ALCHEMY : JOURNAL OF CHEMISTRY

Research Article

Characterization of Gelatin Profile of Chicken Broiler (*Gallus domestica*) Bone Using SDS-PAGE Electrophoresis

Dewi Yuliani, Dhienda Risa Awalsasi, Akyunul Jannah

Chemistry Department, Science and Technology Faculty, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Malang, Indonesia, 65144

ARTICLE INFO

Article History
Revised 11 March 2019
Accepted 30 March 2019
Available online 23 July 2019

* Email of author corresponding:
dewi.yuliani@kim.uin-malang.ac.id

ABSTRACT

Gelatin, a proteinaceous additive, is obtained from hydrolysis of collagen in the bone, hide and skin of animals. As natural product, gelatin has been applied in many industries with various functions. This study attempt to characterize gelatin profile of broiler chicken (*Gallus domestica*) using SDS-PAGE electrophoresis. The chicken bone was pretreated using a strong base, sodium hydroxide, producing type B gelatin. The gelatin was purified through precipitation using the variation of ammonium sulfate concentrations (40-70%) and dialysis using cellophane membrane. The purified gelatin was characterized through SDS-PAGE electrophoresis. Based on electrophoresis visualization, reduction of band intensity by ammonium sulfate 40% showed removal of small peptide fragments. The remained gelatin showed two major bands, α-chains and a β-chain with the respective molecular weight of ~135 and ~245 kDa. The protein content of the unpurified gelatin (E1) was 71.65±0.60 mg/L. The purified E1 gelatins by 40-70% of ammonium sulfate addition contained 61.42±3.90, 60.45±1.36, 59.89±0.24, and 55.32±1.05 mg/L of protein concentration, respectively.

Keywords: chicken bone, gelatin profile, protein electrophoresis

1. Introduction

Gelatin is one of natural products produced of proteinaceous material and applied extensively in food, pharmaceutical, photographic, textile and paper industries [1, 2]. In food industry, gelatin is used as a gelling agent, whipping agent, stabilizer and emulsifier [3]. Gelatin is manufactured through extraction of collagen occurs in various raw material sources such as bone, cattle hides and pigskin. The raw material of gelatin was traditionally pretreated with acid or alkaline solvent to remove non-collagenous components. The material was extracted by water at high temperature and adjusted to neutral condition to obtain gelatin solution. The solution is filtered to remove impurities from the material and extraction process [1, 4]. The obtained gelatin has various functional properties, for instance, gel strength, gel forming, emulsifying, viscosity, and foaming properties.

The properties of gelatin correspond to gelatin structure in the composition. The gelatin comprises of a mixture of different polypeptide chains including α-chain, β-chain (dimer of α-chain) and γ-chain (trimer of α-chain) with a broad range of molar mass (molecular weight) [3]. Extracted gelatin may have low and high molecular weight depending on the process extraction. The gelatin containing high molecular weight possesses better functional properties than that of low molecular weight. The peptide fragments (low molecular weight) are associated with lower viscosity, melting point and Bloom strength of gelatin. These molecular characteristics contribute to their functional properties [5].

The highly purity of gelatin is required in some industries. The extracted gelatin may contain the number of contaminant which is needed to be purified. Ultrafiltration is a common purification method of gelatin that has been conducted and patented since 1990s [1, 2, 4]. The principle of UF is separation of solutions under pressure through semipermeable membrane and capable to remove colloidal materials, organic polymeric molecules, and inorganic polymeric molecules [6]. In this research, gelatin purification was conducted with two different methods such as precipitation and dialysis. These methods have been applied in many enzymes to obtain purified product [7]. The objective of this study was to characterize gelatin profile after purification.

2. Materials and Methods

2.1. Materials

Broiler chicken bone (*Gallus domestica*) was purchased from market in Malang. The chemicals used were sodium hydroxide, 5% acetic acid, distilled water, ammonium sulfate (40-70%), cellophane membrane, 0.5 M phosphate buffer pH 7, 0.2 M phosphate buffer pH 7, and commercial gelatin (bovine).

