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Research Paper / Araştırma Makalesi

Collagen/Gelatin Extraction from Poultry Skin and Mechanically Deboned Meat (MDM) Residues

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ABSTRACT

Collagen is a structural protein found naturally in high amounts in poultry skin and bones. Gelatin is obtained by a partial hydrolysis of collagen under controlled conditions. It is a pure protein with many functional and technological properties such as gelation, thickening, film-forming and emulsification. Besides skin and bones, mechanically deboned meat (MDM) residues are considered good sources of collagen as well. This study aimed to extract collagen and gelatin from poultry skin and MDM residues of neck, shinbone, breastbone, wingtip, shanks, upper/lower backbone and their mixture through pre-treatment (using with dilute alkali and acid), demineralization, degreasing and thermal extraction processes, and evaluate their properties comparatively. Based on Kjeldahl analysis, the protein content of poultry skin was about 15% and that of the MDM residues varied between 15-21% before the extraction process. The SDS-PAGE profiles of the extracted collagen in skin and MDM residues comprised γ, β, α, and sub-α chain protein units. Collagen solution of the upper backbone and mixed MDM residues had the highest protein content with 11.98 mg/mL and 11.33 mg/mL, respectively. The extraction yield of collagen and gelatin significantly differred ($p<0.05$) within the range between 1.32 and 2.16%, and 1.03 and 1.89%, respectively. The viscosity of collagen/gelatin solutions decreased with an increase in shear rate and gelatin obtained from mix MDM residues indicated higher viscosity than that of skin. Results of this study showed successful recovery of collagen and gelatin from poultry processing by-products and residues, which could contribute to the production of high value-added alternative sources for various applications.

Keywords: Poultry, Collagen, Gelatin, Mechanically deboned meat (MDM), Extraction, Electrophoresis

Kanatlı Deri ve Mekanik Olarak Eti Sıyrılmış Kemik (MKS) Artıklarından Kollajen/Jelatin Ekstraksiyonu

ÖZ

Kollajen kanatlı deri ve kemiklerinde oldukça yüksek oranda bulunan yapısal bir proteindir. Kollajenin kontrollü şartlar altında kısmi hidrolizi ile jelatin elde edilir. Jelatin jelleştirme, kıvam artırma, film oluşturma ve emülsifiye etme gibi birçok fonksiyonel ve teknolojik özelliğe sahip saf bir proteindir. Tavuk derisi ve kemiklerinin yanısıra, mekanik olarak eti sıyrılmış kemik (MKS) artıkları da iyi bir kollajen kaynağı olarak kabul edilir. Bu çalışmanın amacı kanatlı deri ve boyun, incik kemiği, göğüs kemiği, kanat ucu, ayak takozu, alt/üst sırt kemiği ve bu kemiklerin karışımının MKS artıklarında, ön işlem (seyreltik alkali ve asit ile muamele), mineral ve yağdan arındırma ve ısıl ekstraksiyon işlemleriyle kollajen ve jelatin ekstrakt etmek ve karşılaştırmalı olarak bazı özelliklerini incelemektir. Khjeldahl analizine göre, örneklerin ekstaksiyon öncesi protein içerikleri tavuk derisinde yaklaşık %15 ve MKS artıklarında ise %15-21 oranları ile önemli farklılıklar ($p<0.05$) göstermektedir. Deri ve MKS artıklarından ekstrakt edilen kollajenin SDS-PAGE profilleri γ, β, α, and sub-α zinciri protein unitelerini içermektedir. Sırt kemiği ve kemik karışımı MKS

artıklarından elde edilen kollajen solüsyonu en yüksek protein içeriğine sahiptir. Kollajen ve jelatin extraksiyon verimi sırasıyla %1.32 ila 2.16 ve %1.03 ila 1.89 arasında değişmektedir. Kollajen/jelatin viskozitesi artan kayma oranı ile azalmıştır ve karışım MKS artıklarından elde edilen jelatin deriden elde edilen jelatinden daha yüksek viskoziteye sahiptir. Bu çalışmanın sonuçları çeşitli uygulamalar için yüksek katma değerli alternatif kaynakların üretimine katkı sağlayabilecek, kanatlı işleme yan ürünleri ve artıklarından kollajen ve jelatinin başarılı şekilde geri kazanımını göstermiştir.

