.3	76.8	80.6	82.3	83.9
	50	0.3	29.4	69.4	76.2	79.6	81.7	82.5
	75	0.3	29.5	69.5	75.3	79.0	80.8	82.7
Brownstripe	0	0.3	26.2	73.4	80.0	84.1	85.7	86.8
Red snapper	25	0.3	29.0	73.2	78.5	82.6	83.7	84.4
skin	50	0.3	27.5	72.5	78.7	82.5	83.6	84.4
	75	0.3	27.8	72.3	78.9	82.8	84.3	84.9
Synthetic films								
LDPE[a]		13.1	67.5	79.9	83.4	85.6	86.9	83.6
OPP[a]		4.6	80.0	86.2	87.9	88.8	89.1	89.6
PE[a]		0.3	0.3	68.3	73.6	82.1	83.5	84.9
PVDC[a]		0.3	79.1	83.8	86.6	87.5	90.0	84.9

[a] From Shiku *et al.* (2003), LDPE, low-density polyethylene; OPP, oriented polypropylene; PE, polyester; PVDC, polyvinylidene chloride.

Film digestibility

The protein digestibility of gelatin films from the skin of both species by α-chymotrypsin ranged from 64 to 69%, regardless of the glycerol concentration (data not shown). The result suggested that glycerol levels did not affect the digestibility of films. Apart from α-chymotrypsin, other digestive proteolytic enzyme also involved in the digestion of proteins in the digestive system (Shiku *et al.*, 2003). From the result, fish gelatin film could be easily digested. Furthermore, films were mostly dissolved in water, resulting in the ease of enzymatic hydrolysis.

Thermal properties

Transition temperature and transition enthalpy of films from bigeye snapper and brownstripe red snapper skin gelatin at different glycerol concentrations are shown in Table 12. Generally, transition temperature and transition enthalpy of gelatin films from both species decreased with increasing glycerol concentration. Transition temperature, observed from the maximum of the endothermic transition peak, of films plasticized with glycerol at 0, 25, 50 and 75% were 96.42, 72.97, 61.08 and 53.14°C, respectively, for bigeye snapper skin gelatin films, and were 100.28, 91.58, 67.30 and 59.89°C, respectively, for brownstripe red snapper skin gelatin films. The observed transition temperature of the film indicated the temperature causing the disruption of the protein interaction formed during film preparation. Transition temperature of films from brownstripe red snapper skin gelatin was relatively higher than that of bigeye snapper skin gelatin at any glycerol levels used. The higher transition temperature might be due to the greater amount of imino acids composition (proline and hydroxyproline) of brownstripe red snapper skin gelatin compared to bigeye snapper skin gelatin (Jongjareonrak *et al.*, 2005a, 2005b). The amount of imino acids showed the direct positive correlation to the thermal stability of protein via hydrogen bond (Sikorski *et al.*, 1984). Moreover, the integrity and molecular weight of protein chains might contribute to the network structure of films obtained (Shiku *et al.*, 2004). Films from bigeye snapper skin gelatin was composed of proteins with lower molecular weight and greater content of degradation peptides, compared with those of brownstripe red snapper skin gelatin (Figure 20). This might be associated with the weaker film network. As a consequence, melting at the lower temperature was found as indicated by the lower T_m in the film from bigeye snapper skin gelatin.

Table 12. Transition temperature and transition enthalpy of fish skin gelatin films as affected by glycerol concentration

Source of gelatin	Glycerol conc (%)	Transition temperature (°C)*	Transition enthalpy (J/g)*
Bigeye snapper skin	0	96.42 ± 1.59^{f}**	265.98 ± 6.60^{e}
	25	72.97 ± 2.70^{d}	137.24 ± 1.75^{c}
	50	61.08 ± 0.73^{b}	46.50 ± 4.50^{b}
	75	53.14 ± 0.49^{a}	29.23 ± 3.12^{a}
Brownstripe red snapper skin	0	100.28 ± 1.02^{g}	217.88 ± 10.02^{d}
	25	91.58 ± 2.84^{e}	144.01 ± 14.86^{c}
	50	67.30 ± 0.38^{c}	31.39 ± 4.77^{a}
	75	59.89 ± 2.10^{b}	18.80 ± 0.83^{a}

*Values are given as mean ± SD from tripicate determinations. **Different superscripts in the same column indicate significant differences ($P<0.05$).

