

IMPACTS OF PLASTICIZER AND PRE-HEATING CONDITIONS ON PROPERTIES OF BOVINE AND FISH GELATIN FILMS FABRICATED BY THERMO-COMPRESSSION MOLDING TECHNIQUE

K. CHUAYNUKUL¹, M. NAGARAJAN¹, T. PRODPRAN^{*1}, S. BENJAKUL²
and S. PRASARPRAN¹

¹Department of Material Product Technology, Faculty of Agro-Industry, Prince of Songkla University,
15 Kanchanawanich Road, Hat Yai, Songkhla 90112, Thailand

²Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University,
15 Kanchanawanich Road, Hat Yai, Songkhla 90112, Thailand

*Corresponding author. Tel.: +66 74286357; fax: +66 74558866

E-mail address: thummanoon.p@psu.ac.th

ABSTRACT

Bovine and fish gelatin films were prepared by thermo-compression molding technique. This study investigated the effects of glycerol levels (15-35%) and resin pre-heating conditions including pre-heating temperatures (120, 140 and 160°C) and times (5 and 10 min) on film properties. Tensile strength (TS), elastic modulus (EM) and yellowness of films decreased, but elongation at break (EAB), water-vapor permeability (WVP) and transparency increased with increasing glycerol level. The gelatin films generally had decreased TS, EM, WVP and transparency, but increased yellowness as the pre-heating temperature and time increased. Therefore, the glycerol level and condition used for resin pre-heating directly influenced the film properties.

Keywords: compression molding, film, gelatin, glycerol level, pre-heating conditions, WVP

1. INTRODUCTION

Proteins are natural polymers frequently used for preparing bio-degradable/edible films. These films are alternatives to synthetic plastic films, which serve as one of the ways of reducing environmental pollution (GOMEZ-GUILLEN *et al.*, 2009). In general, protein films exhibit excellent barrier properties to oxygen, lipids and aromas, and have been reported to possess moderate mechanical properties, as well as a high nutritional value, but with low water vapor barrier property due to the hydrophilic character of these macromolecules (GENNADIOS, 2002; OU *et al.*, 2004). 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 an animal protein derived from the partial hydrolysis of native collagens, which are the most abundant structural proteins found in skins, bones and connective tissues (KARIM and BHAT, 2009). It possesses a good film-forming property and is one of the first materials applied as edible coatings and films (KLOSE *et al.*, 1952; GENNADIOS *et al.*, 1994). Skin and bone from bovine and porcine sources are utilized commercially in gelatin production (JOHNSTON-BANKS, 1990; VENIEN and LEVIEUX, 2005). As a result of religious objections to the consumption of bovine materials and health concerns about the spread of diseases (such as bovine spongiform encephalopathy) to humans, fish gelatin is increasingly gaining attention as an alternative to replace mammalian gelatin (GOMEZ-GUILLEN *et al.*, 2009; KARIM and BHAT, 2009). Among all proteins, gelatin has received considerable attention in the development of edible films owing to its abundance and biodegradability (BIGI *et al.*, 2002; JONGJAREONRAK *et al.*, 2006). In addition, it is unique among hydrocolloids in forming thermo-reversible products with a melting point close to body temperature, which is particularly significant in edible and pharmaceutical applications (NORLAND, 1990; GOMEZ-GUILLEN *et al.*, 2009). With regards to film with many outstanding properties, such as transparency, bio-degradability and barrier properties (gases and aroma), gelatin is suitable for application in bio-degradable packaging (MARTUCCI and RUSECKAITE, 2009; NAGARAJAN *et al.*, 2015). However, the properties of gelatin films depend on the characteristics of the raw materials and manufacturing processes.

In general, there are two main methods used for preparing protein films: 1) the dry and 2) the wet processes. The wet process or solution casting is the most widely used film-forming method (ZHANG *et al.*, 2007; WANG *et al.*, 2009; LIMPISOPHON *et al.*, 2010). The dry process (or thermal processing method), such as compression molding and extrusion, is based on the thermoplastic properties of proteins when plasticized and heated above their glass transition temperature (T_g) under low water content. Heating above T_g produces soft and rubbery material and may permit their incorporation into specific products. 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). This thermal process may affect film properties differently as compared with the casting method, but it enhances the commercial potential for large-scale production of bio-degradable/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 various thermal processing techniques, thermo-compression molding can be applied for film preparation from different polymers. Although this technique is feasible at the laboratory level, it is not a potential technique for realistic packaging applications. This technique is still widely used especially in preliminary studies to ascertain the feasibility of using thermal processing technique and

to standardize the processing conditions prior to developing such biodegradable film via the continuous thermal processing techniques.

However, there is limited information regarding the preparation and properties of gelatin films fabricated by the thermal method. Among thermal processing methods, compression molding is the most common and simple method of molding, and is typically used to investigate the feasibility of converting any polymer to the desired product via thermoplastic processing. One of the requirements of thermal processing in film making from biopolymers including gelatin, is the possession of a sufficient melt flow under the processing conditions. Gelatin normally involves high molecular interaction, which results in high T_g and melt viscosity, but low melt flow-ability. As a consequence, the incorporation of a proper plasticizer to gelatin at suitable levels will allow a sufficient flow of the melt, thereby producing the gelatin film by thermal processing. Moreover, the processing conditions of the thermal technique used are also crucial for obtaining a gelatin film with good properties, which may vary based on gelatin type. The compression molding technique generally involves several fundamental steps including pre-heating (without applied pressure), degassing, heating and pressurizing (compressing), as well as cooling. The conditions used in each step have to be properly manipulated.

Therefore, this study was carried out to investigate the feasibility of gelatin film production by thermo-compression molding technique. In particular, the effects of plasticizer (glycerol) level, pre-heating temperature and time of compression molding on film-forming ability, as well as the properties of films from bovine-hide and fish-skin gelatins were studied.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

This experiment utilized commercial bovine-hide gelatin (~240 bloom) and fish-skin gelatin (~240 bloom), purchased from Halamic Company (Bangkok, Thailand) and LAPI GELATINE S.p.A. (Empoli, Italy), respectively. The glycerol (food grade), used as plasticizer being approved as safe by the Food and Drug Administration (FDA), was purchased from Wako Pure Chemical Industry, Ltd., Tokyo, Japan.