2.2. Gelatin Preparation

Broiler chicken bone (250 g) was pretreated by soaking in sodium hydroxide 5% for 2 days. The ratio of bone to sodium hydroxide was 1:4(v/v). Ossein (pretreated bone) was neutralized by tap water and acetic acid 5%. The ossein was gradually extracted by water in three different temperatures – 55, 65, and 75°C – for 4 h in each. E1, E2, and E3 representing extraction were in 55, 65, and 75°C, respectively. All gelation extracts were dried using oven [8, 9]. Gelatin of 100 mg was dissolved in 10 mL distilled water at 50°C and homogenized by vortex [10]. All gelatins were identified using FTIR spectroscopy.

2.3. Gelatin Purification

Purification of gelatin was conducted by ammonium sulfate precipitation. Ten milliliter of gelatin solution was added with ammonium sulfate in the various concentrations (40-70%). The mixture was left for overnight at ±4°C with continuous stirring to precipitate the gelatin. The mixture was then centrifuged for 30 min, 5000 rpm, to obtained partial purity of gelatin precipitate. The gelatin was further purified by dialysis using cellophane membrane. The gelatin precipitate was dissolved in 5 mL phosphate buffer pH 7 and then the suspense was placed in cellophane membrane. The membrane was immersed in 100 mL phosphate buffer (0.2 M, pH 7) and stirred at 100 rpm for 12 h, ±4°C [11].

2.4. Determination of Profile Gelatin using SDS-PAGE electrophoresis

SDS-PAGE electrophoresis was performed based on Biorad Procedure [12]. The acrylamide concentration used was 12% for the separating gel and 4% for the stacking gel. For sample preparation, 15 µL gelatin solution was mixed with 15 µL buffer sample. The samples were heated at 95°C for 5 min before electrophoresis. Protein bands were stained with comassie brilliant blue R-250. Relative molecular weight of the sample was estimated through comparison with molecular mass standard marker in the range of 10 – 250 kDa (iNtRON Biotechnology, Korea).

2.5. Protein Measurement

Protein concentration was measured using Lowry method [13]. A standard curve was made of BSA (0; 0.01; 0.02; 0.04; 0.06; 0.08 and 0.10 mg/mL) and the absorbance was read at 654 nm. Determination of protein content was conducted on unpurified and purified chicken bone gelatin.

3. Results and Discussion

3.1. Sample Preparation

Gelatin was produced from pretreated broiler chicken bones with 5% sodium hydroxide for 2 days. The gelatin was extracted gradually using hot water in different temperatures. The yield of the gelatin extraction was 4.156, 2.247, and 2.228 g for 55, 65 and 75°C extraction temperature, respectively. The gelatin extracted at 55°C (E1), 65°C (E2), and 75°C (E3) exhibited protein content about 71.65±0.60, 61.03±0.49, and 57.01±1.34 mg/L, respectively (**Figure 1**). The commercial gelatin contained protein two times (150.90±2.99 mg/L) higher than chicken bone gelatin. For purification purpose, solid gelatin was dissolved in distilled water at 50°C.

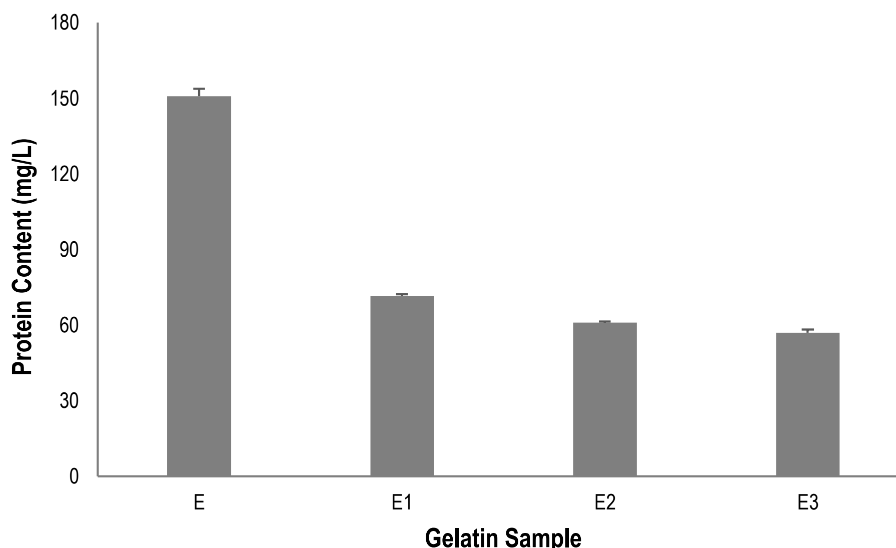

Figure 1. Protein content in chicken bone gelatin. E = commercial gelatin, E1 = gelatin of 55°C extraction temperature, E2 = gelatin of 65°C extraction temperature, and E3 = gelatin of 75°C extraction temperature.

