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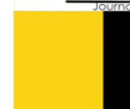
International Food Research Journal 26(2): 499-508 (April 2019)

Journal homepage: http://www.ifrj.upm.edu.my



Properties of chicken head gelatins as affected by extraction method

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Article history

Received: 3 May, 2018
Received in revised form: 25 October, 2018
Accepted: 2 November, 2018

Keywords

Chicken head,
Poultry by-product,
Gelatin,
Bloom strength,
Viscoelastic properties,
Gelling and melting temperatures

Abstract

Malaysia is a surplus poultry producing country with well-established commercial slaughtering and processing plants. Immense quantity of heads, feet, viscera, blood and feathers are usually discarded and not optimally utilized. Chicken heads are rich in protein, and could be a potential source of gelatin. The aim of the present work was therefore to find a simpler, faster, cheaper and greener gelatin extraction technology as compared to current available methods of gelatin extraction from poultry heads. A comparison of three different gelatin extraction methods with alkaline-acid pretreatment (E1), single acid pretreatment (E2) and single alkaline pretreatment (E3) were studied to extract gelatin from chicken heads. E1 and E2 produced gelatins of Type A, while E3 produced gelatin of Type B. High bloom gelatin (>300 g) with <1% of ash content, high gelling (25.8-26.0°C) and melting (30.8-32.3°C) temperatures, good functionality and physical appearance were obtained from E1 and E2 extraction methods. Gelatins of E1 and E2 had higher viscous (G'') and elastic modulus (G') values on cooling and heating as compared to the commercial bovine skin gelatin. FTIR spectra of the gelatins indicated different degrees of structural denaturation. Overall, extraction methods of E1 and E2 produced better gelatin quality than E3. Nonetheless, E1 was the best extraction method for the production of high quality gelatin from chicken heads.

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Introduction

Gelatin is perhaps one of the most versatile ingredients used in the food industries to impact textural related characteristics to the product. Majority of commercial gelatins are manufactured from hides and bones of bovine and porcine. However, safety issues such as bovine spongiform encephalopathy crisis and religious practices resulted in the need to find alternative source of gelatin which should be functionally equal or superior. The global gelatin market is estimated to be worth USD 3.0 billion by 2020, with the food and beverages segment as the largest consumer (Transparency Market Research, 2014). Hence, an additional and sustainable source for gelatin extraction is much needed. Chicken heads could fill in this additional demand of gelatin based on the global increase in the production of poultry as projected jointly by the Organization for Economic Cooperation and Development and the Food and Agriculture Organization of the United Nations (OECD/FAO, 2015).

Chicken heads, which contain skin, comb, wattle, cartilages and bones, have high collagen content (Rivera *et al.*, 2000). Unlike other poultry by-products such as chicken feet and chicken skin, chicken heads are not a coveted raw material for other food applications. The poultry slaughtering industries frequently discard the chicken heads or convert them into feeds. Gelatin extraction from various chicken body parts have been reported such as from chicken feet (Lim *et al.*, 2001; Almeida and Lannes, 2013; Widyasari and Rawdkuen, 2014), chicken skin (Sarbon *et al.*, 2013), mechanically deboned chicken meat residue (Rammaya *et al.*, 2012; Rafieian and Keramat, 2015) and chicken heads (Du *et al.*, 2013). All extraction procedures reported used acid, alkaline or acid and alkaline in combination with extraction temperatures of 45-85°C at varying extraction time. Extraction of gelatin from poultry requires relatively

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less stronger acid/alkaline concentration and shorter extraction time as the poultry are slaughtered at very young age (commercial slaughtering age) and therefore less delicate matrix of the skin.

During the gelatin extraction process, acid or alkaline pretreatment is required to achieve cleavage of some intra- and intermolecular covalent collagen cross-links in addition to remove the non-collagenous proteins and other impurities in gelatin raw material (Djabourov *et al*., 1993; Karim and Bhat, 2009). Depending on the persistence of the cross-links, gelatin with variations in molecular weight distribution will be produced (Jang *et al*., 2002). The efficiency of gelatin extraction and the physico-chemical properties of gelatin are greatly affected by the raw material source as well as the pretreatment and extraction parameters (Montero and Gómez-Guillén, 2000). Depending on the pretreatment, either Type A or Type B gelatin will be produced. Type A gelatin is produced by the acid process and has a broader isoelectric point of pH 7-9, and Type B gelatin produced from alkaline process has an isoelectric point of pH 4.8-5.4 (GMIA, 2013). Bloom strength is one of the main criteria for gelatin selection for applications.

Since chicken head, a by-product of the poultry processing industry, does not contain one homogenous tissue, i.e. either skin or bone alone, therefore, it is beneficial to determine the best extraction method for this raw material. It is commercially viable to extract gelatin from chicken heads given the projected growth in the industries in years to come. Therefore, the objectives of the present work were to determine the effect of different extraction methods on the characteristics of extracted gelatins and to determine the best possible extraction method for producing high quality gelatin from chicken heads.