2.2. Preparation of molding compound resin

Prior to molding, compound resin based on gelatin and plasticizer, which is used as raw material for compression molding, was prepared. From the preliminary investigations of this study, the compound resin in the pellet form could not be successfully prepared by dry blending or melt compounding using twin-screw extrusion technique. For dry blending, the glycerol could not be well dispersed with gelatin powder and as such, the resin could not flow properly upon heat compression and became easily degraded. For melt blending, the gelatin melt was too viscous and tended to stick to the screw surface and became degraded upon mixing, mainly due to the evaporation of water acting as a plasticizer at high temperature. Thus, the plasticized-gelatin molding compound resin was prepared by solution blending. Molding compound resin (a mixture of gelatin and plasticizer) was prepared according to the method of PARK *et al.* (2008) with some modifications. Bovine or fish gelatin powders were first dissolved in hot (65 °C) deionized water to obtain a protein concentration (AOAC, 2000) of 20% (w/v). Glycerol was added to gelatin solution at different concentrations (15, 25 and 35% of protein, w/w). The film-forming solutions (FFS) were then heated at 90 °C for 2 h in a water bath. Upon heat-

pretreatment, the FFSs were gently stirred. Thereafter, 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 ($\sim 0.5 \times 0.3 \times 0.3 \text{ cm}^3$) and further dried in a vacuum oven at $35 \text{ }^\circ\text{C}$ for 48 h. The obtained resins (Fig. 1) were conditioned at $25 \text{ }^\circ\text{C}$ and 60% RH in an environmental chamber (TK120, NUVE, Belgium) for 48 h, before being used for the preparation of films via thermo-compression molding. The conditioned resins obtained had a moisture content of $15 \pm 3\%$.

2.3. Effects of glycerol level on film formation and film properties

2.3.1. Film fabrication by thermo-compression molding

The conditioned bovine and fish gelatin resins containing different glycerol levels (about 3 g) were placed between two stainless steel plates ($10 \times 10 \text{ inch}^2$) covered with Mylar sheets. A spacer with a thickness of 0.1 mm was inserted between the plates. The set was inserted between heating platens of the compression molder previously heated to $120 \text{ }^\circ\text{C}$. To melt the resin, it was pre-heated without applying pressure at the aforementioned temperature for 10 min. The molten resin was subsequently pressed to form a film in the compression molder at that temperature. A pressure of 20 MPa was applied for 2 min followed by the removal of the set from the compression molder. The samples were cooled down to room temperature. The gelatin film was then removed from the plates and subjected to analyses.



Figure 1. Photographs of molding compound resins from bovine and fish gelatins.

2.3.2. Analyses of thermo-compression molded gelatin films

Prior to testing, film samples were conditioned in an environmental chamber for 48 h at $25 \pm 0.5 \text{ }^\circ\text{C}$ and $50 \pm 5\%$ RH.

2.3.2.1 Film thickness

The film thickness was measured using a digital micrometer (Gotech, Model GT-313-A, Gotech Testing Machines Inc., Tawain) (CHUAYNUKUL *et al.*, 2015). Five random thickness measurements were recorded and the average was taken as the result.

2.3.2.2 Mechanical properties

The tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) of the films were measured according to the ASTM-D882-01 (2002a) method, as described by IWATA *et al.* (2000), using a universal testing machine (Lloyd Instruments, Hampshire, UK). The specimen strip (50 x 20 mm²) 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 the 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 the stress-strain curve. Ten specimens were tested for each treatment.

2.3.2.3 Water vapor permeability (WVP)

WVP was determined using a modified ASTM E-96-01 (2002b) method as described by SHIKU *et al.* (2004). The pre-conditioned film was sealed onto the opening of an aluminum permeation cup (30 mm internal diameter) containing dried silica gel (0% RH) with silicone vacuum grease and rubber gasket. The cup was kept in a controlled chamber at 30±0.5 °C and 65±5% RH. The cup was weighed every 1 h until 8 h, under this controlled environment. The WVP of the gelatin film was calculated using the following equation:

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

Where l is the average thickness of the film sample (mm); A is the exposed area of the film (m²); 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.m/m².s.Pa.

2.3.2.4 Color

The color of the film was determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). Also, D_{65} (day light) and a measure cell with an opening of 30 mm were used. Dried film samples were directly placed on the sample compartment and covered with a white standard plate. The color of the film was expressed as L^* - (lightness/brightness), a^* - (redness/greenness) and b^* - (yellowness/blueness) values. The total difference in color (ΔE^*) was calculated according to the equation of GENNADIOS *et al.* (1996a) as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where, ΔL^* , Δa^* and Δb^* are the differences between the color parameter of corresponding film samples and that of the white standard ($L^*= 92.82$, $a^*= -1.29$ and $b^*= 0.51$).

2.3.2.5 Light transmittance and transparency value

The transmission of visible light of gelatin films was measured at a wavelength of 600 nm, using UV-Vis spectrophotometer (Model No. 1601, Shimadzu, Kyoto, Japan) as described

by HAN and FLOROS (1997). The transparency value of the films was then calculated using the following equation:

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

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

2.4. Effects of pre-heating conditions on film formation and film properties

The conditioned bovine and fish gelatin resins containing glycerol at 25% of protein were used to prepare films via compression molding using the same procedure as described above. In this study, different conditions in the pre-heating step prior to pressing were applied, including pre-heating temperatures (120, 140 and 160 °C) and times (5 and 10 min). Thereafter, the pre-heated resins were compressed at the aforementioned temperatures for 2 min with pressure of 20 MPa, followed by cooling as described above. The obtained films from bovine and fish gelatins were then subjected to analyses as mentioned earlier.

2.5. Statistical analysis

All experiments were performed in triplicates ($n=3$) and a completely randomized design (CRD) was used. Analysis of variance (ANOVA) was performed and the mean comparisons were done by Duncan's multiple range tests (STEEL and TORRIE, 1980). Data are presented as mean±standard deviation and the probability value of $p<0.05$ was considered as significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 17.0 for windows, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of glycerol levels on the properties of thermo-compression molded gelatin films

3.1.1. Film forming ability and visualized appearance of films

Glycerol, as a plasticizer, was incorporated into gelatin at different levels (15, 25 and 35% of protein, w/w). The addition of glycerol was expected to influence not only the properties of obtained films, but also the flow-ability and fusion behavior of molding compound resin during thermo-compression. The later effects were important for the feasibility of film-formation via thermal technique. It was found that all levels of glycerol used were able to promote sufficient flow-ability and fusion of the molten resins under hot-pressing (at 120 °C and 20 MPa). Thus, continuous, flexible films with average thickness in the range of 80.2-127.3 μm were formed, regardless of gelatin type. As the glycerol level increased, the average thickness of the films decreased (Table 1). This can be attributed to the fact that an increase in glycerol level resulted in increased flow-ability of the molten gelatin (at 120 °C), probably due to a decrease in its melt viscosity. Theoretically, the addition of a plasticizer to the polymer can also act as a processing aid

which can decrease the melt viscosity of the polymer, thereby enhancing the flow-ability of the polymer melt (PARK *et al.*, 2008; MARTUCCI and RUSECKAITE, 2009). PARK *et al.* (2008) prepared gelatin resins plasticized with 20% glycerol, sorbitol or a mixture of glycerol and sorbitol, and the resins were extruded to produce stretchable films. The results suggested that the film prepared only with sorbitol, as the plasticizer was not suitable for the extrusion film process due to its low fluidity. However, the films can be successfully extruded from resins plasticized with 20% glycerol and the glycerol-sorbitol mixture. Therefore, the glycerol level had an impact on the flow-ability and thus, the film-forming ability via compression molding of gelatin.