FTIR spectra of gelatin gradually extracted at different temperatures from broiler chicken bone is depicted in **Figure 2**. FTIR spectroscopy has been used to monitor the functional groups and secondary structure of gelatin. The typical major amide bands (Amide A, B, I, II, and III) for gelatin were present in the spectra, even though some variations in wavenumber and peak height were detected. The amide I peaks found at 1653.86, 1650.38 and 1650.86 cm^{-1} for E1, E2, and E3 respectively represented chicken bone gelatin structure in the coil form. This result was in agreement with Muyonga [14] that vibration band of amide I between 1,600 – 1,700 cm^{-1} formed spiral structure of gelatin. The peaks of amide II band were noticeable at the wavenumber of 1559.39, 1558.27, and 1542.17 cm^{-1} revealed the change of gelatin secondary structure. The peaks of amide III were detected around the wavenumber of 1241.88, 1244.88, and 1240.80 cm^{-1} for E1, E2, and E3 respectively. The peaks reflected the involvement of NH bending in the random coil of gelatin structure. In addition, amide A band representing the involvement of the NH group of peptide coupled with hydrogen bonding in the primary structure of gelatin appeared at 3417.24, 3456.62, and 3423.05 cm^{-1} for E1, E2, and E3 respectively. According to Muyonga et al. [14], a free NH stretching vibration is normally found in the range of 3400-3440 cm^{-1}. The amide B peaks show the interaction of –NH_3 groups between peptide chains. The wavenumber of amide B peaks in this research was 2922.12, 2925.64, and 2927.62 cm^{-1} for E1, E2, and E3 respectively.

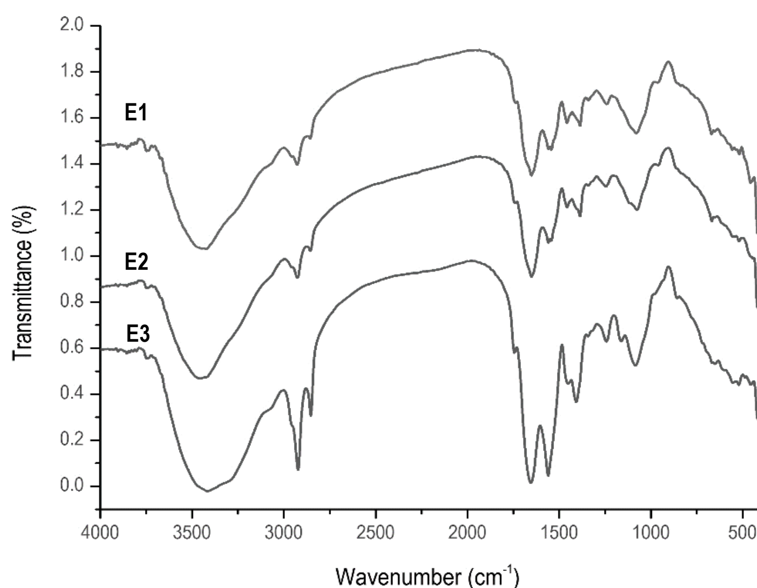

Figure 2. Fourier transform infrared (FTIR) spectra of chicken bone gelatin at different temperature extraction.