Materials and methods

Raw material preparation

A total of 15 kg broiler chicken heads (Cobbs and Ross) were obtained from a wholesale market in Selangor, Malaysia. Upon arrival at the laboratory, chicken heads were washed with tap water and minced using meat mincer with medium coarse cast plate (Hobart 4822, Japan). Minced chicken head were packed in plastic bags and stored immediately at −20°C. The frozen minced chicken head should be analyzed within two weeks. All chemicals and reagents used were of analytical grade.

Gelatin extraction

Frozen minced chicken head was thawed in the cold room prior to extraction. All procedures were later carried out at room temperature. The ratio of the minced chicken head to solution was kept at 1:3 (w/v) unless otherwise specified. The used of NaOH and HCl for pretreatment were modified from Rafieian *et al*. (2013). Three extraction methods, namely E1, E2 and E3 were carried out as shown in Figure 1. The first method, E1 consisted of an initial defatting step whereby the step was carried out by slightly heating the thawed minced chicken head slurry in water at

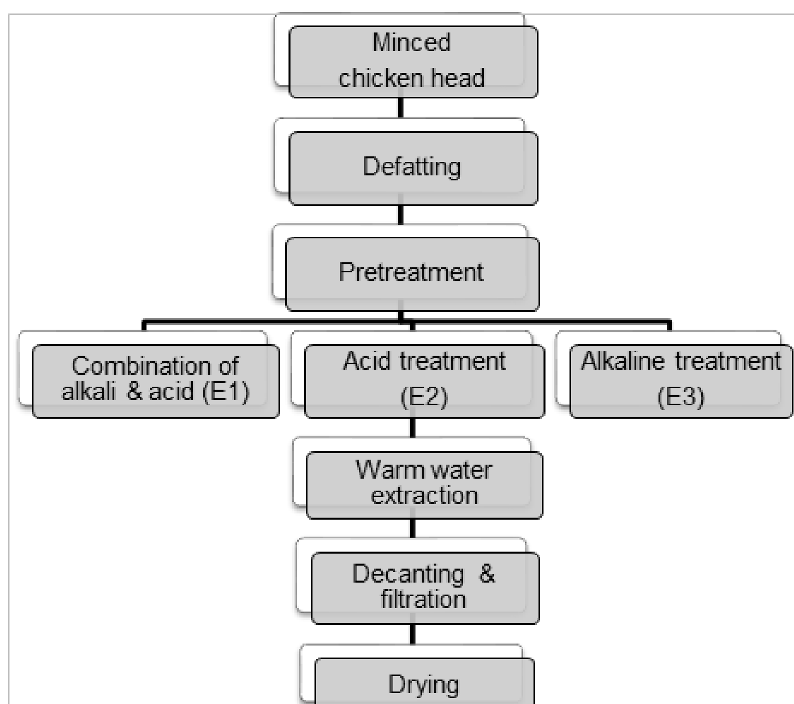

Figure 1. A summarized flowchart for the extraction of gelatin from chicken head

35-40°C for 30 min, after which the slurry was kept at 4°C for 1 h. The solidified fat layer formed at the top of the slurry was then manually skimmed off. The defatted slurry was subjected to alkaline pretreatment with 1 N NaOH in the presence of 1% NaCl for 1 h and the pH of the slurry was kept constant at approximately pH 10.5. The slurry was then subjected to 2% HCl acid pretreatment for 24 h; followed by the gelatin extraction with distilled water at 70°C for 3 h. The extracted gelatin was then filtered and oven-dried at 50°C until dry sheet of gelatin was formed. Washing and decanting were carried out after each individual step to remove residual impurities or until a neutral pH of the wash water was achieved. As for E2, the extraction method was similar to E1, except the alkaline pretreatment step was omitted. For E3, acidic pretreatment step was omitted instead of the alkaline pretreatment.

Characterization of gelatin

Type B bovine skin gelatin (G9382) from Sigma Chemical Co. (St. Louis, Mo., USA) was used as the reference for gelatin comparison. All experiments were run in triplicate.

Extraction yield (%)

The gelatin yields were obtained and calculated based on dry weight basis as follows:

$$\text{Yield (\%)} = \frac{\text{Dry weight of gelatin}}{\text{Dry weight of chicken head}} \times 100$$

Proximate analysis

Proximate composition of extracted chicken head gelatins were analyzed for ash, fat and protein content (micro-Kjedhal, nitrogen conversion factor of 5.55) according to the method of AOAC (2012).

Measurement of bloom strength

Bloom strength was measured according to the GMIA (2013) method. A 6.67% (w/v) gelatin solution was prepared in a standard bloom jar and allowed to hydrate for 1-3 h at room temperature after which the bloom jar was covered and placed in 60°C water bath for 10 min, followed by tempering at 45°C for 15 min. Samples were then allowed to cool at room temperature before being kept at 10°C (16-18 h) for gel maturation. The bloom strength was measured using a texture analyzer (Stable Micro System, TA.XT2i, UK) with a probe (P/0.5R) moving at cross-head speed of 1 mm/s. The maximum force (g) was recorded at a penetration distance of 4 mm into the gelatin gels.

