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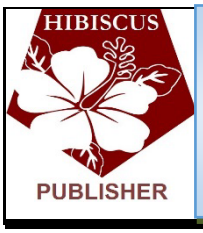

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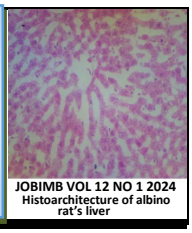

Differentiation of Extracted Gelatins from Porcine, Bovine and Chicken Origins Based on their Physical, Chemical and Structural Properties using Analytical Techniques

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ABSTRACT

Physicochemical properties of extracted gelatins from porcine, bovine and chicken sources compared to that of commercial bovine and porcine gelatins have been determined. Gelatin samples were assessed for their pH, water holding capacity, fat binding capacity, foaming capacity and stability, structural properties, thermal and amino acid profiles and molecular weight distribution. There were significant differences (P<0.05) in pH, water holding capacity, fat binding capacity, foaming stability and foaming capacity between gelatins. Porcine, bovine and chicken gelatins had two prominent bands which were visible in the α-chain region in the range of ~135 to ~100 kDa. Highest gelling point value was observed in extracted chicken gelatin while the highest value of melting point, were in commercial porcine and extracted chicken gelatins. Glycine, hydroxyproline, proline, alanine, arginine and glutamic acid were found to be the major amino acids. Physicochemical properties of gelatins could be characterized using analytical techniques.

INTRODUCTION

Gelatin is a denatured protein obtained by the thermal denaturation of collagen widely used in pharmaceutical, cosmeceutical, photographic and food industries by virtue of its excellent biocompatibility, easy biodegradability and weak antigenicity. In the food industry it is widely used as gel former, whipping agent, protective colloid, binding agent, clarifying agent, film former, thickener, process aid, emulsifier, stabilizer and adhesive agent. It could be obtained not only from the skin and bones of land animals but also from fish and insects. Akhade *et al*., [1] reported that the annual world production of gelatin is nearly 326,000 tonnes with source from pig skin being the most abundant (44%) followed by bovine hides (28%), bones (27%) and other sources (1%). The amount of gelatin used in the food industry worldwide is increasing annually [2]. However a number of zoonotic diseases such as bovine spongiform encephalopathy (BSE) and foot-and-mouth diseases (FMD) are constant threats to human health and thus the use animal by-products are limited in the production of functional foods, cosmetic and pharmaceutical. Further, Islamic religion and Judaism prohibit the consumption and use of any pork related products while the Hindus do not consume beef or beef products [3]. Hence consumer products require strict, genuine labeling to enable consumers to make accurate decisions on the food they purchase.

Due to increasing health concerns and sensitivity among the consumers over the food quality, there is currently a great need for analysis and authentication. Hence, the present study was undertaken to generate information by investigation on physicochemical properties of gelatin extracted from skin of porcine, bovine and chicken consisted of pH, water holding capacity, fat binding capacity, foaming properties, amino acid analysis, melting and gelling points and structural properties using FTIR and SDS-PAGE and compare these results with those of commercial bovine and porcine gelatin.

MATERIALS AND METHODS

Raw materials

Fresh porcine skins were obtained from Pasar Cina, Serdang, Malaysia while fresh chicken and bovine skins were purchased from Pasar Awam, Serdang, Malaysia (January to June, 2019). Commercial gelatins from bovine skin (type B) and porcine skin (type A) were sourced from Sigma Co. (St. Louis, USA). AccQ Tag TM Eluent A and a derivatization reagent, AccQ-fluor reagent kit were purchased from Waters (Massachusetts, USA). Regenerated cellulose nylon filter (0.45 µm) and Minisart RC 15 filter were obtained from Sartorius Stedim Biotech (Goettingen, Germany). Acetonitrile and methanol were of HPLC grade while other chemicals used in this study were of analytical grades.

Extraction of gelatin from bovine, chicken and porcine skins

The gelatins used in this study were derived from bovine, chicken and porcine skin which was extracted using acid process as described by Mohamad *et al.,* [4] with slight modifications. The chicken skins were immersed in 0.2M NaOH for 2h to remove any impurities and excessive oil. The alkaline solution was changed twice every hour and the residues were washed with water until to reach a neutral pH (~ 7.0). For bovine and porcine skins, the skins were washed with water after removing the fur. Skins were chopped into small pieces and one gram of the chopped skin was soaked into 7 mL of hexane at 25 ºC overnight.

The samples were then filtered using Whatman filter paper No. 4 and 7 mL of distilled water was added. The pH of the mixture was adjusted to 2 with concentrated hydrochloric acid solution and the samples were kept at 25 ℃ overnight. The acid solution was then removed and 10 mL of distilled water was added and the pH was adjusted to pH 4 using 0.1 mol/L NaOH. The mixture was then incubated at 50 ℃ for 3 h and dried in an oven at 60 ℃ overnight. The gelatin film was stored at room temperature (25 ºC). The yield of gelatin was calculated based on dry weight [5] using equation (1):

$$\text{Yield (\%)} = \frac{\text{Weight of dry gelatin (g)}}{\text{Weight of initial skin (g)}} \; x\; 100 \qquad \text{(Eqn. 1)}$$

Determination of pH of gelatin solution

pH of the gelatin solution was determined according to British Standard Institute method [6]. Gelatin powder and films were weighed to 1 g and then diluted in 100 mL of distilled water for 30 min to form a 1% (w/v) of gelatin solution. After that, the solution was heated at 60 °C for 30-60 min and cooled to room temperature before measuring pH using a pH meter (Mettler Toledo Ohio, USA). All data were collected from three independent replicates.

Determination of water holding capacity of bovine, chicken and porcine gelatins

Water holding capacity (WHC) of gelatins was determined according to the method described by Rasli and Sarbon, [7] with slight modification. 0.5 g of gelatin samples were dissolved in 10 mL of distilled water, vortexed for 30 s and centrifuged at 2800 ×g for 25 min at 25 °C. The solution was filtered using Whatman No.1. The difference between the initial volume of distilled water added to the sample and the volume of the supernatant was recorded. The test was made in triplicate. Results were reported as volume of water (mL) absorbed per weigh (g) of sample and WHC was calculated as equation (2):

$$\text{Water holding capacity (WHC)} = \frac{\text{V0}-\text{V1 (mL)}}{\text{Weight of gelatin (g)}} \qquad \text{(Eqn. 2)}$$

Where;
V0 = initial volume of distilled water,
V1 = final volume of solution and
W= weight of sample

Determination of fat binding capacity of bovine, chicken and porcine gelatins

Fat binding capacity of gelatins was determined as described by Rasli and Sarbon [7]. 0.5 g of gelatin was added to 10 mL of palm oil and vortexed for 30 s. The oil dispersion was centrifuged at 2800 ×g for 25 min at 25 °C after which the free oil was pour off. The analysis was done triplicate. The fat binding capacity was calculated according to equation (3):

$$\text{Fat binding capacity (FBC)} = \frac{\text{V0}-\text{V1 (mL)}}{\text{Weight of gelatin (g)}} \qquad \text{(Eqn. 3)}$$

Where;
V0 = initial volume and
V1 = volume of the supernatant

Determination of foaming capacity and foaming stability of bovine, chicken and porcine gelatins

Foaming capacity (FC) and foaming stability (FS) of gelatin samples were measured as described by Rasli and Sarbon [7]. Briefly, 1 g of gelatin sample was added to 50 mL of distilled water and dissolved at 60 °C. The solution was homogenized at 10,000 ×g for 5 min to form foam. The analysis was performed in triplicate and the average was recorded. FC and FS were expressed using equations (4) and (5):

$$\text{FC} = \frac{\text{Volume of foam+ Volume of liquid (mL)}}{\text{Initial volume of solution (mL)}} \qquad \text{(Eqn. 4)}$$

$$\text{FS} = \frac{\text{Initial volume of foam+ Volume of liquid (mL)}}{\text{Volume of foam +Volume of liquid (after 30 min) (mL)}} \qquad \text{(Eqn. 5)}$$

Rheology measurements of extracted gelatins from porcine, bovine and chicken

The method described by Sarbon *et al.,* [8] with slight modification was used to measure dynamic oscillatory using a controlled stress rheometer (AR-G2 Magnetic Bearing Rheometer , TA Instrument, US) with cone-plate geometry (60 mm, angle = 1° and gap =31 µm). The gelatin solutions (6.67% w/w) were prepared to determine its viscoelastic properties. They were cooled down from 40 to 5 °C and warmed again from 5 to 40 °C with heating/cooling rate of 1 °C/min. The elastic modulus (G′) and loss modulus (G″) were listed as a function of temperature. The values for the elastic modulus (G′) decrease and those for the loss modulus (G″) increase when melting occurred and the melting temperatures were obtained through it. However, the gelling temperatures were obtained from temperatures at which the elastic modulus (G′) began to dramatically increase in value.

