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# Rheological and molecular properties of chicken head gelatin as affected by combined temperature and time using warm water rendering

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**ABSTRACT**

Physicochemical properties of gelatin extracted from chicken head at 60, 75, and 90 °C for 3 and 6 h, respectively were determined. Increased in extraction temperature (ETE) from 60 to 75 °C and extraction time (ETI) contributed to higher yield and bloom. ETE was highly correlated with yield (r = 0.954). Despite a higher yield, the gelatin bloom and viscoelastic properties began to drop at 90 °C. Gelatin extracted at 75 °C exhibited superior properties with high bloom strength of > 309 g, high in G′, G″, gelling (27–28 °C), and melting temperatures (33–34 °C) with great viscoelastic properties. All gelatins were of type A with α-chains as major protein. FTIR amide bands indicated different degrees of structural changed. Glycine was the main amino acid in G75/6 (20.69%) with total imino acid of 23.19%. Regression models were significant ($p < .05$), and highly fitted for yield and a* ($R^2 > 0.9090\%$). Present findings suggest the feasibility to extract high quality gelatin from chicken head by manipulating ETE and ETI.

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## Introduction

Poultry industry is one of the important and fast growing agriculture subsector globally with annual increase in the broilers production. Broiler processing produce tremendous amount of by-product such as offal, fatty tissues, feather, head, feet, and blood. These by-products are currently processed into feeds or fertilizers, but not fully exploited for the gelatin production. The head about 2% of the body parts, are high in protein; therefore, it could be a valuable source of protein for gelatin production. Commercial gelatin are currently extracted from bovine and porcine sources and these animals require a longer growth period prior to slaughter besides the safety and religious issues associated with them. Production of gelatin from chicken head could provide a more sustainable gelatin supply due to a higher turnover as poultry has shorter growing period prior to slaughter compared to bovine and porcine.

Gelatin extraction involves the hydrolysis of both the intra- and intermolecular bonds of collagen molecules. Various extraction methods to obtain gelatin from different body parts of chicken and other poultry had been reported, but with solvent effect such as liming,[1,2] acid,[3] or combinations of both.[4] Chakka et al.[5] extracted gelatin from chicken feet using acetic, citric, and lactic acid at different concentrations. For gelatin obtained from poultry body parts, only a few reports had closely examined the combination effect of extraction temperature (ETE), time (ETI), and their correlations.

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Santana et al.[6] extracted chicken feet gelatin at 70–76.82 °C for 1–3 h using acetic acid, whereas Mokrejš et al.[7] employed endoprotease aided extraction for chicken feet gelatin extraction at 80 °C (1–4 h). Gelatin had been extracted from mechanically deboned chicken meat[1,8] at different time-temperature and acid/alkali concentrations. Gelatin extracted from chicken skin and bone had also been reported.[9,10]

However, limited studies on gelatin produced from poultry heads are noted such as by Du et al.,[11] Ee et al.,[12] and Gál et al.,[13] It is well known that gelatin has very broad application in many industrial fields due to its unique thermo-reversible properties. Production of gelatin from chicken head is commercially viable and provides an additional and sustainable gelatin to meet the needs of markets that are not amenable to bovine or porcine gelatin. The quality and properties of gelatin are known to be affected by the characteristics of the raw material and the extraction conditions. Effects of warm water extraction procedures, which is the novelty of the work, have not been reported before. Gelatin could be extracted more effectively and efficiently from chicken head by manipulating ETE and ETI. Therefore, this study aimed at determining the combined effect of temperature-time using warm water rendering on the properties of extracted chicken head gelatins. The effect of the process was determined using statistical model fitting. It is hypothesized that ETE and ETI influenced the physicochemical properties of extracted gelatin. This knowledge is required for the upscaling process in the development of a sustainable gelatin extraction industry based on poultry.

## Materials and methods

### Raw material preparation

A total of 10 kg broiler chicken heads (Cobbs and Ross) were purchased from the wholesale market in Selangor, Malaysia. Chicken heads were washed under running tap water to remove impurities. The head were then minced using commercial meat grinder (Hobart 4822, Japan) and collected for storage at −20 °C until use. All chemical and reagents used were of analytical grade.

### Gelatin extraction

Gelatin extraction was done according to Ee et al.[12] with some modifications. The ratio of the minced chicken head to extraction solution was kept at 1:3 (w/v) throughout the procedures. All procedures were carried out at room temperature unless otherwise specified. Chicken head mince was thawed overnight in the chiller was firstly defatted using warm water rendering followed by 5% ethanol solution with stirring for 18 h. After which, slurry was pretreated with 1 N NaOH (in 1% NaCl, pH 10.5) and 2% HCl for 24 h. Gelatin extraction was carried out with distilled water at 60, 75, and 90 °C for 3 and 6 h, respectively. The treatments were labeled as G60/3, G60/6, G75/3, G75/6, G90/3 and G90/6 for gelatin extracted at 60 °C for 3 h, 60 °C for 6 h, 75 °C for 3 h, 75 °C for 6 h, 90 °C for 3 h, and 90 °C for 6 h, respectively. Extracted gelatin slurry was then filtered and oven dried at 50 °C until gelatin sheets were formed. The sheets were then ground into powder and stored in sealed container for further analysis.