Color measurement

The color of extracted gelatin powder was measured using a calibrated Hunter Lab UltraScan PRO colorimeter attached with EasyMatch QC software (Hunter Associate Laboratory Inc., Reston, USA). Values of L^*, a^* and b^* indicate lightness, redness/greenness and yellowness/blueness of samples, respectively.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was performed according to Laemmli (1970) with modification to visualize protein patterns of gelatin samples. Gelatin powder were dissolved in sample reducing buffer and heated at 95°C for 5 min. An 8% separating gel and 4% stacking gel were casted in Mini Protein unit (Bio-Rad Laboratories Inc., Richmond, CA, USA). Next, 10 µL aliquot were loaded on the gel, and a constant voltage of 110 V was applied for the electrophoretic run. Gels were then stained with Coomassie Blue R-250 followed by de-staining procedure overnight. Pre-stained SDS-PAGE standards broad range molecular weight marker (BIO-RAD Cat#161-0318, 6-202 kDa) was used to estimate the molecular weight of polypeptide bands.

Rheological measurement

A controlled stress rheometer (AR-G2 TA Instrument, USA) equipped with a 60 mm cone-plate geometry (angle = 1° and gap = 23 µm) was used to perform small oscillatory tests (Giménez *et al.*, 2005a). Prior to temperature sweep test, a strain sweep test was performed to determine the suitable linear viscoelastic region at the tested temperature. A 6.67% w/v gelatin solution was prepared and a temperature sweep was carried out from 40 to 10°C (gelation) and back to 40°C (melting) at the rate of 2°C/min. The frequency of oscillation was 1 Hz and 1% of strain amplitude was applied. The elastic modulus (G'), viscous modulus (G'') and phase angle (δ) were recorded as a function of temperature. Gelling and melting temperatures were determined from phase angle versus temperature plot (obtain from the viscoelastic measurement above) at its transition point when tan $\delta = 1$ and $\delta = 45°$ (Nikoo *et al.*, 2013).

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy of gelatin was performed using Nicolet 6700 spectrometer model (Thermo-Nicolet, USA), equipped with a DTGS-KBR detector. Disc made from gelatin powder and KBr were placed on the

crystal cell of the FTIR spectrophotometer for measurement. Spectra were acquired from 4000-500 cm^{-1} at room temperature and automatic signals were collected in 32 scans at a resolution of 4 cm^{-1}.

Isoelectric point (IEP)

The zeta potential of gelatin solutions (10 mL) at different pH's were performed according to Khong *et al*. (2018) with modification. Zetasizer Nano-ZS instrument (Malvern Instruments Ltd., U.K.) equipped with a pH autotitrator unit (MPT-2) was used. Gelatin solutions of 0.05% (w/v) were freshly prepared and filtered prior to the automatic titration with 0.25 M HCl, 0.025 M HCl or 0.025 M NaOH under constant stirring. The change of zeta potential was plotted against the pH, and the isoelectric point was estimated from pH when the zeta potential was zero.

Determination of amino acid composition

Determination of amino acid composition was carried out for the gelatin with the highest bloom in comparison with the commercial bovine skin gelatin. Separation of amino acids were achieved by Amino Acid Analyzer High Performance Liquid Chromatography (Waters 501 Millipore Corporation, USA) equipped with a 3.9 × 150 mm AccQ Tag RP-column (Waters Co., Milford, USA) and a fluorescence detector (Waters 2475, Waters Co., Milford, USA). Gelatin samples were hydrolyzed with 6 N HCl at 110°C for 24 h prior to measurement. Internal standard of α-amino butyric acid (AABA) was added into filtrates and were made up to 100 mL volume by deionized water. Derivatization step was carried out using Waters AccQ Fluor™ derivatizing reagent kit (Waters Co., Milford, USA), which consisted of AccQ Fluor borate buffer, AccQ Fluor reagent powder (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and AccQ Fluor reagent diluent. AccQ Tag Eluent A and B were used as the mobile phase at the flow rate of 1 mL/min (Azilawati *et al*., 2014).

Statistical analysis

All data presented are the means of triplicates. Minitab statistical package version 16 was used to run the analysis of variance (ANOVA), followed by Tukey's Multiple Comparison Test to determine the significance difference among the mean values at a significant level of $p < 0.05$.