Anahtar Kelimeler: Kanatlı, Kollajen, Jelatin, Mekanik olarak eti sıyrılmış kemik (MKS), Ekstraksiyon, Elektroforez

INTRODUCTION

Collagen is the main fibrous protein in the skin and tendons of animals [1]. It is a high molecular weight protein and insoluble in water due to its hydrophobic structure [2]. Collagen-containing tissues such as skin, bones and tendons are hydrolyzed at temperatures above 40°C after treatment with dilute acid and alkali. The fibrillar structure of collagen is irreversibly broken down through these treatments [3, 4]. Collagen contains 9% hydroxyproline, 11% alanine, 12% proline and 35% glycine [5]. Proline and hydroxyproline are not found in other proteins as much as collagen [6]. These two amino acids are low in cold-blooded animals and high in warm-blooded animals [5]. The gelatin of cold-blooded animals with a low content of proline and hydroxyproline shows a poor gelling property compared to the gelatin of warm-blooded animals of the same molecular weight [5].

Upto date, nearly 28 collagen types have been identified. Among those, type I collagen is commonly found in skin, bones, tendon, ligaments and organs, whereas type II collagen specifically present in cartlige [6]. Gelatin is formed due to partial hydrolysis of collagen from slaughtering and processing by-products of cattle, pigs, fish and poultry. Unlike collagen, it dissolves in water [7]. Gelatin consists of 7% hydrogen, 17% nitrogen, 25% oxygen and 51% carbon molecules [8]. The protein content of gelatin is between 85 and 92% and the rest is composed of minerals, water and salts [9]. Gelatin is widely used in industry due to its functional properties such as thickening, water-retaining, gelling, and adhesiveness. Gelatin can be considered a multi-functional structural form of an ordinary collagen molecule [10].

Gelatin production is achieved in the following basic stages: treatment of collagen-containing tissues with dilute acid and alkali (breaking the cross-links of collagen by cutting into small pieces, washing and treatment with dilute acid and alkali), gelatin extraction (keeping the tissue in water at temperatures above 40°C by mixing), filtration and evaporation of gelatin solution after extraction (freeze-drying or heating between 40 and 80°C). In the pre-treatment stage, if the washing solution is dilute acid, type A gelatin is obtained, if it is dilute alkaline, type B gelatin is obtained [4, 7, 11]. If the tissue used for gelatin production is skin, type A gelatin is obtained; if that is bone, type B gelatin is obtained [4]. Since it reduces the quality and yield of the gelatin to be obtained, mineral substances such as calcium in bone tissues should be removed before the extraction process [13]. Moreover, production conditions including time and temperature of the extraction process, the acid and alkali concentrations greatly affect the quality of the gelatin to be obtained [12, 14].

Due to its various use in industry, the demand for gelatin is increasing in the world. Gelatin production in the world is approximately 400,000 tons annually, mostly from pig derivatives (80%), cattle (15%), and other sources (5%) [15]. The annual use of gelatin in Turkey is around 5,000 tons and almost 90% of this amount is imported [15]. Poultry processing by-products are considered perfect alternative sources for the production of collagen and gelatin products. Current literature indicates that poultry skin, bones, head, feet are the most popular and rich sources to be used for collagen and gelatin extraction [17-20]. However, limited researches are focusing on MDM residues for protein recovery [21]. Therefore, investigation of collagen/gelatin recovery from these poultry wastes could be highly desirable.

The purposes of this study are to extract collagen and gelatin from the skin and MDM residues of poultry processing, and to determine some properties of the poultry samples (raw materials) and extracted proteins, comparatively through physicochemical analysis, SDS-PAGE profiling and rheological measurements.

MATERIALS and METHODS

Materials

In this study, poultry skin and MDM residues were kindly provided by a national poultry processing company, Gedik Piliç A.Ş. (Uşak, Turkey). MDM samples were obtained from the neck, shinbone, breastbone, wingtip, shank, upper and lower backbone residues separately and as a mixture of the neck, shinbone, breastbone, wingtip, shank, upper and lower backbone residues and used to extract collagen/gelatine. All the samples were immediately stored at -18°C in plastic containers until further use. Electrophoresis standard marker and chemicals were purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA). All chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO, USA).

