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THE EFFECTS OF PREPARATION CONDITIONS ON THE PROPERTIES OF GELATIN FILM FROM HORSE MACKEREL (*Trachurus japonicus*) SCALE

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ABSTRACT

The effects of protein and glycerol concentrations on properties of gelatin film from horse mackerel (Trachurus japonicus) scale were studied. Increasing protein concentration leaded to increasing mechanical properties of gelatin film, while tensile strength (TS) decreased and elongation at break (EAB) increased with increase of glycerol content. Horse mackerel scale gelatin film showed the greatly lower water vapor permeability (WVP) comparable with that of mammalian, cold- and warm-water fish, possibly due to its containing a higher level of hydrophobic amino acid content. Gelatin films from different preparation conditions showed excellent UV barrier properties at wavelength of 200 nm, although the films were transparent at visible wavelength. It appeared that gelatin film derived from horse mackerel scale can be applied as food packaging material due to its low WVP value and excellent UV barrier properties.

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1 INTRODUCTION

Gelatin is a protein derived by partial hydrolysis of collagen extracted from skin, bones, connective tissues, organs and some intestines of mammalian species (Johnston-Banks, 1990). Nevertheless, concerns regarding bovine and porcine health problems in recent years lead to increasing attention on fish gelatin (Gómez-Guillén *et al*., 2011). Gelatin has wide application in food processing industries, such as a gelling agent, stabilizer, thickener, or texturizer of food (Irwandi *et al*., 2009; Gómez-Guillén *et al*., 2011). Another important application of fish gelatin is the production of biodegradable films. Fish gelatin films are considered as a vital eco-friendly packaging material to reduce the environmental impact of synthetic plastic materials (Nagarajan *et al*., 2012). Furthermore, fish gelatin films used as a foodstuff covering have the capacity to protect food against drying, light and oxygen (Gómez-Guillén *et al*., 2009).

Horse mackerel (*Trachurus japonicus*) is one of the most important marine fishes in Japan, with an annual catch of 133,915 tonnes in 2012 (Production figures of fishery and aquaculture industry, Ministry of Agriculture, Forestry and Fisheries, 2012). This fish is used as a raw material of frozen surimi and in the preparation of raw fresh *sashimi* and *sushi*, which are popular foods in Japan (Yamanaka and Tanaka, 2007). However, during fish processing, a large amount of byproduct (about 50-70% of fish weight, consisting of skin, scales and bone) is considered as waste, resulting in serious environmental problems (Kittiphattanabawon *et al*., 2005). Utilization of marine byproduct such as

fish scales, as a raw material in gelatin production, represents a potential strategy to can reduce the environmental pollution, and on the other hand can help to improve the value of fish product.

Fish gelatin films from the skin of brownstripe red snapper and bigeye snapper (Jongjareonrak *et al*., 2006), blue shark (Limpisophon *et al*., 2009), Nile perch (Muyonga *et al*., 2004), halibut (Carvalho *et al*., 2008), tuna (Gómez-Guillén *et al*., 2007), trout (Kim and Min, 2012) and cuttlefish (Hoque *et al*., 2011) were prepared and characterized. However, little information is available on gelatin films made from fish scales, except the reports on lizard fish (Wangtueai *et al*., 2010) and grass carp (Zhang *et al*., 2011) scale gelatin films. Thus, the purpose of this study was to develop edible gelatin films by exploring the effects of protein and glycerol concentrations on formed gelatin film from horse mackerel (*Trachurus japonicus*) scale.

2 MATERIALS AND METHODS

2.1 Fish scale preparation

Horse mackerel scale was collected in Nagasaki Prefecture, Japan. The scale was collected, kept cold, and transported to the laboratory using ice. The fish scales were manually cleaned up with chilled water, and then kept in plastic bags and stored at – 30 ± 2°C until use.

