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Research Article

Optimization Chicken Bones Gelatin Extraction Using Hydrochloric Acid Immersion and Multi-Stage Thermal Treatment

Deva Krisna Kadarani*, Akyunul Jannah

Study Program of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, East Java, Indonesia, 65144

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* Author Corresponding:
devakrisna@kim.uin-malang.ac.id

ABSTRACT

The growing poultry consumption rate yearly includes chicken with significantly increased amounts of by-products like skin and bones. Chicken bones are unused properly even if the bone is rich in collagen which is the primary material to produce gelatin. Gelatin usually is generated by bovine and porcine, but some health and religious issues have successfully forbid using both resources. Chicken bones could be an alternative material for gelatin production. This research aimed to investigate chicken bones as a substitute resource for gelatin manufacturing using hydrochloric acid submersion in collaboration with multi-stage thermal treatment. Chicken bones were proceeded in several steps such as degreasing, decontamination, resizing, demineralization using a hydrochloric acid immersion (1.50, 3.00, 4.50, and 6.00% v/v) for 24 and 48 hours, addition gelatin extraction using multi-stages thermal process (55, 65, and 75°C) for 4 hours each temperature subsequentially, evaporating, drying, and shaping a gelatin powder. This study produced type A gelatin which investigated yield number, moisture and ash content, gel strength, acidity level, and functional group using Fourier-transform Infrared (FTIR) spectroscopy. The sample gelatin has obtained at least 2% up to 8% yields. The moisture and ash contents were suitable to the commercial specification range, 4-12%, and 0.1-0.4%, respectively. The acid conditioning process has an impact on acidity with pH levels 4.40-5.44. Based on gelatin standards, this study declared that processing chicken bones using 6.00% hydrochloric acid submersion for 24 hours was optimal for gelatin extraction. Those optimal condition has formed gelatin with more than 8% yields. It was considered great gelatin with 260.57 g Bloom of gel strength and 90.18% of emulsion stability. Sample gelatin has a quite reasonable acidity level at 4.5. Protein structures confirmation using the vibration of the best gelatin sample has also shown essential components such as O-H, N-H, and C=O on the FTIR spectrum.

Keywords: chicken bones *(Gallus domesticus*), hydrochloric acid immersion, gel strength, emulsion stability, FTIR spectroscopy

1. Introduction

Annual worldwide poultry consumption especially chicken is fairly categorized as a high rate. Chickens (*Gallus domesticus*) are commonly found on daily food intake, where it has 144,874 thousand tonnes yearly consumption number in the last 5 years [1]. The chicken as a nutrition source around the globe has impacted a high number of chicken by-products on the surrounding, particularly skin and bones. Unutilized chicken bones as major waste food production have a considerable high amount of nutrition. Almost one-third of chicken bones are collagen as protein structural were able to partially hydrolyze and alter to be a gelation conformation [2].

Gelatin is a functional ingredient as a food hydrocolloid that can be gelling and encapsulating. These characteristics could be applied to broad areas such as medicine, pharmacy, food, photo, and cosmetic industry. Gelatin is generally produced from bovine and porcine, though emerging health issues like bovine encephalopathy cases. Common sources of gelatin are also controversial among the public because it is strictly forbidden in some religions [3]. Regarding those problems, researchers initiate numerous studies to substitute common gelatin resources and begin to look at other materials like fish and chicken. Fish might have the potential to be food allergens, then this study used chicken as a gelatin source.

Current research is using an acid method for manufacturing gelatin from chicken to produce gelatin type A. Main extraction solvent is hydrochloric acid (HCl). Earlier studies using the same solvent have been done by a few researchers and found good gelatin properties, 6% HCl immersion processed-gelatin of chicken bones has above 250 g Bloom value which describes how great its gel strength is [4]. Another researcher finding also indicated that 3% HCl submersion for 24 hours of chicken intestine could synthesize gelatin with 157.48 g Bloom as the best gel strength among other treatments (48 and 72 hours) [5]. This study used chicken bone and proceeded with different hydrochloric acid (1.50, 3.00, 4.50, and 6.00%) submersion for various times, which were 24 and 48 hours [6]. Similar research has been conducted using different HCl concentrations and immersion time, commencing gelatin process by degreasing, demineralization using HCl, neutralization, addition heating treatment, filtration, hot drying, and lastly forming a powder of dried gelatin [5].