Table 1. Thickness and mechanical properties of thermo-compression molded gelatin films from resins containing different glycerol levels.

Gelatin	Glycerol (%)	Thickness (μm)	EM [#] ($\times 10^2 \text{MPa}$)	TS [#] (MPa)	EAB [#] (%)
Bovine	15	127.3 \pm 7.1 ^c	5.22 \pm 0.20 ^e	26.68 \pm 0.79 ^e	6.86 \pm 1.57 ^{a*}
	25	98.5 \pm 8.2 ^b	3.84 \pm 0.24 ^c	21.65 \pm 0.55 ^d	36.29 \pm 14.67 ^c
	35	83.2 \pm 7.4 ^a	2.05 \pm 0.16 ^b	10.24 \pm 0.85 ^b	125.18 \pm 11.83 ^d
Fish	15	122.7 \pm 8.3 ^c	4.38 \pm 0.24 ^d	25.45 \pm 1.69 ^e	4.98 \pm 0.87 ^a
	25	94.2 \pm 9.4 ^b	3.46 \pm 0.30 ^c	19.74 \pm 1.00 ^c	24.10 \pm 8.73 ^b
	35	80.2 \pm 8.4 ^a	1.30 \pm 0.22 ^a	7.83 \pm 0.68 ^a	124.21 \pm 6.79 ^d

[#]Mean \pm SD (n=3).

^{*}Different superscripts in the same column indicate significant differences (p<0.05).

3.1.2. Mechanical properties

The mechanical properties of thermo-compression molded (120 °C, 20 MPa) bovine and fish gelatin films from resins incorporated with glycerol at various levels are shown in Table 1. As shown in the table, gelatin films had decreased TS and EM, but increased EAB with increasing glycerol levels, regardless of gelatin type (p<0.05). Film containing 35% glycerol had the highest EAB (125.18 and 124.21% for bovine and fish gelatin films, respectively). However, it exhibited a rather sticky surface, which could be attributed to the excessive addition of glycerol. These results indicated the plasticizing effect caused by glycerol, as well as some adsorbed water in the film matrix. The addition of a miscible plasticizer can reduce inter-chain interactions to overcome the brittleness of films and improve film flexibility (LIMPISOPHON *et al.*, 2010). When glycerol is used, its hydroxyl groups could interact with the amino acid groups in the protein, thereby decreasing inter- and intra-molecular interactions between protein chains, such as hydrogen bonds, and improving the motion ability of protein macromolecules, thereby resulting in increased flexibility (i.e. reducing the EM) of the films. As a result, the behavior of gelatin films changed from brittle to flexible. Moreover, glycerol, a small molecule with highly hygroscopic characteristic, is easily inserted between protein chains and attracts more water to the structure of the film, thereby becoming more flexible. Similar results have been reported for protein films prepared using thermal techniques (CUNNINGHAM *et al.*, 2000; ZHANG *et al.*, 2001; SOTHORNVIT *et al.*, 2007).

However, the required amount of plasticizer, which is appropriate for the film-forming ability and better properties of film prepared from thermal processing, is dependent on protein type, technique and condition used (SOTHORNVIT *et al.*, 2007; PARK *et al.*, 2008). PARK *et al.* (2008) produced a gelatin film from resin that was plasticized with 20%

glycerol by extrusion. Thus, it was found that the extruded film could not be produced from resin plasticized with sorbitol, since it had low fluidity during processing. SOTHORNVIT *et al.* (2007) prepared a protein sheet from mixtures of whey protein isolates (WPI) and glycerol (30, 40 and 50%) using the compression molding method. They revealed that the increasing glycerol content not only decreased TS and EM, but also increased the EAB of the WPI-sheet. They suggested that by using the compression molding method, a minimum of 30% glycerol should be added in the production of WPI-sheets, since lower glycerol levels produce sheets which are too brittle to handle and test. ZHANG *et al.* (2001) prepared soy protein sheets with various glycerol levels (10, 20, 30, 40 and 50%) by extrusion. They reported that films with 10% glycerol were very difficult to produce and very brittle after losing moisture. When glycerol of 20 to 30% was added, the strength of the sheet decreased a little, but it was good in terms of greater EAB. An increase in glycerol level (above 30%) resulted in a large decrease in yield stress and continuous increase in EAB. From the results, it was found that the effect of the added glycerol on the compression-molded gelatin film showed a similar trend to the gelatin film developed using the casting technique (VANIN *et al.*, 2005; JONGJAREONRAK *et al.*, 2006; HOQUE *et al.*, 2011).

For the same level of glycerol used, bovine gelatin films showed greater mechanical resistance than fish gelatin films as indicated by the higher TS, EM and EAB of the films. This can be attributed to the higher imino acid content in bovine gelatin (ARVANITTOYANNIS *et al.*, 2002; AVENA-BUSTILLOS *et al.*, 2006). The proline and hydroxyproline contents are approximately 30% for mammalian gelatins, 22-25% for warm-water fish gelatins (tilapia and Nile perch), and 17% for cold-water fish gelatins (cod) (AVENA-BUSTILLOS *et al.*, 2006; CHIOU *et al.*, 2008). Mammalian gelatins commonly have better physical properties and thermal stability than most fish gelatins (ARVANITTOYANNIS *et al.*, 2002; MUYONGA *et al.*, 2004; AVENA-BUSTILLOS *et al.*, 2006). Nevertheless, the incorporation of glycerol at 25% protein, produced continuous gelatin films with sufficient flexibility to be handled, irrespective of gelatin type. Thus, the glycerol levels used had a significant influence on the film-forming ability and properties of the resulting compression-molded gelatin films.

3.1.3. Water vapor permeability (WVP)

The WVP of compression-molded bovine and fish gelatin films from resins incorporated with different glycerol levels is shown in Fig. 2. Generally, the WVP of all gelatin films increased with increasing glycerol levels ($p < 0.05$), regardless of gelatin type. Glycerol, a highly hygroscopic substance, which consists of 3 hydroxyl groups, was able to attract water to the plasticized protein system (SOTHORNVIT *et al.*, 2001). Plasticizers not only improve the mechanical properties of protein films, but also increase the film permeability (CUQ *et al.*, 1997; SOTHORNVIT *et al.*, 2001).