2.2 Gelatin extraction

Gelatin was extracted from horse mackerel scales by the method described by Wangtueai and Noomhorm (2009) with minor modifications. Briefly, frozen scales were thawed and soaked in 0.1 M NaOH solution at a ratio scales:NaOH of 1:8 (w/v) for 6 h (NaOH was replaced once after 3 h) to remove non-collagenous proteins. Then scales were washed with cold distilled water (dist. H_2O) to get neutral pH. The fish scales were drained using a cheesecloth, manually dehydrated and then immersed in 10 x dist. H_2O (w/v) at 70°C for 1 h stirring to extract gelatin. The coarse solids were removed by filtration with two layers of cheesecloth and the liquid was centrifuged at 16,400 *g* for 30 min at 20°C. Gelatin in the supernatant was freeze-dried (DC 401; Yamato Scientific Co., Ltd., Japan).

2.3 Analysis of amino acid content of horse mackerel scale

Twenty milligrams of extracted gelatin powder from horse mackerel scale were hydrolyzed in 6 M HCl at 110°C for 22 h *in vacuo*. The hydrolysate was neutralized with 6 N and 0.6 N NaOH, and filtrated using a 0.45 µm cellulose membrane filter (Toyo Roshi Kaisha Ltd., Tokyo, Japan). The amino acid content in the filtrate was determined by using HPLC system (LC-10A amino acid analysis system, Shimadzu, Kyoto, Japan) with a Shim-pack Amino-Li column (100 mm x 6.0 mm, Shimadzu) and Shim-pack SC-30/S0504 Li pre-column (150 mm x 4.0 mm, Shimadzu).

2.4 Studying the effects of protein and glycerol concentrations on formed gelatin film

2.4.1 The effect of protein concentration

Gelatin powder was dissolved in dist. H_2O at 60°C for 30 min to obtain film forming solution (FFS), the protein concentrations was varied at 1, 2, and 3% (w/v) (protein concentration was determined by Lowry's method (Lowry *et al.,* 1951). To the FFS, glycerol as a plasticizer was added till 20% (w/w), the mixture was stirred continuously at room temperature for 15 min, air bubbles were excluded by using a mixer (HM-500; Keyence Co., Tokyo, Japan). The prepared FFS (4 g) was put onto a rimmed silicone plate (50 x 50 mm) and dried in a chamber (EYELA, KCL-2000A; Tokyo Rikakikai Co., Ltd., Japan) at 25 ± 0.5°C and 50 ± 5% relative humidity (RH) for 48 h. The obtained films were manually peeled off.

2.4.2 The effects of glycerol concentration

Glycerol in a range of 0, 10, 15, 20 and 25% (w/w) was added to the FFS with the appropriate protein concentration obtained from the experiment 2.4.1. The FFS was prepared, cast and dried as previously described.

2.4.3 Determining physical properties of formed gelatin film

Film thickness

Film thickness was determined by using a dial-type thickness gauge (Series 7300; Mitsutoyo Co., Kanagawa, Japan). The measurement was performed at six random sites on each sample the film.

Tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and elongation at break (EAB) of formed gelatin films were determined by the Tensipresser TTP-50BX II (Taketomo Electric Inc., Tokyo, Japan) following the ASTM standard method D 882-97 (ASTM, 1999). The examined film (20 x 45 mm) was placed on specific tensile grip with an initial grip distance of 30 mm and a cross-head speed of 1 mm/s until the films were

disrupted. TS and EAB were calculated by the following equations:

TS (MPa) = (Maximum load (N) samples)/(Initial cross-sectional area (m^2))

EAB (%) = (Film length (mm) at rupture x 100)/(Initial grip length of (mm))

Water vapor permeability

Water vapor permeability (WVP) of formed gelatin films was determined by the ASTM standard test method (1983) as reported by Gontard *et al*. (1992). Each film was hold onto a glass cup (diameter of 5 cm) containing silica gel (0% relative humidity) with silicon vacuum grease and a plastic band. The cups were placed in a desiccator with saturated water (100% relative humidity) and put in ventilated chamber at 30°C. The cups with films were weighed at initial time and every 1 h during 9 h. Six replications was performed for each sample. Water vapor permeability of gelatin film (expressed as a $gm^{-1}s^{-1}Pa^{-1}$) was calculated by the following equation (McHugh *et al*., 1993):

$$WVP = w.\ x.A^{-1}.t^{-1}.\Delta P^{-1}$$

Where *w* is weight gain (g) of the cup, *x* is film thickness (m), *A* = area of exposed film (m^2), *t* is time of gain (s), ΔP is vapor pressure gradient (Pa) between inner and outer surface of the film.

