Properties of thermo-compression molded bovine and fish gelatin films as influenced by resin preparation condition

[1]Chuaynukul, K., [1]*Prodpran, T. and [2]Benjakul, S.

[1]*Department of Material Product Technology, Faculty of Agro-Industry, Prince of Songkla University. 15 Kanchanawanish Road, Hat Yai, Songkhla 90112, Thailand*
[2]*Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University. 15 Kanchanawanish Road, Hat Yai, Songkhla 90112, Thailand*

Abstract

Effect of resin preparation, heat-pretreatment of bovine and fish-gelatin solutions (20%, w/w) containing 25% glycerol (based on protein) at different temperatures (70 and 90°C) for various time (1, 2 and 3 h) prior to semi-drying, on the properties of resulting films was investigated. Tensile strength (TS) and elastic modulus of the films increased, whereas the elongation at break (EAB) decreased when resin was prepared with increasing heating temperature ($p<0.05$). Resin prepared by using heat-pretreatment for 2 h yielded the films with the lowest EAB ($p<0.05$), while an increased EAB was found as resin was prepared from solution heat-pretreated for 3 h, regardless of heating temperature used ($p<0.05$). Heat-pretreatment at high temperature (90°C) and long time (3 h) significantly resulted in degradation of gelatin molecules, leading to loss of films strength. However, heat-pretreatment condition in resin preparation step had no marked effect on water vapor permeability (WVP), color and transparency of resulting films. Films from bovine gelatin had the higher EAB and WVP, compared with fish-gelatin film. The former had the lower L^* and a^*-values, but possessed the higher b^*-value than the latter. Gelatin films exhibited the potential barrier property to UV light. Thus, heat-pretreatment condition for resin preparation had the direct effect on the properties, especially mechanical properties, of resulting films fabricated by thermo-compression molding.

Introduction

Biodegradable packaging has been paid more increasing attention, especially those prepared from alternative materials for petrochemical and plastic products which are non-biodegradable and have the negative impact on environment. Proteins are biopolymers capable of forming the film. Protein-based films generally have excellent barrier properties to gases, lipids and aromas, have moderate mechanical properties, together with a high nutritional value but have high water-vapor permeability due to the hydrophilic character of these macromolecules (Gennadios, 2002; Jongjareonrak *et al.*, 2006). Protein films can be produced from several protein sources including vegetable proteins (corn zein, wheat gluten, soy protein, peanuts and cottonseed protein) and animal proteins (milk proteins, collagen, gelatin, keratin, egg albumin and myofibrillar protein) (Cuq *et al.*, 1998).

Gelatin is animal protein derived from the partial hydrolysis of native collagens, which are the most abundant structural proteins found in the skins, bones and connective tissues (Norland, 1990; Gómez-Guillén *et al.*, 2009). Skin and bone from bovine and porcine sources have usually been utilized commercially in gelatin production (Karim and Bhat, 2009). Due to religious objections to consumption and health concerns about the spread of diseases such as bovine spongiform encephalopathy to humans, fish gelatin as an alternative gelatin to replace mammalian gelatin is gaining increasing attention (Muyonga *et al.*, 2004; Zhou and Regenstein, 2004; Gómez-Guillén *et al.*, 2009; Karim and Bhat, 2009). Among all proteins, gelatin has received attention for the development of edible films because of its abundance and biodegradability (Gennadios, 2002; Jongjareonrak *et al.*, 2006). Gelatin films generally have excellent barrier properties to oxygen and carbon dioxide (McHugh *et al.*, 1994). In addition, gelatin is unique among hydrocolloids in forming thermo-reversible with a melting point close to body temperature, which is particularly significant in edible and pharmaceutical applications (Achet and He, 1995). Considering the film with many outstanding properties such as transparency, biodegradability and barrier properties (gases and aroma), gelatin is appropriate to apply in biodegradable packaging (Cuq *et al.*, 1998; Martucci and Ruseckaite, 2009). The properties of gelatin films depend on characteristic

*Corresponding author.
Email: *thummanoon.p@psu.ac.th*
Tel: 66-7428-6357

of raw materials and manufacturing processes. Commonly, mammalian gelatins have better physical properties and thermostability than most fish gelatins (Ledward, 1986), and this has been related mainly to their higher proline and hydroxyproline amino acid contents (Norland, 1990).

