

Characterization on the mechanical and physical properties of chicken skin gelatin films in comparison to mammalian gelatin films

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Abstract. The purpose of this study was to investigate the mechanical and physical properties of chicken skin gelatin film as compared to mammalian gelatin films in terms of tensile strength (TS), elongation at break (EAB), puncture force, water vapour permeability (WVP), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), light barrier, thermal properties and microstructure. Three film formulations using 4g gelatin of three different types (chicken skin, bovine, and porcine gelatin) and 1.5g glycerol were prepared under mechanical stirring at a temperature of 45°C. The use of different types of gelatin resulted in different mechanical and physical properties. Results revealed that chicken skin gelatin film was optimal due to its high tensile strength (5.57 MPa) and low WVP (1.29×10^{-9} kPa) rate as compared to bovine and porcine gelatin films. The carboxyl group was revealed to be stronger in FTIR assay for chicken skin gelatin film, while XRD revealed amorphous characteristics at a peak $2\theta=20^\circ$. These results contributed to its superior physical characteristics. These desirable characteristics mean that chicken skin gelatin film has remarkable potential as a biodegradable film material as compared to commercial gelatin. It may become a key preferred alternative for producing gelatin films for edible film purposes.

Keywords: Biodegradable films, gelatin films, chicken skin gelatin, mammalian gelatin, characterization.

1. Introduction

Food packaging is used to preserve product quality while minimizing product–packaging interactions. Recently, a wide variety of packaging materials offering these desired effects have been employed to interact with food [1]. Additionally, studies have found that plastics are widely used as edible film materials due to considerations of low cost and availability [2]. Although conventional packaging materials such as plastics and their derivatives are effective for food preservation, however this materials has led to a serious environmental problems [3]. Therefore, the biodegradable packaging from biopolymers is an effective alternative to synthetic packaging material due to its eco-friendly and non-toxic characteristics. Studies have shown that biodegradable films have more desirable physicochemical characteristics than synthetics [4].



Proteins and polysaccharides are the main biopolymers used in edible packaging films [5]. Among proteins, gelatin has recently been studied extensively in manufacturing packaging films due to its excellent physical and mechanical properties ([4]; [6]). Gelatin is a water-soluble protein derived from animal sources, obtained from the hydrolysis of bone collagen or connective tissues skin of mammalian and fish [7]. The acceptance of gelatin as a “Generally Recognized as Safe” (GRAS) substance in the area of food additives by the U.S. Food and Drug Administration (FDA), along with its excellent film forming ability, gel-forming properties around 35°C, excellent versatility due to its alpha-amino acid composition, abundance, low cost, and biodegradability, all make gelatin an attractive protein in the design and development of functional films with potential application in the food sector [8].

In general, films composed of gelatin show good mechanical properties but have also been found to be highly sensitive to moisture and exhibit poor barrier properties against water vapour [9]. Due to the strong cohesive properties of the gelatin polymer, thus the gelatin film produced are brittle and susceptible to crack [4]. Plasticizing additives address this inherent brittleness by reducing intermolecular forces that increase polymeric chain elasticity, thus enhancing film flexibility [10]. Plasticizers also decrease the process temperature, sticking in moulds and enhance wetting. As a final product modifier, plasticizers increase the temperature range of treatment, increase toughness, and lower the glass transition temperature [11]. Good plasticizers used in plasticizing gelatin based film are often cited as polyols. However, the most widely used is glycerol ([12]; [11]).

Gelatin has been extensively studied in terms of tensile strength [10], elongation at break [13], water vapour permeability (WVP) ([14]; [15]), light transmission [16], Fourier transform infrared spectroscopy (FTIR) [17]; [13]), X-ray diffraction [18], and microstructure [19]. [18] reported that film with 60% bovine gelatin exhibited lowest WVP (1.06×10^{-7} kPa) and its X-ray diffraction of the film produced amorphous characteristics, explaining its high biodegradability rate (4.3%). A film derived from pork gelatin was reported to have lower water solubility than those formulated using bovine and fish gelatin [13]. FTIR spectra presented the increased of Amide-A wave number in films as higher gelatin concentrations were used, which resulted from greater interaction occurring between the gelatine's functional groups [13].