Modification of the existing method has been done to process chicken bones for gelatin extraction. Particularly combining acid submersion and multi-stage thermal treatment to obtain the optimal method of gelatin extraction, three different temperatures are used respectively on a production cycle, 55, 65, and 75°C. This adjustment method has considerably improved gelatin quality in some commercial aspects such as proximate, gel strength, acidity level, emulsifying properties, and also protein conformation which is illustrated by a graph of Fourier-transform infrared (FTIR) spectroscopy.

2. Materials and Methods

2.1. Materials

The material utilized in this research was a by-product of chicken bones from traditional chicken noodles in Malang City, East Java, Indonesia, distilled water, hydrochloric acid, corn oil, KBr powder, and sodium bicarbonate.

2.2. Chicken Bones Gelatin Extraction Experimental Design

Accumulation of chicken bones in 2 kilograms was used to investigate the influence of acid pre-treatment and multi-stage thermal processing on gelatin extraction and characterization. Hydrochloric acid immersed on chicken bones was 1.50, 3.00, 4.50, and 6.00% (v/v). Moreover, gelatin chicken bones were prepared for 2 different lengths of acid submersion (24 and 48 hours) [7]. After the acid conditioning process, multi-stages of thermal treatment were conducted progressively, 55, 65, and 75°C for 4 hours [8]. Characterization of gelatin was carried out with three replications.

2.3. Acid Conditing Process

Chicken bones as many as 2 kilograms boiled at 70°C for 1 hour. The treated bone was filtered, washed, and sun-dried for 24 hours. Then, the bones were resized into smaller pieces with 2-4 cm. Dried-bones of chicken were immersed by hydrochloric acid solution 1.5 % (v/v). The ratio of samples and solvents was 1:4 (w/v). Duration for this treatment was 24 and 48 hours and stirred a few times. This procedure has triple repetition using several concentrations of hydrochloric acid, 3.00, 4.50, and 6.00% (v/v). Then, submersion of the bones on saturated sodium bicarbonate aimed to remove excessive acid. Moreover, neutral pH could be reached after watering its bones. In the end, the sample was dried at room temperature [7].

2.4. Multi-Stage Thermal Process

Acid-conditioned chicken bones were extracted from gelatin on the boiling water 55, 65, 75°C as a multi-stage thermal extraction method. Acidified bones as many as 250 grams immersed in boiling water 55°C for 4 hours. The acid treatment produced ossein residues. The ossein was immersed in the boiling water with a ratio of 1:4 (w/v) for 4 hours, and then it stirred a few times. The sample was filtered to get the first step of gelatin solution yield. First-step chicken bones were continued with re-immersion in boiling water at 65°C for 4 hours with a few times of agitation. Therefore, the first-step solution was filtered to get the second step of gelatin solution yield. Those solutions were mixed into the first step gelatin solution. Second-step bones were soaked into boiling water at 75°C for 4 hours and agitated a few times. Then, the mixture was filtered to obtain third-step of gelatin solution. The solution from third-step was mixed into first- and second-step gelatin solution [8].

2.5. Chicken Bones Gelatin Final Process

The mixture of gelatin solution was concentrated through vacuum frying at 50°C for 5 hours (gelatin concentration on 25-35%). Concentrated-gelatin extracts were cooled at 5°C for solidifying gelatin solution. The solid gelatin was dried at 45°C for 24 hours to reduce moisture content [9].