In general, there are two main methods used for preparing protein-based films: the wet and the dry processes. The wet process or casting is the most often use film-forming method (Zhang *et al*., 2007; Wang *et al*., 2009; Guerrero *et al*., 2010). Water or water-ethanol solutions or dispersions of the protein materials are spread on a suitable substrate and later drying. On other hand, the dry process (or thermal possessing method) such as compression molding and extrusion is based on thermoplastic properties of proteins when they were plasticized and heated above their glass transition temperature (T_g) under low water content. Cooling to room temperature can reconvert rubbery material to glassy materials, giving more or less rigid forms with the desired structure (Cuq *et al*., 1997). Thermoplastic processing generally consists of mixing proteins and plasticizer to obtain a dough-like material or compound resin (Guerrero *et al*., 2010). The use of plasticizers reduces the intermolecular forces and increases the mobility of polymeric chains, thereby improving the flexibility and the extensibility of the film (McHugh *et al*., 1994).

However, the major approach widely used to prepare films from protein, especially gelatin, has been limited to the solution casting. The thermal process may affect film properties differently as compared to the casting method, but it enhances the commercial potentiality for large-scale production of biodegradable/edible films. Moreover, this method allows a much shorter period of time for film preparation and the use of conventional techniques which are more convenient for industrial applications than casting. Among thermal processing method, compression molding is the most common and simple method of molding, which is typically used to investigate the feasibility of converting any polymers to designed product via thermoplastic processing (Zhang *et al*., 2007; Guerrero *et al*., 2010). Prior to molding, preparation of compound resin based on gelatin and plasticizer, which is used as raw material for compression molding is of importance (Park *et al*., 2008). From our preliminary investigations, the compound resin in the pellet form could not be successfully prepared by dry blending or melt compounding using twin-screw extrusion technique. The gelatin melt was too viscous which tended to stick to the screw surface and became degraded upon mixing, due mainly to the evaporation of water

acting as plasticizer at high temperature. Thus, the plasticized-gelatin molding compound resin was prepared by solution blending with the aid of heat-pretreatment of the gelatin solution. The time and temperature used for gelatin solution pretreatment could affect the resulting resins and the films properties.

Therefore, this study was aimed to study the feasibility of gelatin film production by using thermo-compression molding. The effect of resin preparation condition including heat-pretreatment temperature and time on film-forming ability and the properties of resulting films from bovine hide (cow's skin) and fish skin gelatins was investigated.

Materials and Methods

Materials and chemicals

Commercial bovine hide gelatin (~240 bloom) and fish skin gelatin (~240 bloom) were purchased from Halamic company (Bangkok, Thailand) and LAPI GELATINE S.p.A. (Empoli, Italy), respectively. Glycerol and L-leucine were procured from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of molding compound resin

Molding compound resin (called in brief as 'resin'), a mixture of gelatin and plasticizer, was prepared according to the method of Park *et al*. (2008) with some modifications. Gelatin powders were first dissolved in hot deionized water (65°C) to obtain the protein concentration of 20% (w/v). Glycerol as a plasticizer was added to gelatin solution at 25% of gelatin. The film-forming solutions (FFS) were then heated at different temperatures (70 and 90°C) and various times (1, 2 and 3 h) in a water bath. Upon heat-pretreatment, the FFSs were gently stirred. Then, the FFSs were poured onto a stainless tray and partially dried for 12 h at ambient temperature. These semi-dried resins were cut into small pellets (~0.5 cm x 0.3 cm x 0.3 cm) and further dried in vacuum oven at 35°C for 48 h. The resins were conditioned at 25°C and 60% RH in an environmental chamber (TK120, NUVE, Belgium) for 48 h before used for preparation of films via thermo-compression molding.

Film preparation by thermo-compression molding

The conditioned resins (about 3 g) were placed between two stainless steel plates (10 x 10 inch²) covered with Mylar sheets. The set was inserted between heating platens of the compression molder (LCC-140, Tang-Master Co., Ltd, Thailand) prior heated to 120°C and the resin was then preheated at this temperature for 10 min without pressure. The

molten resin was subsequently pressed to form a film in the compression molder at 120°C. A pressure of 20 MPa was applied for 2 min, followed by removal of the set from the compression molder and allowing to cool down to ambient temperature within 3 min. The gelatin film with the average thickness of 0.120 mm could be easily removed from the plates and was subjected to analysis.

Analyses of compression-molded films

Film thickness

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

Mechanical properties

Prior to testing the mechanical properties, films were conditioned for 48 h at 25 °C and 50 ± 5% RH. Tensile strength (TS), elongation at break (EAB) and elastic modulus (EM) of the films were measured according to the ASTM-D882-01 (ASTM, 2002a) method as described by Iwata *et al.* (2000) using a universal testing machine (Lloyd Instruments, Hampshire, UK). The specimen strip (50 mm x 20 mm) was clamped between the grips with initial separation of 30 mm and then pulled apart at a cross-head speed of 30 mm/min until it was broken. The TS was calculated by dividing the maximum force at break by cross-sectional area of the film. The EAB was calculated by dividing the length extended (ΔL) by the original length (L_0) of the film. EM was derived from the initial slope of the linear portion of stress-strain curve. Ten specimens were tested for each treatment.