Recently, chicken skin has been considered the best alternative in producing gelatin film due to its excellent thermal properties ($T_m = 33.57^\circ\text{C}$) and high gel strength (355 ± 1.48 g), which is greater than that of bovine gelatin (229 ± 0.71 g) ([20]; [10]). This will improve the strength and permeability of gelatin films. Research on the comparison of potential materials for the manufacture of biodegradable and edible films from different gelatin sources has been carried out ([21]; [13]). However, to date no studies have compared the physical and mechanical properties of films manufactured from alternative gelatin (chicken skin gelatins) and mammalian gelatin (bovine and porcine gelatins). Therefore, the aims of this study was to investigate the properties of gelatin films manufactured from bovine, porcine, and chicken skin gelatin via both mechanical and physical testing.

2. Material and methods

2.1 Materials

Chicken skins were freshly obtained from the local poultry industry (TD Poultry SDN. BHD., Kuala Terengganu) and were kept in ice while transporting to the laboratory. The chicken skins were washed thoroughly in excessive water and stored at -80°C until further use. Commercial type B bovine skin gelatin, type A porcine skin gelatin, and glycerol (MW = 92.09) were obtained from Sigma-Aldrich Company Ltd., Poole, Dorset, UK. All chemicals used in this study were of analytical grade.

2.2 Sample preparation

Visible fat on the skin was mechanically removed. The skin was then thoroughly rinsed in excessive water to remove impurities. Skins were cut into 2-3 cm pieces and freeze-dried. Completely dried skins were grinded before being defatted using the Soxhlet method [22].

2.3 Preparation of chicken skin gelatin

Extraction of chicken skin gelatin was conducted following previous described method [20] by using acid-alkaline pretreatment. The defatted chicken skin was grinded and soaked in sodium hydroxide (0.15%, w/w), sulphuric acid (0.15%, w/v), and citric acid (0.7%, w/w) solutions, consecutively. Gelatin solution was stirred slowly at room temperature (30 min) before centrifugation (Multi-purpose centrifuge, GYROZEN 1580, Korea) at 3500 x g (10 min). Each solution was repeated three times and the supernatant was removed followed by thoroughly rinsed with distilled water. The precipitate obtained were then washed with excessive water before extracted in distilled water for overnight at 45 °C. The clear extract was filtered, concentrated by evaporation under vacuum, and lyophilized. Dried 'gelatin powder' obtained was ground, weighed, and stored for further use.

2.4 Development of gelatin films

Gelatin films were prepared using the casting technique as described by [23] with slight modifications. Three filmogenic solutions of gelatin and glycerol were prepared according to the optimized formulation generated by response surface methodology software [23]. For film preparation, 4.0 gelatin powders (chicken skin, bovine and porcine) were mixed with 100 ml of distilled water under mechanical stirring until completely dissolved. The film forming solution was thus obtained. Then, 1.5 g glycerol was added into the film forming solution as a plasticizer. All mixtures were stirred at 45°C for 20 min obtain a homogeneous gelatin film solution. Lastly, approximately 25 g of filmogenic solution was poured into the Petri dish and dried at 45°C in an oven for 2 days. Dried films were then removed from Petri dish and conditioned in a desiccator prior to investigation of tensile strength (TS), elongation at break (EAB), puncture force, water vapour permeability (WVP), differential scanning calorimetry (DSC), light transmission, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).

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

Tensile strength (TS) and elongation at break (EAB) of the films were determined using a texture analyser (TA.TX Plus, Stable Micro System, USA) with the ASTM technique 0882-97 [24]. A 20 x 100 mm film strips with uniform thickness of 0.20 mm were prepared using a cutting blade and placed onto grip pairs of AT/G probe which was attached to the texture analyser with a 5-kg load cell with 20 mm of initial gap between the up and down parts. The film strips were stretched by moving the headspace at 50 mm/min until breaking. The TS (MPa) was calculated using the following equation:

$$\text{Tensile Strength (TS)}(\text{MPa}) = \frac{F_{\max}(\text{N})}{A(\text{m}^2)} \quad (1)$$

F_{\max} is max load (N) needed to pull the sample apart, A is cross sectional area (m^2) of film sample. Meanwhile, the elongation at break (EAB) (%) was calculated as below:

$$\text{Elongation at break } (\%) = \frac{l_{\max}}{l_o} \times 100 \quad (2)$$

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

2.6 Determination of puncture force

The breaking force and the breaking deformation of the films were determined via puncture force test of the same texture analyser. Films placed in a 5.6 cm diameter cell were punched to the breaking point with a round-ended stainless-steel plunger (3 mm in diameter) at a cross-head speed of 60 mm/min. Breaking force was expressed in terms of N and breaking deformation in terms of percentage (%).