Besides the contribution from solubility, polymer permeability is also dependent on its diffusion coefficient. The diffusion coefficient and thus, the permeability increased with an increase in free volume, as well as the interstitial space between the polymer molecules. From the result, the increase in WVP of both gelatin films with increasing glycerol level could also be explained by an increase in free volume of the film matrix. The insertion of plasticizers between protein molecules increased the free volume of the system and favored the mobility of polymeric chains. Consequently, the network structure of the film became less dense and more permeable (JONGJAREONRAK *et al.*, 2006). ORLIAC *et al.* (2003) reported that an increase in hydrophilic plasticizer levels enhanced the absorption of more water to the film network and increased water vapor transfer.

The results of this study are in agreement with the reports of various protein films prepared using solution casting such as gelatin films from cuttlefish (*Sepia pharaonis*) skin (HOQUE *et al.*, 2011), brown stripe red snapper and big-eye snapper skin (JONGJAREONRAK *et al.*, 2006), pig skin (VANIN *et al.*, 2005), muscle protein of Nile tilapia (PASCHOALICK *et al.*, 2003), wheat gluten (GONTARD *et al.*, 1993) and protein films prepared using thermal method such as sun flower protein film (ORLIAC *et al.*, 2003), soy protein sheet (ZHANG *et al.*, 2001) and soy protein isolate film (CUNNINGHAM *et al.*, 2000).

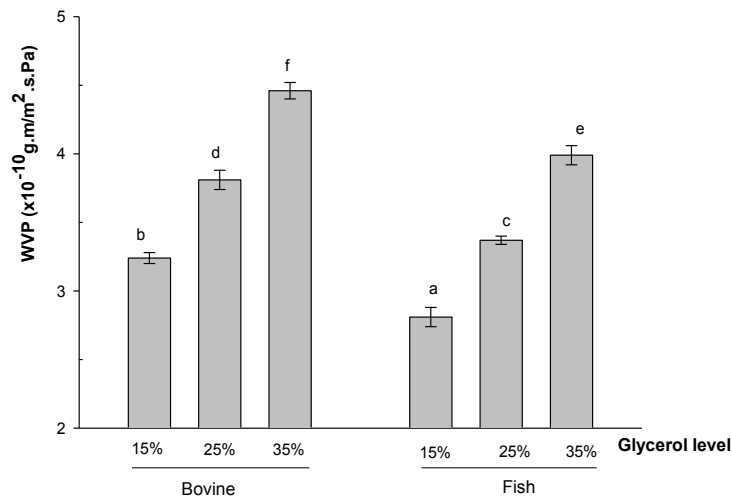


Figure 2. The WVP of thermo-compression molded gelatin films from resins containing different glycerol levels. Each bar represents the standard deviation (n=3). Different letters indicate significant differences (p<0.05).

At the same glycerol level, fish gelatin films showed lower WVP than bovine gelatin films (p<0.05). This may be attributed to the lower concentrations of proline and hydroxyproline in fish gelatins as compared with mammalian gelatins (NORLAND, 1990; GOMEZ-GUILLEN *et al.*, 2009). Moreover, it has been reported that the lower WVP of fish gelatin films could be useful, particularly for applications related to reducing water loss from encapsulated drugs and refrigerated or frozen food systems (NORLAND, 1990). Therefore, the amount of glycerol added influenced not only the flow-ability, film-forming ability and mechanical properties but also the barrier properties of the compression-molded gelatin films.

3.1.4. Color and transparency value

The color of the thermo-compression molded gelatin films from resins containing different glycerol levels is shown in Table 2. In general, as the glycerol concentration increased from 15 to 25%, both types of gelatin films exhibited almost similar L^* - and a^* -values (p>0.05). When the glycerol content was further increased to 35%, the fish gelatin film showed higher L^* - and a^* -values (p<0.05). However, the b^* - and ΔE^* -values of films from both gelatins tended to decrease with increasing glycerol level. The behavior of total color difference (ΔE^*) was mainly as a result of the variation in b^* -value. Overall, the results indicated that when the concentration of glycerol increased, the film showed higher

lightness and less color, especially yellowness. This can be attributed to the effect of the dilution of proteins by the addition of glycerol, which is a colorless plasticizer (PASCHOALICK *et al.*, 2003; SOBRAL *et al.*, 2005). However, VANIN *et al.* (2005) did not observe the effect of plasticizer type (glycerol, polypropylene glycol, ethylene glycol and di-ethylene glycol) and plasticizer concentration (10, 15, 20, 25 and 30%) on the color difference and opacity of gelatin-based films. The different observations might be due to differences in amino acid composition and molecular weight of gelatin, as well as the alignment of gelatin molecules in the film network. At the same glycerol levels, bovine gelatin films were darker in color and had much higher yellowness and slightly lower lightness as evidenced by a greater b^* -value and lesser L^* -value, respectively, when compared with fish gelatin films ($p < 0.05$). This was mainly due to the differences in color of both gelatin powders used as raw materials.

Table 2. Color of thermo-compression molded gelatin films from resins containing different glycerol levels.

Gelatin	Glycerol (%)	$L^{\#}$	$a^{\#}$	$b^{\#}$	$\Delta E^{\#}$
Bovine	15	88.98±0.02 ^a	-1.58±0.02 ^a	6.70±0.16 ^e	7.95±0.14 ^g
	25	89.56±0.04 ^{ab}	-1.41±0.02 ^b	4.88±0.22 ^d	6.17±0.19 ^d
	35	89.85±0.08 ^{bc}	-1.33±0.04 ^b	3.99±0.18 ^c	5.31±0.18 ^c
Fish	15	90.53±0.08 ^c	-1.21±0.04 ^c	2.46±0.05 ^b	3.81±0.05 ^b
	25	90.46±0.08 ^c	-1.17±0.07 ^c	2.27±0.09 ^b	3.76±0.08 ^b
	35	92.01±0.88 ^d	-0.44±0.10 ^d	0.17±0.08 ^a	1.60±0.20 ^a

^aMean ± SD (n=3).

^gDifferent superscripts in the same column indicate significant differences ($p < 0.05$).

The transparency value of compression-molded gelatin films, plasticized with glycerol at different levels, is shown in Fig. 3.