Light transmission

The transmission effects of ultraviolet (UV) and visible light on the formed gelatin films was measured at different wavelengths from 200 to 800 nm using a UV-Visible Recording Spectrophotometer (UV-160; Shimadzu Co., Kyoto, Japan) following the method described by Fang *et al*. (2002).

SDS-PAGE analysis

SDS-PAGE analysis of formed gelatin films from horse mackerel scale was conducted according to the method of Limpisophon *et al*. (2009) with modifications. Film samples (50 mg) were mixed with 5 ml of 1% SDS solution (w/v) and shaken continuously at ambient temperature for 16 h. Supernatants obtained after centrifugation at 5000 *g* for 5 min at 20°C were subjected to SDS-PAGE. The samples (10 µL) were loaded onto 7.5% running polyacrylamide gel at a constant current 20 mA for 75 min. The gel was fixed in the solution of 25% (v/v) methanol and 5% (v/v) acetic acid for 30 min then stained with 0.1% (w/v) Coomassie blue R – 250 in 30% (v/v) methanol and 10% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. High molecular weight markers (Sigma Chemical Co., St. Louis, Mo, USA) were used to estimate the molecular weights of proteins.

2.5 Data analysis

The obtained data were presented as mean ± standard deviation with 6 replications. An analysis of variance (ANOVA) of the data was performed by using SPSS software (SPSS 11.5 for Windows).

3 RESULTS AND DISCUSSION

3.1 Amino acid content in horse mackerel scale gelatin

The amino acid composition of horse mackerel scale gelatin powder was presented in the Table 1. Glycine, the dominant amino acid in horse mackerel scale gelatin, accounted for 30% of total amino acids. Imino acid contents (proline and hydroxyproline) (178 residues per 1000 residues) were greater than those from grass carp scales (94 residues per 1000 residues) (Zhang *et al*., 2011) but lower than those of lizardfish scale gelatin (205 residues per 1000 residues) (Wangtueai and Noomhorm, 2009) and mammalian skin gelatin (216-225 residues per 1000 residues) (Avena-Bustillos *et al*., 2006). Imino acids have a significant function in the triple helical structure of gelatin and affect the gel strength of gelatin gel (Ward and Courts, 1977; Zhang *et al*., 2011). Most fish gelatins have lower amino acid content than mammalian gelatins, explaining the poor gelling ability in comparison with mammalian gelatins. Notably, horse mackerel scale gelatin contained slightly higher hydrophobic amino acids (653 residues per 1000 residues) compared to commercial mammalian (pork), cold-water fish (haddock) and warm-water fish (catfish) skin gelatins (651, 647 and 649 residues per 1000 residues, respectively) (Avena-Bustillos *et al*., 2006).

3.2 Effect of protein concentration on horse mackerel scale gelatin film

3.2.1 Tensile strength and elongation at break

Tensile strength (TS) and elongation at break (EAB) of horse mackerel scale gelatin films at different protein concentrations were presented in the Table 2. The TS of scale gelatin films increased from 19.4 ± 4.75 MPa to 36.8 ± 4.13 MPa and the EAB increased from 38.8 ± 9.77% to 48.8 ± 9.23% with increasing protein concentration and reached the highest at 2% protein concentration. The increasing protein content might result in the better interaction of proteins forming the films, as the

result it improved flexibility and mechanical properties of films (Jongjareonrak *et al*., 2006). The high values of TS and EAB of horse mackerel scale gelatin films was similar to the skin gelatin films from brownstripe red snapper and bigeye snapper (Jongjareonrak *et al*., 2006).