2.7 Determination of water vapour permeability (WVP)

Water vapour permeability (WVP) was determined by modification of ASTM method as explained by [23]. The films were sealed with silicone vacuum grease onto a cup containing silica gel, and held in place with a rubber band. The cups were individually weighed and placed in desiccators with distilled water at room temperature. The cups were then placed in desiccators containing distilled water at 30 °C. The cups were weighted at 1 hour intervals over an 8 hour period. Three films were used for WVP determination and the measurement was conducted in triplicate. WVP was calculated as follows:

$$WVP (Kg.m/m^2.s.Pa) = w \times x / A t \times (P_2 - P_1) \quad (3)$$

where w is the weight gained by the cup (g), x is the average film thickness (mm), A is the permeation area (m^2), t is the time gained (h) and $P_2 - P_1$ is the difference of partial pressure (Pa).

2.8 Determination of thermal property

The thermal properties of films were evaluated via differential scanning calorimetry (DSC, TA Q2000 Instrument, USA) according to the method described by [25]. Film samples (3 mg) were weighted in aluminium pans. The pans were sealed with a TA sample encapsulation press. The samples were placed in the sample cells, while an empty pan was placed in the reference cell. Conditioned films were scanned between 0°C to 100°C at a heating rate of 10°C/min. Melting temperature (T_m , °C) and enthalpy (ΔH , J/g) were measured in triplicate. The melting temperature (T_m) was calculated as the temperature at which the endothermic peak occurs. The glass transition temperature corresponded to the temperature where a baseline inflexion occurred, and the melting temperature was determined as the peak temperature of the endothermic event of the DSC curves.

2.9 Determination of light barrier and transparency

The light transmission of films was measured at the ultraviolet (200-800 nm) and visible range (200-800 nm) using a UV-vis spectrophotometer (Pharo 300, USA) according to the method by [15]. The transparency value of film was calculated using the following equation:

$$Transparency\ Value = -\log T_{600} x^{-1} \quad (4)$$

where T_{600} is the fractional transmission at 600 nm and x is the film thickness (mm). The greater transparency value represents the lower transparency of film.

2.10 Functional group determination by Fourier transform infrared spectroscopy (FTIR)

Following [10], the functional group that arose from the blending of film materials were investigated via infrared spectra recorded by Thermo Nicolet 380 Spectrometer (Fisher Scientific Inc, USA) using deuterated triglycerine sulphate (DTGS) as detector. The sample holder comprised a multi-ounce horizontal attenuated total reflectance (HATR) plate of zinc selenite (ZnSe) crystal. The background spectrum (without sample) was collected after the plate was cleansed with acetone, followed by affixing the film samples to the plate and recording the spectra obtained. A single beam spectrum for each sample was ratioed against an ambient air single beam background spectrum before conversion to absorbance units. The resolutions used varied from 4000 to 650 cm^{-1} over 32 scans. Each sample was conducted in triplicate.

2.11 Determination of X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis was conducted to measure the crystalline and amorphous characteristic of the films. A Rigaku X-ray Diffractometer (Rigaku Corp, USA) with a copper source using 40 kV and 30 mA current at room temperature was used as described by [18]. The measurements were performed by mounting the sample with size of 4 x 4 cm^2 onto a glass slide before placing in the diffractometer chamber. An angle diffraction range used was $2\theta = 3-80^\circ$, with each sample repeated twice.

2.12 Determination of film microstructure

To study the film microstructure, the cross-sections and surface areas of all gelatin films were viewed at 25,000x magnification employing scanning electron microscopy (SEM) (Hitachi S-4300SE, Hitachi Science System Ltd., Japan) at an accelerating voltage of 5.0 kV. Prior to analysis, films were coated with gold to make the samples conductive. The samples were observed in a superficial position at 100x magnification [13].