Methods

Physicochemical analyses of poultry samples were carried out to determine contents of the dry matter, moisture, ash, fat and protein. After alkali and/or acid treatment, collagen protein was separated from raw materials and transformed into gelatin through a thermal extraction process. The amount and profiles of the

extracted collagen/gelatin protein were determined by Bradford assay and SDS-PAGE analysis. Viscosity and gel hardness measurements were performed in the protein solutions obtained during the extraction process. Experiments were performed in duplicate and average values were considered.

Physicochemical Analyses of Poultry Samples

The frozen (-18°C) MDM bone residue samples, which were mechanically stripped of flesh and cut into small pieces to increase surface area, were allowed to thaw overnight at +4°C before analysis. The dry matter and moisture, ash, protein and fat contents of the skin (~ 1x1 cm) and pieced MDM residue samples were determined according to AOAC (1990, 2000) methods. Moisture content were determined by obtaining the constant weight of the samples after drying in an air circulation oven (Memmert UNB400, Schwabach, Germany) at 105ºC (AOAC method 927.05) [22]. Ash content was determined by burning the samples in an ash furnace (Carbolite, Hope Valey, UK) at 550°C for 7 hours (AOAC method 942.05) [22]. Fat content was determined by the Soxhlet method using Gerhardt Soxtherm system (Königswinter, Germany). Protein content was determined by the Kjeldahl method based on total nitrogen, and a factor of 6.25 was used for nitrogen-protein conversion (AOAC method 984.13) [23].

Pretreatment and Extraction

The extraction procedure of collagen/gelatin was adopted from previous studies [18, 20]. Twenty-five grams of thawed and chopped (~ 1x1 cm) skin samples were treated with 100 mL of dilute acid (0.1 N HCl) and alkali (0.1 N NaOH) solutions for 3 h, respectively at room temperature (RT). Mid-washings and filtration steps were applied between acid and alkali treatments. Then, gelatin extraction was carried out in distilled water at 55°C for 7h, with a subsequent filtration as well. After each treatment step, the solution was filtered through a 4-layer cheesecloth and the skin samples were washed with distilled water. At the end of the extraction process, the final filtrate was kept at 4°C for further analyses. Dissolved pieced MDM samples (25 g) were subjected to cleaning in distilled water (100 mL) at 65ºC for 3 h in a shaking incubator at 150 rpm. After filtration through a 4-layer cheesecloth, the samples were demineralized with the treatment of 4% HCl for 24 h. This was performed to weaken the mineral part of the bone and remove calcium phosphates. In the degreasing process, the fat content of the samples was removed with n-hexane at 35°C for 18h. To remove non-proteinous-nitrogen compounds, they were then treated with 0.1 N NaOH for 24h and poultry bone collagen was obtained. Acid treatment of MDM bone samples was carried out at the demineralization stage. MDM bone samples were treated with 0.1 N NaOH to break the crosslinks in the structure before extraction. After these treatments, gelatin extraction was carried out in water using a shaking incubator at 60ºC for 24h. Gelatin solution obtained as a result of the extraction process was filtered and stored at +4°C for further analyses. The procedures followed in the extraction of collagen/gelatin from chicken skin and bones (including MDM sample) were given in Figures 1 and 2 below.

Determination of Protein Content by Bradford Assay

Protein contents in solutions obtained during extraction processes were determined by Bradford assay as previously described in [24]. Hundred µl of ten-fold diluted collagen/gelatin sample solution were mixed with 5 mL of Bradford reagent and resultant protein/dye solutions were kept in the dark for 5 min. Absorbance measurements were taken at 595 nm in UV-VİS Spectrophotometer (Libra S70, Biochrom Ltd, Cambridge, UK) and the content of protein in the samples was determined with the help of the standart curve prepared previously.