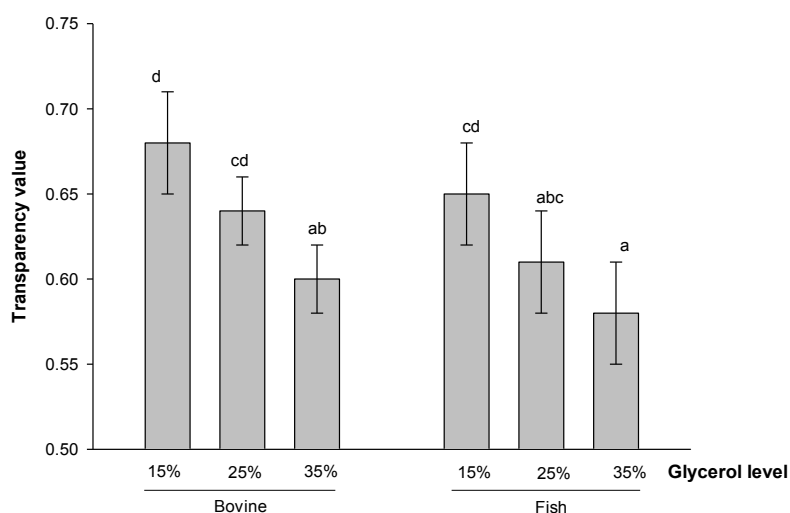


Figure 3. Transparency value of thermo-compression molded gelatin films from resins containing different glycerol levels. Each bar represents the standard deviation (n=3). Different letters indicate significant differences ($p < 0.05$).

Generally, the glycerol content used had a slight impact on the transparency of the compression-molded films of both gelatins. The transparency value of the film tended to decrease with increasing glycerol levels. For both gelatins, films plasticized with 35% glycerol showed a lower transparency value than those with 15% glycerol ($p < 0.05$). Based on the equation used for the calculation of the transparency value, the film sample with lower transparency value was more transparent. From the results, films were more transparent when a high level of glycerol was incorporated. This could be attributed to the fact that the added glycerol decreased protein-protein interactions with concomitant increase in free volume in the film matrix; hence, light could pass through such a network more easily. This result is in accordance with other reports in which films became more transparent with increasing plasticizer level (PASCHOALICK *et al.*, 2003; SOBRAL *et al.*, 2005).

3.2. Effect of pre-heating conditions on properties of thermo-compression molded gelatin films

The result of this study showed that films from bovine and fish gelatins to which 25% glycerol was added had satisfactory mechanical and water vapor barrier properties. Furthermore, the film obtained was not sticky and easy to handle. Therefore, to study the effect of pre-heating conditions on gelatin resins, 25% glycerol level was used.

3.2.1. Film forming ability and visualized appearance of films

To investigate the film forming ability of plasticized gelatins in the melt state via thermo-compression molding, pre-heating of resin prior to pressing was performed at various temperatures (120, 140 and 160 °C) and duration (5 and 10 min). Table 3 shows the overall visualized appearance of films obtained under different pre-heating conditions.

Table 3. Appearance of thermo-compression molded gelatin films prepared under different pre-heating conditions.

Gelatin	Preheating conditions		Visual appearance
	Temp.(°C)	Time (min)	
Bovine	120	5	Film with many voids or pinholes
		10	
	140	5	Continuous, flexible, transparent and yellow film
		10	
	160	5	Opaque and very dark yellow brittle film
		10	Low fluidity of the melt; too brittle film
Fish	120	5	Continuous, flexible, transparent and less yellow film.
		10	
	140	5	Rather opaque and slightly yellow brittle film
		10	
	160	5	Rather opaque and slightly yellow brittle film
		10	

The thickest films with a lot of visualized voids were obtained for both gelatin types when the processing temperatures used were lower than 120 °C. This might be due to the fact that the molten resins could not flow properly because of its high melt viscosity. Moreover, processing temperatures higher than 160 °C resulted in dramatic degradation. It was found that pre-heating of bovine gelatin resin at 120°C for 5 min rendered the film with numerous visualized voids or pin-holes. Under this pre-heating condition, the plasticized-bovine gelatin melt might still possess high viscosity. As a result, the air bubbles could not be evacuated from the melt before compressing and thus, were entrapped in the film after cooling. However, it was not noticed for fish gelatin resin pre-heated under the same condition, possibly due to the lower melt viscosity of fish gelatin as compared with bovine gelatin. Continuous and flexible films without visualized pin-holes were formed when the pre-heating temperature and time were increased up to 160 °C and 5 min, regardless of gelatin type.

The melt viscosity of both gelatin resins might decrease sufficiently and allowed the gelatin melt to flow easily. As a result, the gelatin molecules could form a continuous network structure of film. Generally, the melt viscosity of the polymer decreased with increasing processing temperature, which is a significant factor in determining the flowability and processibility of the polymer via molding processes (CUNNINGHAM *et al.*, 2000; ZHANG *et al.*, 2001; PARK *et al.*, 2008). However, very brittle films with obvious degradation were obtained for both gelatins when the pre-heating of resins at 160 °C for 10 min was implemented. The application of a higher temperature for a longer period of time resulted in a dramatic degradation of gelatin, as well as the evaporation of bounded water. CHUAYNUKUL (2011) reported that partial degradation was noticed in the gelatin film compression molded at 120 °C, as evidenced by the increased free-amino group content. Therefore, the condition used in the pre-heating step was crucial for determining the gelatin film processibility via compression molding. The studied pre-heating conditions also affected the properties of bovine and fish gelatin films differently.

3.2.2. Mechanical properties

The mechanical properties of gelatin films prepared by different resin pre-heating conditions are shown in Table 4. The TS and EM of the films decreased, whilst the EAB increased with increasing pre-heating temperature and time ($p < 0.05$), regardless of gelatin type. Gelatin films exhibited the highest values of TS and EM at pre-heating temperature of 120 °C (20.82 MPa, 6.17×10^5 MPa and 18.90 MPa, 4.76×10^5 MPa for bovine and fish gelatin films, respectively). The TS and EM decreased when the pre-heating temperature was increased. This was probably due to the partial degradation of gelatin, which took place at higher temperature and extended time. The shorter gelatin molecules formed the lower intermolecular interactions, resulting in films with lower strength and stiffness (that is lower TS and EM, respectively). CHUAYNUKUL (2011) observed that the partial degradation of gelatins took place in resins and the resulting films when the resins were subjected to higher temperature prior to molding, as reconfirmed by the increased free-amino group content of the gelatin. The impact of heating on the degradation of gelatin has been investigated and reported by HOQUE *et al.* (2010). It was pointed out that the partial degradation of gelatin occurred when the gelatin solution was heated at a temperature higher than 70°C. This caused a decrease in the TS of the resulting films, because the shorter chains of gelatin molecules could not form the strong film network. The presence of degradation of protein molecules upon being processed at higher temperature was similarly observed in various protein-based films fabricated using

thermal processing techniques (SOTHORNVIT *et al.*, 2007; KATOH *et al.*, 2004; JANSSENS *et al.*, 2013).