2.13 Statistical analysis

All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by the Duncan's multiple range test. Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

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

The results obtained for tensile strength (TS) of gelatin films are presented in Table 1. The results ranged from 2.97 ± 0.06 MPa to 5.57 ± 0.11 MPa, suggesting significant differences ($p < 0.05$) among the tensile strengths of gelatin film of different gelatin source. The data clearly show that chicken skin gelatin film possesses the highest TS value. This was supported via the compact and organized structure of chicken skin gelatin film, as shown by scanning electron microscopy. Variation in TS value may also be due to the levels water content in the gelatin. In general, different types of gelatin contain different levels of OH group. FTIR results showed that chicken skin gelatin film possessed lower levels of OH group than commercial gelatin. Also, chicken skin gelatin demonstrated higher gel strength (355 ± 1.48 g) than bovine gelatin (229 ± 0.71 g), in which a high gel strength property would also contribute to the tensile strength of the film [20]. These findings are in the agreement with those of [13], who reported that porcine and bovine gelatin films possess lower TS value compared to chicken skin gelatin films, which were 4.04 and 4.46 MPa, respectively.

Elongation at break (EAB), also known as fracture strain, is the ratio between changed length and initial length after breakage of the test specimen. It is an important factor in biodegradable film, as it describes the capability of the film to resist changes of shape without crack formation [26]. The results obtained for EAB of gelatin films are presented in Table 1. The results ranged from 122.78 ± 0.29 % to 135.60 ± 0.44 %, suggesting a significant difference ($p < 0.05$) in the EAB of gelatin films made with different gelatin sources. From the data obtained, it is clearly shown that chicken skin gelatin film possessed the lowest EAB value, while bovine gelatin film possessed the highest EAB value. X-ray diffraction revealed that bovine and porcine gelatin films exhibited broader amorphous peak at $2\theta = 20^\circ$ due to high levels of water content as generated by FTIR spectra. This amorphous character and high water content contributed to the flexible and stretchable film structure. This is in agreement with the results of [15], who found that the amorphous character of gelatin-CMC film incorporated with xanthan gum improved the EAB of the film.

3.2 Puncture test

The results obtained for the puncture test (PT) of gelatin films (Table 1) showed that chicken skin gelatin film possessed the highest PT value. The amount of imino acid proline and hydroxyproline content in the gelatin would also affect the PT of the film. [20] reported that imino acid proline and hydroxyproline content is higher in chicken skin gelatin (proline: 13.42%, hydroxyproline: 12.13%) than in bovine gelatin (proline: 12.66%, hydroxyproline: 10.67%). A high amount of imino acid proline and hydroxyproline content indicated that the film possessed rigid hydrogen bonding and a compact molecular structure [20]. Thus, more force is needed to punch through the film membranes. This finding explains the highest PT values being obtained were in chicken skin gelatin and low PT values obtained in bovine and porcine gelatin.

3.3 Water vapour permeability (WVP)

The results for water vapour permeability (WVP) of chicken skin, porcine, and bovine gelatin films are presented in Table 1. The results ranged from $1.29 \times 10^{-9} \pm 0.03$ kPa to $2.25 \times 10^{-9} \pm 0.13$ kPa, suggesting a significant difference ($p < 0.05$) in the WVP of gelatin films of different gelatin sources. Chicken skin gelatin film possessed the lowest WVP value at $1.29 \times 10^{-9} \pm 0.03$ kPa, while porcine gelatin had the highest WVP value ($2.25 \times 10^{-9} \pm 0.13$ kPa). The highest WVP rate indicates a high amount of water vapour passing through the film membrane. High WVP rate is an undesirable characteristic in packaging film. This is because a film with high WVP would hasten the food deterioration process, shortening the shelf life of food products.