Water vapour permeability (WVP)

Water vapor permeability (WVP) was determined using a modified ASTM E-96-01 method (ASTM, 2002b) similar to that reported by Shiku *et al.* (2004). The preconditioned film was sealed onto an aluminum cup with 30 mm internal diameter which contained dried silica gel to ensure 0% relative humidity inside the cup. The cup was kept in a controlled chamber (NUVE, TK120, Belgium) at 30°C and 65% RH. The cup was weighed every 1 h until 8 h under this controlled environment. WVP of gelatin film was calculated using the following equation:

$$WPV = \frac{q \cdot l}{t \cdot A \cdot \Delta P}$$

Where *l* is the average thickness of the film sample (mm); *A* is the exposed area of the film (m^2); ΔP is the difference of the partial vapor pressure (Pa) across the film and the term *q/t* was calculated by linear regression from the plot of weight gain and time, in the constant rate period. The WVP value was expressed in g.mm/m^2.s.Pa.

Color

Color of the film was determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). D65 (day light) and a measure cell with opening of 30 mm were used. The color of the films was expressed as L*, a* and b* values.

Light transmittance and transparency value

The transmission of UV and visible light of gelatin films were measured at selected wavelengths ranging from 200-800 nm, using UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) as described by Han and Floros (1997). The transparency value of the films was calculated by the following equation:

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

Where T_{600} is the fractional value of transmittance at 600 nm and x is the film thickness (mm). According to this equation, higher transparency value indicates lower degree of film transparency.

Free amino group content

Free amino group content of gelatin powders, resins and films was determined by the method of Benjakul and Morrissey (1997) with some modifications. Films (~ 2 cm x 5 cm) were cut into small pieces and dissolved in an appropriate amount of deionized water. The gelatin solution of each sample was adjusted to obtain an equal protein concentration (5 mg/ml), determined by Biuret method (Robinson and Hodgen, 1940), before the analysis. The gelatin solution (125 µl) was mixed with 2 ml of 0.2121 M phosphate buffer (pH 8.2) and 1 ml of 0.01% TNBS solution. The mixtures were placed in water bath at 50°C for 30 min in the dark. The reaction was terminated by adding 2 ml of 0.1 M sodium sulfite. After being cooled down at room temperature for 15 min under the dark surrounding, the absorbance was measured at 420 nm using an UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The standard curve was prepared using L-leucine in the range of 0-5 mM. The activity was expressed as mM L-leucine.

Statistical analysis

Experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test. For pair comparison, T-test was used. Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL).

Results and Discussion

Mechanical properties

Mechanical properties of the films fabricated from the resins prepared from gelatins solubilized at different heat-pretreatment temperatures (70 and 90°C) and times (1, 2 and 3 h) are shown in Table 1. For both bovine and fish gelatin films, TS and EM increased but EAB decreased when the time of heat-pretreatment in resin preparation step increased up to 2 h (p<0.05), regardless of temperatures used. Moreover, at the same heat-pretreatment time (up to 2 h) films obtained from resin pretreated at 9°C had higher TS and EM but lower EAB than did those obtained from resin pretreated at 70°C. Heat-pretreatment of gelatin solution in resin preparation step possibly enhanced unfolding of the coil structure of gelatin molecules (Hoque *et al.*, 2010). The more stretched gelatin molecules could result in higher degree of molecular interaction upon molecular inter-diffusion in the melt state, which led to the formation of film network with greater inter-molecular interactions during thermal compaction under heat press. In general, the proteins must be in an open or stretched form to allow the molecular interaction that is necessary for film formation. The extent of this interaction depends on the protein structure (degree of chain extension) and the sequence of hydrophobic and hydrophilic amino acid residues in the protein. At appropriate high temperatures, proteins become fully unfolded and are able to rearrange effectively all non-polar amino acids. Heating modified the functional properties of protein, especially in term of protein structure, causing exposition of the SH groups in certain protein and promoting the exposition of hydrophobic groups which can be interacted with themselves via hydrophobic interactions during the protein film formation (Perez-Gago and Krochta, 2001). For gelatins from many sources, they had a very low concentration of cystine (approximately 0%) with negligible disulfide bonds (S-S) (Avena-Bustillos *et al.*, 2006); therefore, a film network of gelatin is mostly stabilized and strengthened by hydrogen bonds and hydrophobic interactions.