### Extraction yield (%)

The gelatin yield was obtained by calculating the weight differences where percentage yield (dry weight basis) = (dry weight of gelatin)/(dry weight of chicken head) × 100%.

### Bloom strength

Bloom strength was measured by preparing a 6.67% (w/v) gelatin solution in standard bloom jar.[14] The bloom jar was covered and placed in 60 °C water bath for 10 min, followed by tempering at 45 °C for 15 min. Gelatin solution was then left to cool to room temperature before being kept at 10 °C (16–18 h) for

gel maturation. Bloom strength (maximum force, g) was measured using a P/0.5 R probe of texture analyzer (Stable Micro System, TA.XT2i, Surrey, UK) with a 4 mm penetration distance at the speed of 1 mm/s.

### Color

The lightness (L*), redness (a*), and yellowness (b*) values of gelatin powder were measured using a precalibrated Hunter Lab UltraScan PRO colorimeter (Hunter Associate Laboratory Inc., Reston, USA).

### Isoelectric point (IEP)

Gelatin solution of 0.05% (w/v) was freshly prepared and continuously stirred in distilled water until completely solubilized. The solution was filtered through Whatman No. 1 prior to the automatic titration with 0.25 M HCl, 0.025 M HCl, or 0.025 M NaOH under constant stirring. Zeta potential of gelatin solution (10 ml) was measured from pH value 3.0–9.0 using Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, U.K.) equipped with a pH autotitrator unit (MPT-2) at room temperature. Zeta potential was plotted as a function of pH and the isoelectric point (zero zeta potential) was estimated.

### Viscoelastic properties measurement

The dynamic viscoelastic behavior of 6.67% (w/v) gelatin sample was performed using a controlled stress rheometer (AR-G2 TA Instrument, Castle, DE, USA) equipped with 60 mm cone-plate geometry (1° angle with 23 μm gap). Silicon oil was applied over the outer edge of the sample holder to prevent evaporation of gelatin samples during heating. Linear viscoelastic region for the gelatin sample was determined using a strain sweep test. Temperature sweep was employed by cooling gelatin samples on a Peltier plate from 40 to 10 °C (gelation) and heating back to 40 °C (melting) at the scanning rate of 2 °C/min, a strain of 1% and a frequency of 1 Hz. The elastic modulus (G'), viscous modulus (G"), and phase angle (δ) were measured as a function of temperature.

### Gelling and melting temperatures

Gelling and melting temperatures of gelatin were determined from the phase angle versus temperature plot at its transition point when tan δ = 1 and δ is 45°.[15]

### SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Peptide pattern of gelatin samples were analyzed using SDS-PAGE analysis with modification.[16] Gelatin powders were dissolved in sample reducing buffer and heat-denatured 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). A 10 μl aliquot was loaded onto the casted gel and subjected to electrophoretic run at a constant current of 110 V. Bio-Rad prestained broad range SDS-PAGE marker (BIO-RAD Cat#161-0318, 6–202 kDa, Hercules, CA, USA) was also loaded onto the gel for molecular weight estimation. Protein bands of gelatin samples were visualized after staining (Coomassie Brilliant Blue R250) and destaining procedure.

### Fourier transform infrared (FTIR) spectroscopy analysis

FTIR analysis of extracted gelatin from chicken head was performed using Nicolet 6700 spectrometer model (Thermo-Nicolet, Waltham, USA). Briefly, finely ground gelatin powder and potassium bromide was pressed to form a pellet, which was then placed in the sample holder for measurement. FTIR spectra were acquired in the range of 4000–500 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ for 32 scans.

### Determination of amino acid composition

Amino acid composition of gelatin with the highest bloom strength (G75/6) was analyzed and compared to the commercial bovine skin gelatin (G9382, Sigma Chemical Co., St. Louis, Mo., USA). Gelatin sample was hydrolyzed in a screw-cap tube with 6 N HCl at 110 °C for 24 h. AABA (alpha amino butyric acid) was added as an internal standard. All samples was derivatized using Waters AccQ Fluor™ derivatizing reagent kit (Waters Co., Milford, USA), before being separated 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 fluorescence detector (Waters 2475, Waters Co., Milford, USA). Mobile phase (AccQ Tag eluent A and B) were filtered and used at the flow rate of 1 ml/min.[17]

### Statistical analysis

All data were means of triplicate. Minitab statistical package (version 17) was used to perform the analysis. Data were subjected to one-way analysis of variance (ANOVA), followed by Tukey Multiple Comparison Test to determine the significance difference among the mean values at ($p < .05$). Two-way ANOVA was also conducted to test the significant evidence of interactions effects. Pearson's correlation was used to analyze the correlations among the variables. Those significantly correlated parameters were further analyzed using regression analysis for best fitting model estimation.