Transition enthalpy of films from skin gelatin of both species decreased with increasing glycerol levels (Table 12). The decrease in thermal stability of films was possibly affected by the presence of plasticizer, glycerol, which reduced the interaction of proteins, such as hydrogen bonds, ionic-interactions, hydrophobic-hydrophobic interactions, which stabilized the network structure of film (Barreto *et al.*, 2003). Furthermore, the presence of plasticizer between protein chains could hinder the reorganization of random gelatin strands to the typical triple-helix structure via hydrogen bond (Arvanitoyannis *et al.*, 1997). Renaturation of random gelatin strands to the triple-helix structure during film formation was most likely associated with the increased thermal stability of gelatin film (Arvanitoyannis *et al.*, 1997). Therefore, gelatin films plasticized with higher glycerol concentration required the lower enthalpy for destroying the inter-chain interactions. The low transition enthalpy observed in the film containing a greater concentration of glycerol was coincidental with the low transition temperature. Thus, thermal properties of fish skin gelatin film were markedly affected by the level of plasticizer used.

Protein patterns

Protein patterns of gelatin films containing different glycerol levels from both fish skins are shown in Figure 20. For the same fish skin gelatin film, no differences in protein patterns were observed between the films with different glycerol levels used. The result indicated that no differences in non-disulfide covalent bond were found with different glycerol levels. Protein patterns of gelatin film from brownstripe red snapper was similar to those of gelatin. However, protein pattern of gelatin films from bigeye snapper skin showed the marked differences, compared with those of gelatin. Band intensity of high MW cross-links decreased with the concomitant increase in small MW peptide fragments, especially with the MW of 70.2, 61.5 and 55.4 kDa. Additionally, α_1-chain was mostly degraded. The decrease in high MW cross-links and α-chain might result in the lowered mechanical properties of films from bigeye snapper skin gelatin.

From the result, similar degradation patterns of gelatin films from bigeye snapper skin was observed between film containing different glycerol levels, suggesting that protein molecules in all films were specifically cleaved and certain peptides with similar

molecular weight were generated. Some proteinase or collagenase might involve in the degradation of gelatin from bigeye snapper skin.

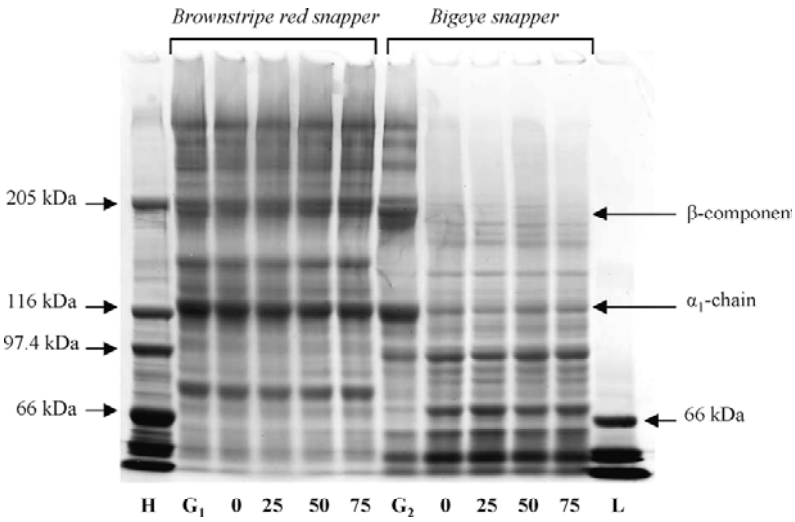

Figure 20. Protein pattern of gelatin films. H, High MW protein markers; L, Low MW protein markers; G_1 and G_2, gelatin powder of brownstripe red snapper and bigeye snapper skins; respectively, The numbers designate glycerol concentration (% of protein).

Effect of proteinase inhibitors on protein degradation and the properties of fish skin gelatin film

Protein patterns

Protein patterns of FFS of bigeye snapper skin gelatin with and without proteinase inhibitors and incubated at 60°C for 30 min are shown in Figure 21. High MW cross-links were observed in gelatin samples, however FFS incubated at 60°C without inhibitor had no high MW cross-link with the decreased band intensity of α-chains. With heating at 100°C, band intensity of high MW cross-links component at 205 kDa of film forming solution was retained to high extent. In presence of soybean trypsin inhibitors at the concentrations of 0.01 and 0.1 mM, degradation of cross-links and α-chains was mostly inhibited. Interestingly, no degradation was found in FFS added with 10 or 20 mM EDTA. Therefore, it could be concluded that collagenase or proteinase played a vital role in

degradation of film solution, especially at 60°C. The degradation of gelatin caused by proteolysis might be associated with the poor mechanical properties of gelatin films from bigeye snapper skin. Since these enzymatic hydrolysis was mostly inhibited by soybean trypsin inhibitor and totally inhibited by EDTA, it was suggested that heat-activated metallo- and/or serine collagenase might be presented in bigeye snapper skin gelatin. The degradation of collagens by matrix metallo-proteinases has been reported in the biological system (Hasty *et al*., 1987). Therefore, this metallo proteinase/collagenase might exist in the bigeye snapper skin and was not removed or inactivated by gelatin extraction process. The remaining collagenase was activated during FFS preparation and effectively hydrolyzed the gelatin.