The effect of thermal treatment on the mechanical properties of protein films has been previously

Table 1. Mechanical properties of gelatin films thermo-compression molded from resins obtained from different preparation conditions

Sample	EM[#] (x10² MPa)	TS[#] (MPa)	EAB[#] (%)
B/70/1[*]	2.92 ± 0.20^c	16.08 ± 0.66^c	$55.00 \pm 8.70^{e¥}$
B/70/2	3.28 ± 0.19^d	17.50 ± 0.40^d	67.20 ± 7.40^{fg}
B/70/3	2.98 ± 0.15^c	16.31 ± 0.70^c	70.80 ± 5.80^g
B/90/1	3.22 ± 0.23^d	18.56 ± 0.70^e	49.80 ± 7.12^{de}
B/90/2	3.52 ± 0.16^e	20.10 ± 0.69^f	39.04 ± 5.98^{bc}
B/90/3	2.80 ± 0.14^{bc}	14.50 ± 0.73^b	73.00 ± 8.97^g
F/70/1	2.67 ± 0.14^{ab}	14.68 ± 0.70^b	44.60 ± 7.20^{cd}
F/70/2	2.92 ± 0.13^c	15.88 ± 0.39^c	34.40 ± 7.30^{bc}
F/70/3	2.82 ± 0.16^{bc}	14.88 ± 0.55^b	41.60 ± 6.88^{bcd}
F/90/1	2.94 ± 0.13^c	17.31 ± 0.88^d	33.60 ± 5.15^{ab}
F/90/2	3.26 ± 0.18^d	18.85 ± 0.77^e	26.00 ± 5.03^a
F/90/3	2.52 ± 0.11^a	12.62 ± 1.24^a	58.40 ± 8.20^{ef}

*-/--/- refers to gelatin type (B: bovine gelatin, F: fish gelatin)/heat-pretreatment temperature in °C /heat-pretreatment time in h.
#Values are given as mean ± SD (n=3).
¥The different superscripts in the same column indicate the significant differences (p<0.05).

reported (Perez-Gago and Krochta, 2001; Choi and Han, 2002). Perez-Gago and Krochta (2001) observed that the increase in thermal treatment temperatures from 70°C to 80°C for 20 min increased the tensile strength of whey protein isolate films (from 5 to 14 MPa), but more intense treatment of 90°C and 100°C for 20 min caused a reduction of tensile strength of the films (to 13 and 9 MPa). Choi and Han (2002) treated pea protein solution (10%) at 90°C for 5 to 50 min and observed that treatment time had strong influence on the mechanical properties of films. They suggested that the thermal treatment increased the tensile strength of the film, compared to that without treatment. Garcia and Sobral (2005) reported that muscle protein films prepared from a higher thermal treatment (60°C for 30 min) were more resistant to tensile deformation and more rigid than the films treated at 40°C for 30 min.

However, as heat-pretreatment time was further increased to 3 h for both temperatures used, the resulting films generally had decreased TS and EM but increased EAB (p<0.05) (Table 1). This was plausibly because partial degradation of gelatin molecule in the solution was more pronounced during heating at extended time. Hoque *et al.* (2010) studied the effect of heat treatment on the properties of cuttlefish *(Sepia pharaonis)* skin gelatin films. They reported that at thermal treatment temperatures of over 80°C, gelatin chains underwent partial degradation. As a result,

Table 2. Water vapor permeability (WVP) of gelatin films thermo-compression molded from resins obtained from different preparation conditions

Samples	WVP[#] ($\times 10^{-7}$ g.mm /m^2.s.Pa)
B/70/1[*]	3.38±0.15$^{cd,¥}$
B/70/2	3.32±0.18^c
B/70/3	3.44±0.11^{cd}
B/90/1	3.56±0.15^d
B/90/2	3.36±0.19^{cd}
B/90/3	3.35±0.15^d
F/70/1	2.74±0.18^{ab}
F/70/2	2.86±0.18^{ab}
F/70/3	2.96±0.11^b
F/90/1	2.76±0.18^{ab}
F/90/2	2.70±0.19^a
F/90/3	2.90±0.10^{ab}

*-/--/- refers to gelatin type (B: bovine gelatin, F: fish gelatin)/ heat-pretreatment temperature in °C /heat-pretreatment time in h.
#Values are given as mean ± SD (n=3).
¥The different superscripts in the same column indicate the significant differences (p<0.05)

Table 3. Color of gelatin films thermo-compression molded from resins obtained from different preparation conditions

