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Effects of plasticizer concentrations on functional properties of chicken skin gelatin films

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Abstract

The aim of this study was to investigate the functional properties of chicken skin gelatin films with varied concentrations of a hydrophilic plasticizer. Gelatin film solutions with different glycerol concentrations A(control), B(5%), C(10%), D(15%) and E(20%), were stirred at 45°C for 20min and oven dried at 45°C. Film characterization determination were included, tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP), solubility, transparency, moisture content, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (X-RD). Glycerol added resulted in improvement of TS and WVP properties. Film B (5% glycerol) demonstrated low EAB (106%), WVP (0.0175 g.mm/h.m2.k.Pa) and solubility (58.64%), but with high TS (3.64 MPa), moisture content (16.0%), UV light transmission (0.04%) and transparency (0.81) compared to films C, D and E. FTIR spectrum analyses demonstrated an aliphatic alcohol group only for Film E (20% glycerol). Hence, chicken skin gelatin film at 5% glycerol concentration showed the most promising potential for industrial food processing applications.

Introduction

Natural biodegradable polymers have been used extensively during the last decade because they offer many advantages over synthetic or non-biodegradable polymers. Thus, biopolymer-based packaging materials from naturally renewable origins have become a major research area (Kokoszka *et al*., 2010). Packaging materials based on biodegradable biopolymers guarantee biodegradability and environmental compatibility (Debeaufort *et al*., 1998). Such biodegradable films are fabricated from different polymer types, which include (i) polysaccharides such as cellulose and starch as well as chitosan, exudates, gums and pectin derivatives; (ii) lipids such as acetoglyceride, wax and paraffin; (iii) and proteins such as gelatin, casein, whey and soy (Bourtoom, 2008). Proteins from different sources, especially extracted gelatins, have impressive potential for biodegradable film applications due to relative abundance and excellent film-forming abilities (Arfat *et al*., 2014). Gelatins have harnessed significant interest due to excellent filmogenic properties, film-forming abilities and use as outer wraps to protect packaged food from dehydration, light and oxygen (Arvanitoyannis, 2002).

Biodegradable films from different gelatin sources include gelatin extracted from fish skin (Gómez-Estaca *et al*., 2009); pigskin (Sobral *et al*., 2001); bovine bone (Cao *et al*., 2009); and bovine hide (Gómez-Estaca *et al*., 2009). However, gelatin film brittleness predisposes them to cracking because of the polymer's strong cohesive energy density (Arvanitoyannis *et al*., 1998). Plasticizing additives have since helped to decrease this inherent brittleness by reducing intermolecular forces that increase polymeric chain elasticity, which then enhances the film's flexibility (Thomazine *et al*., 2005). Consequently, gelatin film tensile strength (TS) and elongation at break (EAB) are reduced, which improves mechanical resistance.

Moreover, prior studies found more desirable properties in gelatin based films such as water vapor permeability (WVP), moisture content and film solubility compared to lipid and polysaccharide based films. Physical property differences between mammalian and fish gelatin films have also been recorded, with the former reported as stronger and more permeable to water vapor, and the latter more elastic (Sobral *et al*., 2001).

Although mammalian based gelatin films have been available for a while, due to Judaic, Islamic and Hindu religious preference and safety concerns, some of these are religiously forbidden to consumers. The prejudice includes porcine- and bovine-related products. Therefore, producing gelatin films from

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alternative gelatin sources has attracted many researchers while food processors increasingly demand the development of gelatin alternatives, especially as the global market for Halal certified foods expands (Karim and Bhat, 2009). Thus, the development of alternative gelatins sourced from (i) fish (Cheow *et al.*, 2007); and (ii) poultry (skin, feet and bone) (Sarbon *et al*., 2013) have been extensively explored as mammalian alternatives. Characterization on chicken skin gelatin has been successfully conducted by Sarbon *et al.* (2013) with yield of extracted gelatin obtained was 16% (based on dry weight basis). The gel strength of extracted chicken gelatin (6.67%, w/v) was significantly higher (355 ±1.48g) in Bloom value as compared to bovine gelatin (229 ± 0.71g). Furthermore, amino acid composition which contribute to the chicken gelatin properties such as proline, hyrdroxyproline, glycine were 13.42%, 12.13% and 33.7%, respectively. In addition, the Imino acids (proline and hydroxyproline) value of chicken skin gelatin were reported higher than bovine gelatin (12.66 and 10.67%, respectively). Therefore, there is a need in investigation on potential of chicken skin gelatin as food film packaging. Hence, the present study examined biodegradable chicken skin gelatin film by characterizing functional properties of films with different hydrophilic plasticizer (glycerol) concentrations.