Characterization of chicken feet gelatin was conducted by de Almeida et al. [15]. The FTIR spectra of chicken gelatin showed the vibration peak at the wavenumbers of 1652.01 cm^{-1} to the amide I, of 1539.87 cm^{-1} to the amide II, of 1241.29 cm^{-1} to the amide III, of 3399.56 cm^{-1} to the amide A and of 2923.72 cm^{-1} to the amide B. Commercial gelatin showed FTIR spectra of amide I at 1651.32 cm^{-1}, amide II at 1556.53 cm^{-1}, amide A at 3391.84 - 3467.09 cm^{-1} and amide B at 2921.49 cm^{-1}. The wavenumber of the amide peaks in chicken feet gelatin is similar to that of broiler chicken bone gelatin in this research.

3.2. Protein Profile of Chicken Bone Gelatin

Gelatin purification was performed only on E1 sample by precipitation and dialysis method. During precipitation process, the gelatin precipitate in small part and the partial gelatin gathered to form a layer on the surface. The precipitate gelatin still contained high amount of ammonium sulfate which is needed to purify by dialysis. Protein pattern of unpurified and purified gelatin is shown in **Figure 3**.

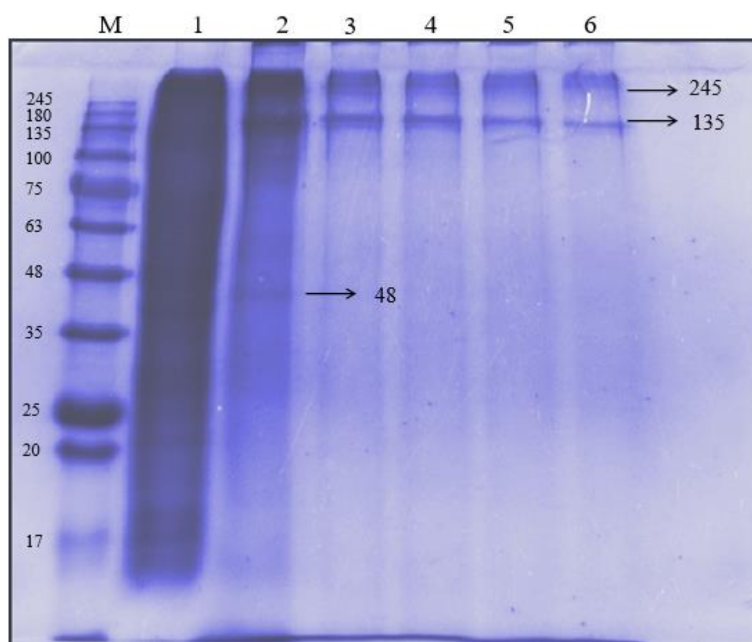

Figure 3. Protein pattern of unpurified and purified chicken bone gelatin. M = protein marker, 1 = commercial gelatin, 2 = unpurified gelatin, 3 = gelatin purified with 40% ammonium sulfate, 4 = gelatin purified with 50% ammonium sulfate, 5 = gelatin purified with 60% ammonium sulfate and 6 = gelatin purified with 70% ammonium sulfate.

Gelatin is made up of amino acids joining to form linear polymer varying in molecular weight from 15 to 300 kDa. Gelatin comprises of α-chains, β-chains (dimer of α-chain) and γ-chains (trimer of α-chain) and some lower molecular fragments [3]. Molecular weight determination of chains and fragments in gelatin was used as a marker for molecular weight of standard protein. Commercial gelatin was used as comparison to chicken bone gelatin.

Unpurified chicken bone gelatin exhibited a broad range of molecular weight, from ~15 to ~300 kDa (**Figure 3**, Line 1). Two major bands with high intensity in chicken bone gelatin are composed of α-chains (α_1 upper and α_2 lower), and a β-chain. The respective α-chain and β-chain has ~135 and ~245 kDa molecular weight. The findings are in agreement with Du et al [16], and Abdullah [17]. Small peptide fragments were also found with molecular weight under 100 kDa and the highest intensity of small peptide fragment had ~48 kDa molecular weight.

The broad range of molecular weight of the extracted gelatin indicates that the unpurified gelatin consisted of low and high molecular weight protein. Molecular weight of gelatin produced is affected by extraction temperature. In this research, extraction temperature of 55-75°C is sufficient to degrade α-chain, β-chain and γ-chain to form various lengths of product. According to Muyonga et al. [14], elevated temperature promotes the cleavage of gelatin chains leading to the formation of lower molecular components.