Samples	L*[#]	a*[#]	b*[#]
B/70/1[§]	89.39±0.10^a	-1.54±0.03^b	6.00±0.29$^{a¥}$
B/70/2	89.65±0.40^a	-1.53±0.04^b	6.19±0.57^a
B/70/3	89.45±0.24^a	-1.60±0.06^{ab}	5.86±0.48^a
B/90/1	89.57±0.08^a	-1.59±0.04^{ab}	5.84±0.03^a
B/90/2	89.38±0.05^a	-1.63±0.08^a	6.08±0.19^a
B/90/3	89.61±0.03^a	-1.59±0.01^{ab}	6.03±0.06^a
F/70/1	90.52±0.07^b	-1.28±0.03^c	2.48±0.10^b
F/70/2	90.52±0.06^b	-1.25±0.06^c	2.45±0.19^b
F/70/3	90.53±0.11^b	-1.26±0.08^c	2.78±0.11^b
F/90/1	90.59±0.06^b	-1.24±0.03^c	2.51±0.13^b
F/90/2	90.53±0.08^b	-1.27±0.03^c	2.59±0.13^b
F/90/3	90.60±0.16^b	-1.25±0.03^c	2.62±0.19^b

§-/--/- refers to gelatin type (B: bovine gelatin, F: fish gelatin)/ heat-pretreatment temperature in °C /heat-pretreatment time in h.
#Values are given as mean ± SD (n=3)
¥The different superscripts in the same column indicate the significant difference (p<0.05)

three-dimension network with weak interconnection between gelatin molecules was formed as evidenced by continuous decrease in TS. In general, degradation led to short chains which most likely established the weaker chain-to-chain interaction or less junction zones mainly via hydrogen bond (Hoque *et al.*, 2011). A review on fish gelatin by Gómez-Estaca *et al.* (2009) indicated that the molecular weight distribution and amino acid composition were the main factors influencing the physical and structural properties of gelatin. The amino acid composition depends on specific species while the molecular weight distribution depends mainly on processing conditions. Films with a higher proportion of high-molecular weight fractions exhibited higher TS and lower EAB compared to films obtained from gelatins containing lower proportion of high-molecular weight fragments (Gómez-Estaca *et al.*, 2009).

From the results, at the same heat-pretreatment condition used for resin preparation, bovine gelatin films had higher TS, EM and EAB than fish gelatin films (p<0.05). This might result from the higher amount of proline and hydroxyproline residues in the α-chain of bovine gelatin, compared to that of fish gelatin (Norland, 1990). Hydroxyl group of hydroxyproline most likely involved in inter-chain hydrogen bonding (Jongjareonrak *et al.*, 2006; Gómez-Estaca *et al.*, 2009), contributing to stabilization of the film network.

Water vapor permeability (WVP)
WVP of the films compression-molded from the resins prepared from gelatins solubilized at different heat-pretreatment temperatures and times is shown in Table 2. WVP of bovine and fish gelatin films was in the range 3.32 - 3.56 x10^{-7} g.mm/m^2.s.Pa and 2.70-2.96 x 10^{-7} g.mm/m^2.s.Pa, respectively. Irrespective of gelatin types, heat-pretreatment condition used in resin preparation step generally had no marked impact on WVP. However, Hoque *et al.* (2010) reported the decrease in WVP of the fish-skin gelatin films prepared by solution casting of FFS pre-heated at higher temperatures, which might be due to the exposure of hydrophobic domains of gelatin chains.

Fish gelatin film exhibited lower WVP than did bovine gelatin film obtained from the resin prepared at the same heat-pretreatment condition (p<0.05). This more likely related to higher hydrophobicity, due to lower amount of hydroxyproline in fish gelatins compared to mammalian gelatins (Avena-Bustillos *et al.*, 2006; Karim and Bhat, 2009). Hydroxyl group of hydroxyproline residue could interact with water molecules via hydrogen bonding (Karim and Bhat, 2009). Lower WVP of fish gelatin films compared to bovine gelatin films prepared by thermo-compression molding found in this work was similar to that observed on cast gelatin films (Gómez-Estaca *et al.*, 2009). Gómez-Estaca *et al.* (2009) reported that the WVP of bovine gelatin film (2.20 x 10^{-8} g.mm/h.cm^2. Pa) was higher than that of fish gelatin film (1.65 x 10^{-8} g.mm/h.cm^2.Pa). Avena-Bustillos *et al.* (2006) studied WVP of different types of mammalian and warm- and cold-water fish skin gelatin films. They reported that the cold-water fish gelatin films had the

Table 4. Light transmittance and transparency value of gelatin films thermo-compression molded from resins obtained from different preparation conditions