Results and discussion

Yield

Gelatin yields are frequently reported based on weight of raw material or by the collagen content, such as by Du *et al*. (2013). The yield always varies with the source and the actual parameters of extraction involved in the whole extraction process. The yields of extracted chicken head gelatin ranged from 7.7% to 10.3% (Table 1). Highest yield was obtained from acid pretreatment (E2), but no significant different ($p > 0.05$) were found between E2 and E1 (combination of alkaline-acid pretreatment). These results indicated that both extraction methods could produce high yield. The lower yield from extraction method E3 could be due to incomplete hydrolysis of the collagen (Jamilah and Harvinder, 2002). Du *et al*. (2013) reported gelatin extraction for chicken heads based on collagen content; therefore, no direct comparison on yield could be made. Gelatin obtained from chicken feet with acetic acid extraction and ultrasonic assisted extraction methods (Widyasari and Rawdkuen, 2014) had 2-3% higher yield than E1 and E2 extractions. Sarbon *et al*. (2013) obtained a yield of 16% for gelatin extracted from freeze dried chicken skin using an alkaline and subsequent citric acid pretreatment. However, ultrasonic assisted extraction and freeze drying of skin prior to extraction are comparatively expensive steps to carry out.

Proximate composition analysis

All extracted chicken head and bovine skin gelatins evaluated had approximately 83 to 85% protein (Table 1). The extracted chicken head gelatins had similar protein content to those gelatins reported for chicken deboner residues (Rafieian and Keramat, 2015), chicken feet (Widyasari and Rawdkuen, 2014), and chicken heads (Du *et al*., 2013). Good quality gelatins have low ash content of not exceeding 3% (Food Chemicals Codex, 1996). Therefore, all of our extracted gelatins could be considered as high quality gelatin, especially gelatin of E1 and E2 with their ash content <1% (Table 1). The ash contents in the gelatin of E1 and E2 were lower than chicken feet gelatin extracted using acetic acid (Almeida and Silva Lannes, 2013) and gelatin extracted from mechanically deboned chicken meat residues (Rammaya *et al*., 2012). Hence, E1 and E2 could be superior extraction methods for chicken head gelatin. Although only warm water fat rendering and no solvent was used in the fat removal process in the present work, all extracted gelatins had fat contents within the acceptable range.

Bloom strength

Bloom strength is one of the most important attributes reflecting the gelling quality of gelatin and specific application. Gelatin with high bloom strength

Table 1. Characteristics of extracted chicken head gelatins in comparison with commercial bovine gelatin.

Characteristic	Extraction conditions			Bovine skin gelatin
	E1	E2	E3	
Yield (%) dry weight basis	10.04 ± 0.37^{A}	10.29 ± 0.36^{A}	7.67 ± 0.52^{B}	–
Proximate (%)				
Ash	0.44 ± 0.03^{A}	0.57 ± 0.03^{B}	2.53 ± 0.02^{C}	0.98 ± 0.04^{D}
Protein	83.85 ± 1.99^{A}	83.46 ± 1.10^{A}	84.95 ± 0.23^{AB}	83.98 ± 2.54^{A}
Fat	2.74 ± 0.14^{A}	3.59 ± 0.09^{B}	4.56 ± 0.06^{C}	0.16 ± 0.01^{D}
Gel bloom (g)	355.77 ± 0.33^{A}	332.40 ± 4.28^{B}	38.62 ± 3.25^{C}	190.64 ± 1.86^{D}
Color				
*L**	81.44 ± 0.74^{A}	77.57 ± 0.36^{B}	67.38 ± 0.27^{C}	70.46 ± 0.80^{D}
*a**	0.65 ± 0.07^{A}	1.35 ± 0.06^{B}	1.30 ± 0.05^{B}	3.32 ± 0.21^{C}
*b**	15.58 ± 0.06^{AB}	15.05 ± 0.16^{A}	14.13 ± 0.31^{B}	15.32 ± 0.57^{A}
Temperatures (°C)				
Gelling	26.00 ± 0.00^{A}	25.80 ± 0.35^{A}	15.17 ± 0.29^{B}	21.40 ± 0.53^{C}
Melting	32.30 ± 0.10^{A}	30.77 ± 0.25^{B}	24.10 ± 0.17^{C}	28.97 ± 0.06^{D}
Isoelectric point	7.47	7.42	4.78	4.98

Means within the same row with different superscripts are significantly different ($p < 0.05$). E1: alkaline-acid pretreatment; E2: acid pretreatment; M3: alkaline pretreatment

generally has higher melting and gelling points, and shorter setting time of the final product (Schrieber and Gareis, 2007). Bloom strengths obtained in the present work were significantly difference (p < 0.05) among the chicken head and bovine skin gelatins (Table 1). Gelatin of E1 (355.8 g) exhibited the highest bloom strength followed by E2 (332.4 g). These two gelatins showed significantly higher (p < 0.05) bloom values than the commercial bovine skin gelatin (190.6 g). Gelatin of E3 had the lowest gel strength. The short alkaline extraction period might have resulted in incomplete gelatin hydrolysis yielding less α-chains and higher molecular weight peptide chain, thus having less ability to align orderly to form a strong network to give a high bloom. Du *et al*. (2013) reported a much lower bloom (200.4-247.9 g) in their chicken head gelatins. This indicated that the combination of alkaline-acid pretreatment steps in the present work were effective to destabilize the bonding between α-chains in the native collagen matrix and convert the tissue collagen into a suitable form for hot water extraction. Gelatins of E1 and E2 had higher bloom than chicken feet gelatin obtained by acid extraction (Almeida and Lannes, 2013; Widyasari and Rawdkuen, 2014), although comparable to chicken skin gelatin (Sarbon *et al*., 2013).