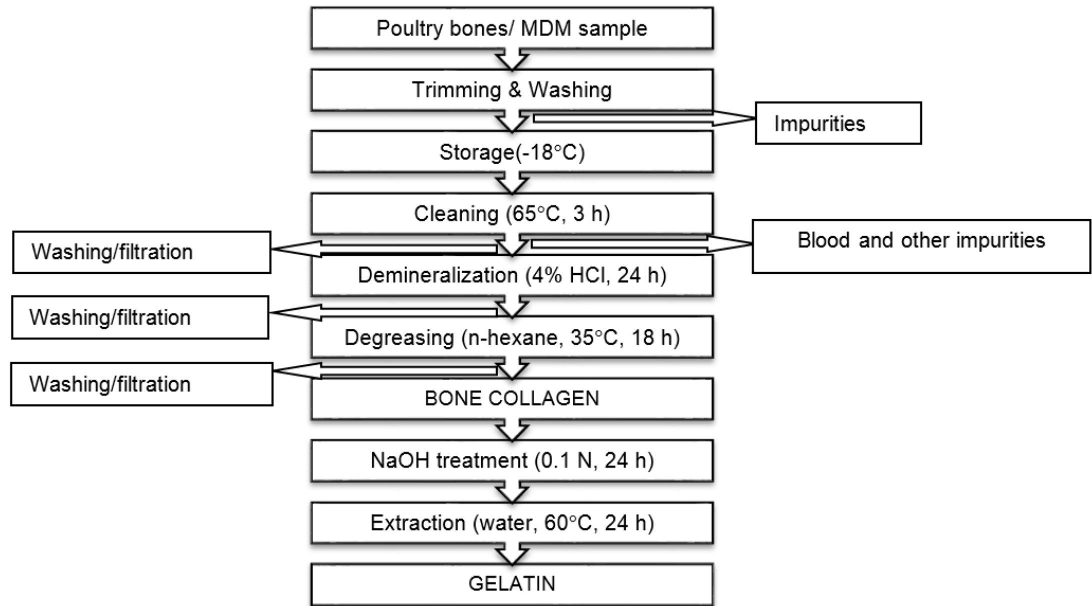


Figure 1. Extraction procedure for collagen/gelatin from poultry MDM residues

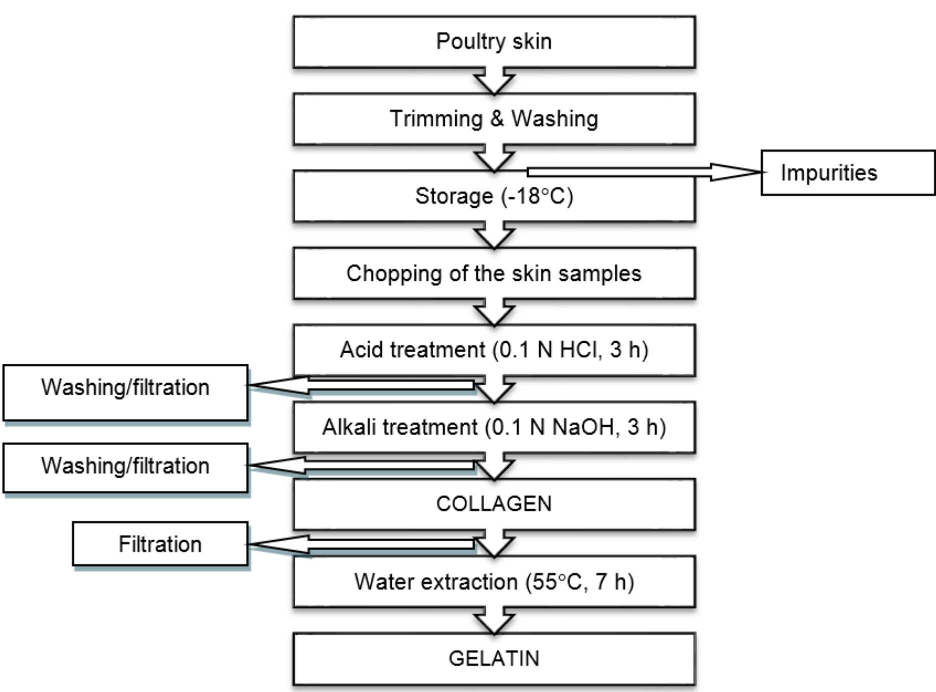


Figure 2. Extraction procedure for collagen/gelatin from poultry skin

Yield of Extraction

By considering protein content based on Bradford assay, the yield of extraction was calculated with the following formula;