Materials and Methods

Materials

Fresh chicken skins were obtained from TD Poultry Sdn. Bhd. (Terengganu, Malaysia) and were chilled in ice during transportation to the laboratory. Upon arrival, visible fat was mechanically removed, after which the skins were washed and weighed (wet weight) before storage at -80°C until use. All chemicals used in this study were of analytical grade.

Preparation of sample

Frozen chicken skins were thawed overnight in a chiller at 4–5°C. They were copiously rinsed to remove gross impurities, and then cut into 2–3 cm^2 pieces and freeze-dried for about two days. Completely dried skins were ground and then defatted following the Soxhlet method (AOAC, 2006). The defatted skin then were stored in chiller (4-5°C) before being used in extraction method.

Extraction of chicken skin gelatin

Gelatin extraction followed the method described by Sarbon *et al.* (2013), with slight modification. Approximately 15 g of defatted skin was mixed with 400 ml of sodium hydroxide (NaOH) (0.15%, w/v), and then stirred at room temperature (22°C) for 30 min before centrifuging (Multi-purpose centrifuge, GYROZEN 1580, Korea) at 6,500 x *g* for 10min at 4°C. Alkaline treated pellets were rinsed with water and pretreatment steps were repeated with 400 ml of sulphuric acid (H_2SO_4) (0.15%, v/v), followed by 400 ml of citric acid ($C_6H_8O_7$) (0.7%, w/v). Each pretreatment step was repeated three times. The pellets were then subjected to a final wash in distilled water and centrifuged for 15 min at 4°C. Final extraction using distilled water at controlled temperature (45°C), was conducted overnight (17 hours). These solubilized gelatin solutions were then filtered with Whatman No. 4 filter paper. Gelatin solutions were then evaporated under a vacuum rotary at 45°C, which reduced final solution volumes to 1/10. Final solutions were then freeze-dried and gelatin powder were obtained.

Film formation

For gelatin film preparation, the casting technique was utilized as described by Jahit *et al*. (2015), with slight modification. Filmogenic solutions were prepared by mixing 4g of chicken skin gelatin with 100 ml of distilled water with varied concentrations of glycerol as plasticizer (0, 5, 10, 15 and 20%, w/w). The glycerol concentration used (w/w) was based on total filmogenic solution. Respective concentrations are designated 'Formulations A–E'. To prepare for film fabrication, the gelatin powders were mixed with distilled water with mechanical stirring using magnetic stirrer and completely dissolved. All mixtures were stirred at 45°C for 20min to obtain homogeneous solutions. Approximately 25 ml of each filmogenic solution was then poured into a Petri dish and oven dried at 45°C. The dried films were conditioned in desiccator contained of silica gel for 24h before films characterization were conducted.

Determination of tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and elongation at break (EAB) reflect the mechanical durability (resistance) of a film. These trials followed methods described by Rivero *et al*. (2010). Five rectangular (1 cm x 6 cm) films strips were prepared from each formulation. Each film strip was affixed to a pair of grips on the AT/G probe attached to the texture analyzer (Stable Microsystem, TAXT Plus, USA), bearing a 5 kg load cell. The initial gap between upper and lower parts of the grip was set at 40 mm. The film strip was stretched by moving the upper grip at a head speed

of 1mm/min until the film broke. Tensile strength (TS) was calculated as the maximum load each film sustained before failure, using the following formula:

$$\text{Tensile strength (MPa)} = \frac{F_{max}\ (N)}{A\ (m^2)}$$

F_{max} is the maximum load (N) required to pull the sample apart; A is the cross sectional area (m^2) of the film sample.

The percentage of elongation at break (EAB) is calculated as follows:

$$EAB\ (\%) = \frac{l_{max} \times 100}{l_o}$$

Where l_{max} is film elongation (mm) at the moment of rupture; and l_o is the initial grip length (mm) for each sample.