Color

The color of gelatin depends on the raw material and extraction condition, and usually does not affect its functional properties except for consumers' acceptance (Ockerman and Hansen, 2000). Generally, there were significant differences (p < 0.05) in color attributes among extracted gelatins. Gelatins of E1, E2 were lighter in color (higher L* value) as compared to that of E3 and bovine skin (Table 1), which is very desirable. Similar finding was reported by Jang *et al*. (2002), whereby the color of acid-treated chicken feet gelatin gel was more desirable than that of the alkali-treated one. Bovine gelatin was brownish in color and had the highest *a** values significantly (p<0.05). The low *L** values for gelatin of E3 could be due to the short alkaline extraction period that was unable to remove the unwanted pigments effectively. All the extracted gelatins had higher *L** values than the turkey head gelatin but similar to the chicken gelatin of Du *et al*. (2013) and chicken feet gelatin of Almeida and Lannes (2013).

SDS-PAGE

The SDS-PAGE peptide profile of extracted chicken head and commercial bovine skin gelatins is as shown in Figure 2. Extraction conditions influenced the peptide profiles of the resulting gelatins. All extracted gelatins had one β-chain (approximately 202 kDa) and two α-chains (approximately 113 kDa), as major peptides constituents. This pattern is similar to that of chicken feet gelatin (Widyasari and Rawdkuen, 2014), and chicken and turkey head gelatins (Du *et al*., 2013). Gelatins of E1 and E2 had intense peptide bands visually, followed by bovine skin gelatin. However, β-chain and α-chains in gelatin E3 were less obvious. The presence and intensity

of α-chains and higher molecular mass proteins contributed positively to the higher bloom strength in extracted gelatin (Muyonga *et al*., 2004; Gomez-Guillen *et al*., 2011), which was also observed in the results of the bloom strength of their gelatin. Acid extraction seemed to give a stronger influence on the recovery of the α-chain. The decrease of α-band intensity along with the low bloom strength in gelatin E3 suggested that the alkaline gelatin extraction alone was not sufficient for gelatin extraction from chicken head. An hour of alkaline treatment in the present work seemed insufficient to destabilize the bonding between α-chains in the native collagen matrix thus less amount of free α or β-chains could be released and extracted.

Viscoelastic properties of gelatin

The dynamic viscoelastic properties of chicken head and bovine skin gelatin solutions are shown in Figure 3. A rapid increase in elastic/storage modulus, G' (Figure 3a) and viscous/loss modulus, G'' (Figure 3b) as well as a sharp decrease in phase angle (Figure 3c) were observed during cooling in all gelatins especially gelatin of E1 and E2, thus indicating rapid formation of junction zones to form gel network. Similar behavior was reported by Du *et al*. (2013) for their extracted gelatin. During the heating ramp, a gradual decrement in G' (Figure 3d) and G'' (Figure 3e) as well as a sharp increment in phase angle (Figure 3f), were observed, as a result of the gelatin transition from gel to solution (the melting of the

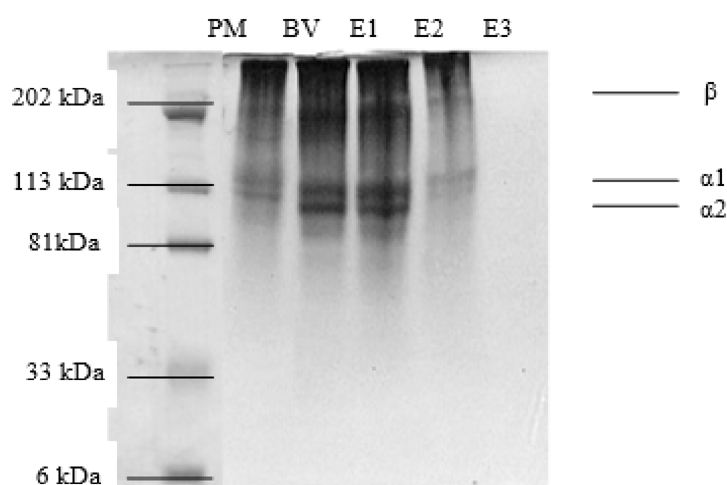

Figure 2. SDS-PAGE peptide pattern of extracted chicken head and commercial bovine gelatins (PM: peptide marker; BV: bovine gelatin; E1: alkaline-acid pretreatment; E2: acid pretreatment; E3: alkaline pretreatment).