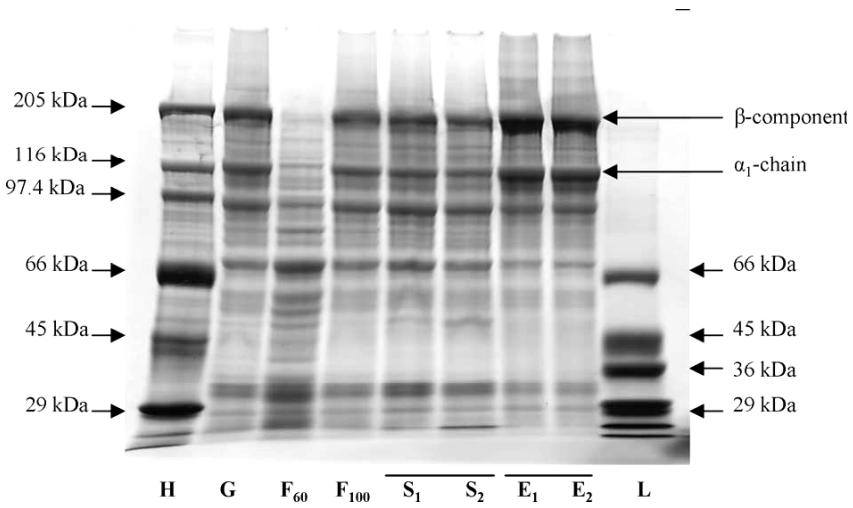

Figure 21. Protein patterns of FFS of gelatin from bigeye snapper skin. H, High MW protein markers; G, gelatin powder of bigeye snapper skin; F_{60}, FFS incubated at 60°C for 30 min; F_{100}, FFS heated at 100°C for 10 min, S1 and S2, FFS added with 0.01 and 0.1 mM soybean trypsin inhibitor; respectively, and incubated at 60°C for 30 min, E_1 and E_2, FFS added with 10 and 20 mM EDTA; respectively, and incubated at 60°C for 30 min, L, Low MW protein markers.

Mechanical properties

TS and EAB of gelatin films prepared from FFS incubated at 60°C for 30 min with and without EDTA and from FFS heated at 100°C for 30 min are shown in Table

13. No differences in TS were observed among all samples (P>0.05). However, EAB of gelatin films added with 10 mM EDTA were 2-folds higher than that of films prepared from FFS incubated at 60°C without EDTA. This result indicated that films prepared from gelatin with a larger content of high MW cross-links component gave the superior mechanical properties, especially EAB of gelatin films. The high positive correlation between film strength/EAB of films and the content of high MW component of gelatin have been reported (Muyonga *et al*., 2004b). Therefore, mechanical properties of gelatin films were obviously affected by molecular weight distribution of proteins.

Table 13. Mechanical and WVP properties of bigeye snapper skin gelatin films prepared from FFS with and without proteinase inhibitors

Treatment	TS (MPa)*	EAB (%)*	WVP (10^{-10} g m^{-1} s^{-1} Pa^{-1})**
1	$15.41 \pm 2.93^{a***}$	24.42 ± 6.68^{a}	2.61 ± 0.15^{a}
2	15.68 ± 2.01^{a}	33.69 ± 3.65^{ab}	2.67 ± 0.05^{a}
3	15.52 ± 2.92^{a}	42.34 ± 5.38^{b}	2.58 ± 0.10^{a}

1: Films prepared from FFS incubated at 60°C for 30 min; 2: Films prepared from FFS incubated at 100°C for 30 min; 3: Films prepared from FFS incubated with 10 mM EDTA at 60°C for 30 min. *Values are given as mean ± SD from ten determinations. **Values are given as mean ± SD from five determinations. ***Different superscripts in the same column indicate significant differences (P<0.05).

Water vapor permeability

WVP of all film samples was not different (P>0.05) (Table 13). Therefore, the degradation of protein in gelatin film did not affect the WVP of the film, suggesting that molecular weight distribution had no influence on the water barrier of film. The composition of protein and the amount of plasticizer would be the essential factors determining the properties of gelatin film.

5.5 Conclusion

Transparent and relatively strong edible films were prepared from fish skin gelatins. TS and EAB of film from both species increased with increasing protein content. EAB increased with increasing glycerol content, while TS decreased. WVP of films increased and films became more transparent with increasing glycerol content. Increase in glycerol content also reduced transition temperature and transition enthalpy of film. Mechanical properties of film from brownstripe red snapper skin gelatin were generally higher than that from bigeye snapper skin gelatin at any protein and glycerol concentrations tested. Heat activated metallo- and/or serine- proteinases/collagenase lowered the mechanical properties of gelatin film via the cleavage of peptide chain, especially during film preparation. The addition of EDTA inhibited the proteolysis with the concomitant increase in mechanical properties of film.