## Results and discussion

### Gelatin yield

Conversion of collagen into gelatin is very much dependent on the collagen source and the severity of extraction process.[18] Gelatin yield ranged from 5.9 to 11.7% with the highest yield was obtained at G90/3, followed by G90/6; G75/6; G75/3; G60/6; G60/3 in descending order. The yield of chicken head gelatin increased significantly ($p < .05$) with the increase of ETE and ETI except for gelatin of G90/6 (Figure 1). Exposure to high heat treatment for longer time may cause degradation to the gelatin. At 60 °C and 75 °C, higher gelatin yields were obtained from 6 h extraction. Direct comparison on yield could not be made with Du et al.[11] as their gelatin yield was reported based on collagen content. Higher yield (20–36%) were reported for enzyme-aided gelatin extraction from chicken head.[13] Santana et al.[6] reported gelatin yields of 1.7–8.6% from the chicken feet depending on the extraction method.

Two-way analyses showed that there was a significant ($p < .05$) interaction effect for ETE*ETI on the gelatin yield ($R^2 = 0.9981$ and $p = .000$). The positive effect of ETE and ETI on the gelatin yield was also reported by Kittiphattanabawon et al.[19] Similar observation was noted for gelatin from seabass skin[20] and skate skin.[21] Higher ETE provides more energy to destabilize the bonding in the collagen; hence more gelatin can be extracted out from the skin complex into the medium effectively.

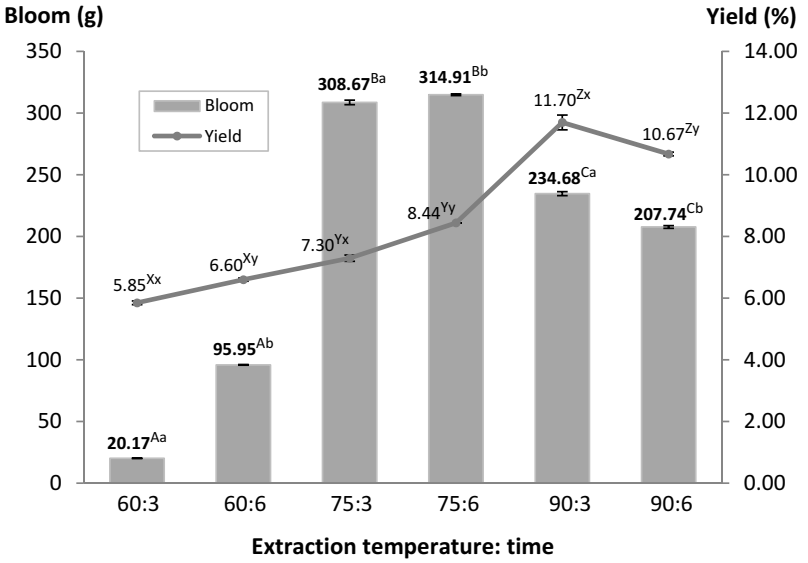


**Figure 1.** Effect of ETE and ETI on the yield (dry weight basis) and bloom values of chicken head gelatin. Bars (means ± SD, n = 3) with the uppercase letters show differences among ETEs and lowercase letters show differences between the ETIs for the same temperature ($P < .05$).

Pearson's correlation at Table 2a shows that gelatin yield was significantly ($p < .05$) correlated with ETE (r = 0.954). Regression analysis indicated that ETE was the only significant predictor for gelatin yield with $R^2$ of 0.9090 (Table 2b). Gelatin yield was not significantly influenced by ETI (r = 0.068, $p = .387$). This suggests a simple linear relationship of gelatin and ETE (Yield = – 3.97 + 0.165 Temperature) as the best fitted function.

## *Bloom strength*

Gel forming ability is one of the most important physical attribute that reflects gelatin quality. Gelatin with higher bloom strength is usually associated with good viscoelastic properties exhibit higher melting and gelling point. Higher bloom gelatin has higher market value and lesser amount is needed in the application. The bloom strengths of chicken head gelatin extracted under varying ETE and ETI (Figure 1) were significantly difference ($p < .05$). Gelatin with the highest bloom values (> 300 g) were produced at 75 °C, followed by those extracted at 90 °C (> 200 g) and 60 °C (< 100 g).

Gelatins extracted at 60 °C were low in bloom strength and had difficulty to set well. This indicates incomplete gelatin hydrolysis due to insufficient heat and extraction, which possibly yields less α and higher molecular weight peptide chain for a strong network formation. At 60 °C and 75 °C, gelatin extracted for 6 h had higher bloom than those extracted for 3 h. Higher ETE with longer ETI, contributed to the higher gelatin bloom as more α or β-chains were released from the skin; however, not true for extraction at 90 °C. At 90 °C, the longer extraction period resulted in lower bloom, although highest gelatin yield was obtained (Figure 1). Acid process at 75 °C was reported to produce gelatin with better gel strength from chicken skin than those extracted at 100 °C.[9]

The interaction effect of ETE*ETI was significant ($p < .05$) for gelatin bloom strength. Gelatin bloom had significant positive correlation with ETE (moderate, r = 0.613) and ETI (weak, r = 0.505). Multiple regression combining ETE and ETI resulted in the equation of Bloom = – 421 + 36.5 Time + 5.44 Temp, ($R^2$ = 0.6310) as the best fit for the prediction of gelatin bloom from experimental data. Chicken head gelatin extracted at 75 °C had a gel bloom comparable to gelatin reported by other researchers. Du et al.[11] obtained chicken head gelatin with bloom strength of 200–248 g at 50–60 °C.