Table 4. Mechanical properties and water vapor permeability (WVP) of thermo-compression molded gelatin films prepared under different pre-heating conditions.

Gelatin	Pre-heating conditions		EM [#] (x10 ² MPa)	TS [#] (MPa)	EAB [#] (%)	WVP [#] (x10 ⁻¹⁰ g.m/m ² .s.Pa)
	Temp. (°C)	Time (min)				
Bovine	120	5	ND	ND	ND	ND
		10	6.17±1.37 ^f	20.82±2.32 ^f	33.61±6.75 ^a	3.29±0.26 ^f ‡
	140	5	4.33±0.87 ^{de}	17.15±0.43 ^{de}	57.87±12.96 ^b	3.07±0.30 ^{ef}
		10	3.48±0.71 ^c	13.85±1.33 ^c	126.29±11.49 ^e	2.80±0.04 ^{cd}
	160	5	2.07±0.73 ^c	11.56±1.40 ^a	90.12±9.63 ^d	2.47±0.11 ^{ab}
		10	ND	ND	ND	ND
Fish	120	5	4.76±0.52 ^e	18.90±1.41 ^e	24.68±5.94 ^a	3.12±0.05 ^{ef}
		10	3.63±0.07 ^{cd}	16.67±1.63 ^d	31.50±5.79 ^a	2.97±0.26 ^{de}
	140	5	2.79±0.25 ^{bc}	13.52±0.92 ^{bc}	79.90±7.18 ^{cd}	2.61±0.08 ^{bc}
		10	1.91±0.57 ^{ab}	11.81±1.72 ^{ab}	105.52±15.1 ^e	2.50±0.07 ^{ab}
	160	5	1.60±0.36 ^a	11.42±0.56 ^a	82.24±12.27 ^c	2.25±0.06 ^a
		10	ND	ND	ND	ND

[#]Mean ± SD (n=3).

[‡]Different superscripts in the same column indicate significant differences (p<0.05).

ND = Not determined.

For the EAB value, pre-heating at higher temperature resulted in increased EAB of the films in both gelatins (p<0.05). At the same pre-heating temperature, films pre-heated for 10 min had higher EAB than those pre-heated for 5 min (p<0.05), regardless of gelatin type. This suggested an increase in film extensibility or stretch-ability. From the result, films prepared from the same type of gelatin had the highest EAB when pre-heating at 140 °C for 10 min was implemented. An increase in EAB was observed with a concomitant decrease in TS (GENNADIOS *et al.*, 1996b; JONGJAREONRAK *et al.*, 2006). Additionally, with increasing temperature from 120 to 140°C, gelatin might undergo partial degradation, leading to the formation of shorter chains. This may result in lower and/or weaker interaction between gelatin molecules (resulted in decreased TS), which could allow the molecules to slip around each other with more ease and a larger degree upon tensile deformation before breaking of the film specimen (leading to increased EAB) (HOQUE *et al.*, 2010). MUYONGA *et al.* (2004) observed that the gelatin extracted from Nile perch bones, which consisted of a higher proportion of low-molecular weight fractions, had considerably lower tensile strength, but higher percentage of elongation. However, when the pre-heating temperature was further increased to 160 °C, both gelatin films exhibited decreased EAB. At this temperature, a higher degradation of gelatin might take place resulting in shorter chains. These shorter gelatin chains may be unable to form a strong network, leading to a decrease in both TS and EAB.

When the films were compared based on different gelatin sources, fish gelatin films had lower TS, EM and EAB as compared with bovine gelatin films fabricated under the same pre-heating condition. This might be due to the presence of a higher number of imino acid residues in the α-chain of bovine gelatin, which contributed to the high number of

hydrogen bonds (NORLAND, 1990). CHIOU *et al.* (2008) prepared fish gelatin films from Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*) and compared their mechanical properties to bovine and porcine gelatin films. They reported that pollock and salmon gelatin films had lower TS and EAB than mammalian gelatin films. Therefore, the pre-heating temperature and time used in thermo-compression molding are essential to controlling the mechanical properties of the resulting films.

3.2.3. Water vapor permeability (WVP)

The WVP of the thermo-compression molded gelatin films prepared from different pre-heating conditions is shown in Table 4. The WVP of the films seemed to decrease with an increase in pre-heating temperature and time used, irrespective of gelatin type ($p < 0.05$). At the same pre-heating temperature, films prepared with pre-heating time of 10 min showed lower WVP than those prepared with pre-heating time of 5 min ($p < 0.05$). Films obtained from higher pre-heating temperature also exhibited lower WVP ($p < 0.05$). These results indicated that pre-heating at higher temperature led to increase in the water vapor barrier of the compression-molded gelatin films. This may be attributed to the exposure of the hydrophobic domains of gelatin chains upon heating, resulting in increased hydrophobic interaction and hydrophobicity of the films; thus, lowering the tendency of water absorbance by the film matrix (KIM *et al.*, 2002). Furthermore, the regular arrangement of shorter chain gelatin obtained after heating at higher temperatures, plausibly led to a compact matrix of gelatin during film formation (KIM *et al.*, 2002). As a result, the diffusion of water molecules could be retarded, which resulted in decreased WVP. KATOH *et al.* (2004) reported that molding at higher temperature caused the formation of a more compact structure of keratin film, which prevented the film from absorbing water. Moreover, the high temperature treatment of proteins might provoke additional interaction and crosslinking of protein molecules. JANSSENS *et al.* (2013) recorded an increase in water absorption of the compression-molded gluten protein film, as higher processing temperatures and longer times were used. This is mostly due to the presence of a higher degree of protein cross-linking during compression molding. A similar result was also reported by KIM *et al.* (2002) for heated soy protein films, in which the WVP of the film decreased during heating, and this was probably caused by the formation of crosslinks.

Under the same pre-heating condition, fish gelatin film had lower WVP as compared with bovine gelatin film ($p < 0.05$). This can be attributed to the higher hydrophobicity of fish gelatin, which had lower concentrations of proline and hydroxyproline as compared with mammalian gelatins (NORLAND, 1990; KARIM and BHAT, 2009). AVENA-BUSTILLOS *et al.* (2006) reported the WVP of fish-gelatin films to be significantly lower than that of films made from mammalian gelatin. They explained the tendency of fish-gelatin films to exhibit lower WVP values than land animal-gelatin films in terms of amino acid composition. Fish gelatins, especially cold-water fish gelatins, are known to contain higher amounts of hydrophobic amino acids and lower amounts of hydroxyproline (AVENA-BUSTILLOS *et al.*, 2006).