Determination of light transmission and film transparency

Ultraviolet (UV) and visible (Vis) light barrier properties were measured using a UV-Vis spectrophotometer (50 Probe, Cary®, USA). Filmstrips of 1 cm × 4 cm were cut and placed directly into a test cell. Transmittance at selected wavelengths (200–800 nm) were measured. An empty cell test was used as reference (Han and Floros, 1997). Opacity of the chicken skin gelatin film was evaluated according to the method of Abdollahi *et al.* (2013) with a slight modification. Film opacity was calculated as follows:

$$\text{Opacity} = -\log T/x$$

where T is transmission (%) at 600 nm and x is film thickness (mm). Film thickness were measured using Digimatic Micrometer (Mitutoyo, Japan) following method by Li *et al.* (2014). All determinations were recorded as the mean of three measurements.

Determination of water vapor permeability (WVP)

Water Vapor Permeability (WVP) were measured using the method described by Suderman *et al.* (2015), with some modification. Circular aluminum cups (2 cm x 2 cm) containing 10g of silica gel were individually sealed for each of three samples per formula. These cups were weighed and placed in a desiccator containing distilled water at 30ºC. Each cup were weighed hourly for six hours. Water vapor permeability (WVP) was calculated using the following equation:

$$WVP\ (g.mm.h^{-1}.cm^{-2}.pa^{-1}) = \frac{\Delta W\ (g) \times \text{Film thickness (mm)}}{\text{Times (h)} \times \text{Test area (cm}^2) \times \Delta P\ (Pa)}$$

Determination of water solubility

Film samples (2 cm^2) were dried at 105°C for 24 h and then immersed in 30 ml of distilled water at 22°C for 24 h. Each sample was then filtered through No.1 Whatman filter paper. Papers containing insoluble film were then dried at 105°C for 24h. Water solubility was determined by using the following equation:

$$\text{Water Solubility}\ (\%) = (W_0 - W_1) / W_0 \times 100$$

Where, W_0 and W_1 are initial and insoluble dry matter weights, respectively. All tests per formula were repeated with three separate samples and results averaged (Krittika *et al*., 2010).

Determination of moisture content

Film samples were weighed (W_1) and placed in an oven at 105°C and weighed again after 24h (W_2). Water content was determined as the percentage of initial film weight lost after drying, reported on a wet basis as follows:

$$\text{Moisture content}\ (\%) = (W_1 - W_2) / W_1 \times 100$$

As above, for each formulation, trials were repeated three times with separate samples and results averaged (AOAC, 2006).

Determination of structure via fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopic (FTIR) determined intermolecular cross- linking of biomaterial and monitor the changes in the functional groups and secondary structure. The sample structure was determined by FTIR, following the method described by Jahit *et al.* (2016) with some modification. Three film samples per formulation were cut into 1 cm^2 pieces and placed on a film holder. The light barrier property was measured at wavelengths between 4000–500nm at 4 cm^{-1} resolution for 32 scans. Each determination was repeated three times (as above) and averaged per formulation.

X-ray diffraction (XRD)

X-Ray diffraction (XRD) measurements were undertaken (MiniFlex II, Rigaku, Japan) at room temperature with voltage and current generated at 30 kV and 15 mA, respectively, following the method described by Jahit *et al.* (2015). Relative intensity recorded scattering over an angular range (2θ) of 10–30°. Films were placed on a 2 cm^2 metal slide and secured by tape for a scanning period of about 20min per slide.

Table 1. Light transmittance and transparency of chicken skin gelatin films with different concentrations of glycerol Film Formulations: A (0% glycerol); B (5% glycerol); C (10% glycerol); D: (15% glycerol); E: (20% glycerol). Different superscripts ($^{a–d}$) in same row represent significant differences ($p < 0.05$). Data reported as mean values ± standard deviation