**Table 1.** Effect of ETE and ETI on color values, gelling and melting temperatures of chicken head gelatins.

| Parameter | Temperature (°C) | Time (h) | |
|---|---|---|---|
| | | 3 | 6 |
| L* | 60 | $77.37 \pm 0.00^{Aa}$ | $73.94 \pm 0.01^{Ab}$ |
| | 75 | $78.15 \pm 0.02^{Ba}$ | $77.82 \pm 0.02^{Bb}$ |
| | 90 | $77.47 \pm 0.01^{Ca}$ | $74.54 \pm 0.01^{Cb}$ |
| a* | 60 | $1.15 \pm 0.01^{Aa}$ | $2.76 \pm 0.01^{Ab}$ |
| | 75 | $1.54 \pm 0.06^{Ba}$ | $2.55 \pm 0.02^{Bb}$ |
| | 90 | $2.25 \pm 0.01^{Ca}$ | $3.56 \pm 0.02^{Cb}$ |
| b* | 60 | $14.78 \pm 0.00^{Aa}$ | $19.09 \pm 0.01^{Ab}$ |
| | 75 | $18.44 \pm 0.06^{Ba}$ | $19.34 \pm 0.02^{Bb}$ |
| | 90 | $19.55 \pm 0.02^{Ca}$ | $19.45 \pm 0.01^{Cb}$ |
| Gelling temperature | 60 | - | $22.67 \pm 0.51^{A}$ |
| | 75 | $27.33 \pm 0.15^{Ba}$ | $27.47 \pm 0.40^{Ba}$ |
| | 90 | $24.87 \pm 0.81^{Ca}$ | $24.13 \pm 0.25^{Ca}$ |
| Melting temperature | 60 | - | $29.07 \pm 0.45^{A}$ |
| | 75 | $34.23 \pm 0.32^{Ba}$ | $33.10 \pm 0.17^{BCb}$ |
| | 90 | $31.73 \pm 0.29^{Ca}$ | $30.13 \pm 1.10^{Aa}$ |

*Averages of triplicate analysis.
*Values in the row with different lowercase (a–c) were significantly different ($p < 0.05$).
*Values in the column with different uppercase (A–F) were significantly different ($p < 0.05$).
*Measurement of gelling and melting temperatures for gelatin G60/3 cannot be performed as it failed to gel.

While Gal et al.[13] reported a gelatin bloom of 113–355 g for his enzyme aided extraction. Various bloom strength values had been reported for gelatins obtained from poultry skin and feet using different extraction methods.[4–7,22]

## Color

Gelatin is faintly yellow to light tan color which is dependent on the gelatin source and extraction conditions.[14] Color contributed to consumer acceptance but it does not affect its functional properties,[23] although the light color gelatin is more desirable for food application. All L*, a*, and b* color values of extracted chicken head gelatin were affected by the ETE and ETIs significantly ($p < .05$, Table 1). Differences in color were observed among the extracted gelatin. Two-way ANOVA analyses showed significant ($p < .05$) evidence for interaction effect ETE*ETI in all three color values with high $R^2 \geq 0.9992$.

Gelatin of G75/3 exhibited the highest L* value. Generally, gelatin extracted for 6 h had lower L* values compared to those extracted for 3 h, which indicated a darker color of the gelatin. Longer ETI may increase the rate of amino acid-sugar Maillard reaction. L* value had moderate negative relationship (r = – 0.671) with ETI significantly ($p < .05$). ETI was the only significant predictor for L* values of gelatin with $R^2$ of 0.4503 (L = 79.893–0.743 Time). At the same time, L* color values was negatively correlated with a* color values (r = – 0.702). Chicken head gelatin were brighter than those extracted from turkey head (57.72–62.04)[11] and chicken feet (19.92),[22] respectively. Similar L* values were reported for gelatin obtained from chicken deboner residue[24] and chicken head.[11]

Both a* and b* color values of gelatin increased significantly ($p < .05$) with the increase in ETE and ETI. The color of gelatin was changed from a pale translucent yellow brown to a slightly darker brown hue. Extraction at higher ETE for longer duration was more likely to cause more extensive non-enzymatic browning to occur among those amino and carbonyl groups in the gelatin source material. Overall, gelatin extracted at higher ETE (90 °C) had higher a* and b* values compared to those extracted 60 °C and 75 °C. Nagarajan et al.[25] reported a similar color change for squid skin gelatin. Both a* and

b* color values exhibited positive correlations with ETE and ETI significantly. High $R^2$ (0.9234) obtained for a* color values (a* = – 2.033 + 0.4363 Time + 0.03161 Temp) indicated the model fits the data well.