2.6. Yield of Gelatin

Gelatin yield was attained by comparison of weight between dried gelatin powder and raw material (cleaned chicken bones) following **Equation 1** [7].

$$\text{Yield (\%)} = \frac{\text{Weight of Dried Gelatin Powder}}{\text{Weight of Chicken Bones}} \text{ x } 100\% \quad (1)$$

2.7. Moisture Content

Five grams of sample was put into an evaporating dish. The sample on the dish was heated in an oven at 105°C for 30 minutes. Then, the heated sample was directly cooled in a desiccator and weighed. The moisture content of gelation was calculated using **Equation 2** [7].

$$\text{Moisture Content (\%)} = \frac{\text{Weight of Wet Sample - Weight of Dry Sample}}{\text{Weight of Wet Sample}} \text{ x } 100\% \quad (2)$$

2.8. Ash Content

A sample of 2 grams was put into an evaporating dish and furnaced at 600°C for 6 hours (indicator: sample turned white). Then, the heated sample is directly cooled in a desiccator and weighed. Ash content could be determined using **Equation 3** [7].

$$\text{Ash Content (\%)} = \frac{\text{Dry Weight}}{\text{Wet Weight}} \text{ x } 100\% \quad (3)$$

2.9. Acidity Level (pH)

Sample as many as 0.2 grams was dissolved into 20 mL distilled water at 80°C and homogenized using a magnetic stirrer. The solution was determined acidity level by pH meter (Mettler Toledo) at room temperature [10].

2.10. Gel Strength

Gelatin solution with concentration 6.67% (w/v) was prepared using distilled water. Gelatin mixture homogenization has been completed on agitation by magnetic stirrer and heating at 80°C for 15 minutes. Subsequently, the sample was decanted into standard bloom jars (a bottle with diameter 56-60 mm, height 85 mm), closed the jar, and left for 2 minutes. The sample was incubated at 10°C for 17±2 hours and gel strength was determined using a texture analyzer. **Equations 4** and **5** were used to calculate gel strength [10].

$$D \text{ (dyne/cm}^2\text{)} = F/G \text{ x } 980 \quad (4)$$

$$\text{Gel Strength (g Bloom)} = 20 + ((2{,}86 \text{ x } 10^{-3}) \text{ x } D) \quad (5)$$

Where, F= height of curve (Newton), D = gel strength (Dyne/cm^2), and G = Constanta (0.07).

2.11. Emulsion Stability

Sample as many as 10 grams was suspended into 150 mL distilled water. Corn oil as many as 150 mL was added into the sample and mixed for 2 minutes. The mixture was decanted into centrifuge tubes and heated up to 80°C for 30 minutes. The solution which did not form emulsion was separated and weighed. Emulsion stability was defined as a mixture that persistently formed emulsion after the heating process. Emulsion stability was calculated following **Equation 6** [11].

$$\text{Emulsion Stability (\%)} = \frac{\text{Mass of Emulsion}}{\text{Mass of Total Mixture}} \text{ x } 100\% \quad (6)$$

2.12. Identification of Gelatin Using FTIR Spectroscopy

Gelatin powder 0.02 grams was mixed with 0.01 grams of KBr powder and ground until smooth texture. Afterward, the mixture was pressed into a tray using a hydraulic pump to create a pellet. Characterization of the pellet was determined by FTIR spectroscopy (Shimadzu) at 4000-400 cm^{-1} wavelength [12].

3. Result and Discussion

Exploration of by-product chicken bones as gelatin raw material was impressive to study in the last decades. Manufacturing of gelatin required five basic steps - washing, extraction, purification, concentration, and drying [13]. In practice, the chicken bones were treated with several phases of gelatin production such as degreasing, decontamination, resizing, demineralization, chemical pretreatment, neutralization, multi-stage thermal, evaporation, drying, and shaping.