Film formulation	Light Transmission (%)								Transparency Values (T_{600})
	200 nm	280 nm	350 nm	400 nm	500 nm	600 nm	700 nm	800 nm	
A	0.03±0.01^{a}	4.48±0.80^{a}	61.77±1.50^{a}	72.10±1.01^{a}	72.70±0.17^{a}	78.10±1.13^{a}	79.27±2.00^{a}	80.63±1.01^{a}	0.77±0.04^{c}
B	0.04±0.01^{a}	4.29±0.73^{a}	62.47±1.36^{a}	72.77±1.40^{a}	76.90±4.16^{a}	77.03±1.33^{a}	82.67±2.31^{a}	78.90±0.50^{a}	0.81±0.05^{c}
C	0.03±0.01^{a}	2.68±0.18^{b}	42.90±2.86^{b}	54.73±7.51^{b}	54.70±3.24^{b}	60.63±2.17^{b}	63.13±3.70^{b}	67.37±0.15^{b}	1.55±0.11^{b}
D	0.03±0.01^{a}	1.65±0.41^{b}	42.40±2.23^{b}	64.53±4.72^{ab}	61.10±9.52^{b}	60.50±6.92^{b}	64.03±5.66^{b}	73.30±6.17^{b}	1.57±0.35^{b}
E	0.02±0.01^{a}	1.14±0.20^{c}	40.50±7.98^{b}	47.03±7.13^{b}	29.47±9.01^{c}	30.90±7.21^{c}	54.70±7.88^{b}	65.80±4.56^{b}	3.97±0.28^{a}

Film Formulations: A (0% glycerol); B (5% glycerol); C (10% glycerol); D: (15% glycerol); E: (20% glycerol). Different superscripts ($^{a–d}$) in same row represent significant differences ($p < 0.05$). Data reported as mean values ± standard deviation.

Statistical analysis

The One-way ANOVA was applied to all results using the Minitab 16 program for Windows (Minitab 213 Inc., USA). When differences between analyzed groups were significant the mean pairs were assessed on the basis of the Fisher's test with a level of significance of 0.05 ($p<0.05$).

Results and Discussion

Light transmission and film transparency

Table 1 represent UV (200-280) and visible light (600-800) results. The chicken skin gelatin films exhibited low UV light transmission. However, no significant difference ($p > 0.05$) for transmission at 200nm was noted as glycerol content increased. By contrast, light transmission at 280 nm showed a significant difference ($p < 0.05$) between formulations. The increased of glycerol concentration resulted in lower light transmission at 280 nm. Lower UV light transmission (200–280 nm) is likely due to the different molecular weight, composition and size of glycerol that impedes the light transmission properties of these films (Orliac *et al*., 2003). Reports indicate that films made from animal gelatin block UV light more efficiently than films of synthetic origin (Hoque *et al*., 2011). In addition, higher contents of aromatic amino acids in protein-based structures in gelatin film are more capable of absorbing UV-light (Limpan *et al.*, 2010).

Transparency values of all film formulation were presented in Table 1. The results showed that the transparency value of films decreased as the percentage of glycerol increased. The lower the transparency value, the higher the opacity of the films. Therefore, from the results obtained, film E (with transparency value of 3.97±0.28) is more opaque as compared to film A (with transparency value of 0.77±0.04) due to present of glycerol in film E. This findings can be concluded that, a lower transparency value which means a higher absorbance of films could be an excellent barrier to prevent light-induced lipid oxidation when applied in food system (Gómez-Guillén *et al.*, 2007).

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

Table 2 depicts tensile strength (TS) values (33.66, 3.64, 2.22, 1.78 and 1.75 Mpa), respectively, for formulations (A–E) of the gelatin films produced with different glycerol concentrations (0, 5, 10, 15 and 20%). A significant difference ($p < 0.05$) between Film A and Films B–E was observed. TS values for films C, D and E decreased slightly as glycerol content increased. However, no significant difference ($p > 0.05$) occurred between films (C, D and E) with glycerol contents of 10, 15 and 20%, respectively.

The highest TS (Film A) decreased by 89.19% with the addition of 5% glycerol (Film B). Mechanical behavior of Film A (0% glycerol) was typically brittle and rigid. This characteristic may be attributed to higher interactions and proximity between proteins chains in the absence of a plasticizer. This result agreed with Yang and Paulson (2000) who found that films without plasticizer are extremely brittle and shattered when handled. Polar groups (-OH) along a plasticizer's chain are believed to develop polymer-plasticizer hydrogen bonds that replace polymer-polymer interactions in biopolymer films. Small quantity of plasticizer could be easily inserted between polymer chains, producing a "cross-linker" effect that would decrease the free volume and the segmental mobility of the polymer, decreasing the

Table 2. Tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP), film solubility and moisture content of chicken skin gelatin films with different concentrations of glycerol. Formulations: A (0% glycerol); B (5% glycerol); C (10% glycerol); D: (15% glycerol); E: (20% glycerol). Different superscripts ($^{a–d}$) in same row represent significant differences ($p < 0.05$). Data reported as mean values ± standard deviation