Sample	Light transmittance (%) at different wavelengths (nm)								Transparency value#
	200	280	350	400	500	600	700	800	
B/70/1*	0.00	18.23	63.98	77.61	85.21	86.95	87.88	88.38	0.59±0.03[b¥]
B/70/2	0.00	18.63	64.07	77.37	85.05	86.73	87.69	88.14	0.60±0.01[b]
B/70/3	0.00	19.96	65.49	78.35	85.70	87.37	88.28	88.74	0.59±0.02[b]
B/90/1	0.00	18.40	63.90	77.20	84.82	86.58	87.56	88.09	0.59±0.03[b]
B/90/2	0.00	24.41	67.62	78.64	85.11	86.72	87.61	88.11	0.59±0.03[b]
B/90/3	0.00	21.29	65.91	78.67	85.91	87.53	88.36	88.76	0.61±0.01[b]
F/70/1	0.01	21.59	82.42	87.36	89.74	90.36	90.64	90.83	0.54±0.02[a]
F/70/2	0.01	24.53	84.90	87.34	88.74	89.25	89.57	89.72	0.55±0.03[a]
F/70/3	0.01	26.57	82.11	86.70	89.00	89.64	89.97	90.13	0.55±0.03[a]
F/90/1	0.01	26.09	83.40	87.66	89.76	90.21	90.66	90.81	0.53±0.01[a]
F/90/2	0.01	22.86	82.60	87.28	89.66	90.30	90.62	90.76	0.54±0.01[a]
F/90/3	0.01	26.12	81.87	86.50	88.94	89.69	90.10	90.29	0.55±0.01[a]

*-/--/- refers to gelatin type (B: bovine gelatin, F: fish gelatin)/ heat-pretreatment temperature in °C /heat-pretreatment time in h.
#Values are given as mean ± SD (n=3)
¥The different superscripts in the same column indicate the significant differences (p<0.05).

Table 5. Free-amino group content of the resins obtained from different preparation conditions and of the corresponding compression-molded films

Sample	Free-amino group content# (mmol/g of protein)	
	Resin	Film
B/70/1*	0.302±0.003[b,A]	0.372±0.004[b,B¥]
B/70/2	0.304±0.004[b,A]	0.370±0.003[b,B]
B/70/3	0.308±0.006[b,A]	0.374±0.005[b,B]
B/90/1	0.304±0.004[b,A]	0.378±0.004[b,B]
B/90/2	0.306±0.004[b,A]	0.384±0.004[b,B]
B/90/3	0.340±0.003[c,A]	0.425±0.005[c,B]
F/70/1	0.298±0.003[b,A]	0.362±0.004[b,B]
F/70/2	0.298±0.003[b,A]	0.368±0.004[b,B]
F/70/3	0.302±0.005[b,A]	0.372±0.004[b,B]
F/90/1	0.304±0.004[b,A]	0.376±0.003[b,B]
F/90/2	0.304±0.003[b,A]	0.384±0.003[b,B]
F/90/3	0.335±0.003[c,A]	0.418±0.004[c,B]
**Bovine gelatin powder	0.280±0.003[a]	-
**Fish gelatin powder	0.274±0.003[a]	-

*-/--/- refers to gelatin type (B: bovine gelatin, F: fish gelatin)/ heat-pretreatment temperature in °C /heat-pretreatment time in h.
#Values are given as mean ± SD (n=3)
¥The different lowercase superscripts in the same column indicate the significant differences (p<0.05). The different uppercase superscripts in the same row indicate the significant differences (p<0.05)
**Commercial bovine or fish gelatin powders used as raw materials for preparing the resins

lowest WVP (0.932 g.mm/m²h.k.Pa) and followed by warm-water fish (1.309 g.mm/m²h.k.Pa) and mammalian gelatin films (1.884 g.mm/m²h.k.Pa), respectively.

Color and transparency of films

L*, a* and b* values of the films compression-molded from the resins prepared from gelatins solubilized at different heat-pretreatment temperatures and times are presented in Table 3. In general, no differences in color of the films obtained from resins with different heat-pretreatment temperatures and times used were found, regardless of gelatin types. However, the colors of gelatin films obtained were dependent on gelatin types. The bovine gelatin films had lower L* and a* values but had higher b* value (p<0.05), compared to the fish gelatin films obtained from the same resin preparation condition. This indicated that the bovine gelatin films had less lightness and more yellowness than the fish gelatin films. The differences in color of both gelatin films were most likely attributed to the different color of their gelatin powders used as raw materials. The difference in lightness of gelatin raw material might be due to the different purification procedure used in preparation of commercial gelatin. Normally, purification methods including filtration, clarification and deionization, etc. have been used commercially to increase the purity of gelatin (Jones, 1987). Additionally, the different color of

films from both gelatins possibly resulted from the gelatin powders which were probably prepared from different drying processes. A commercial grade gelatin is usually dehydrated by progressive increases in air temperature. The dried bed is then broken into pieces that are ground to the required particle size. The yellow color of gelatin powder might be developed during thermal process and varied with varying chemical composition in each gelatin. A small amount of sugar and carbohydrate and amino group possibly underwent Maillard reaction, resulted in yellow pigment (Sikorski, 2001).