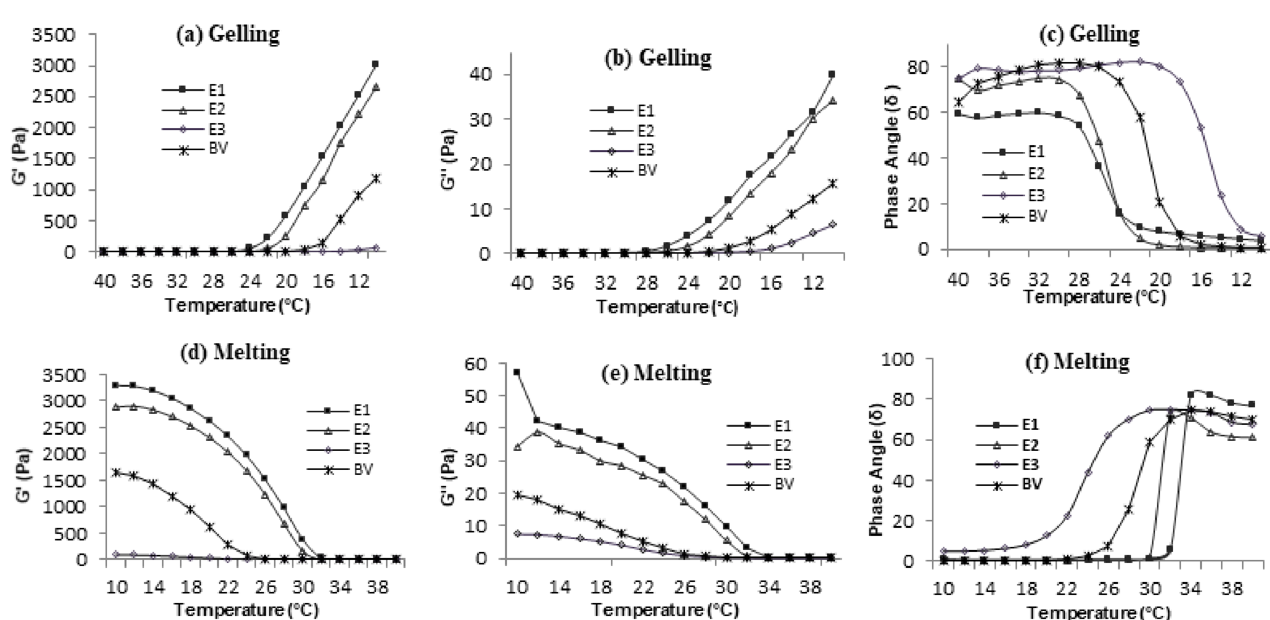

Figure 3. Changes in elastic modulus (G'), viscous modulus (G'') and phase angle of 6.67% chicken head gelatin solutions in comparison with bovine skin gelatin solution during cooling (a, b and c) from 40 to 10°C, and during heating (d, e and f) from 10 to 40°C (E1: alkaline-acid pretreatment; E2: acid pretreatment; E3: alkaline pretreatment)

gelatin). Gelatins of E1 and E2 had better gelling ability than E3 and bovine gelatin as indicated by their higher increment of G' and G'' during cooling. Overall, gelatins of E1 and E2 had better viscoelastic properties than gelatin of E3 and bovine. This result indicated that both the alkaline-acid as well as acid pretreatments were able to yield sufficient α-chains and higher molecular weight peptide chain that manage to align orderly to form a strong network. These gelatins also exhibited higher G' values at low gelling temperature as compared to bovine skin gelatin, thus suggesting the enhanced ability to refold into a triple helix (Gómez-Guillén *et al*., 2002) and to give more heat stable structure. Melting and gelling temperatures of gelatin solutions were obtained from rheological runs above and as shown in Table 1. The gelling and melting temperatures of chicken head gelatins of E1 and E2 were significantly higher ($p < 0.05$) than those of bovine skin and gelatin of E3. Both the gelling (15.2-26.0°C) and the melting (24.1-32.3°C) points of the gelatins were in the order of E1>E2 >BV>E3. All gelatins showed significant ($p < 0.05$) difference in their melting temperatures, although no significant ($p > 0.05$) difference was observed in the gelling temperature between gelatins of E1 and E2. Similar range of melting and gelling temperatures were reported for gelatin from chicken skin (Sarbon *et al*., 2013), and chicken and turkey head (Du *et al*., 2013). The relatively high content of the amino acids in chicken head gelatins (E1) might have positively contributed to its stronger viscoelastic properties by promoting triple helix formation and stabilization of gelatin at low temperature.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra for proteins were noticeable at amide region. Amide bands are associated with the degree of molecular order as well as the triple helical structure of collagen. Among all the bands, amide I and II bands are the two most prominent vibrational bands related to the protein backbone conformation and for protein secondary structural prediction. The FTIR spectra of chicken head and bovine skin gelatins are as shown in Figure 4. All gelatins exhibited major absorption bands in amide band region. Differences in relative intensity of the peaks as well as in amide frequencies were observed among the gelatins. Generally, gelatin of E2 and E3 showed the similar spectra with the highest peak relative intensity followed by gelatin of E1 and bovine skin gelatin. These differences could be attributed to the pretreatments used in the extraction procedure, and it is an indication of degradation of the collagen. The FTIR spectra of commercial bovine gelatin amide I, II, III, A and B were noticeable at 1638.6 cm^{-1}, 1546.2 cm^{-1}, 1241.2 cm^{-1}, 3442.7 cm^{-1}, and 2917.2 cm^{-1}, respectively. Amide I band (1600-1700 cm^{-1}) is linked to the C=O stretch vibrations along the polypeptide backbone (Kong and Yu, 2007). For the chicken head gelatins, amide I position were obtained at 1652.3 cm^{-1} (E1) and 1643.9 cm^{-1} (E2) and 1643.6 cm^{-1} (E3), fitting well within the range of 1600-1700 cm^{-1}, which was also similar to that of chicken feet gelatin (Almeida *et al*., 2012). The amide II band (1550-1600 cm^{-1}) is responsible for the arrangement of NH bending and CN stretching vibration. The amide II band readings for all gelatins were less