### Isoelectric point (IEP)

Isoelectric point is related to the surface charge, protein solubility, and amino acid residues. Charge characteristics will influence the functional properties of gelatin. At the isoelectric point, protein in the aqueous system has a zero net charge. At pHs below the IEP, zeta potential tended to increase and most of the amino groups are positively charged. Gelatin solution with pH adjusted near its IEP will form more compact and stiffer gel.[26] All extracted gelatin were of type A gelatin with an IEP in the pH range of 6–9. The IEPs for gelatin extracted at different ETEs and ETIs were observed at pH 5.9 (G60/3), 6.93 (G60/6), 6.47 (G75/3), 7.24 (G75/6), 6.78 (G90/3), and 6.3 (G90/6), respectively. Varying zeta potential values were observed, suggesting the different degree of unfolding or exposure of charged amino acids. Varying ETEs and ETIs may cleave the gelatin telopeptide region at different sites and lead to conformational changes in gelatin.

### Viscoelastic properties of gelatin

Temperature sweep test was performed in the range of 10–40 °C to investigate the dynamic viscoelastic properties of chicken head gelatin (Figure 2). Gelatin of G60/3 was excluded from the oscillatory test as it failed to gel. The elastic/storage modulus (G') of all gelatin were more prominent than their corresponding viscous/loss modulus (G") over the studied temperature in both heating and cooling scans. During cooling scan, all gelatin showed similar viscoelastic behavior with the G', G" and phase angle of gelatin remained fairly static at first followed by a sharp increase in G' and G" (Figure 2 c and e) and a sharp decrease in phase angle (Figure 2 a). These sharp changes indicated thermal transition of gelatin from solution to gel through formation of junction zones for gel network to occur. Similarly during heating, all gelatin samples demonstrated a considerable fall in G' and G" values and significant increase in phase angle initially and these values became relatively stable near the end of heating ramp (Figure 2 b, d and f). These changes indicated thermal transition of gelatin gel which melted into solution at the studied temperatures.

Gelatin extracted at 75 °C for 3 and 6 h were observed to have similar value of G', G", and phase angle values. Both had the highest values of G' and G", hence indicating greater thermo-stability that required higher thermal transition during cooling and heating process. This is followed by gelatin of G90/3> G90/6> G60/6, respectively. Gelatin with higher bloom strength usually exhibited better viscoelastic properties. Our data on viscoelastic properties of chicken head gelatin were in agreement with their bloom strength. Gelatins extracted at 90 °C exhibited greater viscoelastic properties than gelatin extracted at 60 °C. Extraction at higher temperature may probably contribute to relatively higher amount of free amino group content in the gelatin.

### Gelling and melting temperatures

Melting and gelling temperatures of gelatin were obtained from above rheological runs (Table 1). The gelling and melting temperatures of chicken head gelatin were in the range of approximately 22.7–27.5 °C and 29.1–34.2 °C, respectively. Gelatins extracted at 75 °C were observed to have the highest values of gelling and melting temperatures. This is followed by gelatin of G90/3> G90/6> G60/6 respectively. The melting temperature of gelatin G75/3 was significantly ($p < .05$) higher than gelatin of G75/6 but no significant ($p > .05$) different existed between their gelling temperatures. ETE of 75 °C for 3 and 6 h might encourage the yield of sufficient Proline rich regions in gelatin molecules that lead to a gelatin