Commercial gelatin showed higher WVP than chicken skin gelatin film. High WVP obtained in porcine and bovine gelatin films may be due to its bigger and scattered films molecular structure as compared to chicken skin gelatin film. This is because, bigger film molecular structure would allow more water vapour to pass through the film, thus increasing its WVP. High amount of water content would also increase the WVP of the gelatin films. High water content also would contribute to the hydrophilic character of the gelatin, as it will attract more water, thus increasing the WVP value. As reported by [28], porcine films contained higher amounts of tyrosine (2.6%) and serine (3.5%) than those from chicken skin (tyrosine: 1.22%, serine: 2.20%) and bovine (tyrosine: 1.16%, serine: 2.93%) gelatin [20]. Low amino acid tyrosine and serine content in chicken skin gelatin explains its hydrophobic character, contributing to its low WVP value as compared to commercial gelatin films. The results obtained were in the same agreement with the study by [27] and [29] who found high WVP value of pigskin gelatin film which ranged from 1.38 to 1.95 kPa. In another study, [30] found that film from fish skin gelatin had a low WVP value, ranging from 0.44 to 1.23 kPa. These results may be due to the low water content of fish skin gelatin.

3.4 Thermal property

The results obtained for melting temperature (T_m) and glass transition (T_g) of chicken skin, porcine and bovine gelatin are presented in Table 1. The results for T_m ranged from $60.42 \pm 0.34^\circ\text{C}$ to $76.26 \pm 0.73^\circ\text{C}$, while T_g ranged from $47.23 \pm 0.69^\circ\text{C}$ to $51.12 \pm 0.55^\circ\text{C}$ ($p < 0.05$). Data shows that chicken skin gelatin film had the highest T_m ($76.26 \pm 0.73^\circ\text{C}$) and T_g ($51.12 \pm 0.55^\circ\text{C}$), while bovine gelatin film showed the lowest T_m ($60.42 \pm 0.34^\circ\text{C}$) and T_g ($47.23 \pm 0.69^\circ\text{C}$). This demonstrates that chicken skin gelatin film melts at higher temperatures than commercial gelatin films.

These results may be due to the amount of imino acid content in the gelatin. [20] found that the enthalpy change between T_m and T_g depends on the stability of the collagen structure. Imino acids proline and hydroxyproline are believed to influence the rigidity of gelatin through hydrogen bonding that stabilizes the triple-helix structure. Chicken skin gelatin contains high levels of proline (13.42%) and hydroxyproline (12.13%) as compared to bovine (proline: 12.66%, hydroxyproline: 10.67%) and porcine (proline: 13.1%, hydroxyproline: 10.8%) gelatins ([20]; [31]). High imino acids proline and hydroxyproline indicated that the films have strong hydrogen bonding and stable triple helix structure. Thus, more energy is needed to break the hydrogen bonds in the gelatin. The phenomenon explains the T_m and T_g value of chicken skin gelatin film was similar to the commercial gelatin films. These results are supported by the previous findings of [20], who found that the difference in thermostability between chicken and bovine gelatin may be attributed to the higher proline and hydroxyproline content of chicken gelatin. Thus, more energy is needed to break hydrogen bonds and for helix to coil transitions.

Table 1. Tensile strength (TS), elongation at break (EAB), puncture test (PT), water vapor permeability (WVP), glass transition (T_g) and melting point (T_m) of chicken skin, bovine and porcine gelatin films

Gelatin films	TS (MPa)	EAB (%)	PT (N)	WVP (kPa)	T_g (°C)	T_m (°C)
Chicken skin	5.57 ± 0.11^a	122.78 ± 0.29^b	29.06 ± 0.06^a	$1.29 \times 10^{-9} \pm 0.03^b$	51.12 ± 0.55^a	76.26 ± 0.73^a
Bovine	2.97 ± 0.06^c	135.60 ± 0.44^a	18.03 ± 0.09^b	$1.75 \times 10^{-9} \pm 0.06^{ab}$	47.23 ± 0.69^b	60.42 ± 0.34^b
Porcine	3.21 ± 0.08^b	130.06 ± 0.31^{ab}	16.55 ± 0.11^b	$2.25 \times 10^{-9} \pm 0.13^a$	50.00 ± 0.91^{ab}	61.71 ± 0.89^b

Different letter (^{a-c}) indicated significantly different ($p < 0.05$) within the column. Data reported are mean value \pm standard deviations.

3.5 Light barrier and transparency

Though the films turned to white when plasticizer was added, observation showed that the gelatin films produced were transparent. This may be due to the light-scattering effect of the emulsion formed by the composite gelatin film blends. The result obtained for light barrier and transparency of chicken skin, porcine and bovine gelatin are presented in Table 2. The results showed that there was a significant difference ($p < 0.05$) on the light barrier and transparency of gelatin film of different gelatin source. Results showed that chicken skin gelatin film obtained the lowest transparency (1.31%), whereas the highest transparency value was obtained by porcine gelatin film (1.74%).