3.2.2 Water vapor permeability (WVP)

The effect of various protein concentrations on the WVP values of scale gelatin films is shown in Table 2. The WVP of scale gelatin films increased from 0.46 ± 0.05 to 1.51 ± 0.07 $x10^{-10}$ $gm^{-1}Pa^{-1}s^{-1}$ with increasing protein concentration. Notably, greater WVP values were observed in gelatin films with increased thickness. However, the mean WVP value of horse mackerel scale gelatin films (0.98 ± 0.07 $x10^{-10}$ $gm^{-1}Pa^{-1}s^{-1}$) was lower than that of skin gelatin films from brownstripe red snapper and bigeye snapper (1.22 ± 0.11 $x10^{-10}$ $gm^{-1}Pa^{-1}s^{-1}$ and 1.33 ± 0.04 $x10^{-10}$ $gm^{-1}Pa^{-1}s^{-1}$, respectively) (Jongjareonrak *et al*., 2006) at the same protein concentration (2%). We cannot find critical reasons for the lower WVP values of horse mackerel gelatin film when compare with others gelatin film at similar protein content in FFS. However, it might be explained by the slightly higher amount of hydrophobic amino acids in horse mackerel scale gelatin comparable with commercial mammalian, cold-water fish and warm-water fish skin gelatins (Avena-Bustillos *et al*., 2006).

Table 1: Amino acid composition of gelatin from horse mackerel scale

Amino acid	Residues/1000 residues
Aspartic acid	49 ± 2
Threonine	25 ± 2
Serine	33 ± 2
Glutamic acid	71 ± 3
Glycine	322 ± 5
Alanine	131 ± 3
Valine	21 ± 2
Methionine	17 ± 2
Isoleucine	10 ± 2
Leucine	23 ± 3
Tyrosine	2 ± 1
Phenylalanine	18 ± 2
Hydrolysine	10 ± 1
Lysine	32 ± 3
Histidine	7 ± 2
Arginine	51 ± 4
Hydroxyproline	67 ± 3
Proline	111 ± 7
Imino acids*	178 ± 9
Hydrophobic amino acids**	653

Data are expressed as mean ± standard deviation (n=3)

** indicates total hydroxyproline and proline*

*** indicates total glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine and proline*

Table 2: Effect of FFS* protein concentration on thickness, tensile strength (TS), elongation at break (EAB) and water vapor permeability (WVP) of scale gelatin films

Protein concentration	Thickness (µm)	TS (MPa)	EAB (%)	WVP ($x10^{-10}gm^{-1}Pa^{-1}s^{-1}$)
1%	10.7 ± 0.33^{a}	19.4 ± 4.75^{a}	38.8 ± 9.77^{a}	0.46 ± 0.05^{a}
2%	23.6 ± 0.52^{b}	36.5 ± 3.66^{ab}	46.1 ± 7.44^{ab}	0.98 ± 0.07^{b}
3%	38.5 ± 0.71^{c}	36.8 ± 4.13^{b}	48.8 ± 9.23^{b}	1.51 ± 0.07^{c}

**1 FFS means film-forming solution*

Data are expressed as mean ± standard deviation (n=6)

Different superscripts in the same column indicate statistical differences ($p < 0.05$)

3.2.3 Light transmission of scale gelatin film (%)

The effect of protein concentration on the light transmission of gelatin films from horse mackerel scale is shown in Table 3. Light transmission at each wavelength from 200 to 800 nm decreased with increasing protein concentration. A likely explanation is that gelatin films with greater thickness would absorb the light more effectively than those with lower thickness, leading to low light transmission in both the UV and visible ranges (Jongjareonrak *et al*., 2006).