Two ways of pretreatment of chicken bones to produce a high yield of gelatin used acid solvent for type A and alkaline solvent for type B [14]. Production gelatin from chicken bones in this study was undertaken on acid condition pretreatment instead of alkaline because it took only a shorter extraction time than basic circumstances [6]. Gelatin as a nutritious protein was naturally derived from collagen, as a result of partial hydrolysis of parent collagen structures. Chemical pretreatments allowed releasing free alpha-chains through cleavages a sufficient number of covalent cross-links on collagen, to transform insoluble collagen into soluble gelatin [13]. This experiment used various concentrations of hydrochloric acid as pretreatment solutions, in 1.50; 3.00; 4.50; 6.00% (v/v). Harris [13] stated the higher concentration of hydrochloric acid, the more substantial number of cross-links broke-up assist to the shorter time is a prerequisite for gelatin extraction.

Table 1. Quality Parameters on Chicken Bones Gelatin Type A

Hydrochloric Acid Concentration (% v/v)	Soaking Time (Hour)	Quality Parameter					
		Yield (%)	Moisture Content (%)	Ash Content (%)	pH	Gel Strength (g Bloom)	Emulsion Stability (%)
1.50	24	2.00±0.45	6.18±0.85	0.25±0.02	5.44±0.03	224.01±0.00	63.28±8.31
	48	2.00±0.31	6.40±0.57	0.28±0.08	5.40±0.35	229.86±5.07	93.47±4.89
3.00	24	2.39±0.40	4.89±2.26	0.30±0.05	5.22±0.03	248.87±3.35	75.93±0.45
	48	2.06±0.37	8.81±2.63	0.36±0.06	5.03±0.02	251.80±2.53	85.86±2.52
4.50	24	5.45±0.58	11.59±1.69	0.18±0.01	4.79±0.02	256.92±8.77	74.51±2.84
	48	3.41±0.38	11.40±2.12	0.30±0.04	4.62±0.03	258.38±12.47	91.38±0.86
6.00	24	8.35±0.49	8.19±1.13	0.15±0.00	4.51±0.01	260.57±5.52	90.18±6.84
	48	6.94±0.35	10.80±1.02	0.17±0.01	4.40±0.01	265.69±4.39	98.02±2.31

Values of yield, moisture content, ash content, pH, gel strength, and emulsion stability showed mean±SD (Standard Deviation)

Soaking (immersion) time was an influential factor on gelatin number of yields. This study used two different soaking times, 24 and 48 hours. The result showed 24 hours acid pre-treatment was more optimum for gelatin production (**Table 1**). The earlier study also revealed that 24 hours acid submersion was the best condition in gelatin manufacturing with approximately 4% yields [5]. Gel strength aspect was affected by acid submersion time which found in this study that 48 hours acid-immersed was greater than 24 hours acid-immersed. Although previous research found out differently where 48 hours treatment has lower gel strength than 24 hours, it was an insignificant deviation between both treatments [5]. Therefore, another prior research using a different concentration of chloride acid for 24 hours immersion time was detected a parallel connection of acid percentages and gel strength. When 2% hydrochloric acid has only around 63 g Bloom, but on the contrary, 6% hydrochloric acid has 252.43 g Bloom [4].

Gelatin processing methodology in this current research has applied three divided temperature-dependent extraction processes called multi-stage thermal mechanisms. The commencing process started with only 55°C, then continued at 65°C and completed the last extraction with 75°C. During thermal-dependent chicken bones gelatin production, it was potentially dropping down gel strength aspect because of excessive hydrolysis within gelatin structure [13].

Other aspects such as moistures and ash content were also crucial issues for trading the final product of gelatin. This study found different values of moisture contents, from 4 up to 12% (**Table 1**). This condition also revealed a correlation between hydrogen bonds and non-helix structure stability of gelatin which were inversely proportional. When a gelatin product has a low moisture content, it meant fewer hydrogen bonds on it that influenced the stability of gelatin structure and altered it into a more stable complex. Hence, in this study, the most stable gelatin was gelatin produced by 3.00% hydrochloric acid immersion for 24 hours (**Table 1**). The ash content of the gelatin in this current study was just below 0.5% because of a phenomenon called deionization of mineral component chicken bones as long as strong acid immersion processing [3]. Gelatin industry-standard stated ash content on final product allowed with only less than 2% but could differ relies on regional regulatory [15].