Table 4 shows transmission of UV and visible light at selected wavelength in the range of 200-800 nm of bovine and fish gelatin films compression-molded from resins prepared from solutions with different heat-pretreatment temperatures and times. Light transmission in visible range (350-800 nm) of bovine and fish gelatin films was in the range of 63.98 - 88.76% and 81.87 - 90.83%, respectively. The transmission of UV light at 280 nm of bovine and fish gelatin films was in the range of 18.23 -

24.41% and 21.59 - 26.57%, respectively. Very low transmission (0.00 - 0.01%) was found at 200 nm, suggesting that gelatin films were good barrier to UV light, especially in the region of UVC.

Transparency value of resulting films is shown in Table 4. The lower transparency value, the higher transparence of films was obtained. From the results, the bovine gelatin films were more transparent than fish gelatin films ($p < 0.05$). A little difference in light transmission between bovine and fish gelatin films might be due to the differences in composition, density and the aggregation or alignment of gelatin molecules in the films between both gelatins. However, for both gelatin films, the resin preparation condition, heat-pretreatment of gelatin solutions at different temperatures and times, did not affect the light transmission and transparency of the resulting films ($p > 0.05$).

Free-amino group content

Table 5 shows free-amino group content of resins prepared from gelatin solutions heat-pretreated at different temperatures (70 and 90°C) and times (1, 2 and 3 h) and their corresponding compression-molded films. The free-amino group content was determined as for the indicative of degradation of gelatin in the resin and film upon heating. For both gelatin types, heat-pretreatment at 70°C for 1-3 h and at 90°C for up to 2 h provided the resins with similar free-amino group contents (0.298 - 0.306 mmol/g) ($p > 0.05$), while the resins prepared at 90°C for 3 h had higher free-amino group contents (0.335 - 0.340 mmol/g). A similar trend of free-amino group content was also observed in the corresponding compression-molded films. Higher free-amino group correlated with greater degree of peptide bond breakage, representing degradation of gelatin. This result indicated that degradation of gelatin molecules was more pronounced in resin prepared with heat-pretreatment at high temperature (90°C) for long time (3 h). This result would explain the lower TS and EM of the film compression-molded from the resin prepared under this condition, compared to the others (Table 1). According to Hoque *et al.* (2010), excessive temperature (over 80°C) for treatment of gelatin film-forming solution led to decreased mechanical properties of resulting films since the films were mainly stabilized by the weak bond from shorten chains.

The results showed that the compression-molded films obtained exhibited higher free-amino groups than the corresponding resins and the gelatin powder, respectively. This revealed that degradation of gelatin more likely took place during heat-pretreatment of gelatin solution in resin preparation step and additionally during film fabrication at high temperature, which contributed to varying mechanical properties of the resulting films.

Conclusion

Gelatin films were successfully prepared by thermo-compression molding technique. Conditions including heat-pretreatment temperature and time used to prepare the molding compound resin influenced the properties of resulting bovine and fish-gelatin films differently. Generally, the resin preparation condition showed mark effect on mechanical properties, while it did not significantly influence water-vapor permeability and optical properties of the resulting films. The compression molded films obtained from resin heat-pretreated at 90°C for 2 h had the highest TS and EM. However, heat-pretreatment for longer time (3 h) caused significant degradation of gelatin in resins and films, leading to loss of films mechanical properties.

Acknowledgements

References

Achet, D. and He, X. W. 1995. Determination of the renaturation level in gelatin films. Polymer 36: 787-791.

ASTM. 2002a. Standard test method for tensile properties of thin plastic sheeting (D 882-01). In Annual Book of ASTM Standards. p. 162-170. Philadelphia: American Society for Testing and Materials.

ASTM. 2002b. Standard test method for water vapor transmission of materials (E 96-01). In Annual Books of ASTM Standards. p. 1048-1053. Philadelphia: American Society for Testing and Materials.

Avena-Bustillos, R. J., Olsen, C. W., Chiou, B., Yee, E., Bechtel, P. J. and McHugh, T. H. 2006. Water vapor permeability of mammalian and fish gelatin films. Journal of Food Science 71: 202-207.

Benjakul, S. and Morrissey, M. 1997. Protein hydrolysates from Pacific whiting solid waste. Journal of Agricultural Food and Chemistry 45: 3423-3430.

Choi, W. S. and Han, J. H. 2002. Film-forming mechanism and heat denaturation effects on the physical and chemical properties of pea-protein-isolate edible films. Journal of Food Science 67: 1399-1406.

Cuq, B., Gontard, N. and Guilbert, S. 1997. Thermal properties of fish myofibrillar protein-based films as affected by moisture content. Polymer 38: 2399-2405.