Film formulation	Tensile strength (Mpa)	Elongation at break (%)	Water vapour permeability (g.mm/h.m^2.k.Pa)	Solubility (%)	Moisture content (%)
A	33.66^a	3.87^d	0.015^b	55.60^c	7.86^e
B	3.64^b	106.43^c	0.0175^b	58.64^c	16.0^d
C	2.22^c	107.73^c	0.0235^a	66.48^b	18.5^c
D	1.78^c	137.98^b	0.022^a	67.10^b	21.0^b
E	1.75^c	148.33^a	0.024^a	86.75^a	24.4^a

Film formulations: A (0% glycerol); B (5% glycerol); C (10% glycerol); D: (15% glycerol); E: (20% glycerol). Different superscripts ($^{a–d}$) in same row represent significant differences ($p < 0.05$). Data reported as mean values ± standard deviation.

mechanical strength of the films (Ghasemlou *et al*., 2011).

Observations from this study recorded good tensile strength with increased glycerol concentration. Bergo and Sobral (2007) also showed that the addition of glycerol reduced formations of junctions between adjacent chains in a biopolymer—which otherwise are responsible for gelatin's crystallinity—thus, increasing the film's mobility and flexibility. Table 2 also presents elongation at break (EAB) data, corresponding to the film's breaking point property. EABs for chicken skin gelatin films under study exhibited an inverse trend to TS results such that films with the highest TS had the lowest EAB and vice versa. EAB results for glycerol concentrations of (0, 5, 10, 15 and 20%) were 3.87, 106.43, 107.73, 137.98 and 148.33%, respectively, for formulations A–E. Hence, EAB increased as glycerol concentration increased, with a significant difference between Film A ($p < 0.05$) and Films B–E. This could indicate that glycerol's presence causes a reduction in interactions between biopolymer chains (Arvanitoyannis, 2002), resulting in higher EAB values. Therefore, the interactions between proteins chains are reduced which permits increased macromolecular movements and also decrease the free volume and the segmental mobility of the polymer, thus enhancing film extensibility (Jongjareonrak *et al.*, 2006; Ghasemlou *et al*., 2011).

Water vapor permeability (WVP)

Water vapor permeability (WVP) also increased with increased glycerol concentrations (Table 2). However, no significant difference ($p > 0.05$) in WVP between Films A and B was observed. Similarly, there were no significant differences ($p > 0.05$) between Films C, D and E. Film E had the highest WVP (0.024 g.mm/h.m^2.kPa), and Film A presented the lowest WVP (0.015 g.mm/h.m^2.kPa). The lowest permeation of water vapor in Film A indicated that the absence of glycerol allowed stronger interactions and a higher degree of protein molecular organization. Therefore, Film A is highly compacted structure more effectively prevented water vapor penetration. By contrast, Film E showed the highest WVP value which indicated higher water vapor permeation, likely due to its highest concentration of glycerol (20%), which increased the film structure's free volume and thus, favored the mobility of polymeric chains. Consequently, the film's less dense network became more permeable (Gontard *et al*., 1993). In addition, increase water vapor transmission through a protein-based film positively correlates with a higher content of polar amino acid residues in the film's structure, as well as the presence of hydrophilic plasticizers such as glycerol (Arfat *et al*., 2014).

Furthermore, the low WVP in chicken skin gelatin films obtained in this study plausibly correlate with higher tensile strength as demonstrated by Film A's stronger interactions and higher organization of protein molecules in the film's network (Arfat *et al*., 2014). In conclusion, higher WVP indicates higher potential for water vapor permeability. Since a major function of food packaging is to avoid or decrease moisture transfer between processed food and its immediate environment, WVP should be as low as possible (Gontard *et al*., 1992).