Acidity characteristic of gelatin as the final product was also an essential aspect of broad application areas that the current study explored and determined various pH levels about 4.40 up to 5.44. The numbers matched commercial specifications on 3.8–5.5 [16]. This aspect described heterogeneity for positive charges distribution in gelatin molecules and altered pH level of gelatin. When hydrochloric acid concentration on processing was high, then more positive charges were in gelatin formation and distributed its divergency for enhancing the pH level of gelatin theoretically [3]. **Table 1** showed that 6.00% hydrochloric acid (48 hours) has the highest acidity level at pH 4.40. Similar to the previous experiment has a 3.42 up to 4.14 range of pH level for using chloride acid in chicken bones manufacturing [4].

This gelatin study focused on gel strength as the most powerful criteria in chicken bones gelatin quality. Rigidity and firmness were highly correlated with gel strength properties demonstrated as Bloom values. Bloom values represented the total energy and duration required to create a firmness gel [3]. It was also associated with the gelling capacity of gelatin as the final product that could be applied in wide-ranging areas [13]. Due to gelatin protein consisting of mostly α and β chains, the proportion of both components determined molecular weight distribution affecting the gelling power of gelatin [17]. According to this current study, Bloom values at 250 up to 300 g were suitable for commercial gelatin from 100 to 300 g. Previous observation of chicken bones gelatin with a similar method using 24 hours of 9.00 % phosphoric acid was 372.29 g Bloom value [18]. The other investigation found 252.43 g Bloom for gel strength of 6.00 % chloride acid submersion using different process steps [4].

Emulsion stability of gelatin was important information for the market as an emulsifier and stabilizer. Chicken bones gelatin proceeded with 48 hours immersion of 6.00% hydrochloric acid has the best emulsion stability on about 98%. Furthermore, on 24 hours of treatment of 6.00% hydrochloric acid, it has just 90% emulsion stability which remained considered the best quality of gelatin product (**Table 1**). The phenomenon of surface-active related to molecules-charges, non-ionic group, and molecular weight distribution could be explained by how gelatin stabilized an emulsion [13]. Interfacial tension in the middle of two different phases was claimed as an emulsion stability influential factor that has a power or tendency to break them apart. Increasing their tensions created a greater susceptibility of dissociation in each phase shown by chicken bones gelatin emulsified corn oil and water in this experiment. Reduction of interfacial tension led to stable emulsion oil-in-water (O/W) [19]. The mechanism starting with gelatin imbibes oily dispersion of corn oil, then initiated synthesis of a thin film to be both protector and stabilizer for that emulsion. It occurred through retarding breakdown via coalescence, a fusion of oil drops into a separated-liquid phase [3]. As additional information, there was another factor that feasibly diminishes interfacial tension of emulsification, such as a heat treatment [20].