Determination of functional groups of gelatins

FTIR spectra of all gelatin samples were obtained using Nicolet 6700 spectrometer model (Thermo-Nicolet, USA) with deuterated triglycine sulphate (DTGS) and KBr detectors. The sample was placed in contact with ATR accessory equipped with ZnSe cell at controlled ambient temperature (25 °C).

All spectra were recorded within a range of 4000-650 cm^{-1} with 4 cm^{-1} resolution and 32 scans. A single beam spectrum was obtained for all samples. These spectrums were subtracted against a background air spectrum and the results were presented in absorbance units. Spectrum acquisition of each sample was repeated thrice under the same conditions and an average spectrum obtained. Commercial gelatins from bovine skin (type B) and porcine skin (type A) were used as the standard reference.

Determination of polypeptides pattern of bovine, chicken and porcine gelatins using dodecyl sulphate polyacrylamide gel electrophoresis

Protein patterns of all gelatins were determined using a sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) made up of 4% (v/v) stacking gel and 6% (v/v) separating gel according to the method described by Hafidz *et al.,* [9]. 5 mg/mL of gelatin was mixed with sample buffer (0.5 M Tris-HCl, pH 6.8); 10% SDS; glycerol, 0.5% bromophenol blue, 2-mercaptoethanol) at a ratio of 1 to 1 (v/v). The mixture was heated in a water bath at 95 °C for 4 min. The gels were run in a Mini-PROTEAN Tetra electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) at 110 V for ~90 min. 0.05% The gel was then stained with coomassie brilliant blue R250 and destained with 30% (v/v) methanol mixed with 10% acetic acid. A Bio-Rad protein marker (USA) with molecular masses ranging from 45 to 200 kDa was used in this study.

Amino acid analysis of bovine, chicken and porcine gelatins

Amino acid was analyzed using the method described by Abdullah *et al*., [10] . ~ 0.1 to 0.2 g of gelatin samples were mixed with 5 mL of concentrated hydrochloric acid (6N) and the mixture was then hydrolyzed at 110 °C for 24 h in an oven. Prior to further dilution using distilled water, 4 mL of α-aminobutyric acid (AABA) was added to the mixture and homogenized. The sample was filtered using a 0.45 µm cellulose acetate membrane and 10 µL of the aliquot was used for derivatization. 70 µL AccQ.Fluor borate buffer was added to the aliquot to generate the proper pH (~ 8.2-10.0) where the excellent derivative yields occur. It was followed by an additional of 20 µL AccQ.Fluor reagent that will be reacted with both primary and secondary amino acids within seconds.

The derivatized compound was heated in the heating block at 55 °C for 10 min before injected into high performance liquid chromatography (HPLC, Waters Model 2695, Massachusetts, USA). The HPLC system was equipped with Aminex HPX-87H, 300 x 7.8 mm (Bio-Rad, CA) column, online degasser, auto injector and a multi-wavelength Waters 2475 fluorescence detector (Massachusetts, USA). The injection volume was 10 µL and HPLC system flow rate set at 1 mL/min. Waters Empower™ Pro software was used for system control and data acquisition [11]. All measurements were performed in triplicates.

Statistical analysis

Each experiment was repeated three times, and the results were reported as standard deviation (SD) of triplicate independent experiments. Data obtained were analyzed by one-way analysis of variance (ANOVA) using Minitab software (version 16.0, Minitab Inc., USA). All statistics were based on a confidence level of 95%, and $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Yield of the extracted gelatin from skins of bovine, chicken and porcine

The yield is amount of dry gelatin produced from a number of raw materials through extraction process [12]. Alkali and acid treatment was conducted to weaken the collagen structure, solubilize the non-collagen proteins and hydrolyze some of the peptide bonds and keep the consistency of the collagen fibers [8]. **Table 1** shows the yield values of extracted gelatin from bovine, porcine and chicken skin. Extracted porcine gelatin shows the highest percentage yield (12.5%) while the lowest percentage yield was from extracted chicken gelatin (8.19%). The highest yield of gelatin observed with increasing time due to cavitation and mechanical effect of ultrasound [13]. The lower yield of the gelatin may be due to different age of skin samples used for extraction as reported by Cole and McGill [14].

Table 1. Yield of extracted gelatin from skins of bovine, chicken and porcine.

Type of gelatin	Yield (%)	Range yield (%) in other study	
Chicken	8.19	9.25 - 15.0	Taufik [15] Rasli and Sarbon [16] Widyasari and Rawdkuen [17]
Bovine	12.0	7.09 - 23.52	Ahmad *et al*., [18] Ahmad *et al*., [19]
Porcine	12.5	10.22 - 12.67	Sompie *et al*., [20]

Widyasari and Rawdkuen [17] reported that the yield of extracted gelatin from chicken feet using acid extraction was 12.64% on dry weight basis, while the using of ultrasonic assisted extraction method was 12.37% on dry weight basis. The lower yield of the gelatin may be due to the loss of extracted collagen through leaching during washing steps in the pretreatment process or due to the incomplete of hydrolysis of collagen [8].

Yield of porcine gelatin in this study was in line with that of Sompie *et al.,* [20] who reported that the yield of pig skin gelatin were 10.22 to 12.67%. Protein content of collagen in the skin of animals affected by their age in which, the mature and stronger collagen fiber will obtain by increasing the age of the animals [21]. The differences in gelatin percentage obtained were influenced by species, age of animals, proximate composition, collagen content and methods of the extraction [22].

pH of gelatin solution

Table 2 shows the pH values of commercial bovine, commercial porcine, extracted bovine, extracted porcine and extracted chicken gelatins. Highest pH value was obtained from commercial porcine gelatin (7.18 ± 0.02) while the lowest pH value observed in extracted bovine gelatin (3.44 ± 0.02). There were significant differences ($P<0.05$) among the pH for all the gelatin samples. Ninan *et al*., [23] have reported that porcine skin gelatin have a pH of 7.5 which are consistent with the result of this study. Commercial bovine gelatin had pH of 5.32 since it was a type B gelatin. Gelatin consist of type A and type B. Type A was derived using acid pre-treatment while type B was derived from alkaline pre-treatment [24].

Table 2. pH Value, water holding capacity value and fat binding capacity value of the gelatins.

Type of gelatins (n=3)	pH value	WHC value (mL/g)	Fat binding capacity value (mL/g)
Commercial bovine	5.32 ± 0.02 [b]	9.3 ± 0.1[d]	5.7 ± 0.1[d]
Commercial porcine	7.18 ± 0.02 [a]	21.7 ± 0.12 [b]	4.3 ± 0.06[e]
Extracted bovine	3.44 ± 0.02 [e]	7.8 ± 0.1[e]	12.8 ± 0.06 [a]
Extracted porcine	4.06 ± 0.02 [c]	20.8 ± 0.2[c]	8.6 ± 0.15 [c]
Extracted chicken	3.54 ± 0.02 [d]	22.3 ± 0.12 [a]	9.2 ± 0.1[b]

Mean (± SD) of results from three separate experiments.
Values with the different superscript letters (a, b, c, d and e) within the same column are statistically significantly different (P<0.05).