3.2.4. Color and transparency value of films

The color and transparency value of compression-molded gelatin films prepared under different pre-heating conditions are shown in Table 5. As the pre-heating temperature and time increased, the films had increased yellowness (b^* -value) and total color difference (ΔE^* -value), as well as slightly decreased film transparency (as indicated by an increase in

the transparency value) ($p < 0.05$). The increased yellowness of the films pre-heated at higher temperature and longer time was plausibly due to Maillard reaction, which is associated with the heat-induced process (HOQUE *et al.*, 2010).

As the pre-heating temperature and time increased, the lightness (L^*) of the bovine gelatin films showed a slight decreasing trend, whereas fish gelatin films showed no difference in L^* -value (90.12-90.62). PASCHOALICK *et al.* (2003) suggested that the increase in temperature caused a slight increase in the color of films, possibly due to the occurrence of a reaction among the glycerol molecules and the reactive group of lysine. For films fabricated under the same pre-heating condition, the fish gelatin films exhibited a lighter color but they were slightly more transparent than the bovine gelatin films. The differences in color and lightness of both films observed from the results were most likely influenced by the different gelatin types and manufacturing processes. The slight difference in transparency value of bovine and fish gelatin films might be due to differences in the intrinsic characteristics of each gelatin type, such as composition, density, aggregation or alignment of gelatin molecules in the films, as well as type of extraction treatments used to prepare the gelatin (BAILEY *et al.*, 1989). The purification procedure might increase the lightness of gelatin powder (NORLAND, 1990).

Table 5. Color and transparency values of thermo-compression molded gelatin films prepared under different pre-heating conditions.

Gelatin	Pre-heating conditions		$L^{\#}$	$a^{\#}$	$b^{\#}$	$\Delta E^{\#}$	Transparency value [#]
	Temp. (°C)	Time (min)					
Bovine	120	5	ND	ND	ND	ND	ND
		10	89.78±0.07 ^{cd}	-1.55±0.06 ^{de}	5.09±0.14 ^d	6.05±0.12 ^c	0.64±0.03 ^{ab[†]}
	140	5	89.10±0.05 ^{bc}	-1.82±0.03 ^c	7.49±0.11 ^e	8.42±0.09 ^d	0.70±0.03 ^c
		10	88.65±0.13 ^b	-1.97±0.16 ^b	8.36±0.71 ^f	9.4±0.65 ^e	0.71±0.03 ^c
	160	5	87.16±1.41 ^a	-3.06±0.12 ^a	12.04±0.62 ^g	13.43±1.22 ^f	0.81±0.02 ^d
		10	ND	ND	ND	ND	ND
Fish	120	5	90.62±0.03 ^d	-1.27±0.02 ^f	1.73±0.08 ^a	3.53±0.06 ^a	0.58±0.04 ^a
		10	90.59±0.03 ^d	-1.30±0.01 ^f	1.90±0.06 ^a	3.60±0.05 ^a	0.60±0.03 ^a
	140	5	90.50±0.06 ^d	-1.43±0.01 ^e	3.32±0.18 ^b	4.62±0.20 ^b	0.63±0.02 ^{ab}
		10	90.47±0.05 ^d	-1.46±0.03 ^e	3.49±0.14 ^b	4.69±0.05 ^b	0.63±0.03 ^{ab}
	160	5	90.12±0.07 ^d	-1.64±0.05 ^d	4.17±0.07 ^c	5.46±0.07 ^{bc}	0.68±0.02 ^{bc}
		10	ND	ND	ND	ND	ND

[#]Mean ± SD (n=3).

[†]Different superscripts in the same column indicate significant differences ($p < 0.05$).

ND = Not determined.

4. CONCLUSIONS

Films from bovine and fish gelatins were successfully prepared using the thermo-compression molding technique. Increasing the glycerol level resulted in decreased TS, EM and yellowness, but increased EAB, WVP and transparency of the resulting films. Gelatin films had generally decreased TS, EM, WVP and transparency, whilst increased yellowness occurred as pre-heating temperature and time increased. The compression-molded gelatin films incorporated with 25% glycerol (based on protein) had sufficient

flexibility (that is, EAB). Film processing conditions had significant impact on the processibility and properties of obtained compression-molded gelatin films. Among the major steps involved in the compression-molding of gelatin films, the pre-heating of resin prior to molding seemed to be the most crucial step in determining the processibility and properties of the obtained films. Therefore, the plasticizer level, pre-heating temperature and time have to be controlled for optimal flow-ability and fusion of the molten gelatin before compression/molding into a film.

ACKNOWLEDGEMENTS

The authors would like to thank the Prince of Songkla University, Thailand for the financial support. The TRF Distinguished Research Professor Grant is also acknowledged. PSU – Postdoctoral Fellowship from Prince of Songkla University for Muralidharan Nagarajan is acknowledged.

REFERENCES

- AOAC. 2000. "Official methods of analysis" 17th Ed. Association of Official Analytical Chemists, Gaithersberg.
- Arvanitoyannis I.S. 2002. Formation and properties of collagen and gelatin films and coatings. In "Protein-Based Films and Coatings" A. Gennadios (Ed.) pp. 275-304. CRC Press, Boca Raton, Florida.
- ASTM. 2002a. Standard test method for tensile properties of thin plastic sheeting (D882-01). In: "Annual Book of ASTM Standards" (Ed.). pp. 162-170. ASTM (American Society for Testing and Materials), Pennsylvania.
- ASTM. 2002b. Standard test method for water vapor transmission of materials (E96-01). In: "Annual Book of ASTM Standards" (Ed.). pp. 162-170. ASTM (American Society for Testing and Materials), Pennsylvania.
- 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. *J. Food Sci.* 71:202-207.
- Bailey A.J. and Light N.D. 1989. *Connective Tissue in Meat and Meat Products*. Elsevier Applied Science, London.
- Bigi A., Cojazzi G., Panzavolta S., Roveri N. and Rubini K. 2002. Stabilization of gelatin films by cross-linking with genipin. *Biomaterials*. 23:4827-4832.
- Chiou B.S., Avena-Bustillos R.J., Bechtel P.J., Jafri H., Narayan R., Imama S.H. *et al.* 2008. Cold water fish gelatine films: effects of cross-link on thermal, mechanical, barrier and biodegradation properties. *Eur. Polym. J.* 44:3748-3753.
- Chuaynukul K., Prodpran T. and Benjakul S. 2015. Properties of thermo-compression molded bovine and fish gelatin films as influenced by resin preparation condition. *Int. Food Res. J.* 22:1095-1102.
- Chuaynukul K. 2011. *Properties of Gelatin Film Prepared by Thermo-Compression Molding Technique as Affected by Forming Conditions*. Master of Science Thesis, Prince of Songkla University, Thailand.
- Cunningham P., Ogale A.A., Dawson P.L. and Acton J.C. 2000. Tensile properties of soy protein isolate films produced by a thermal compaction technique. *J. Food Sci.* 65:668-671.
- Cuq B., Gontard N. and Guilbert N. 1998. Proteins as agricultural polymers for packaging production. *J. Cereal Chem.* 75:1-9.
- 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.
- Gennadios A. (Ed.) 2002. *Protein-Based Films and Coatings*. CRC Press, Boca Raton, Florida.
- Gennadios A., Weller C.L., Hanna M.A. and Froning G.W. 1996a. Mechanical and barrier properties of egg albumen films. *J. Food Sci.* 61:585-589.
- Gennadios A., Ghorpade V.M., Weller C.L. and Hanna M.A. 1996b. Heat curing of soy protein films. *T. ASAE*. 39:575-579.