Table 3: Effect of protein concentration on the light transmission (%) of gelatin films

Protein concentration	Light transmission at different wavelength (nm)						
	200	280	350	400	500	600	800
1%	0.3	23.2 ± 0.89^{b}	34.3 ± 1.51^{b}	59.4 ± 1.07^{b}	69.7 ± 0.88^{c}	75.1 ± 0.82^{b}	80.6 ± 0.87^{b}
2%	0.3	16.4 ± 2.51^{a}	30.4 ± 2.78^{a}	52.7 ± 1.14^{a}	62.6 ± 1.58^{b}	69.4 ± 2.12^{a}	75.8 ± 2.03^{a}
3%	0.3	11.8 ± 1.57^{a}	29.0 ± 1.91^{a}	47.2 ± 0.96^{a}	55.2 ± 1.79^{a}	78.6 ± 1.24^{c}	80.0 ± 1.43^{b}

Data are expressed as mean ± standard deviation (n=6)

Different superscripts in the same column indicate statistical differences ($p < 0.05$)

3.2.4 SDS-PAGE

Protein patterns of gelatin films prepared with different protein concentrations in Fig.1 showed that all gelatin films consisted of two different α chains, α1 and α2, as well as a β component. Furthermore, there were no differences in patterns among the films. Limpisophon *et al*. (2009) reported no differences in the protein patterns of gelatin films from under-utilized blue shark skin prepared with various protein concentrations.

From this study, it was revealed that gelatin film prepared with 2% protein concentration had similar values of TS and EAB and the lowest WVP values comparable with film had 3% protein concentration. Therefore, gelatin film from a 2% protein concentration was used to study the effect of glycerol concentration on properties of gelatin film.

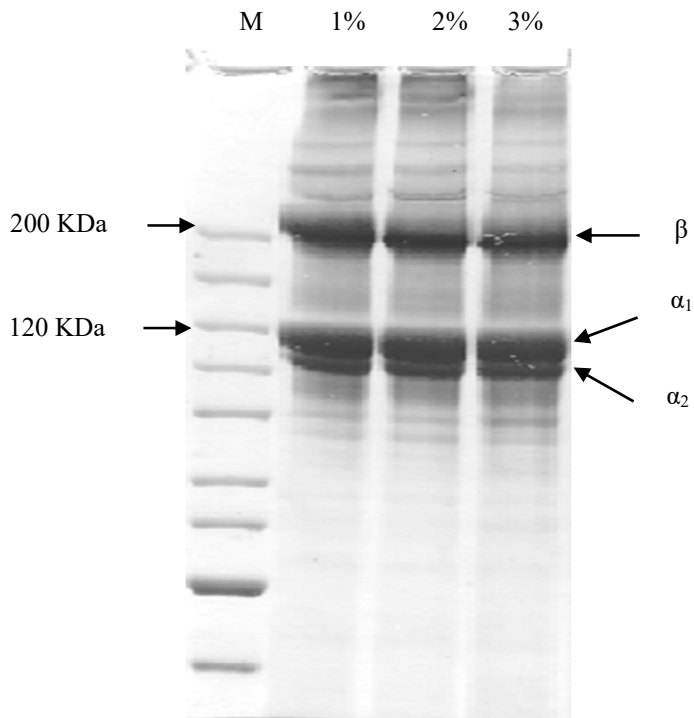

Fig. 1: SDS-PAGE patterns of gelatin films at varying protein concentrations (1, 2 and 3%) in film-forming solution (FFS)

"M" means protein marker

3.3 Effect of glycerol concentration on properties of gelatin film

3.3.1 Mechanical properties

TS and EAB of gelatin film with 2% protein at various glycerol concentrations are shown in Table 4. TS decreased from 48.1 ± 1.94 to 29.6 ± 4.12 MPa with increasing glycerol concentration from 0 to 25%. These results were similar to those of the gelatin film from shark skin (Limpisophon *et al*., 2009) and brownstripe red snapper and bigeye snapper (Jongjareonrak *et al*., 2006) at various glycerol concentrations. Gelatin film is hydrophilic

and glycerol also adopted hydrophilic characteristics, when adding glycerol into FFS, it could be incorporated in the gelatin film network by establishing hydrogen bonds with polar groups of protein (Gontard *et al*., 1993), leading to the reduction of tensile strength of the film.