Water solubility

Water solubility is an important property of edible/biodegradable films, especially since potential applications require low water solubility to enhance

product integrity and water resistance (Turhan and Sahbaz, 2004). Table 2 demonstrated the solubility results of chicken skin gelatin films with different glycerol concentrations. Film E (20% glycerol) had the highest solubility (86.57%) with a significant difference ($p < 0.05$) from Films A–D (55.60, 58.64, 66.48 and 67.10%), respectively. However, no significant differences ($p > 0.05$) occurred between Films A and B, or Films C and D. The solubility of the films obtained was highly affected by the functional group that obtained in each material of the film samples. This finding was supported with the results obtained in FTIR determination. Film which contained high glycerol concentration show higher intensity in Amide A (3291.51 to 3295.51 cm^{-1}). This was due to high contain of –OH group in glycerol as well as gelatin, therefore it will increase the –OH group in the film. Thus it will initiate higher interaction with –OH group in water and leads to a higher water solubility. Furthermore, the increase of films water solubility is related to an increase in the proportion of soluble solids in films formulations. In addition the solubility properties also contributed by glycerol which is a hydrophilic and highly soluble compound. In addition, WVP value also contributed to the water solubility of the films. A higher film solubility can be an advantageous property of importance. Potential applications can require water insolubility to enhance product integrity and water resistance.

Moisture content

Moisture content is another packaging film property of great importance in food packaging applications, because it helps retain moisture levels within packaged products. The moisture content of films obtained in this study showed increases with increasing glycerol content (Table 2). Furthermore, the authors observed significant differences ($p > 0.05$) in moisture content between all tested films. Moisture content of Film E was the highest (24.4%), in which Film E also had the highest glycerol concentration (20%). Film A, with no glycerol content, had the lowest moisture content (7.86%). The results indicates that glycerol acts as a water-holding agent. This is in the same agreement with Tapia-Blácido, do Amaral Sobral, and Menegalli (2011), which found that film plastisized with glycerol have a higher moisture content after conditioning, as compared to sorbitol.

The differences in moisture content is likely relate to water solubility and the film's chemical structure, which were affected by different glycerol concentrations as previously discussed. As film solubility increased, moisture content also increased,

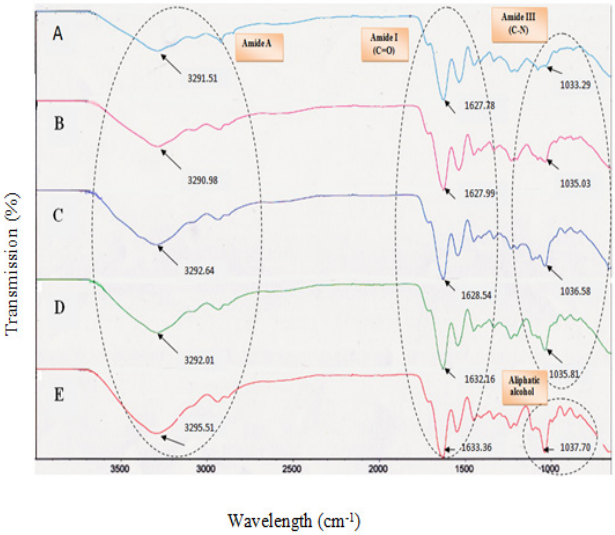

Figure 1. FTIR spectra of chicken skin gelatin based film formulations: A (0 % glycerol); B (5% glycerol); C (10% glycerol); D (15% glycerol); E (20% glycerol)

possibly due to a concentration of monomers that directly affect reaction rate and the volume of hydrophilic groups in a polymer network (Wang *et al*., 2010). In addition, Glycerol is the smallest straight chain molecule and is the most hygroscopic among all plasticizers tested (Sothornvit and Krochta, 2001). The hygroscopic properties of glycerol itself may contributed to the absorption of moisture and increase the moisture content of film. Moreover, from FTIR result, the intensity of Amide I peak wavenumber increased, with increased glycerol content. This is mainly because C=O and N-H bands easily form intermolecular hydrogen bonds with the O-H of a glycerol compound. Therefore, films gain significant numbers of O-H groups in their matrices from the increase in hydroxyl groups that can increase hydrogen bond and attract moisture (Hu *et al*., 2009).

Fourier transform infrared (FTIR) spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy studies depicted bands formed by four individual peaks marked Amide A, Amide I, Amide III and Aliphatic alcohol (Figure 1). Amide A peaks were observed associated with stretching vibrations of N-H bands between 3291.51 to 3295.51 cm^{-1}. These peaks waxed more intensely, and both widen and sharpen with the increase of glycerol content in the films possibly due to -OH group contribution made by the plasticizer.