- Gennadios A., Mchugh T.H., Weller C.L. and Krochta J.M. 1994. Edible coatings and films based on proteins. In: "Edible Coatings and Films to Improve Food Quality" J.M. Krochta E.A. Baldwin and M. Nisperos-Carriedo (Ed.). pp. 210-278. Technomic Publishing Co. Inc., Lancaster, Pennsylvania.
- Gomez-Guillen M.C., Perez-Mateos M., Gomez-Estaca J., Lopez-Caballero E., Gimenez B. and Montero P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. *Trends Food Sci. Tech.* 20:3-16.
- Gontard N., Guilbert S. and Cuq J.L. 1993. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J. Food Sci.* 58:206-211.
- Han J.H. and Floros J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *J. Plast. Film Sheet.* 13:287-298.
- 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 Hydrocolloid.* 25:82-90.
- 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. *J. Food Eng.* 96:66-73.
- Iwata K.I., Ishizaki S.H., Handa A.K. and Tanaka M.U. 2000. Preparation and characterisation of edible films from fish water-soluble proteins. *Fish Sci.* 66:372-378.
- Jansens K.J.A., Hong N.V., Telen L., Brijs K., Lagrain B. and Vuure A.W.V. 2013. Effect of molding conditions and moisture content on the mechanical properties of compression molded glassy, wheat gluten bioplastics. *Ind. Crop. Prod.* 44:480-487.
- Johnston-Banks F.A. 1990. Gelatin. In "Food Gels" P. Harris (Ed.). pp. 366-394. Elsevier Applied Science, London.
- 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 Hydrocolloid.* 20:492-501.
- Karim A.A. and Bhat R. 2009. Fish gelatin: properties, challenges, and prospect as an alternative to mammalian gelatins. *Food Hydrocolloid.* 23:563-576.
- Katoh K., Shibayama M., Tanabe T. and Yamauchi K. 2004. Preparation and physicochemical properties of compression-molded keratin films. *Biomaterials.* 25:2265-2272.
- Kim K.M., Weller C.L., Hanna M.A. and Gennadios A. 2002. Heat curing of soy protein films at selected temperatures and pressures. *LWT-Food Sci. Technol.* 35:140-145.
- Klose A.A., Machi E.P. and Hanson H.L. 1952. Use of antioxidants in the frozen storage of turkeys. *Food Technol.* 6:308-11.
- Limpisophon K., Tanaka M. and Osako K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionaceglauca*) skin gelatin. *Food Chem.* 122:1095-1101.
- Martucci J.F. and Ruseckaite R.A. 2009. Tensile properties, barrier properties and biodegradation in soil of compression-molded gelatin-dialdehyde starch films. *J Applied Polym. Sci.* 112:2166-2178.
- Muyonga J.H., Cole C.G.B. and Duodu K.G. 2004. Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Latesniloticus*). *Food Chem.* 86:325-332.
- Nagarajan M., Benjakul S., Prodpran T. and Songtipya P. 2015. Effects of bio-nanocomposite films from tilapia and squid skin gelatins incorporated with ethanolic extract from coconut husk on storage stability of mackerel meat powder. *Food Pack. Shelf Life.* 6:42-52.
- Norland R.E. 1990. Fish gelatin. In: "Advances in Fisheries Technology and Biotechnology for Increased Profitability" M.N. Voight and J.K. Botta (Ed.). pp. 325-333. Technomic Publishing Co. Inc., Lancaster, Pennsylvania.
- Orliac O., Rouilly A., Silvestre F. and Rigal L. 2003. Effect of various plasticizers on the mechanical properties, water resistance and aging of thermo-moulded films made from sunflower proteins. *Ind. Crop. Prod.* 18:91-100.
- Ou S.Y., Kwok K.C. and Kang Y.J. 2004. Changes in vitro digestibility and available lysine of soy protein isolate after formation of film. *J. Food Eng.* 64:301-305.
- 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 Sci. Technol.* 41:692-700.

- Paschoalick T.M., Garcia F.T., Sobral P.J.A. and Habitante A.M.Q.B. 2003. Characterization of some functional properties of edible films based on muscle proteins of Nile tilapia. *Food Hydrocolloid*. 17:419-427.
- 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 Chem*. 86:493-499.
- Sobral P.J.A., Santos J.S. and Garcia F.T. 2005. Effect of protein and plasticizer concentrations in film forming solutions on physical properties of edible films based on muscle proteins of a Thai Tilapia. *J. Food Eng*. 70:93-100.
- Sothornvit R., Olsen C.W., McHugh T.H. and Krochta J.M. 2007. Tensile properties of compression-molded whey protein sheets: determination of molding condition and glycerol-content effects and comparison with solution-cast films. *J. Food Eng*. 78:855-860.
- Sothornvit R. and Krochta J.M. 2001. Plasticizer effect on mechanical properties of β -lactoglobulin films. *J. Food Eng*. 50:149-155.
- Steel, R.G.D. and Torrie, J.H. (Ed.). 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill, New York.
- Vanin F.M., Sobral P.J.A., Menegalli F.C., Carvalho R.A. and Habitante A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. *Food Hydrocolloid*. 19:899-907.
- Venien A. and Levieux D. 2005. Differentiation of bovine from porcine gelatins using polyclonal anti-peptide antibodies in indirect and competitive indirect ELISA. *J. Pharmaceut. Biomed*. 39:418-424.
- 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. *J. Food Eng*. 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. *J. Food Sci*. 72:498-503.
- Zhang J., Mungara P. and Jane J. 2001. Mechanical and thermal properties of extruded soy protein sheets. *Polymer*. 42:2569-2578.

Paper Received September 3, 2016 Accepted December 30, 2016