On the other hand, EAB of gelatin film from horse mackerel scale increased sharply from 4.22 ± 1.01 to 51.2 ± 4.99%. The result was in agreement with Limpisophon *et al*. (2009), who reported that the EBA of gelatin film from shark skin increased from 1.57 to 80.4% when glycerol content increased from 0 to 50%. The presence of plasticizer caused a reduction of intermolecular interaction and also increased the mobility of macromolecules (Gontard *et al*., 1993). Furthermore, the moisture content of gelatin film increased with increasing the plasticizer concentration because of its high hygroscopic characteristics (Sobral *et al*., 1999).

Table 4: The effects of glycerol concentrations on thickness, tensile strength (TS), elongation at break (EAB) and water vapor permeability (WVP) of scale gelatin film

Glycerol concentration (%)	Thickness (µm)	TS (MPa)	EAB (%)	WVP ($x10^{-10}gm^{-1}Pa^{-1}s^{-1}$)
0%	18.9 ± 0.36^{a}	48.1 ± 1.94^{e}	4.22 ± 1.01^{a}	0.74 ± 0.07^{a}
10%	19.7 ± 0.45^{b}	45.1 ± 2.41^{d}	15.1 ± 1.99^{b}	0.79 ± 0.06^{a}
15%	21.6 ± 0.38^{c}	39.9 ± 3.12^{c}	28.1 ± 2.59^{c}	0.89 ± 0.03^{b}
20%	23.6 ± 0.52^{d}	36.5 ± 3.66^{b}	46.1 ± 7.44^{d}	0.98 ± 0.07^{c}
25%	24.4 ± 0.33^{e}	29.6 ± 4.12^{a}	51.2 ± 4.99^{e}	1.08 ± 0.11^{d}

Data are expressed as mean ± standard deviation (n=6)

Different superscripts in the same column indicate statistical differences ($p < 0.05$)

3.3.2 Water vapor permeability

WVP of films prepared from gelatin of horse mackerel scale at different glycerol contents is presented in Table 4. WVP values of films with 10% glycerol and that without glycerol were no significant difference (p>0.05). WVP of films increased with increasing glycerol levels (p<0.05). Glycerol, a hygroscopic plasticizer with three hydroxyl groups (-OH), was able to attract water to the gelatin film system, leading to increasing WVP of the film (Sobral *et al*., 1999; Limpisophon *et al*., 2009). Furthermore, when glycerol level increased the free volume of the gel system also elevated, this enhanced the mobility of polypeptide chains due to the insertion of glycerol molecules between them. Therefore, the network structure of films became less dense and more permeable (Gontard *et al*., 1993). The increase of WVP of horse mackerel scale gelatin films with increasing glycerol concentration was also observed in skin gelatin films from shark (Limpisophon *et al*., 2009), brownstripe red snapper and big snapper (Jongjareonrak *et al*., 2006) and cuttlefish (Hoque *et al*., 2011).

3.3.3 Light transmission

Transmission of UV and visible light at selected wavelength in the range of 200-800 nm to gelatin film from scale of horse mackerel at different glycerol contents is shown in Table 5. Transmission in visible range (350-800 nm) of gelatin films was from 28.1 ± 1.12 to 79.6 ± 1.15%. The transmission of UV light at 280 nm was in the range of 13.8 ± 0.53 – 17.0 ± 1.41%. Very low transmission (0.3%) was found at 200 nm. The similar results were also observed for skin gelatin film from shark (Limpisophon *et al*., 2009), brownstripe red snapper and big snapper skin (Jongjareonrak *et al*., 2006).

Table 5: Effect of glycerol concentrations on light transmission (%) of horse mackerel scale gelatin films