Widyasari and Rawdkuen [17] reported that the pH value of the extracted gelatin from chicken feet using acid extraction method was higher than that of ultrasound assisted extraction method. Commercial bovine gelatin showed pH of ~5.32 which is in agreement with the findings by Widyasari and Rawdkuen [17]. Mohtar *et al.*, [25] reported that the pH of commercial bovine gelatin (type B) was 5.48. The difference in the pH value of gelatins could be due to the type and strength of chemicals used during the extraction procedure [22].

Ekasary *et al.*, [26] reported that the pH values obtained from the three porcine gelatin samples were 5.23, 5.35, and 5.22, within the range of 3.8-5.5 which is specified for gelatin type A according to the Gelatin Manufacturers Institute of America (GMIA) standard method [27]. The result obtained from this study for extracted porcine gelatin was in the range of the result reported by GMIA standard method. Furthermore, the low pH obtained for extracted samples could be due to HCl solution used for swelling the skin [19]. The relationship between the processing method used to extract gelatin and the pH of gelatin has yet not been established [28].

Water holding capacity value of bovine, chicken and porcine gelatins

Water-holding capacity (WHC) is a functional property which was closely related to interactions between water and gelatin components [29]. Results from WHC of gelatin samples are as shown in Table 2. There were significant differences (P<0.05) among the WHC for all gelatin samples with different species. The highest WHC (22.3 mL/g) was observed in extracted chicken gelatin, whereas the extracted bovine gelatin exhibited a lowest WHC (7.8 mL/g).

Nurul and Sarbon [30] reported that the WHC was affected by the amount of hydrophilic amino acid content. WHC has been reported to decrease with a decrease in hydrophilic amino acid and hydroxyproline content [31]. Similar results were observed for grey triggerfish skin gelatin, which showed lower water holding capacity and hydroxyproline as compared to that of bovine gelatin [32].

WHC value of extracted bovine and commercial bovine gelatin were 7.8 and 9.3 mL/g which were significantly difference (P<0.05). However, the WHC value of bovine gelatin presented in this study was lower than others which could be due to high value of hydrroxyproline and arginine contents in porcine and chicken gelatin [7]. Furthermore, this finding concurred with a previous report by Sarbon *et al.,* [8] who claimed that chicken skin gelatin contained highe amounts of arginine (5.57%) and hydroxyproline (12.13%) rather than bovine gelatin. It has been reported that high percentage of hydroxyproline contents can affect the WHC value [33]. Results from this study showed that extracted chicken gelatin has the highest WHC as it contains the highest amount of hydroxyproline content (14.89%) rather than porcine and bovine gelatin. This is in agreement with results obtained from studies by Rasli and Sarbon [7] who claimed that WHC of freeze-dried and vacuum oven dried chicken skin gelatins were higher as compared to that of bovine gelatin. In a study by Ninan *et al.*, [23] chicken skin gelatin showed higher amounts of WHC compared to that of bovine gelatin. The WHC results for commercial porcine and extracted porcine gelatin were 21.7 and 20.8 mL/g which were lower than that of chicken gelatin (22.3 mL/g) which may be due to the content of arginine. Rali and Sarbon [7] claimed that high content of arginine in gelatin result in lower WHC.

Fat binding capacity value of bovine, chicken and porcine gelatins

Results of fat binding capacity (FBC) for bovine, porcine, and chicken gelatins are as shown in **Table 2**. Highest FBC value was from extracted bovine gelatin (12.8 ± 0.06) while the lowest value was obtained from commercial porcine gelatin (4.3 ± 0.06). There is a significant difference (P<0.05) between the FBC among all type of gelatins. Nurul and Sarbon [30] reported that functional property of FBC is related to gelatin texture and dependent on interactions between oil and gelatin components.

Cho *et al*., [34] claimed that higher FBC was related to the high content of tyrosine. George *et al*., [33] also reported that rohu skin gelatin had the highest percentage of hydrophobic residue (tyrosine) that could contribute to the higher capacity for fat binding. In this study, extracted bovine gelatin has the higest value of tyrosine (1.2%) which has contributed to the highest FBC value (12.8 mL/g) as compared to other gelatins.

It was reported that the degree of exposure of hydrophobic residue and higher numbers of non-polar side chains in amino acids like tyrosine, leucine, valine and isoleucine related to an increase in FBC [23]. In this study, the content of leucine and isoleucine are consistent with the result of FBC where extracted bovine gelatin contained the highest amount of leucine, isoleucine and valine (3.96, 1.84 and 3.08%) and commercial porcine gelatin had the lowest amount of leucine, isoleucine and valine (3.14, 1.05 and 2.41%).

Based on the results from this study, extracted chicken gelatin has higher FBC rather than commercial bovine gelatin which is in agreement with results obtained from Sarbon *et al.,* [8]. Results from this study have also demonstrated that higher FBC corresponds to lower WHC where extracted bovine and porcine gelatins had the higher FBC and the lower WHC. This is in accordance with a study by Ninan *et al*., [23] who reported that rohu skin gelatin had the highest FBC and the lowest WHC.

Foaming capacity and foaming stability ratio of bovine, chicken and porcine gelatins

Results from the ratio of foaming capacity (FC) and foaming stability (FS) of porcine, bovine, and chicken gelatins are summarized in **Table 3**. The highest FC ratio was observed in commercial porcine gelatin (3.36) while the lowest ratio was obtained from extracted porcine gelatin (1.34). There was a significant difference (P<0.05) between the FC in all type of gelatins. The highest FS ratio was from extracted bovine gelatin (1.75) while the commercial porcine gelatin (1.15) showed the lowest ratio of FS. There was a significant difference (P<0.05) between the FS in all type of gelatins. However, there was no significant difference (P>0.05) between extracted chicken and extracted porcine gelatin.

Table 3. Foaming capacity and foaming stability ratio of gelatins.

Type of gelatins (n=3)	Foaming capacity ratio	Foaming stability ratio
Commercial bovine	2.88 ± 0.03[b]	1.19 ± 0.02[d]
Commercial porcine	3.36 ± 0.03[a]	1.15 ± 0.02[d]
Extracted bovine	1.87 ± 0.02[c]	1.75 ± 0.03[a]
Extracted porcine	1.34 ± 0.03[e]	1.39 ± 0.03[c]
Extracted chicken	1.50 ± 0.03[d]	1.41 ± 0.02[b]

Mean (± SD) of results from three separate experiments.
Values with the different superscript letters (a, b, c, d and e) within the same column are statistically significantly different (P<0.05).

It was reported that the particle size of gelatin affected the FC ratio as obsereved in a study by Rasli and Sarbon [7]. In this study, the ratio of FC for commercial porcine and commercial bovine gelatins were 3.36 and 2.88, respectively which were higher than FC ratio of extracted gelatins. Jellouli *et al.,* [32] claimed that the FC of grey triggerfish skin gelatin (123%) was slightly higher than bovine gelatin (119%) which has indicated a difference in foaming ability between both gelatins due to higher hydrophobic amino acid content (alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine and tyrosine) in the grey triggerfish skin gelatin. It was also reported that the foaming capacity of bovine gelatin lower than chicken skin gelatin samples may be due to the latter's higher content of hydrophobic amino acids such as proline, isoleucine, leucine and phenylalanine [8].

Benjakul *et al*., [35] reported that higher FS of chicken skin gelatin was possibly due to the greater existence of highly hydrophobic groups which resulted in the molecular reactions in the foam lamella. In this study, the content of hydrophobic groups (leucine, isoleucine and valine) is consistent to the result of FC and FS for extracted gelatins where the extracted bovine gelatin has higher ratio of FC and FS rather than extracted porcine and extracted chicken gelatin. Extracted gelatin from bovine contained the higher amount of leucine, isoleucine and valine (3.96, 1.84 and 3.08%), compared to that of chicken (3.45, 1.65 and 2.49%) and porcine (3.14, 1.05 and 2.41%). The determination of foaming properties has a crucial impact on the processing and quality of some products such as cake batters, milk, salad dressings and bakery products [30].