Yield (%) = (protein amount of recovered collagen or gelatin / wet weight of raw material) * 100

SDS-PAGE Analysis

Protein fractions in the samples were analyzed by SDS-PAGE according to Laemmli protocol [25]. Collagen/ Gelatin samples were mixed with sample buffer (1:1 v/v) and boiled for 5 minutes to denaturate proteins. Twenty microliters of each sample was loaded into each well. Electrophoresis was carried out on a 4-12% polyacrylamide gel using MiniProtean Tetra System (Biorad Lab Inc, USA). It was run at 75 V in the first 15 minutes, then at 150 V for an hour within tris glycine buffer. Following protein staining performed using Coomassie Brillant Blue R250 for 30 min, destaining was carried out for a few hours and protein bands were examined.

Viscosity Measurements

Apparent viscosity values of collagen/gelatin solutions was determined at room temperature by using DV2T Brookfield Viscometer (MA, USA). A wide-mouth 50 mL tube containing 40 mL of sample and stainless steel RV-5 spindle (ranging between 400 and 800.000 cP) was used for the measurements. Viscosity measurements were carried out at four different spindle speeds (25, 50, 100, 200 rpm) at RT. Average values of two replicates were considered.

Gel Hardness Measurements

Gel hardness measurements of collagen/gelatin solutions were carried out using Brookfield CT3 Texture Analyzer (MA, USA) with a load cell of 4.5 kg. A flat bottom cylinder (clear acrylic), with a diameter of 25.4 mm, probe (TA11-1000) was used forced to penetrate 10 mm into the sample with a penetration speed of 0.5 mm/s to determine the maximum mass in g. Hardness (g) and hardness work done (mJ) were recorded in the 1st and 2nd cycle penetration tests.

Statistical Analysis

One-way ANOVA was applied using Tukey’s test to compare means of moisture, fat, ash, dry matter and protein contents of poultry samples viscosity and hardness of collagen and gelatin obtained. IBM© SPSS© V23 software was used to perform statistical tests and the level of significance was 0.05.

RESULTS and DISCUSSION

Composition of Poultry Skin and MDM Residue Samples

Chemical properties of poultry skin and MDM residue raw materials were given in Table 1. Among the samples, the poultry skin had the lowest moisture content with ~44% ($p<0.05$). The sample with the highest fat content was the skin sample with ~16% and followed by the wingtip sample with ~9.6% ($p<0.05$). However, both indicated the lowest ash contents in comparison to the other samples ($p<0.05$). The shinbone residue had the highest ash content with ~20.5%, while having the lowest fat content with ~3.3% ($p<0.05$). Although the shinbone residue seemed to have the highest protein content (~21.7%), the

difference of protein content among the samples were not significant (p>0.05). In a previous study concerning MDM residues of neck, breast and backbone for gelatine production, the protein, ash, fat and moisture contents were reported as 18.92%, 12.67%, 6.17% and 60.26%, respectively [26]. In another study concerning Alaska pollocks indicated that their frames had the moisture content of 64.0±2.9, crude protein content of 18.4±08, crude fat content of 0.7±0.2 and ash content of 14.9±0.07 per 100 grams [27]. The approximate chemical composition of the pork skin was 34.9% protein, 22.6% fat, and 44.4% moisture [28]. Ferraro et al. [29] demonstrated that the moisture, dry matter and Kjeldahl protein content of femur and tibia bones were 10.8±0.1, 89.2±0.3 and 29.5±0.2 for the 4 and 7 year-old milk cows. The results of the presented study were comparable with the relevant literature.