Glycerol content	Light transmission at different wavelength (nm)						
	200	280	350	400	500	600	800
0%	0.3	13.8 ± 0.53^{c}	28.1 ± 1.12^{a}	48.2 ± 0.62^{c}	64.2 ± 5.03^{a}	74.3 ± 1.97^{a}	79.6 ± 1.15^{a}
10%	0.3	14.4 ± 1.86^{bc}	29.6 ± 1.60^{a}	49.4 ± 3.00^{bc}	63.9 ± 3.05^{a}	72.2 ± 0.42^{b}	78.1 ± 0.50^{ab}
15%	0.3	15.8 ± 1.31^{abc}	30.3 ± 4.32^{a}	51.1 ± 1.31^{abc}	63.5 ± 0.59^{a}	70.0 ± 0.52^{c}	76.6 ± 0.82^{bc}
20%	0.3	16.4 ± 2.51^{ab}	30.4 ± 2.78^{a}	52.7 ± 1.14^{ab}	62.6 ± 1.58^{a}	69.4 ± 2.12^{c}	75.8 ± 2.03^{c}
25%	0.3	17.0 ± 1.41^{a}	30.8 ± 0.66^{a}	53.5 ± 5.56^{a}	60.2 ± 6.38^{a}	69.0 ± 1.80^{c}	75.1 ± 1.37^{c}

Data are expressed as mean ± standard deviation (n=6)

Different superscripts in the same column indicate statistical differences ($p < 0.05$)

3.3.4 SDS-PAGE

SDS – PAGE patterns of gelatin films from horse mackerel scale at different glycerol concentrations are presented in Fig. 2. The SDS-PAGE patterns showed that all gelatin films at all glycerol concentrations consisted of two different α chains, α1 and α2, as well as a β component, and there were no differences in patterns among the glycerol content. Limpisophon *et al.* (2009) observed no differences in the protein patterns of gelatin film from under-utilized blue shark skin at various glycerol concentrations. It can be explained that films of all gelatin samples were most likely stabilized by weak bond, especially hydrogen bond. These bonds were destroyed in the presence of SDS as well as mercaptoethanol used for electrophoresis, leading to no differences in protein pattern of gelatin films at different glycerol levels.

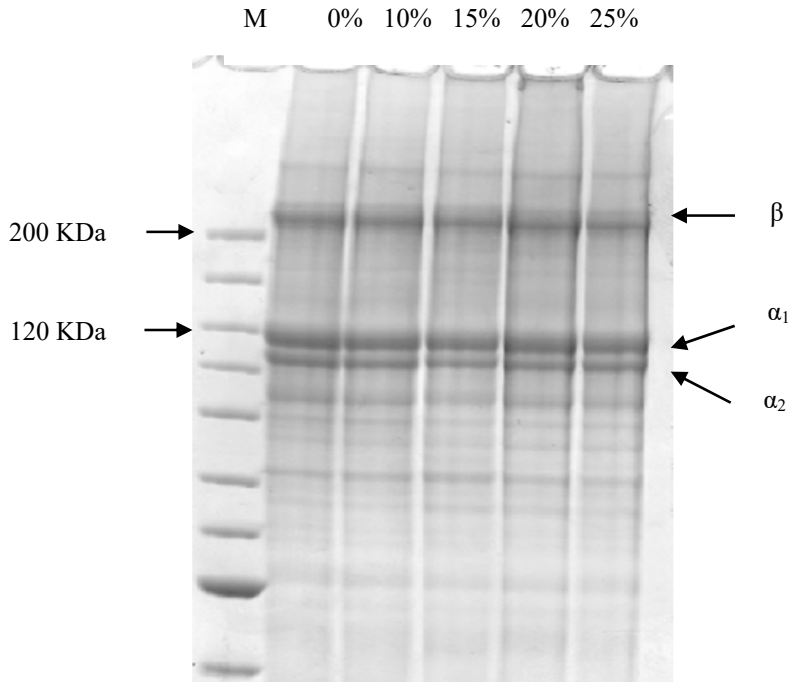

Fig. 2: SDS-PAGE patterns of gelatin films at different glycerol concentrations (0, 10, 15, 20 and 25%) in film-forming solution (FFS)

"M" means protein marker

4 CONCLUSIONS

Properties of gelatin film from horse mackerel scales depend on protein concentration and glycerol content in FFS. Gelatin film with 2% protein concentration and adding 20% glycerol as a plasticizer had high TS and EAB and low WVP values when comparable with gelatin films from the skin of other fishes or mammals do. This film can be modified according to the intended purpose as packaging material in food industry.

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