Cuq, B., Gontard, N. and Guilbert, N. 1998. Proteins as agricultural polymers for packaging production. Journal of Cereal Chemistry 75: 1-9.

Garcia, F. T. and Sobral, P. J. A. 2005. Effect of the thermal treatment of the filmogenic solution on the mechanical properties, color and opacity of films based on muscle proteins of two varieties of Tilapia. LWT - Food Science and Technology 38: 289-296.

Gennadios, A. 2002. Protein-based films and coatings. Florida: CRC Press. Boca Raton.

Gómez-Estaca, J., Montero, P., Fernandez-Martin, F. and Gómez-Guillén, M. C. 2009. Phycico-chemical and film-forming properties of bovine and tuna-skin gelatin: a comparative study. Journal of Food Engineering 90: 480-486.

Gómez-Guillén, M. C., Perez-Mateos, M., Gómez-Estaca, J., Lopez-Caballero, E., Giménez, B. and Montero, P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. Trends in Food Science and Technology 20: 3-16.

Han, J. H. and Floros, J. D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. Journal of Plastic Film and Sheeting 13: 287-298.

Hoque, M. S., Benjakul, S. and Prodpran, T. 2010. Effect of heat treatment of film-forming solution on the properties of film from cuttlefish *(Sepia pharaonis)* skin gelatin. Journal of Food Engineering 96: 66-73.

Hoque, M. S., Benjakul, S. and Prodpran, T. 2011. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish *(Sepia pharaonis)* skin gelatin. Food Hydrocolloids 25: 82-90.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish-water soluble proteins. Fisheries Science 66: 372-378.

Jones, R. T. 1987. Gelatin: structure and manufacture. In Ridgway, K. (Ed.). Hard Capsules Development and Technology, p. 13-30. London: The Pharmaceutical Press.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloids 20: 492-501.

Karim, A. A. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospect as an alternative to mammalian gelatins. Food Hydrocolloids 23: 563-576.

Ledward, D. A. 1986. Gelation of gelatin. In Mitchell, J. R. and Ledward, D.A. (Eds). Functional Properties of Food Macromolecules, p. 171-201. London: Elsevier.

Martucci, J. F. and Ruseckaite, R. A. 2009. Tensile properties, barrier properties and biodegradation in soil of compression-molded gelatin-dialdehyde starch films. Journal of Applied Polymer Science 112: 2166-2178

McHugh, T. H., Aujard, J. F. and Krochta, J. M. 1994. Plasticized whey protein edible films: water vapor permeability properties. Journal of Food Science 59: 416-423.

Muyonga, J. H., Cole, C. G. B. and Duodu, K. G. 2004. Extraction and physico-chemical characterisation of Nile perch *(Lates niloticus)* skin and bone gelatin. Food Hydrocolloids 18: 581-592.

Norland, R. E. 1990. Fish Gelatin. In Voprimight, M.N. and Botta, J.K. (Eds). Advances in Fisheries Technology and Biotechnology for Increased Profitability, p. 325-333. Lancaster: Technomic Publishing.

Park, J. W., Whiteside, W. S. and Cho, S. Y. 2008. Mechanical and water vapor barrier properties of extruded and heat-pressed gelatin films. LWT - Food Science and Technology 41: 692-700.

Perez-Gago, M. B. and Krochta, J. M. 2001. Denaturation time and temperature effects on solubility, tensile properties and oxygen permeability of whey protein edible films. Journal of Food Science 66: 705-710.

Robinson, H. W. and Hodgen, C. G. 1940. The biuret reaction in the determination of serum protein. I. A study of condition necessary for the production of the stable color which bears a quantitative relationship to the protein concentration. Journal of Biological Chemistry 135: 707-725.

Shiku, Y., Hamaguchi, P. Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska Pollack. Food Chemistry 86: 493-499.

Sikorski, Z. E. 2001. Chemical and functional properties of food proteins. Lancaster: Technomic Publishing Company, Inc.

Guerrero, P., Retegi, A., Gabilondo, N. and de la Caba, K. 2010. Mechanical and thermal properties of soy protein films processed by casting and compression. Journal of Food Engineering 100: 145-151.

Wang, L., Auty, M. A. E., Rau, A., Kerry, J. F. and Kerry, J. P. 2009. Effect of pH and addition of corn oil on the properties of gelatin-based biopolymer films. Journal of Food Engineering 90: 11-19.

Zhang, S., Wang, Y., Herring, J. L. and Oh, J. H. 2007. Characterization of edible film fabricated with channel catfish *(Ictalurus punctatus)* gelatin extract using selected pretreatment methods. Journal of Food Science 72: 498-503.

Zhou, P. and Regenstein, J. M. 2004. Optimization of extraction conditions for pollock skin gelatin. Journal of Food Science 69: 393-398.