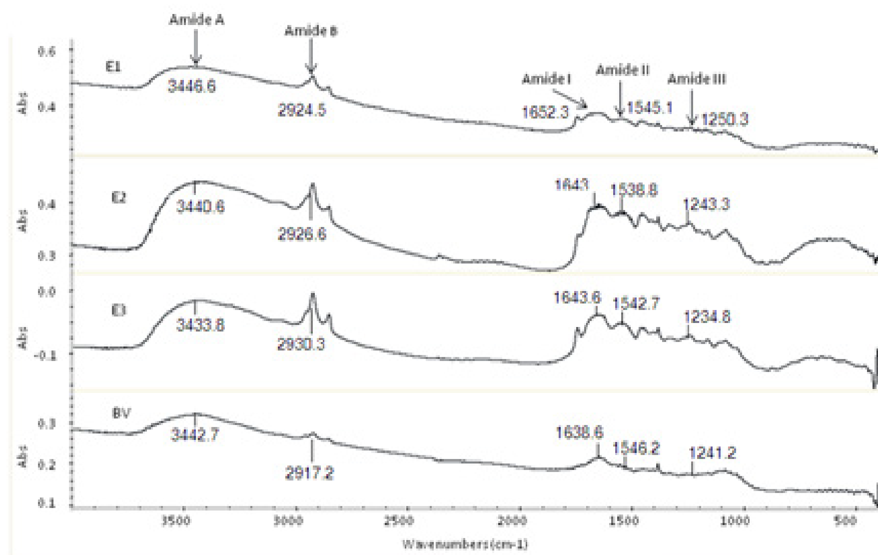

Figure 4. Comparison of FTIR spectra of chicken head and bovine gelatins (E1: alkaline-acid pretreatment; E2: acid pretreatment; E3: alkaline pretreatment; BV: bovine gelatin).

than 1550 cm^{-1} (1538.8-1545.1 cm^{-1}). Amide I and II absorption peak for gelatin of E1 (combination of alkaline-acid pretreatment) appeared at higher wavenumber as compared to gelatins of E2 and E3, thus suggesting a higher degree of molecular order and better stability in gelatin of E1 (Payne and Veis, 1988). Amide III (1220-1320 cm^{-1}) represents the combination of C-N stretching and N-H deformation from the amide linkages (Widyasari and Rawdkuen, 2014), which is associated with the loss of triple helix state as a result of denaturation of collagen to gelatin. The helical structure of chicken head gelatin was detected at 1234.8-1250.3 cm^{-1}. According to Albu *et al*. (2009), the triple helical structure integrity can be reflected by AIII/A1450 ratio that has to be higher or equal to 1. In the present work, the AIII/A1450 ratio for all the extracted gelatins was less than 1 (0.85-0.86); therefore indicating denaturing of triple helix which is expected due to the disruption of the acid labile cross-link at the telopeptide region and amide bonds of the triple helical structure of collagen during gelatin extraction. Gelatin of E1 and bovine skin gelatin had lower amide I, II and III intensities as compared to gelatins of E2 and E3 which might indicate a greater degree of peptide cleavage and deamidation of asparagine and glutamine leading to the alteration of the triple helix (Friess and Lee, 1996). Amide A (3400-3440 cm^{-1}) is associated with the N-H stretching vibration indicating the existence of hydrogen bonds. The position is shifted to lower frequency (usually 3300 cm^{-1}) if the N-H group of shorter peptides are involved in a hydrogen bonding (Ghica *et al*., 2009). The shifting might be due to the interaction of free amino group from the degraded gelatins with other reactive group (Benjakul *et al*., 2009). The shifting of Amide A to lower frequency was not noticeable in all the extracted chicken head gelatins of E1 (3446.6 cm^{-1}), E2 (3440.6 cm^{-1}), and E3 (3433.8 cm^{-1}), hence suggesting no excessive gelatin degradation contributed by the pretreatments. Amide B which correspond to the CH2 stretching band was observed at wavenumbers of 2924.5 cm^{-1}, 2926.6 cm^{-1} and 2930.3 cm^{-1} for gelatins of E1, E2 and E3, respectively. A significant higher peak was observed for gelatins of E2 and E3. This peak was less predominant for gelatin of E1, and slightly merged with the amide A band indicated better stability of this gelatin (Kemp, 1987). Thus, it can be concluded that combination or single alkaline or acid pretreatment might have induced the changes in secondary structure and functional groups of resulting gelatin, associated with the increased intermolecular interactions and denaturation of gelatin.