Gelling and melting temperature of bovine, chicken and porcine gelatins

Results from gelling and melting temperature of bovine, chicken and porcine gelatins are as in **Table 4**. It was observed that the storage modulus (G′) values were greater than the loss modulus (G″) in both the heating and the cooling scans. **Figs. 1** and **2** show the melting point and gelling point of gelatin in heating scan (5-40 °C) and cooling scan (40-5 °C). The gelling and melting temperatures are determined at which G′/G″ crossover occurs as indicated from the cross points of G′ and G″ in the viscoelastic profiles.

Table 4. Gelling and melting temperatures of different gelatins.

Type of gelatins	Gelling point (°C)	Melting point (°C)
Commercial bovine	20.8 ± 0.03[e]	28.3 ± 0.18[d]
Extracted bovine	22.8 ± 0.01[d]	28.8 ± 0.02[c]
Commercial porcine	24.3 ± 0.15[c]	31.8 ± 0.03[a]
Extracted porcine	24.9 ± 0.17[b]	30.9 ± 0.04[b]
Extracted chicken	25.4 ± 0.02[a]	31.8 ± 0.25[a]

Mean (± SD) of results from three separate experiments.
Values with the different superscript letters (a, b, c, d and e) within the same column are statistically significantly different (P<0.05).

Highest gelling point value was observed for extracted chicken gelatin (25.4 °C) while the lowest value was for commercial bovine gelatin (20.8 °C). There was a significant difference (P<0.05) between the gelling point among all type of gelatins. Highest value of melting point was obtained from both commercial porcine and extracted chicken gelatin (31.8 °C) while the lowest value was for commercial bovine gelatin (28.3 °C). There was a significant difference (P<0.05) among the melting point in all type of gelatins. However, there was no significant difference (P>0.05) between commercial porcine and extracted chicken gelatin was observed.

In this study, melting points of extracted bovine and extracted porcine gelatin were 28.8 and 30.9 °C while for commercial bovine and porcine gelatin were 28.3 and 31.8 °C. It was reported that the melting point of commercial bovine gelatin is at 28.8 °C [36] which is in accordance with the results obtained from the current study. The melting point of both extracted chicken gelatin and commercial porcine gelatin were 31.8 °C which both were considered as high melting points. It was reported that melting points of mammalian gelatins are in the range of ~ 32.2-32.6 °C which are considered as high melting points [23]. Higher melting points reflect higher gel strength [37] and one of the reasons which most of gelatin used these days are from porcine source.

The melting point of chicken gelatin obtained in this study is in the range of a study by Rasli and Sarbon [7] who claimed that melting points for chicken gelatin through freeze dried and vacuum oven dried samples were 32.64 °C and 29.12 °C. Choi and Regenstein [38] claimed that melting points increase with an increase in maturation time and levels of proline and hydroxylproline contribute to melting point characteristics. In this study, the extracted chicken gelatin shows high melting point (31.8 °C) which could be due to higher content of hydroxyproline (14.89%) as compared to others. Thus, chicken gelatin can be considered as an alternative to porcine gelatin as it has a high melting point.

In this study, the gelling points of extracted bovine and porcine gelatin were 22.8 °C and 24.9 °C while for commercial bovine and commercial porcine gelatin were 20.8 °C and 24.3 °C. It was reported that fish gelatin has lower gelling point compared to that of mammalian counterparts [37]. Prior reports on the gelling point of fish gelatin recorded the ranges of ~4-12 °C and ~18-19 °C for cold- and warm-water fish species, respectively [39]. In this case, the gelling point of mammalian gelatin should be higher than 19 °C and the result reported in this study for both porcine and bovine gelatins were consistent with mammalian group as reported in those studies.

Sarbon *et al*., [8] had concluded that gelling points of chicken skin gelatin was higher compared to that of bovine gelatin possibly due to the higher content of imino acid and hydroxyproline in chicken skin. In this study, the extracted chicken gelatin shows the highest gelling point (25.4 °C) which was confirmed by higher content of hydroxyproline (14.89%) when compared to that of porcine and bovine gelatins. Structural properties of all gelatin samples using FTIR for all gelatin samples are as shown in **Fig. 3**. The absorption bands for all samples are situated in amide regions. FTIR spectroscopy has been used to monitor the functional group and secondary structure of the gelatin [5].

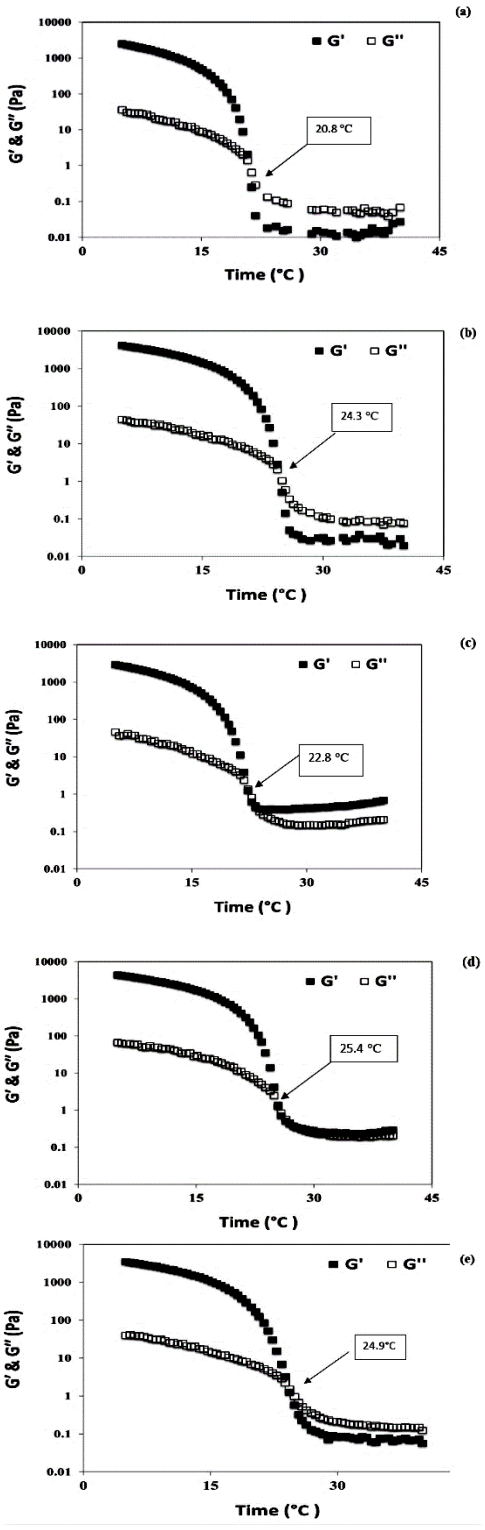

Fig. 1. Gelling temperature of gelatin for: (a) commercial bovine; (b) commercial porcine; (c) extracted from bovine; (d) extracted from chicken; (e) extracted from porcine.