Transparency values were directly influenced by the molecular structure of the gelatin used. Scanning electron microscopy revealed that chicken skin gelatin film had a small, compact and well-organized microstructure compared to bovine and porcine gelatin, which possessed bigger and scattered films microstructure. A smaller film molecular structure would increase the opaqueness of the films, thus inhibiting the amount of light passing through the film. This explains the low transparency value obtained for chicken skin gelatin film as compared to the commercial gelatin films. The results obtained was in agreement with a study by [32], who reported that the transparency of gelatin films decreased as the opacity increased.

Table 2. Light barrier and transparency values of chicken skin, bovine and porcine gelatin films

Gelatin films	Wavelength (nm)								
	200	280	350	400	500	600	700	800	T_{600}
Chicken skin	37.02 ± 1.92^b	26.37 ± 3.22^b	8.60 ± 4.21^{bc}	18.05 ± 7.05^b	39.99 ± 5.59^b	39.49 ± 12.86^a	42.92 ± 12.92^a	42.26 ± 13.34^b	3.52 ± 1.16^a
Bovine	37.31 ± 6.52^b	24.79 ± 6.50^b	4.49 ± 0.88^c	15.50 ± 2.18^b	30.83 ± 2.31^c	34.69 ± 2.67^a	41.03 ± 2.50^a	38.36 ± 5.81^b	3.78 ± 0.28^a
Porcine	31.72 ± 3.48^b	20.24 ± 6.59^b	3.36 ± 0.76^c	11.97 ± 2.01^b	30.58 ± 1.92^c	34.43 ± 1.42^a	37.78 ± 4.85^a	38.37 ± 1.93^b	2.96 ± 0.12^a

Different letter (^{a-b}) indicated significantly different ($p < 0.05$) within the column. Data reported are mean value \pm standard deviations.

3.6 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the chicken skin, bovine, and porcine gelatin films are shown in Table 3. The bands situated around 1624 to 1648 cm^{-1} , 1510 to 1580 cm^{-1} and 3275 to 3320 cm^{-1} , correspond to the amount of water content and also the alcohol, probably from glycerol, which were Amides I, II, and III, respectively. The stretching of COO of proteins amide resulted in Amide I arises, while vibrational of N-H groups and stretching vibrations of C-N groups resulted in Amide II arises. Other than that, the

vibrations in plane of C–N and N–H groups of bound amides, or vibrations of C–H₂ groups of glycine corresponds to Amide III arises [33].

A significant difference ($p < 0.05$) were found at the peaks corresponding to Amides I, II and III. The intensity of Amide III bands was the highest in porcine gelatin film (3300 cm⁻¹) as compared to bovine (3292 cm⁻¹) and chicken skin (3288 cm⁻¹) gelatin films. The same trends were found in the Amide I and II bands. Porcine gelatin film generated the highest wave number at Amide I (1646 cm⁻¹) and II (1558 cm⁻¹) bands, while chicken skin gelatin film generated the lowest wave number at Amide I (1626 cm⁻¹) and II (1539 cm⁻¹) bands. Whereas bovine gelatin film generated moderate wave number at Amide I, II and III bands at 1635 cm⁻¹, 1539 cm⁻¹ and 3292 cm⁻¹, respectively. The increase of amplitudes of amide I groups could be due to that the intermolecular hydrogen bonds formed between the C–O bands and C–N bands from gelatin.

The aliphatic alcohol group content in the film represent the hydroxyl groups (-OH) and C–O stretching. Therefore, results have shown that all gelatin film possessed an aliphatic alcohol group. The values obtained showed that there were no differences between gelatin films was due to the standardize amount of glycerol was used. These behaviours explain the TS, EAB and WVP values obtained by porcine, bovine and chicken skin gelatin films, as discussed earlier. Higher water content would resulted in more flexible films, thus increasing its EAB and WVP value and lowering in TS value of the films. The results obtained were in agreement with a study by [18], who found that FTIR spectra of Amide I, II and III of bovine gelatin film situated around 1630 cm⁻¹, 1539 cm⁻¹ and 3290 cm⁻¹, respectively. While in another study, [33] found that the Amide I, II and III bands of porcine gelatin are situated around 1632 cm⁻¹, 1548 cm⁻¹, and 3288 cm⁻¹, respectively.