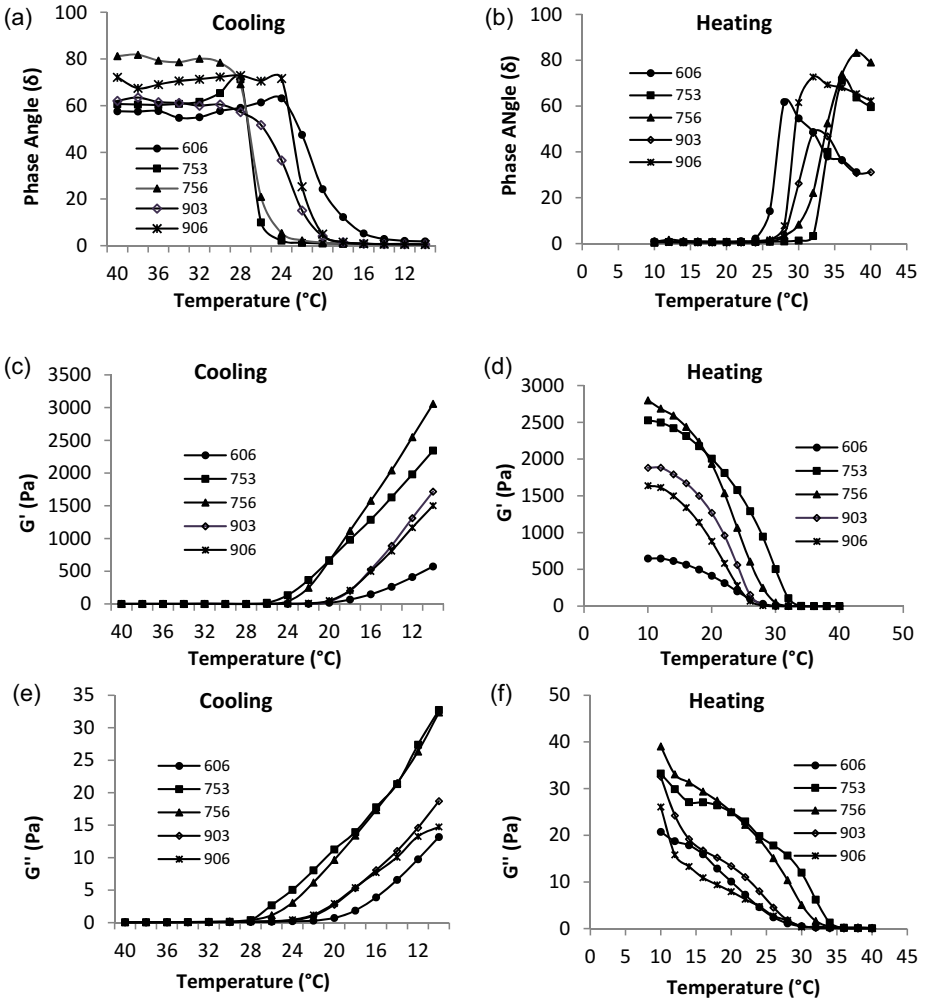


**Figure 2.** Changes in phase angle, elastic modulus (G′) and viscous modulus (G″) of 6.67% chicken head gelatin during cooling from 40 to 10 °C (a, c, and e) and heating from 10 to 40 °C (b, d, and f). Measurement of gelatin G60/3 cannot be performed as the gelatin failed to gel.

with good thermo-stability. Gelatin of G90/3 and G90/6 showed no different ($p > .05$) in their gelling temperatures. The interaction effect between ETE*ETI was not significant ($p > .05$) for gelling and melting temperatures of both gelatin.

Similar range of gelling and melting temperatures were reported for gelatin from chicken skin[4] and chicken and turkey head.[11] However, higher melting point (34.5–42.2 °C) was obtained from chicken head gelatin that extracted with Polarzyme 6.0 T.[13] Bovine and porcine gelatin were reported to have gelling and melting temperatures in the range of 20–25 and 28–31 °C, respectively.[27] Gelatin of G60/6 had the lowest melting and gelling temperatures and viscoelastic properties compared to other gelatin, which could be due to relatively low amount of extracted α-chains, resulting in less ability of renaturation. This behavior is also in good accordance with the low gel bloom observed for G60/6.

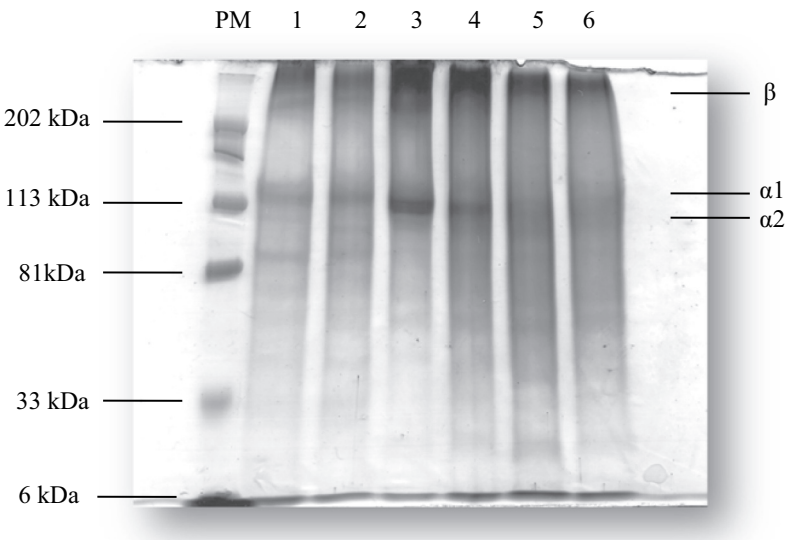


**Figure 3.** SDS-PAGE protein pattern of chicken head gelatins (PM: protein marker; 1: G60/3; 2: G60/6; 3: G75/3; 4: G75/6; 5: G90/3; 6: G90/6.

## *SDS page*

All extracted gelatin had α-chains as the major protein with a molecular weight approximately 113 kDa. A β-chain (~202 kDa) was present in all gelatins except for G90/3 and G90/6 (Figure 3). The disappearance of β-chain in G90/3 and G90/6 was likely due to the thermal hydrolysis at 90 °C. Higher ETE and ETI resulted in greater destabilization of the bonding between α-chains in the chicken head collagen. Similar decrease in β-band intensity was also reported for giant squid skin[28] and splendid squid[25] gelatin obtained from 80 °C extraction. Alpha chains were predominant in gelatin of G75/3 and G75/6. Higher amount of α-chains positively contributed to the bloom strength and viscoelastic properties of gelatin.[28] The high intensity of α-band in gelatin of G75/3 and G75/6 was in accordance with their great bloom strength (Figure 1). Similar α and β-chains were present in gelatin obtained from chicken feet,[29] duck feet,[30] chicken, and turkey head.[11]