Table 1. Chemical composition of poultry skin and MDM residue samples

No*	Samples	Moisture (%)	Fat (%)	Ash (%)	N (%)	Protein (%) (Nx6.25)	Dry matter (%)
1	Poultry skin	44.06±2.07^{B}	16.33±1.18^{A}	0.33±0.07^{E}	2.42±0.19^{A}	15.13±1.17^{A}	55.93±2.07^{A}
2	Neck	62.14±2.14^{A}	5.90±0.86^{C}	12.52±1.72^{BC}	3.00±0.41^{A}	18.76±2.58^{A}	37.85±2.15^{B}
3	Shinbone	55.03±1.58^{AB}	3.28±0.20^{C}	20.51±1.54^{A}	3.47±0.10^{A}	21.68±0.63^{A}	44.95±1.58^{AB}
4	Breastbone	56.28±3.22^{A}	4.18±0.21^{C}	18.47±2.04^{AB}	2.98±0.43^{A}	18.69±2.66^{A}	43.71±3.22^{B}
5	Wing tip	61.78±0.90^{A}	9.57±0.11^{B}	4.31±0.38^{DE}	2.51±0.17^{A}	15.73±1.10^{A}	38.21±0.90^{B}
6	Shanks	63.89±2.17^{A}	5.64±0.97^{C}	11.17±1.55^{BCD}	3.26±0.05^{A}	20.41±0.30^{A}	36.09±2.16^{B}
7	Upper backbone	59.71±0.11^{A}	3.43±0.08^{C}	15.17±0.26^{ABC}	3.34±0.11^{A}	20.92±0.68^{A}	40.28±0.11^{B}
8	Lower backbone	58.47±2.25^{A}	4.71±0.40^{C}	14.42±1.87^{ABC}	2.91±0.21^{A}	18.20±1.31^{A}	41.52±2.25^{B}
9	Bone mixture	64.26±2.99^{A}	6.51±0.19^{BC}	10.04±0.79^{CD}	3.16±0.07^{A}	19.80±0.46^{A}	35.72±3.00^{B}

* Samples from 2 to 8 represent MDM residues of the given bones taken separately and sample 9 represents MDM residue of the bone mixture (the neck, shinbone, breastbone, wingtip, shank, upper and lower backbone). Different letters in the same column represent significant differences (p<0.05).

Protein Content of Collagen/Gelatin Solutions

The protein contents (mg/mL) of collagen/gelatin solutions determined by the Bradford method were given in Figure 3. Dark columns represented collagenous material and light ones represented gelatin extracted. The upper backbone and mixed MDM residue appeared to have the highest protein (collagen) content among all the other samples (p<0.05). In general, the content of protein in collagen solutions were found to be higher (p<0.05) than that in gelatin solutions, indicating protein loss through extraction process. Interestingly, the content of protein measured in collagen solution was slightly lower than that measured in gelatin solutions of the neck and wing tip residue samples (p>0.05). This might be due to triggered protein dissolution and extraction through given process steps. Regarding extracted gelatin, while the neck residues comprised the highest (10.50 mg/mL) gelatin content, breastbone residues revealed the lowest (6.06 mg/mL) gelatin content (p<0.05).

Since the protein contents of recovered collagen and gelatin from poultry and the other sources were mostly expressed in percentage or yield, it was hard to compare the results given here properly with the literature studies. For instance, Almedia et. al [17] obtained gelatin from skin and tendons of chicken feet with the protein content of 85%. In another study concerning multi-step chicken bone collagen recovery, Cansu and Boran [18] reported that protein losses during cleaning, demineralization and degrasing steps were 6.4, 13.4 and 18.6%, respectively.

Protein content in the recovered collagen and gelatin solutions were used in the calculation of extraction yields, which were presented in Table 2. They were expressed in weight of protein in the corresponding solutions, determined by Bradford assay per weight of moist raw material (skin and MDM samples). In current literature, similar calculations are given especially based on the dry weight of powdered gelatin/collagen recovered per weight of raw material [14, 17]. However, in our case, we did not make the final solutions dry and get powder. According to this, there might be differences in extraction yield obtained in comparison to the reported works. For instance, Almedia et al. [17] reported that the yield of collagen recovered from chicken skin was about 7.83%. The yields of collagen and gelatin extracted from skin samples in the presented work were 1.32% and 1.62%, respectively. Regarding bone residues, the highest collagen yield was achieved in upper backbone and mixed MDM samples with ~2.15% (p<0.05), whereas, the highest gelatin yield was obtained in upper backbone and neck residues with ~1.85% (p<0.05).