Table 3. FTIR spectra of chicken skin, bovine and porcine gelatin films

Gelatin films	Amide III	Amide I	Amide II	Aliphatic Alcohol
	stretching vibration of C–N bands and N–H groups of bound amide, vibrations of C–H ₂ groups of glycine	C=O stretching	bending vibration N–H group, stretching vibration of C–N group	hydroxyl groups (-OH), C–O stretching
	3275-3276cm ⁻¹	1648-1624 cm ⁻¹	1510-1580 cm ⁻¹	1250-1020 cm ⁻¹
Chicken skin	3288.97±0.20 ^b cm ⁻¹	1626.20±0.20 ^b cm ⁻¹	1539.13±0.00 ^b cm ⁻¹	1033.80±0.20 ^a cm ⁻¹
Bovine	3292.49±0.20 ^{ab} cm ⁻¹	1635.64±0.40 ^{ab} cm ⁻¹	1540.77±0.20 ^{ab} cm ⁻¹	1034.63±0.40 ^a cm ⁻¹
Porcine	3300.20±0.10 ^a cm ⁻¹	1646.79±0.20 ^a cm ⁻¹	1558.91±0.30 ^a cm ⁻¹	1034.70±0.20 ^a cm ⁻¹

Different letter (^{a-b}) indicated significantly different ($p < 0.05$) within the column. Data reported are mean value ± standard deviations.

3.7 X-ray diffraction (XRD)

The diffractograms of chicken skin, porcine, and bovine gelatin films are shown in Figure 1. The results obtained for all film formulations for various types of gelatin demonstrate similarities. The diffractograms for all samples present an amorphous character, indicating no tendency towards re-crystallization, which probably due to the high stability and high moisture content in gelatin films. The diffractograms of the chicken skin, porcine and bovine gelatin films showed one diffraction peak at approximately $2\theta = 20^\circ$. The more amorphous structure of the composite film at 20° of gelatin film peaks diffraction, attributed to the typical fingerprints for gelatin powder [34]. However, intensity of the peak varied depending to the types of gelatin used. According to the diffractograms, porcine gelatin film possessed the highest intensity at peak $2\theta = 20^\circ$. Chicken skin gelatin film possessed the lowest intensity at peak $2\theta = 20^\circ$.

The results revealed that the amorphousness of the films decreases as the intensity of the peak increased. Therefore, porcine gelatin film has the most amorphous film characteristics as compared to the chicken skin and bovine gelatin film, as it has the highest intensity at peak $2\theta = 20^\circ$. Result obtained was in the same agreement with [10] who found that the diffractograms of chicken skin gelatin film which plasticized with glycerol present an amorphous character.

In addition, the diffractograms of porcine and bovine gelatin films also generated small peaks at $2\theta = 8^\circ$. The peaks indicate that the films possessed crystalline character. Diffractograms of chicken skin gelatin film showed no sign of crystallization, while porcine and bovine gelatin films exhibited small crystalline peak at 8° . This may be due to the molecular structure of the gelatin used. According to [35], the peak at $2\theta = 8^\circ$ indicates the triple helix diameter; thus, the intensity of the gelatin films is associated to the triple-helix content. The intensity of the peak increased as the molecular structure of the gelatin used increased. SEM showed that chicken skin gelatin film possessed the smallest and most organized microstructure than that of commercial gelatin. While porcine and bovine gelatin films possessed bigger and scattered films molecular structure, explaining their crystalline character. A similar effect was also observed in bovine gelatin, with semi-crystalline regions at peaks situated around $2\theta = 7$ to 8° [36].