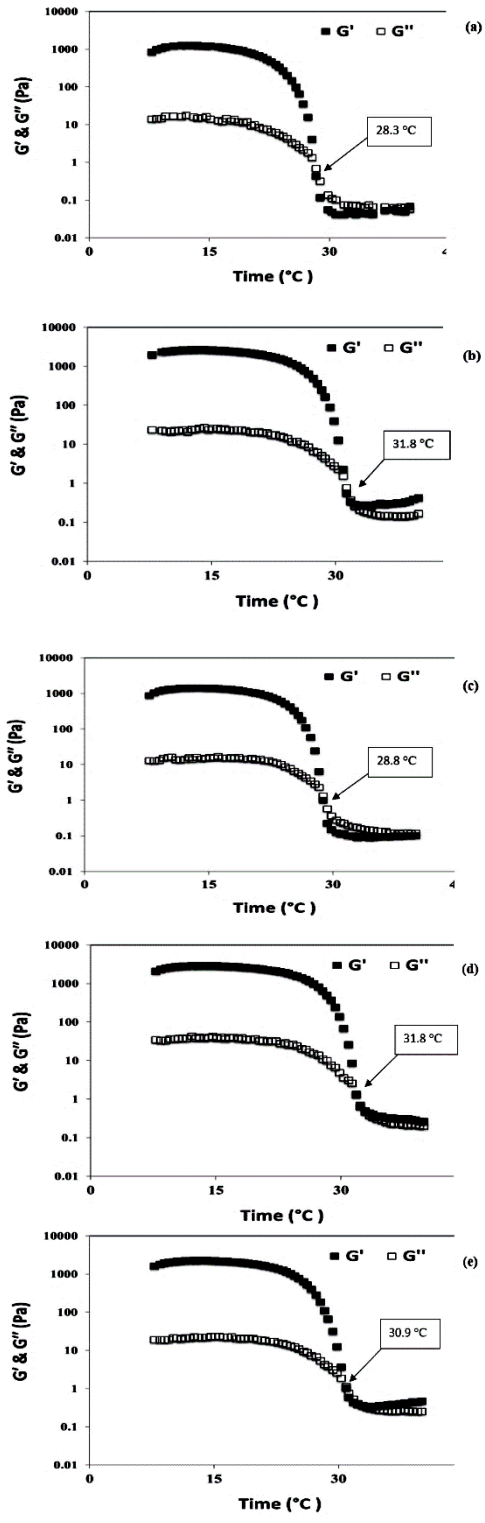

Fig. 2. Melting temperature of gelatin for: (a) commercial bovine; (b) commercial porcine; (c) extracted from bovine; (d) extracted from chicken; (e) extracted from porcine.

This sampling technique allows radiation through the sample without transmission and only small amount of sample was sufficient to allow transmission of the incident radiation cover the entire ATR surface [40]. **Table 5** shows wave number (cm^{-1}) and types of amides found in all gelatin sources which had similar spectral characteristics. Amide I and amide II bands for extracted chicken gelatin were found at 1631.15 cm^{-1} and 1532.04 cm^{-1}; for extracted porcine gelatin were at 1631.33 cm^{-1} and 1530.65 cm^{-1}; for commercial porcine gelatin were at 1631.57 cm^{-1} and 1527.68 cm^{-1}; for extracted bovine gelatin were at 1631.18 cm^{-1} and 1532.95 cm^{-1}; and for commercial bovine gelatin were at 1632.11 cm^{-1} and 1531.92 cm^{-1}.

Bandekar [41] reported that the amide I vibration primarily indicates C=O stretching coupled to contributions from C-N stretching, C-C-N deformation and in-plane N-H bending modes. On the other hand, the strong peaks at 1633.94 cm^{-1} and 1634.72 cm^{-1} represent arginine's C-N stretching [42]. Rahmelow *et al*., [43] stated that peaks at 1633-1636 cm^{-1} represented the presence of arginine which were consistent with the results of appearance peak in amide I in this study. Amide II vibration mode is the combination of CN stretch and in-plane NH deformation mode of the peptide group [44]. Yakimets *et al*., [45] claimed that the appearance of peaks from amides I and II were at 1700-1600 cm^{-1} and 1560-1500 cm^{-1} which were similar to the results of amide I and amide II in this study. It was reported that peaks at 1451.26 and 1451.27 cm^{-1} indicated the presence of C-N stretching from proline, whereas peaks at 1335.45 and 1336.42 cm^{-1} for bovine gelatin may be ascribed to the C-N vibration of tryptophan [42]. Peaks at 1443.68, 1443.13, 1440.81, 1444.36 and 1443.25 cm^{-1} in this study, were consistent with the results reported by Barth and Zscherp [42].

In this study, amide III bands for extracted chicken gelatin were found at 1239.14 cm^{-1} and 1078.57 cm^{-1}; for extracted porcine gelatin were at 1239.31 cm^{-1} and 1078.60 cm^{-1}; for commercial porcine gelatin were at 1237.39 cm^{-1} and 1080.72 cm^{-1}; for extracted bovine gelatin were at 1240.07 cm^{-1} and 1076.57 cm^{-1}; and for commercial bovine gelatin were at 1240.27 cm^{-1} and 1081.73 cm^{-1}. Rasli and Sarbon [7] reported peaks of amide III in vacuum oven and freeze dried of chicken skin were at 1236.66 cm^{-1} and 1235.61 cm^{-1}, respectively. Amide III bands for bovine gelatin ranged at 1031.62–1241.66 cm^{-1}, representing a combination of peaks due to C-N stretching and N-H deformation from amide linkages, as well as absorptions that arose out of wagging vibrations from the CH_2 groups of the glycine backbone and proline side-chains [44].

Nurul and Sarbon [30] reported that amides A and B were less prominent for both bovine gelatin with amide A peaks at 3289.74 cm^{-1} and amide B peaks at 2960.78 cm^{-1}, 3077.20 cm^{-1} and 2942.58 cm^{-1}, respectively.

Ranges for amides A and B were at 3300-3610 cm^{-1} and 2924-3166 cm^{-1}, respectively. Amide A indicates NH-stretching coupled with hydrogen bonding, whereas amide B indicates weak N-H stretching [46]. Amide A and B bands for extracted chicken gelatin were found at 3282.66 cm^{-1} and 2939.51 cm^{-1}; for extracted porcine gelatin were at 3283.42 cm^{-1} and 2930.43 cm^{-1}; for commercial porcine gelatin were at 3286.14 cm^{-1} and 2943.02 cm^{-1}; for extracted bovine gelatin were at 3288.26 cm^{-1} and 2942.96 cm^{-1}; and for commercial bovine gelatin were at 3287.14 cm^{-1} and 2944.88 cm^{-1}. All the results found for amide A and B are consistent with the results reported by Nurul and Sarbon [30]. It was reported that FTIR spectra of gelatins that were originated from bovine, and porcine had similar spectral characteristics [47]. In this study, all the appearance peaks for bovine, chicken and porcine gelatins were similar (at ~1076 to 3288 cm^{-1}) except for extracted bovine gelatin which was contained at 1328.74 cm^{-1}.

Electrophoretic patterns of bovine, chicken and porcine gelatins

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) profile of different gelatin samples is as shown in **Fig. 4**. Commercial available gelatinses have been reported as the heterogeneous compounds of α (100 kDa), β (200 kDa) and γ-chains (300 kDa) with molecular weight less than 300 kDa [48]. The distributions of these chains are determined by the conditionings process and the intensity of hydrolysis used. Commercial bovine skin gelatin in this study contained 2 prominent bands (~ 120 and 106 kDa) which had the molecular weight distributions similar to that of reported by Muyonga *et al.,* [49] who claimed that the major molecular weight fraction of type B gelatin from bovine bone was in the α-chain region. The extracted bovine skin gelatin had 4 prominent bands (~ 118, 103, 59 and 47 kDa) which two of them were found in the α-chain region. The extracted chicken skin gelatin showed 3 prominent bands (~ 202, 126 and 113 kDa) in the β-chain and α-chain regions. It was reported that the molecular weight of chicken feet gelatin showed the major protein band with the molecular weight of 198 kDa and 130 kDa which are in the range of β-chain and α-chain region.