Protein Profiling

The SDS-PAGE profiles of collagen obtained from poultry samples were shown in Figure 4. Protein bands corresponding to different molecular weights represent different fractions of collagen/gelatin. Based on the literature, the high molecular weight proteins in collagen/gelatin are the "γ" chain, in the range of 230-340 kDa, "β" chains, in between 123-230 kDa, and the "α" chains, in between 80-125 kDa. Besides, there are α-chain subunits with lower molecular weight found in the environment and these are subunit-1 (50-80 kDa), subunit-2 (35-49 kDa), subunit-3 (25-35 kDa), and subunit-4 (10-25 kDa) [6]. During the conversion of collagen to gelatin, the protein fragments with various weights are formed by breaking the peptide bonds and the cross-links between the chains [30]. According to our results, three major bands with molecular weights of ~200, 130, 115 kDa were observed in poultry skin (lane

1), representing β, α_1, α_2 collagen. The slight and strong bands at ~130 and between 10 and 80 kDa, indicating α-collagen units and subunits, respectively were detected in the bones samples (lane 2, 3, 4, 5, 6, 7, 8). The mixed MDM residue obtained after the final processing of bones in slaughterhouse exhibited a many protein bands between ~130 and 10 kDa, corresponding to α-collagen units and subunits as well (lane 9). In all of the MDM samples, a high intensity band at ~50 kDa was dominantly observed. However, it was not detected in the collagen profile of the skin sample. In case gelatin extraction, thermal treatment applied for a period (above denaturation temperature) might result in destroyed triple helices and broken down of peptide bonds [30]. Thermal denaturation occurs in gelatin over 43°C, in association with the helix-coil transition as previously reported [31]. This phenomenon was consistent with the observed smear-like appearance of the gelatin profiles (lane 1g - 9g) represented in Figure 4. The mixed MDM residue sample better exhibited most of the protein fractions/units&subunits (lane 9c) when compared to the other residue samples (lane 2c – 8c).