## *Fourier transform infrared spectroscopy analysis*

FTIR spectroscopy has become one of the widely used rapid techniques for structural characterization of protein and peptide. Figure 4 shows that FTIR spectra of all the extracted chicken head gelatin were noticeable at amide region with identical pattern though some variations in intensity and wavenumber were observed. Four main characteristic bands were identified: amide I, II, III and A, with the amide I and II bands are the two most prominent bands correlated with the secondary structure content of protein. Amide I band (1600–1700 $cm^{-1}$) is linked to stretching vibrations of the C=O bond of the amide, while amide II band is associated primarily to bending vibrations of the N–H bond. The amide I, II, III, and A band for gelatin of G75/3 and G75/6 were noticeable at 1631.52 $cm^{-1}$; 1631.30 $cm^{-1}$, 1536.29 $cm^{-1}$; 1538.20 $cm^{-1}$, 1238.54 $cm^{-1}$; 1239.18 $cm^{-1}$, 3280.73 $cm^{-1}$; 3280.65 $cm^{-1}$, respectively. Meanwhile, the FTIR spectra of G90/3 and G90/6 showed amide I at 1632.85 $cm^{-1}$; 1634.61 $cm^{-1}$, amide II at 1532.74 $cm^{-1}$; 1533.02 $cm^{-1}$, amide III at 1237.96 $cm^{-1}$; 1237.75 $cm^{-1}$, and amide A at 3280.39 $cm^{-1}$; 3282.19 $cm^{-1}$, respectively. Gelatin extracted at 75 °C and 90 °C had higher peak intensity than those extracted at 60 °C. Amide III (1220–1320 $cm^{-1}$) represents the C-N stretching vibration and N-H deformation from the amide

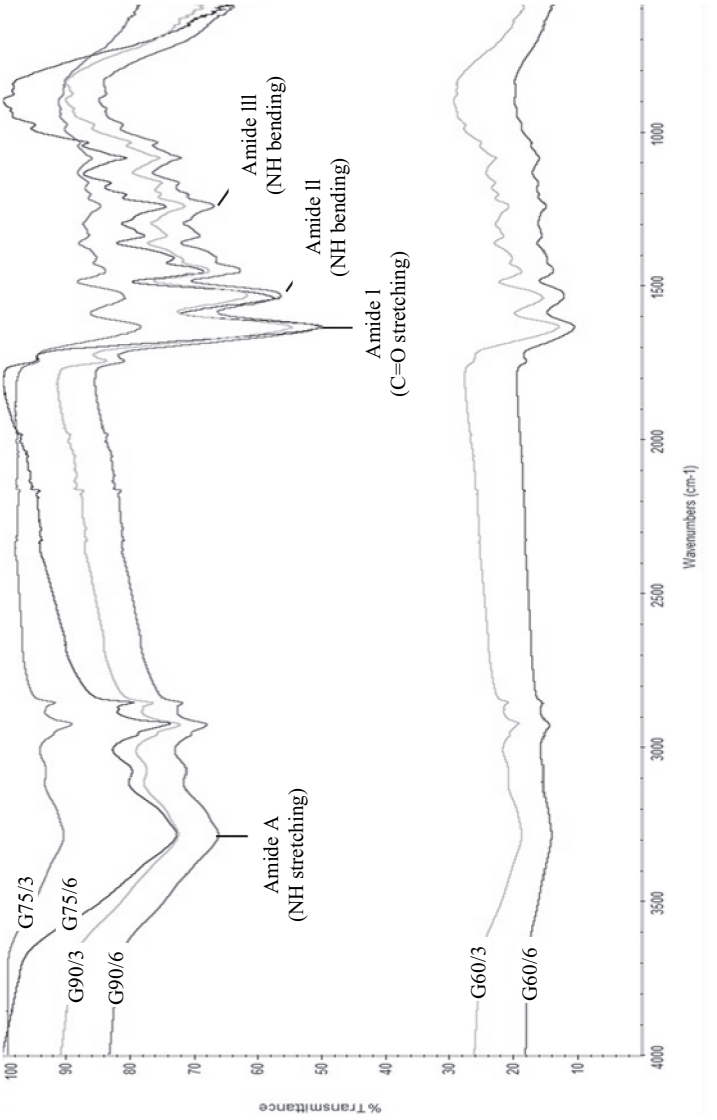


**Figure 4.** FTIR spectra of extracted chicken head gelatins.

**Table 2a.** Pearson correlation matrix of seven variables.

| | Temperature | Time | Yield | Bloom | L* | a* | b* |
|---|---|---|---|---|---|---|---|
| Temperature | 1 | | | | | | |
| Time | 0 | 1 | | | | | |
| Yield | 0.954** | 0.068 | 1 | | | | |
| Bloom | 0.613** | 0.505* | 0.702** | 1 | | | |
| L* | 0.086 | −0.671** | −0.030 | 0.020 | 1 | | |
| a* | 0.489* | 0.827** | 0.559* | 0.623** | −0.712** | 1 | |
| b* | 0.624** | 0.508* | 0.662** | 0.678** | −0.273 | 0.744** | 1 |