The purification of chicken bone gelatin was conducted with different concentrations of ammonium sulfate (40-70%). Lines 3-6 (**Figure 3**) showing purification can remove small peptide fragments significantly. The purification can be observed by the losing of small peptide fragments, especially 48 kDa fragments. The reduction intensity of α-chains and β-chains bands also indicates the loss of gelatin fractions after purification.

3.3. Protein Measurement by Lowry Method

The protein content of purified gelatins was 61.42±3.90, 60.45±1.36; 59.89±0.24; and 55.32±1.05 mg/L using 40, 50, 60, and 70% ammonium sulfate concentration, respectively (**Figure 4**). Higher concentration of ammonium sulfate produced lower protein content in gelatin. However, the reduction of protein concentration in gelatin was no significantly different. Ammonium sulfate of 40% was chosen as the best concentration to purify gelatin due to the concentration enable to eliminate small peptide fragment drastically without losing the major bands during purification. The losing protein in purified gelatin was confirmed by a weak intensity of protein band shown in **Figure 3**.

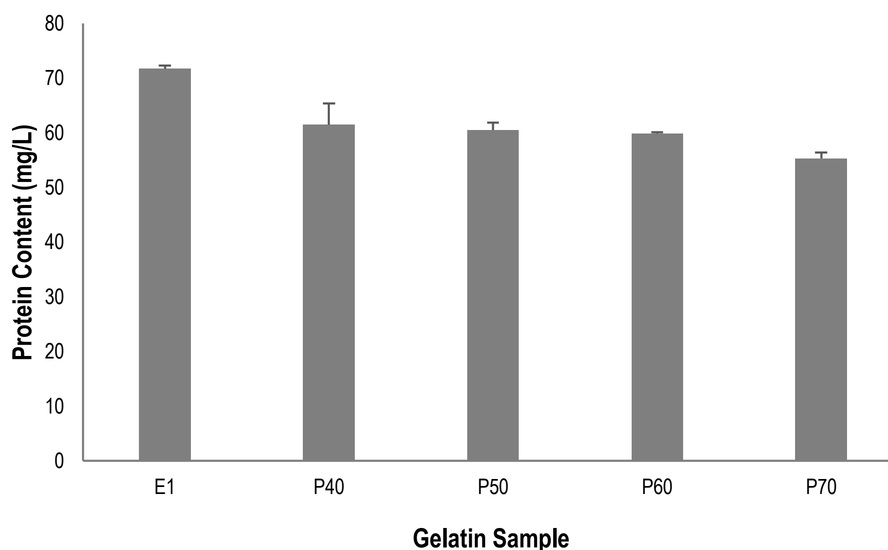

Figure 4. Protein content of unpurified and purified gelatin. E1 = unpurified gelatin, P40 = gelatin purified using 40% ammonium sulfate, P50 = gelatin purified using 50% ammonium sulfate, P60 = gelatin purified using 60% ammonium sulfate, P70 = gelatin purified using of ammonium sulfate.

4. Conclusion

Gelatin purification by precipitation and dialysis enable to remove small peptide fragments of gelatin. The ammonium sulfate of 40% optimally reduced small peptide fragments and maintained two major bands, α-chains and β-chain with molecular weight of ~135 and ~245 kDa, respectively. Precipitation with high concentration of ammonium could remove insignificant concentration of α-chains and β-chains. The extracted gelatins (E1, E2, and E3) contained 71.65±0.60, 61.03±0.49, and 57.01±1.34 mg/L of protein concentration, respectively. The protein content in the E1 gelatin after purification for the respective ammonium sulfate concentration of 40%, 50%, 60% and 70% was 61.42±3.90, 60.45±1.36; 59.89±0.24; and 55.32±1.05 mg/L.

References

[1] B. Chakravorty & D. P. Singh, “Concentration and Purification of Gelatin Liquour by Ultrafiltration,” *Desalination*, vol. 78, pp. 279-286, 1990.

[2] K. Christensson, S. Levin, M. Jansson, & M. Andeasson, “Method for Producing and Purifying Gelatin,” United State Patent 20070249811A1, 25 Oct., 2007.