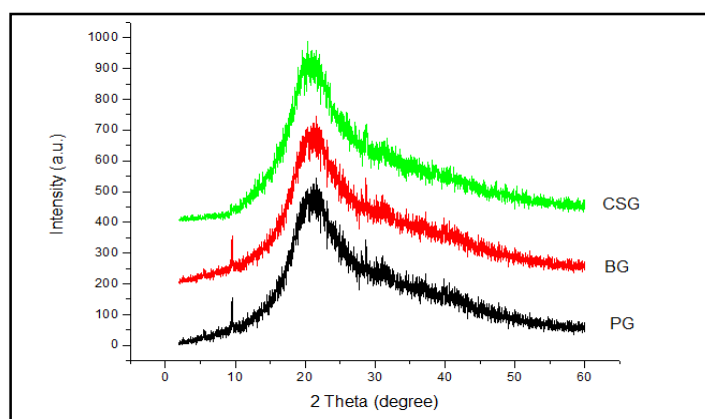


Figure 1. Diffractograms of different gelatin films between $0 - 60^\circ$, where CSG -chicken skin gelatin, BG -bovine gelatin and PG -porcine gelatin films.

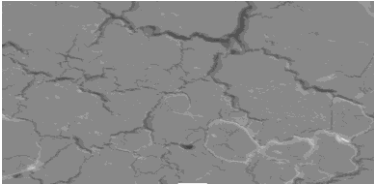
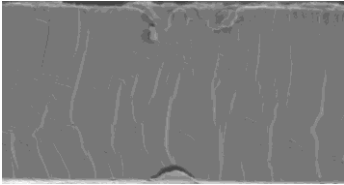
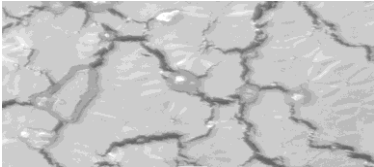
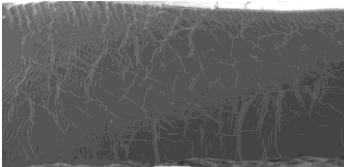
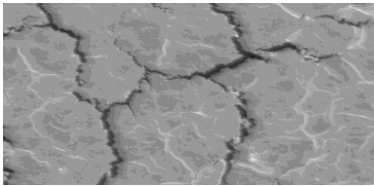
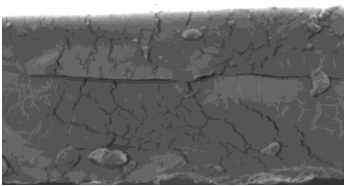
3.8 Film microstructure

Scanning electron microscopy (SEM) was used to study the microstructural changes in gelatin films and to obtain the surface and cross-section topography of the films. The microstructures of chicken skin, porcine, and bovine gelatin films are presented in Table 4. SEM revealed that the cross section of chicken skin gelatin film possessed small, compact and organized microstructure, while bovine and porcine gelatin films possessed bigger and scattered film microstructures with possible signs of a cracked surface area. This may be due to the high levels of hydroxyl group in bovine and porcine gelatin films, as revealed by FTIR. High levels of water content may disrupt the film network and create films with large and disorganized microstructure. This result was in agreement with studies by [37] and [27], who found that the internal structure of pigskin gelatin films revealed discontinuous zones that were characterized by horizontal cracks randomly distributed along the networks.

Additionally, it was observed that the surface area of the chicken skin gelatin film was homogeneous, with no brittle areas or bubbles. The XRD results also show that X-ray diffraction of chicken skin gelatin film displayed amorphous structure with no sign of crystallization, whereas both bovine and porcine gelatin films displayed crystalline peak at $2\theta = 8^\circ$. These findings support the homogenous structure obtained in chicken skin gelatin film. The formation of this homogenous and smaller surface area structure is related to the lower water content in chicken skin gelatin than in bovine and porcine gelatins. The structural difference of films may be responsible for the improvement of WVP obtained in the films.

The results are in agreement with those of [13], who found that bovine and porcine gelatin films displayed the formation of bumps and bubbles, as their bigger microstructures impact the results of oxygen permeability of the films.

Table 4. Cross section and microstructure of chicken skin, bovine and porcine gelatin films

Gelatin films	Surface area	Cross section
Chicken skin		
Bovine		
Porcine		

4. Conclusion

In conclusion the mechanical and physical properties of gelatin films from different sources showed that films from chicken skin gelatin offer better properties than commercial (porcine and bovine) gelatin films. This study found that chicken skin gelatin film possessed good properties for tensile strength, elongation at break and water vapour permeability, thermal properties, as well as film's microstructure. Therefore, chicken skin gelatin could be a potential alternative to commercial gelatin, as biodegradable films packaging.

5. References

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