Isoelectric point (IEP)

Zeta potentials representing the surface charge of gelatin, which could be the result of cleaving the telopeptide region at different sites, leading to differences in the ease of conformational changes (Benjakul *et al*., 2010), were measured as a function of pH for all gelatins. At the isoelectric point (IEP), proteins in the aqueous system have zero net charges and when above their IEPs, have negative charges which also reflect their solubility properties. Gelatin solution with pH adjusted near its IEP will form more compact and stiffer gel (Gudmundsson and Hafsteinsson, 1997). Gelatins of E1 and E2 were of Type A gelatin with IEP at pH 7.47 and 7.42, respectively. Gelatin of E3 was Type B gelatin with IEP at pH 4.78. Bovine gelatin had its IEP at pH 4.98. Gelatin resulted from acidic hydrolysis was more frequently of Type A and alkaline hydrolysis giving Type B. During alkaline pretreatment, both asparagine and glutamine were converted into aspartic and glutamic acids, respectively, causing a decrease in IEP value (Schrieber and Gareis, 2007). The relatively high IEP of Type A gelatin with positively charged droplets, make it possible to create oxidatively stable oil-in-water emulsions since it could repel iron ions from oil droplet surfaces over a wider range of pH value (Surh *et al*., 2006).

Amino acid composition

Amino acids contents reflect and affect the properties of gelatin, especially bloom strength (Benjakul *et al*., 2009) and play crucial role in restabilizing the triple helix of gelatin, via hydrogen bonding ability of its hydroxyl group (Giménez *et al*., 2005b). Amino acid composition of gelatin of E1 was compared to commercial bovine skin gelatin (Table 2). Glycine, proline and hydroxyproline are the abundant amino acids in both gelatins with glycine being the most dominant. Aykin-Dinçer *et al*. (2017) and Kuan *et al*. (2017) also reported that glycine, hydroxyproline and proline are abundant in broiler skin gelatin and duck feet gelatin, respectively. Both gelatins were high in imino acids (proline and hydroxyproline) (22.98-23.12%) and slight differences in other amino acid components. The hydroxyproline content for gelatin of E1 was slightly higher than the commercial bovine skin gelatin. According to Benjakul *et al*. (2009), gelatin with a higher content of hydroxyproline show stronger gel structure and better viscoelastic properties, which was verified in our findings where gelatin of E1 had the highest bloom and viscoelastic properties. Gelatin of E1 showed higher hydroxyproline content than the gelatin of Du *et al*. (2013) even though they were from the same source.

Table 2. Amino acid analysis of chicken head gelatin of E1 and commercial bovine gelatin

Amino acid	Gelatin of E1 (%)	Bovine skin gelatin (%)
Hyp	12.12	11.69
Asp	4.93	5.01
Ser	2.86	3.65
Glu	8.54	8.56
Gly	20.57	20.66
His	4.85	5.43
Arg	8.68	8.24
Thr	2.78	2.60
Ala	8.21	7.80
Pro	10.86	11.43
Cys	0.11	0.05
Tyr	0.69	0.51
Val	2.22	2.44
Met	1.45	1.28
Lys	3.92	3.83
Ile	1.53	1.62
Leu	3.26	3.11
Phe	2.41	2.10
Total imino acid	22.98	23.12

E1: alkaline-acid pretreatment.

Conclusion

Gelatins of Type A with high bloom (>300 g) were successfully extracted from chicken head using a simpler, faster, cheaper and greener extraction method involving pretreatment carried out mainly at ambient temperature, warm water rendering for fat removal and extraction at 70°C for 3 h. Overall, extraction time was much shorter (time-saving) with minimum usage of solvent to substrate ratio (3:1) and no freeze drying step was needed. Extraction methods E1 (combination of alkaline-acid pretreatment) and E2 (acid pretreatment alone) produced gelatin of higher bloom strength, melting and gelling temperatures as compared to commercial bovine skin gelatin. E1 and E2 had gelatin yield of approximately 10%. Combination of alkaline-acid pretreatment (E1) is recommended as the best method for gelatin extraction in this study.

Acknowledgement

The present work was financially supported by the Research University Grant of Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

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