[3] A. A. Mariod & H. F. Adam, “Review: Gelatin, Source, Extraction and Industrial Applications,” *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 12, no. 2, pp. 135-147, 2013.

[4] B. Dutré & G. Trägårdh, “Purification of Gelatin by Ultrafiltration with a Forced Solvent Stream along the Membrane Permeate Side: An Experimental Approach, *Journal of Food Engineering*, vol. 25, pp. 233-244, 1995.

[5] M. Nagarajan, S. Benjakul, T. Prodpran, P.Songtipya, & H. Kishimura, ”Characteristics and Functional Properties of Gelatin from Splendid Squid (*Loligo formosana*) Skin as Affected by Extraction Temperature,” *Food Hydrocolloids*, vol. 29, no. 2, pp. 389-397, 2012.

[6] K. Scott, “Introduction to membrane separations” in *Handbook of Industrial Membrane*, 1st ed. Oxford: Elsevier Science Publishers Ltd., pp. 3-185, 1995.

[7] E. Dako, A. M. Bernier, A. T. Dadie, & C. K. Jankowski, “The Problems Associated with Enzyme Purification” www.intechopen.com, 2012. [Online]. Available: http://cdn.intechweb.org/pdfs/28766.pdf, [Accessed: January 20, 2019].

[8] R. A. Rachmania, F. Nisma, & E. Mayangsari, “Ekstraksi Gelatin dari Tulang Ikan Tenggiri melalui Proses Hidrolisis Menggunakan Larutan Basa,” *Farmasi*, vol. 10, no. 2, pp. 18-28, 2013.

[9] I. N. Simpen, N. M. Puspawati, & A. A. I. R. Prabawanti, ”Karakteristik Mutu Gelatin dari Kulit Ayam Broiler melalui Proses Perendaman Kombinasi Asam-Basa,” *Jurnal Kimia*, vol. 10, no. 2, pp. 204-211, 2016.

[10] M. A. Aina, I. Amin, R. M. Hafidz, & Yakoob, “Identification Polypeptide Biomarkers of Porcine Skin Gelatine by Two-Dimensional Electrophoresis,” *International Food Research Journal*, vol. 20, no. 3, pp. 1395-1399, 2013.

[11] A. K. Wardani & O. L. Nindita, “Purification and Characterization of Protease from Protease-producing Bacteria Isolated from Tofu Whey,” *Jurnal Teknologi Pertanian*, vol. 13, no. 3, pp. 149-156, 2012.

[12] Bio-Rad Laboratories Inc., “A Guide to Polyacrylamide Gel Electrophoresis and Detection,” Bio-Rad Laboratories, Life Science Group, 2019. [Online]. Available: http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf. [Accessed: February 12, 2018].

[13] O. H. Lowry, N. J. Rosebrough, A. L. Farr, & R. J. Randall, “Protein Measurement with the Folin Phenol Reagent,” *the Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265-275, 1951.

[14] J. H. Muyonga, C. G. B. Cole, & K. G. Doudu, “Fourier Transform Infrared (FTIR) Spectroscopic Study of Acid Soluble Collagen and Gelatin from Skins and Bones of Young and Adult Nile Perch (*Lates niloticus*),” *Food Chemistry*, vol. 86, pp. 325-332, 2004.

[15] P. F. de Almeida, S. C. S. Lannes, F. A. Calarge, T. M. B. Farias, & J. C. C. Santana, ”FTIR Characterization of Gelatin from Chicken Feet.” *Journal of Chemistry Engineering*, vol. 6, pp. 1029-1032, 2012.

[16] L. Du, Z Khiari, Z. Pietrasik, & M. Betti, “Physicochemical and Functional Properties of Gelatins Extracted from Turkey and Chicken Heads,” *Poultry Science*, vol. 92, no. 9, pp. 2463-2474, 2013.

[17] M. S. P. Abdullah, M. I. Noordin, S. I. M. Ismail, S. Nyamathulla, M. Jasamai, L. K. Wai, N. M. Mustapha, & A. F. Shamsuddin, “Physicochemical Evaluation and Spectroscopic Characterisation of Gelatin from Shank and Toes of *Gallus gallus domesticus*,” *Sains Malaysia*, vol. 45, no. 3, pp. 435-449, 2016.