Spectral peaks between 1627.78–1633.36 cm^{-1} presented for the Amide I group, indicating stretching vibrations from C=O bands. Intensity of Amide I peak wavenumber increased, with increased glycerol content. This is mainly because C=O and N-H bands easily form intermolecular hydrogen bonds

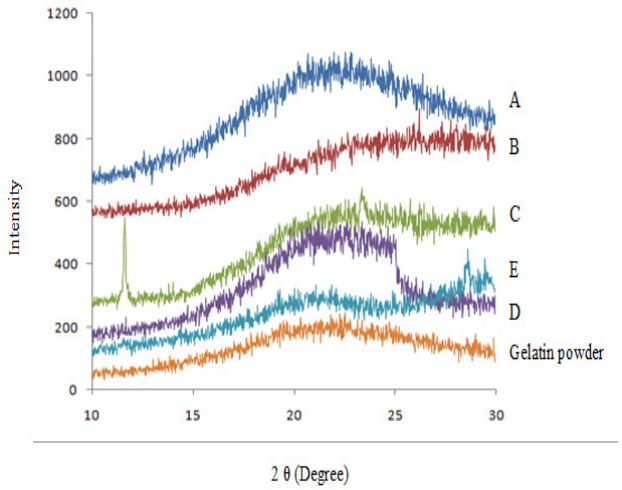

Figure 2. X-Ray Diffragtogram of chicken skin gelatin based film formulations: A (0% glycerol); B (5% glycerol); C (10% glycerol); D (15% glycerol); E (20% glycerol)

with the O-H of a glycerol compound (Ubonrat and Bruce, 2010). The Amide I band (1600–1700 cm^{-1}) is mainly associated with C=O stretching vibrations (70–85%) and C-N groups (10–20%) where the exact position of the band is determined by the backbone's conformation and hydrogen bonding pattern (Hanani *et al*., 2011). The increase of the intermolecular reaction between hydrogen bonding within O-H and C=O resulted in higher intensity in Amide I peak wavenumber. Thus, the Amide I band is the most useful in infrared spectroscopy analysis of protein structure (Surewicz and Mantsch, 1998).

Amide III peaks between 1033.29–1035.81 cm^{-1} were observed in Films A, B, C and D with displacements of increased intensity and wider, sharper peaks. Such displacements are possibly related to additional interactions arising between glycerol and film structure. These bands also reflect the presence of free water. These same peak amplitudes increased with more glycerol content, thus increasing the amount of free water (Bergo and Sobral, 2007). The aliphatic alcohol group presented only with 20% glycerol content at peak 1037.70 cm^{-1} (Film E), indicating that the content of Glycerol in Films E is high enough to exhibit a signal in an infrared spectrum. However, this signal is not such evident with lower amounts of glycerol in the films. Generally, similar spectra for all films were observed, such that different glycerol concentrations appeared to have little effect on protein secondary structures, except for the presence of aliphatic alcohol in Film E.

X-ray diffraction (XRD)

X-Ray diffraction (XRD) studies quantified the crystalline structures of films. Figure 2 presents their XRD diffractograms. Crystallinity measurement results for Films A–E were very similar and, overall, showed an amorphous state. Regarding the amorphous state demonstrated by all films and gelatin powder, intensities observed for gelatin powder presented the most amorphous state, while Film A showed less. The addition of glycerol decreased the intensities of peak at 2θ making the film more amourphous as compared to control film. This is probably due to the high stability of these films when glycerol was added. Furthermore, the amourphous character of film added with glycerol was possible due to increasing moisture in the films, avoiding any tendency to form semi-crystalline regions (Bergo and Sobral, 2007).

Conclusion

In conclusion, this study showed that the addition of different glycerol concentration will affect the properties of chicken skin gelatin film. All measurements conducted in this study showed the increased of glycerol concentration resulted in increased of most film properties value except for tensile strength. From out of five (5) formulation, film B (5% glycerol content) appeared as the best formulation with high tensile strength and low elongation at break (EAB). Film B also demonstrated good barrier properties such as lower moisture content, water solubility, water vapor permeability (WVP) and high thermal stability as influenced by glycerol addition. In addition film B is more opaque as compared to control film due to present of glycerol. This findings can be concluded that a lower transparency value which means a higher absorbance of films could be an excellent barrier to prevent light-induced lipid oxidation when applied in food system. Hence, Film B holds high potential for further food packaging application studies.

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