*P ≤ 0.05; ** P ≤ 0.01

linkages. This changes were related to the loss of triple helix state to a coil structure occurred in association with the treatment performed during transformation of collagen to gelatin. Amide A is associated with the free N-H stretching vibration observed in a limited area of 3400–3440 $cm^{-1}$. Amide A band for chicken head gelatin were shifted to a lower frequency at 3279–3282 $cm^{-1}$, indicating changes in collagen secondary structure and the involvement of the NH group in hydrogen bonding. Differences in spectra of chicken head gelatin thus indicate that the secondary structure of gelatin was influenced by the extraction time and temperature during transformation of collagen to gelatin. Similar amide bands were identified in amide region for gelatin extracted from chicken feet,[31] Pekin duck feet,[32] and quail bone.[3]

### *Amino acid composition*

Physico-chemical properties of gelatin especially bloom strength is affected by amino acid composition. Gelatin of G75/6 showed similar amino acid compositions with bovine gelatin, with the glycine as the predominant amino acid, followed by hydroxyproline and proline (Table 3). Gelatin of G75/6 had higher hydroxyproline (0.64%) than bovine gelatin. Higher amount of hydroxyproline in gelatin is often associated with stronger gel and better viscoelastic properties, which was confirmed by Ee et al.[12] as well as in gelatin of G75/6 that exhibited highest bloom strength and had great viscoelastic properties. Relatively high amount of imino acids in G75/6 may contribute to its strong viscoelastic properties by promoting triple helix formation and stabilization of gelatin at low temperature. Hydroxyproline could strengthen and stabilize the gel network by involving in hydrogen bonding with adjacent chains through its hydroxyl group.[33] Gelatin of G75/6 also had higher amount of hydroxyproline and imino acid than gelatin obtained from chicken head[11] and Chicken feet.[34]

## Conclusion

A total of six Type A gelatin with different bloom strength and yield were successfully extracted from chicken head at 60, 75, and 90 °C for 3 and 6 h, respectively. Gelatin yield, bloom strength, color values and viscoelastic properties were greatly influenced by the ETE and ETI. Longer extraction ETI resulted in higher yield and bloom value for extraction at 60 °C and 75 °C. However, at 90 °C, gelatin bloom, L* color values and viscoelastic properties were reduced compared to 75 °C. Gelatin of G75/3 and G75/6 exhibited high bloom strength of > 300 g, good yield, possesses good viscoelastic properties and had high gelling (27.3–27.5 °C) and melting temperatures (33.1–34.2 °C). Correlation and regression analysis were computed to develop prediction models for gelatin properties. Gelatin yield was significantly correlated with ETE (r = 0.954). All five regression models were significant ($p < 0.05$) and fitted highly for yield and a* with their $R^2$ values > 0.9. It can be concluded that the chicken head is a promising source to produce high bloom gelatin with great rheological and viscoelastic properties at ETE of 75 °C. This finding will enable the poultry industry to expand into a sustainable gelatin manufacturing which will benefit the whole supply chain and the ecosystem. Valorization of waste into

**Table 2b.** Multiple linear regression models fitting.

| Dependent variable | Yield | Bloom | L* | a* | b* |
|---|---|---|---|---|---|
| R-sq | 0.9090 | 0.6310 | 0.4503 | 0.9234 | 0.6470 |
| Rsq (adj) | 0.9040 | 0.5820 | 0.4159 | 0.9132 | 0.5999 |
| F-value | 160.49 | 12.84 | 13.11 | 90.42 | 13.75 |
| P value | 0.000 | 0.001 | 0.002 | 0.000 | 0.000 |
| S | 0.67813 | 72.2343 | 1.30673 | 0.239891 | 1.09215 |
| Constant | −3.9722 | −421 | 79.893 | −2.033 | 9.47 |
| Temperature | 0.16533 | 5.44 | - | 0.03161 | 0.0855 |
| Time | - | 36.6 | −0.743 | 0.4363 | 0.568 |
| Regression equation | Yield = − 3.97 + 0.165 Temp | Bloom = − 421 + 36.5 Time + 5.44 Temp | L = 79.893–0.743 Time | a* = −2.033 + 0.4363 Time + 0.03161 Temp | b* = 9.47 + 0.0855 Temp + 0.568 Time |

**Table 3.** Amino acid composition of chicken head gelatin G75/6 in comparison with commercial bovine gelatin.[12].

| Amino acid | G75/6 (%) | Bovine gelatin (%) |
|---|---|---|
| Hyp | 12.33 | 11.69 |
| Asp | 4.91 | 5.01 |
| Ser | 2.86 | 3.65 |
| Glu | 8.50 | 8.56 |
| Gly | 20.69 | 20.66 |
| His | 4.93 | 5.43 |
| Arg | 8.64 | 8.24 |
| Thr | 2.82 | 2.60 |
| Ala | 8.14 | 7.80 |
| Pro | 10.86 | 11.43 |
| Cys | 0.19 | 0.05 |
| Tyr | 0.71 | 0.51 |
| Val | 2.17 | 2.44 |
| Met | 1.46 | 1.28 |
| Lys | 3.77 | 3.83 |
| Ile | 1.47 | 1.62 |
| Leu | 3.15 | 3.11 |
| Phe | 2.42 | 2.10 |
| Total imino acid | 23.19 | 23.12 |

higher value products for the poultry industry and secondly it provides a cleaner alternative to existing gelatin with less issue of global warming as seen in the livestock industry.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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