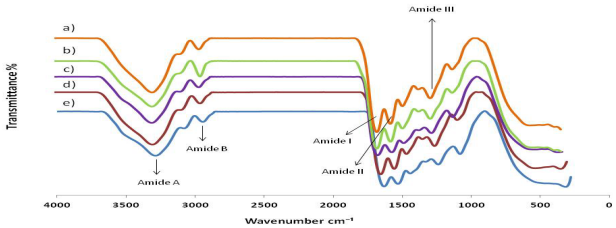

Fig. 3. FTIR spectra of gelatins: (a) extracted chicken, (b) extracted porcine, (c) commercial porcine (d) extracted bovine, (e) commercial bovine**.**

Table 5. Wave number value (cm^{-1}) and types of amides in different gelatins

		Wave number (cm^{-1})				
		Extracted chicken	Extracted porcine	Commercial porcine	Extracted bovine	Commercial bovine
Type of amide	Amide A	3282.66 ± 0.25^{e}	3283.42 ± 0.11^{d}	3286.14 ± 0.21^{c}	3288.26 ± 0.13^{a}	3287.14 ± 0.12^{b}
	Amide B	2939.51 ± 0.11^{d}	2930.43 ± 0.15^{e}	2943.02 ± 0.21^{b}	2942.96 ± 0.23^{c}	2944.88 ± 0.17^{a}
	Amide I	1631.15 ± 0.04^{e}	1631.33 ± 0.25^{c}	1631.57 ± 0.19^{b}	1631.18 ± 0.21^{d}	1632.11 ± 0.25^{a}
	Amide II	1532.04 ± 0.09^{b}	1530.65 ± 0.04^{d}	1527.68 ± 0.14^{e}	1532.95 ± 0.23^{a}	1531.92 ± 0.18^{c}
		1443.68 ± 0.13^{b}	1443.13 ± 0.25^{d}	1440.81 ± 0.17^{e}	1444.36 ± 0.21^{a}	1443.25 ± 0.28^{c}
	Amide III	1239.14 ± 0.23^{d}	1239.31 ± 0.14^{c}	1237.39 ± 0.31^{e}	1240.07 ± 0.25^{b}	1240.27 ± 0.16^{a}
		1078.57 ± 0.13^{c}	1078.60 ± 0.15^{c}	1080.72 ± 0.23^{b}	1076.57 ± 0.26^{d}	1081.73 ± 0.07^{a}

Mean (± SD) of results from three separate experiments.
Values with the different superscript letters (a, b, c, d and e) within the same row are statistically significantly different (P<0.05).

The extracted porcine gelatin was exhibited wider molecular weight distribution compared to that of bovine, chicken and commercial porcine gelatin. Extracted porcine gelatin showed 7 prominent bands (~125, 114, 106, 96, 87, 76 and 57 kDa). The presence of polypeptide bands with molecular weight less than α-chain (100 kDa) in extracted porcine gelatin were in agreement with a reports by Cole and Roberts [50]. Furthermore, all these four bands (125, 116, 114 and 57 kDa) were consistent with the results of Waber *et al*., [51], who found 3 distinct bands with molecular weights of 120, 117 and 60 KDa.

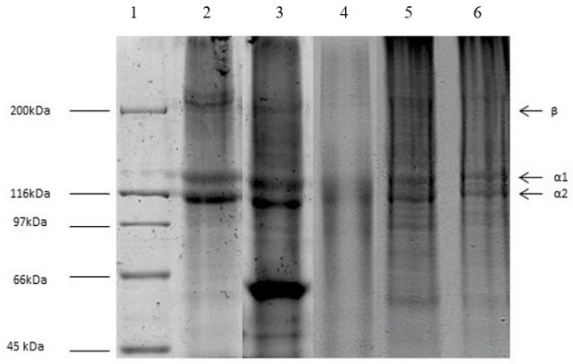

Fig. 4. Electrophoretic polypeptides patterns of different gelatins. Lane 1: standard protein marker with molecular weight of 45- 200 kDa; Lane 2: extracted chicken; Lane 3: extracted bovine; Lane 4: commercial bovine; Lane 5: extracted porcine; Lane 6: commercial porcine.

Determination of amino acid composition of bovine, chicken and porcine gelatins

Amino acid composition and molecular weight distribution determines the properties of gelatin [52]. **Table 6** shows the percentage of total amino acids of commercial bovine, commercial porcine, extracted bovine, extracted porcine and extracted chicken gelatins. Glycine, hydroxyproline, proline, alanine, arginine and glutamic acid were found to be the major amino acids. Glycine able to stabilize the gelatin structure by connecting one hydrogen bond per turn to the carbonyl oxygen of the peptide backbone.

Glycine, the highest amino acids contained in gelatin while hydroxyproline as the prevalent amino acid is specific to gelatin [53]. Furthermore, proline and hydroxyproline contributed to the stability of the triple helix by joining the backbone and bound the rotation of the polypeptide backbone [54].

The percentage of glycine in all gelatin samples in this study are the highest which are in line with the previous studies by Arnesen and Gildberg [55] and Binsi *et al*., [56] who claimed that glycine is the most abundant amino acid in gelatin. Asghar and Henrickson [57] described that the general formula of tripeptides contain Gly-X-Y, where X is generally proline and Y is mainly hydroxyproline which the presence of these three amino acids contribute significantly in gelatin. It has been reported that proline and hydroxyproline play a major role in restabilizing the triple helix of heat-denatured collagen such as gelatin, which is based on hydrogen bonding through hydroxyproline hydroxyl groups [58]. Ahmad *et al.,* [18] reported that the major amino acid content of bovine are glycine, proline and hydroxyproline which was obtained at different time of extraction. In this study, glycine, proline and hydroxyproline content of extracted bovine (20.12%, 13.75% and 13.02%) and commercial bovine gelatins (21.8%, 13.08% and 12.98%), are consistent with those reported by Ahmad *et al.,* [18].

Hafidz *et al.,* [9] reported that both bovine and porcine gelatin had high amount of glycine, proline and arginine. However, porcine gelatin contained higher amount of glycine, proline and arginine compared to bovine gelatin. In this study, porcine gelatin contained higher amount of glycine and arginine compared to that of bovine gelatin which were consistent as with the result reported by Hafidz *et al*., [9] where the percentage of total amino acid for arginine in commercial porcine and extracted porcine (8.77 and 8.56 %) higher than percentage of total amino acid for arginin in commercial bovine and extracted bovine gelatin (8.03 and 8.37%). It was reported that higher content of hydroxyproline, proline and alanine in chicken skin gelatin may contribute to its higher viscoelastic properties by promoting triple helix formation and stabilization of gelatin at low temperature [8]. Norland [59] also reported that gelatin from warm-blooded animal tissues have a higher amount of hydroxyproline contents which can promote triple helix formation and stabilization of the gelatin at low temperatures.

Table 6. Percentage of total amino acid in different gelatins.

Amino acid	Composition (% w/w) Extracted chicken	Extracted porcine	Commercial porcine	Extracted bovine	Commercial bovine
Hydroxyproline	14.89± 0.11	13.19 ± 0.06	14.02 ± 0.32	13.02 ± 0.30	12.98 ± 0.55
Aspartic Acid	4.82 ± 0.10	4.46 ± 0.01	4.90 ± 0.13	4.81 ± 0.21	5.50 ± 0.29
Serine	2.40 ± 0.03	3.03 ± 0.02	3.50 ± 0.08	3.00 ± 0.02	3.31 ± 0.10
Glutamic Acid	8.53 ± 0.15	8.43 ± 0.11	8.89 ± 0.22	8.05 ± 0.03	8.86 ± 0.23
Glycine	20.14 ± 0.22	20.55 ± 0.12	23.17 ± 0.60	20.12 ± 0.18	21.80 ± 0.60
Arginine	8.47 ± 0.12	8.56 ± 0.09	8.77 ± 0.22	8.37 ± 0.03	8.03 ± 0.24
Threonine	2.42 ± 0.05	2.25 ± 0.01	2.01 ± 0.05	2.52 ± 0.01	2.32 ± 0.05
Alanine	9.25 ± 0.30	8.86 ± 0.10	7.87 ± 0.19	8.45 ± 0.04	8.40 ± 0.38
Proline	12.77 ± 0.10	14.26 ± 0.04	12.74 ± 0.26	13.75 ± 0.11	13.08 ± 0.48
Tyrosine	0.98 ± 0.01	0.91 ± 0.01	0.73 ± 0.03	1.20 ± 0.01	0.54 ± 0.03
Valine	2.49 ± 0.01	3.08 ± 0.01	2.41 ± 0.11	3.08 ± 0.02	2.47 ± 0.09
Methionine	1.33 ± 0.01	1.05 ± 0.01	1.06 ± 0.05	1.09 ± 0.01	1.34 ± 0.06
Lysine	3.79 ± 0.01	3.75 ± 0.03	3.31 ± 0.17	3.86 ± 0.04	4.32 ± 0.19
Isoleucine	1.65 ± 0.01	1.42 ± 0.01	1.05 ± 0.10	1.84 ± 0.01	1.32 ± 0.08
Leucine	3.45 ± 0.03	3.44 ± 0.02	3.14 ± 0.13	3.96 ± 0.03	3.27 ± 0.13
Phenylalanine	2.62 ± 0.01	2.76 ± 0.02	2.43 ± 0.1 0	2.88 ± 0.02	2.46 ± 0.09
Cystine	Not detected	Not detected	Not detected	Not detected	Not detected

In this study, extracted chicken gelatin has the highest percentage of hydroxyproline and alanine contents (14.89% and 9.25%) and it has contributes to the highest gelling point properties as compared to that of porcine and bovine gelatin. The percentage of methionine and tyrosine for bovine, chicken and porcine gelatins in this study were lower than other amino acids content. Binsi *et al*., [56] reported that methionine, cystine and tyrosine contents in gelatin from skin of bigeye snapper (*Priacanthus hamrur*) are low. Du *et al.,* [60] claimed that both chicken and turkey heads gelatin had low amount of tyrosine and histidine. In this study, methionine and tyrosine were found the minor amino acids while histidine and cystine were not detected. It was reported that loss of cystine could be due to the acid hydrolysis during sample preparation [61]. Difference in the amino acid composition of gelatin products affect the physical quality, strength, viscosity and melting point of the gel [62].