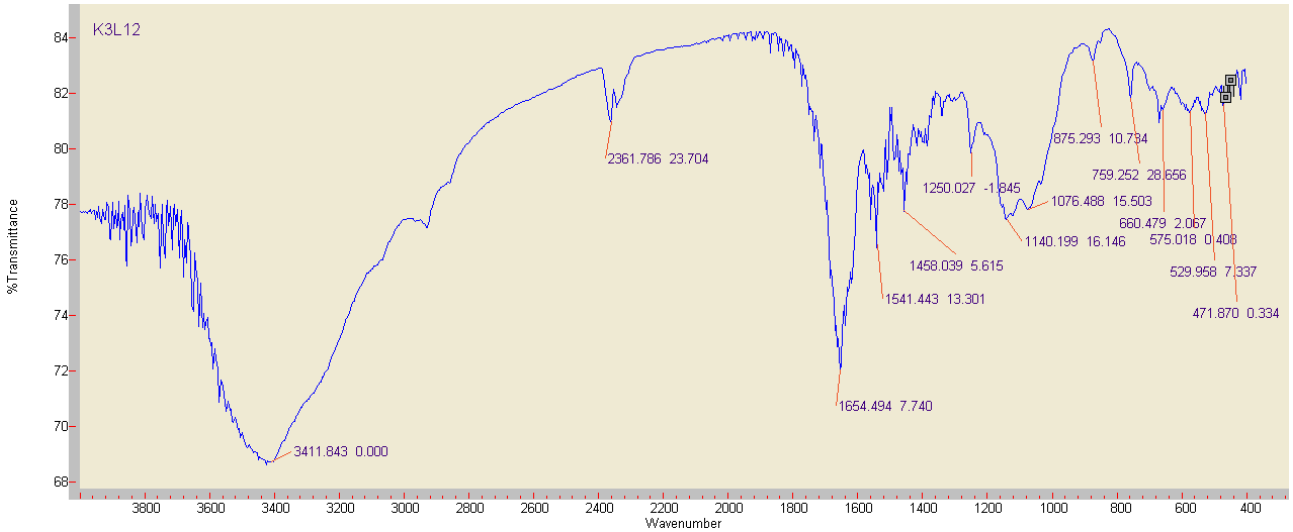

Figure 1. FTIR spectrum of gelatin from chicken bones gelatin with 6.00% hydrochloric acid pretreatment for 24 hours.

Investigation of the functional group in the gelatin sample (6.00% hydrochloric acid; 24 hours) was conducted using FTIR spectroscopy (**Figure 1**). Gelatin was the polymerization of amino acid monomer which is primarily composed of N-H, C-H, C=O, and O-H [21]. The highest frequency region was detected in 3411.84 cm^{-1} representing N-H bond (amide) with participating in hydrogen bonding affected on the broadening band of N-H. The broad bond was probably damaged from temperature treatment during gelatin processing [22]. Furthermore, a similar pattern found on croaker fish skin gelatin has a wavenumber of 3423.03 and 2925.31 cm^{-1} allocated as amide A and amide B, respectively (**Table 1**). Component of amide A on the peak was caused by OH and NH stretching, then amide A combined with CH_2 stretching peak also seen on the fish skin gelatin spectra. On the other hand, NH stretching has also been founded on amide B. Spectra of standard

gelatin have shown double bond CO stretching of amide I at 1640 cm^{-1}, while on this study, it appeared at 1654.49 cm^{-1} of chicken bones gelatin spectra. Then, in standard gelatin peak, N-H bending of amide II and amide III showed at 1540 and 1250 cm^{-1}, respectively, where sample gelatin has 1511.44 and 1250.02 cm^{-1}. Focusing on the existence of important regions of chicken bones gelatin infrared frequency was obvious evidence for verifying protein conformation on the samples.

Table 2. Comparison Absorption Region of Various Gelatins

Functional Group	Type of Amide	Wavenumber (cm^{-1})		
		Standard Gelatin [22]	Fish Skin Gelatin [17]	Chicken Bones Gelatin (This Study)
N-H and O-H bond	Amide A	3350	3423.02	3411.84
N-H bond	Amide B	2922	2925.31	2361.77
C=O stretching	Amide I	1640	1642.05	1654.49
N-H bending	Amide II	1540	1561.61	1511.44
N-H bending	Amide III	1240	1242.75	1250.02

4. Conclusion

This study concluded that 6.00% hydrochloric acid immersion for 24 hours and multi-stage thermal treatment were the optimal chicken bones gelatin type A extraction methods. This research produced gelatin with a good grade for commercialization that yield percentage was more than 8%. It also obtained great gel strength with more than 260 g Bloom and emulsion stability of 90.18%. The gelatin has a good acidity level at pH 4.51. Based on this research, different hydrochloric acid concentrations affected quality assurance aspects such as yields, acidity level, gel strength, emulsion stability, and gelatin conformation.

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