CONCLUSION

Results from this study demonstrated that the highest pH value obtained in commercial porcine gelatin (7.18 ± 0.02) while the highest WHC value observed in extracted chicken gelatin (22.3 ± 0.12). Highest gelling point value was from extracted chicken gelatin (25.4 °C) and the highest value of melting point was observed in both commercial porcine and extracted chicken gelatin (31.8 °C). Hence, chicken gelatin was suggested to present as a probable alternative to substitute usage of porcine and bovine gelatin in industry. Highest value of FBC was from extracted bovine gelatin (12.8 ± 0.06) and the highest foaming capacity ratio was observed in commercial porcine gelatin (3.36). Highest FS ratio was obtained from extracted bovine gelatin (1.75). Extracted bovine gelatin showed to have better foaming properties due to higher content of hydrophobic amino acids. Bovine, chicken and porcine gelatins had similar spectral characteristics, and for extracted bovine gelatin most of the peaks appeared at 1328.74 cm^{-1}. Porcine, bovine and chicken gelatins had similar two prominent bands which were visible in the α-chain region in the range of ~ 135 to 100 kDa. Glycine, hydroxyproline, proline, alanine, arginine and glutamic acid were found to be the major amino acids for extracted and commercial gelatin. Results from this study showed that different types of gelatin have different physicochemical properties which could serve as a basis for the determination of authenticity in food products and detection and quantification of adulteration.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

REFERENCES

1. Akhade AR, Koli JM, Mohite AS, Shirdhankar MM, Patange SB. Optimization process to extract gelatin from the skin of talang optimization process to extract gelatin from the skin of talang queenfish (*scomberoides commersonnianus*) J Exp Zool India. 2020;23(2):1051-5.
2. Montero P, Gómez-Guillén M. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. J Food Sci. 2000;65:434-8. https://doi.org/10.1111/j.365-2621.000.tb16022.x.
3. Karim A, Bhat R. Fish gelatin: Properties, challenges and prospects as an alternative to mammalian gelatins. Food Hydrocoll. 2009;23:563-76. https://doi.org/10.1016/j.foodhyd.2008.07.002.
4. Mohamad NA, Mustafa S, El Sheikh AF, Khairil Mokhtar NF, Ismail A, Ali ME. Modification of gelatin - DNA interaction for optimised DNA extraction from gelatin and gelatin capsule. J Sci Food Agric 2016;96:2344-51. https://doi.org/10.1002/jsfa.7482.
5. Kaewruang P, Benjakul S, Prodpran T, Nalinanon S. Physicochemical and functional properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as affected by extraction conditions. Food Biosci. 2013;2:1-9. https://doi.org/10.1016/j.fbio.2013.03.002.
6. Institution BS. Methods for sampling and testing gelatine (physical and chemical methods). BSI, BS 757, London1975.
7. Rasli N, Sarbon NM. Physicochemical and structural properties of Asian swamp eel (*Monopterus albus*) skin gelatin as compared to bovine gelatin. Int Food Res J. 2015;22:699-706.
8. Sarbon NM, Badii F, Howell NK. Preparation and characterization of chicken skin gelatin as an alternative to mammalian gelatin. Food Hydrocoll. 2013;30:143-51. https://doi.org/10.1016/j.foodhyd.2012.05.009.
9. Hafidz RN, Yaakob CM, Amin I, Noorfaizan A. Chemical and functional properties of bovine and porcine skin gelatin. Int Food Res J. 2011;18:813-7.
10. Abdullah S, Noordin MI, Ismail SI, Shaik N, Jasamai M, Wai L, et al. Physicochemical evaluation and spectroscopic characterisation of gelatine from shank and toes of *Gallus gallus* domesticus Sains Malaysiana 2016;45:435-49.
11. Azilawati MI, Hashim DM, Jamilah B, Amin I. Validation of a reverse-phase high- performance liquid chromatography method for the determination of amino acids in gelatins by application of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate reagent. J Chromatogr A. 2014;1353:49-56. https://doi.org/10.1016/j.chroma.2014.04.050.
12. Said MI, Triatmojo S, Erwanto Y, Fudholi A. Gelatin properties of goat skin produced by calcium hydroxide as curing material. Media Peternakan. 2011;34:184-9.https://doi.org/10.5398/medpet.v34i3.3951.
13. Tu Z-C, Huang T, Wang H, Sha X-M, Shi Y, Huang X-Q, et al. Physicochemical properties of gelatin from bighead carp (*Hypophthalmichthys nobilis*) scales by ultrasound-assisted extraction. J Food Sci Technol. 2015;52:2166-74. https://doi.org/10.1007/s13197-013-1239-9.
14. Cole CGB, McGill AEJ. The properties of gelatins derived by alkali and enzimic conditioning of bovine hide from animals of various ages. J Soc Leather Technol Chem. 1988;72:159-64.
15. Taufik M. Kajian potensi kulit kaki ayam broiler sebagai bahan baku gelatin dan aplikasinya dalam edible film antibakteri. Universitas Gadjah Mada2011.
16. Rasli HI, Sarbon NM. Effects of different drying methods on the rheological, functional and structural properties of chicken skin gelatin compared to bovine gelatin. Int Food Res J. 2015;22(2):584.
17. Widyasari R, Rawdkuen S. Extraction and characterization of gelatin from chicken feet by acid and ultrasound assisted extraction. Food Appl Biosci J. 2014;2:85-97. https://doi.org/10.14456/fabj.2014.7.
18. Ahmad T, Ismail A, Ahmad SA, Abdula Khalil K, Atta Awad E, Kee Leo T, et al. Characterization of gelatin from bovine skin extracted using ultrasound subsequent to bromelain pretreatment. Food Hydrocoll. 2018;80:264-73. https://doi.org/10.1016/j.foodhyd.2018.01.036.
19. Ahmad T, Ismail A, Ahmad SA, Abdul Khalil K, Teik Kee L, Atta Awad E, et al. Extraction, characterization and molecular structure of bovine skin gelatin extracted with plant enzymes bromelain and zingibain. J Food Sci Technol. 2020;57(3):https://doi.org/10.1007/s13197-020-04409-2.
20. Sompie M, Triatmojol S, Pertiwiningrum A, Pranoto Y. The effects of animal age and acetic acid concentration on pig skin gelatin characteristics. Journal of the Indonesian Tropical Animal Agriculture. 2012:3-37.

21. Swatland HJ. Structure and development of meat animals. Englewood Cliffs, New Jersey: Prentice-Hall Inc; 1984.
22. Songchotikunpan P, Tattiyakul J, Supaphol P. Extraction and electrospinning of gelatin from fish skin. Int J Biol Macromol. 2008;42:247-55. https://doi.org/10.1016/j.ijbiomac.2007.11.005.
23. Ninan G, Joseph J, Aliyamveettil ZA. A comparative study on the physical, chemical and functional properties of carp skin and mammalian gelatins. J Food Sci Technol. 2014;51:2085-91. https://doi.org/10.1007/s13197-012-0681-4.
24. Baziwane D, He Q. Gelatin: The paramount food additive. Food Rev Int. 2003;19:423-35. https://doi.org/10.1081/FRI-120025483.
25. Mohtar NF, Perera C, Quek SY. Optimisation of gelatine extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS-PAGE. Food Chem. 2010;122:307-13. https://doi.org/10.1016/j.foodchem.2010.02.027.
26. Ekasary.A, Harmita H, Baitha PM. Optimized high-performance liquid chromatography-fluorescence detection method for the measurement of glycine, proline, and hydroxyproline concentrations in porcine gelatin. Int J Appl Pharm. 2018:1-10.
27. GMIA. Gelatin handbook. New York,USA Gelatine Manufactures Institute of America; 2012.
28. Park JH, Choe JH, Kim HW, Hwang KE, Song DH, Yeo EJ, et al. Effects of various extraction methods on quality characteristics of duck feet. Korean J Food Sci Anim Resour 2013;33:162-9. https://doi.org/10.5851/kosfa.2013.33.2.162.
29. Rawdkuen S, Thitipramote N, Benjakul S. Preparation and functional characterization of fish skin gelatin and comparison with commercial gelatin. Int J Food Sci Technol 2013;48:1093-102. https://doi.org/10.111/ijfs.12067.
30. Nurul AG, Sarbon NM. Effects of pH on functional, rheological and structural properties of eel (*Monopterus* sp.) skin gelatin compared to bovine gelatin. Int Food Res J. 2015;22.
31. Koli JM, Basu S, Nayak BB, Patange SB, Pagarkar AU, Gudipati V. Effect of pH and ionic strength on functional properties of fish gelatin in comparison to mammalian gelatin. Fishery Technology. 2013;50:126-32.
32. Jellouli K, Balti R, Bougatef A, Hmidet N, Barkia A, Nasri M. Chemical composition and characteristics of skin gelatin from grey triggerfish (*Balistes capriscus*). Food Sci Technol. 2011;44:1965-70. https://doi.org/10.016/j.lwt.2011.05.005.
33. George, N, Joseph J, Zynudheen A. Preparation and characterization of gelatin extracted from the skins of rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*). J Food Process Preserv. 2010a;35:143-63. https://doi.org/10.1111/j.745-4549.2009.00467.x.
34. Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, et al. Processing optimization and functional properties of gelatin from shark (*Isurus oxyrinchus*) cartilage. Food Hydrocoll. 2004;18:573-9. https://doi.org/10.1016/j.foodhyd.2003.10.001.
35. Benjakul S, Oungbho K, Visessanguan W, Thiansilakul Y, Roytrakul S. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chem. 2009;116 445-51. https://doi.org/10.1016/j.foodchem.2009.02.063.
36. Sarbon NM, Badii F, Howell NK. Purification and characterization of antioxidative peptides derived from chicken skin gelatin hydrolysate. Food Hydrocoll. 2018;85:311-20. https://doi.org/10.3389/fnut.2022.848289.
37. Boran G, Mulvaney SJ, Regenstein JM. Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources. J Food Sci. 2010;75:E565-71. https://doi.org/10.1111/j.750-3841.2010.01543.x.
38. Choi SS, Regeinstein JM. Physicochemical and sensory characteristics of fish gelatin. J Food Sci. 2008;65:194-9. https://doi.org/10.1111/j.365-2621.000.tb15978.x
39. Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocoll. 2011;25:1813-27. https://doi.org/10.016/j.foodhyd.2011.02.007.
40. Griffiths PR, de Haseth JA. Fourier transform infrared spectrometry. New York, NY, USA: John Wiley & Sons; 2007.
41. Bandekar J. Amide modes and protein conformation. Biochimica et Biophysica Acta. 1992;1120:123-43. https://doi.org/10.1016/0167-4838(92)90261-b.
42. Barth A, Zscherp C. What vibrations tell about proteins. Q Rev Biophys. 2002;35:369- 430. https://doi.org/10.1017/s0033583502003815.
43. Rahmelow K, Hu¨bner W, Ackermann T. Infrared absorbance of protein side chains. Anal Biochem. 1998;257:1-11. https://doi.org/0.1006/abio.997.2502.
44. Almeida PF, Araújo MGO, Santana JCC. Collagen extraction from chicken feet for jelly production. Acta Sci Technol. 2012;34:345-51. https://doi.org/10.4025/actascitechnol.v34i3.10602.
45. Yakimets I, Wellner N, Smith AC, Wilson RH, Farhat I, Mitchell J. Mechanical properties with respect to water content of gelatin films in glassy state. Polymer. 2005;46:12577-85. https://doi.org/10.1016/j.polymer.2005.10.090.
46. Barbara S. Infrared spectroscopy fundamentals and applications. Canada: John Wiley & Sons; 2004.
47. Cebi N, Durak MZ, Toker OS, Sagdic O, Arici M. An evaluation of Fourier transforms infrared spectroscopy method for the classification and discrimination of bovine, porcine and fish gelatins. Food Chem. 2016;190:1109-15. https://doi.org/10.016/j.foodchem.2015.06.065.
48. Zhang Z, Li G, Shi B. Physicochemical properties of collagen, gelatine and collagen hydrolysate derived from bovine limed split wastes. J Soc Leather Technol Chem. 2006;90:23-8.
49. Muyonga JH, Cole CGB, Duodu KG. Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatine. Food Hydrocoll. 2004;18:581-92. https://doi.org/10.1016/j.foodhyd.2003.08.009.
50. Cole CGB, Roberts JJ. Changes in the molecular composition of gelatin due to the manufacturing process and animal age, as shown by electrophoresis. J Soc Leather Technol Chem. 1996;80:136 - 41.
51. Weber P, Steinhart H, Paschke A. Characterization, antigenicity and detection of fish gelatine and isinglass used as processing aids in wines. Food Addit Contam. 2010;27:273-82. https://doi.org/10.1080/02652030903030397.
52. Gómez-Guillén MC, Pérez-Mateos M, Gómez-Estaca J, López-Caballero E, Giménez B, Montero P. Fish gelatin: a renewable material for developing active biodegradable films. . Trends Food Sci Technol 2009;20:3-16.
53. Bailey AJ, Light ND. Connective tissue in meat and meat products. London ; New York Elsevier Appl Sci; 1989.
54. Schrieber R, Gareis H. Gelatine handbook: Theory and industrial practice. Germany: John Wiley & Sons; 2007.
55. Arnesen JA, Gildberg A. Preparation and characterization of gelatine from the skin of harp seal (*Phoca groendlandica*). Bioresour Technol. 2002;82:191-4. https://doi.org/10.1016/s0960-8524(01)00164-x.
56. Binsi PK, Shamasundar BA, Dileep AO, Badii F, Howell NK. Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel-forming ability of fish mince. Food Hydrocoll. 2009;23:132-45. https://doi.org/10.1016/j.foodhyd.2007.12.004.
57. Asghar A, Henrickson RL. Chemical, biochemical, functional, and nutritional characteristics of collagen in food systems. London: Academic Press; 1982. 232-372 p.
58. Gime´nez B, Go´mez-Guille´ n MC, Montero P. The role of salt washing of fish skins in chemical and rheological properties of gelatin extracted. Food Hydrocoll 2005;19:951-7.
59. Norland RE. Fish gelatin. Voght MN, Botta JK, editors. Lancaster, PA: Technomic Publishing Co; 1990.
60. Du L, Khiari Z, Pietrasik Z, Betti M. Physicochemical and functional properties of gelatins extracted from turkey and chicken heads. Poult Sci. 2013;92:2463-74.https://doi.org/10.3382/ps.2013-03161.
61. Lourenço SO, Barbarino E, De-Paula JC, Pereira LODS, Marquez UML. Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycological Res 2002;50:233-41. https://doi.org/10.1046/j.440-835.2002.00278.x.
62. Arnesen JA, Gildberg A. Extraction of muscle proteins and gelatin from cod head. Process Biochem. 2006;41:697–700. https://doi.org/10.1016/j.procbio.2005.09.001.