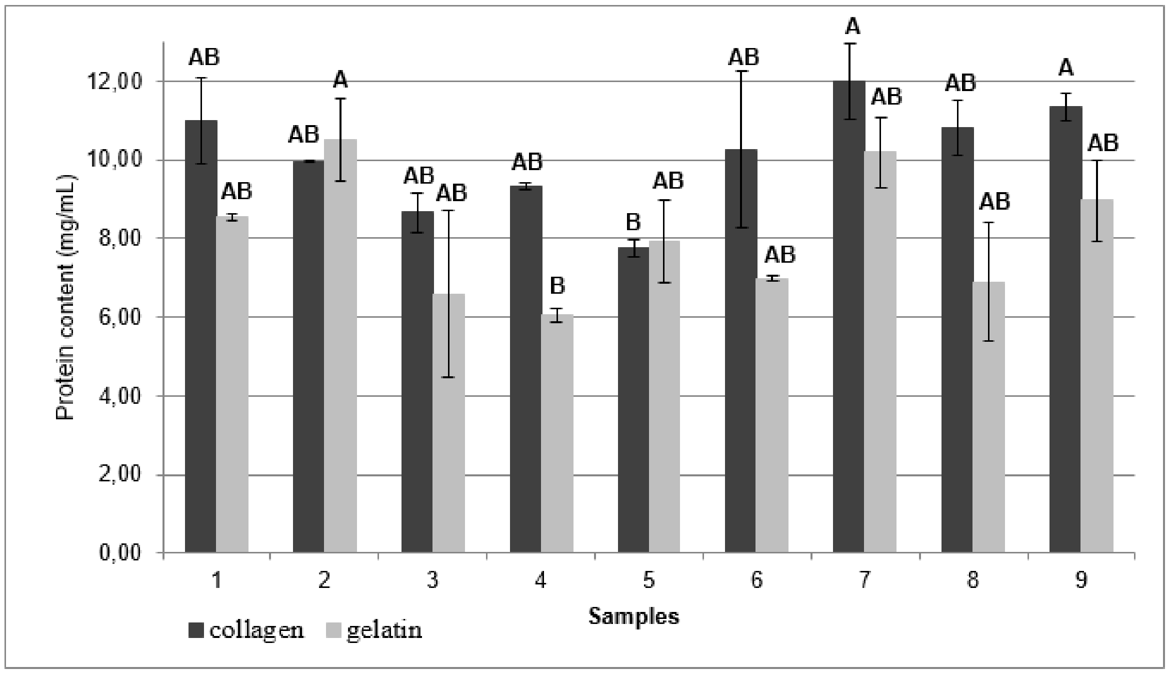


Figure 3. Protein contents in collagen/gelatin solutions determined using Bradford assay. Numbers represent the skin and MDM residue samples as follows. 1: skin; 2: neck; 3: shinbone; 4: breast bone; 5: wing tip; 6: shanks; 7: upper back bone; 8: lower back bone; 9: bone mixture (the neck, shinbone, breastbone, wingtip, shank, upper and lower backbone). Different letters in the same group of the samples represent significant difference ($p<0.05$)

Table 2. The yield of collagen and gelatin obtained from the skin and MDM residue samples

Sample	Raw material*	Collagen yield (%)	Gelatine yield (%)
1	poultry skin	$1.32±0.13^{B}$	$1.62±0.06^{ABC}$
2	neck	$1.89±0.14^{AB}$	$1.89±0.19^{A}$
3	shinbone	$1.72±0.12^{AB}$	$1.15±0.20^{C}$
4	breastbone	$1.86±0.02^{B}$	$1.03±0.06^{C}$
5	wingtip	$1.39±0.04^{AB}$	$1.50±0.09^{ABC}$
6	shanks	$2.02±0.45^{AB}$	$1.26±0.01^{BC}$
7	upper backbone	$2.16±0.17^{A}$	$1.83±0.16^{AB}$
8	lower backbone	$2.06±0.29^{AB}$	$1.24±0.27^{BC}$
9	bone mixture	$2.15±0.09^{A}$	$1.61±0.19^{ABC}$

* Samples from 2 to 8 represent MDM residues of the given bones taken separately and sample 9 represents MDM residue of the bone mixture (the neck, shinbone, breastbone, wingtip, shank, upper and lower backbone). Different letters in the same column represent significant difference ($p<0.05$).

Collagen and gelatin extracted from animal skin and bones can be used for various applications including gelation, thickening, emulsification and film formation. Regarding MDM residues of different bones reported in this study, the neck, upper backbone and the bone mix might serve better functionality for these purposes since they had higher protein content in comparison to the others.

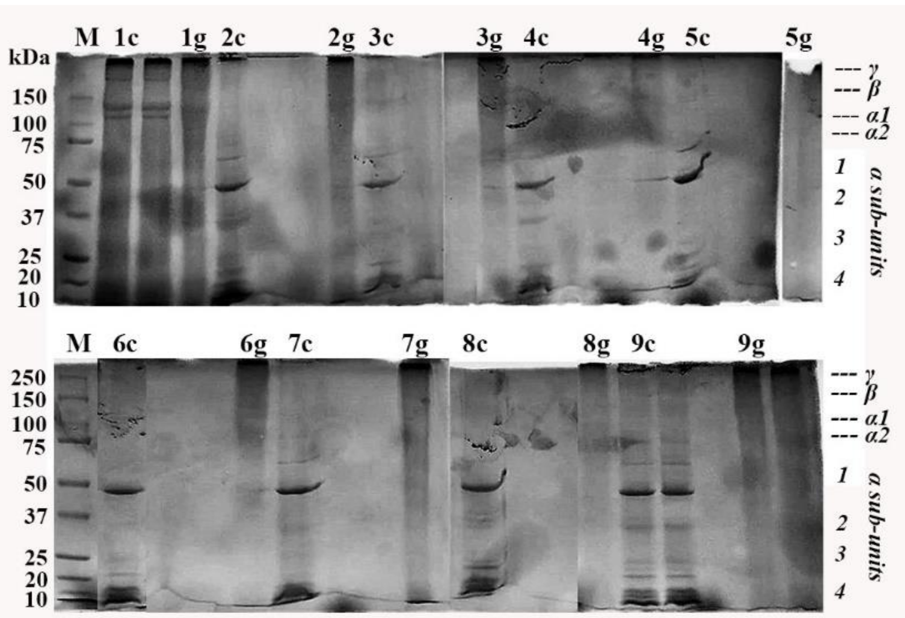


Figure 4. SDS-PAGE profiles of collagen and gelatin from poultry skin and MDM residues. Lane M represents the protein standard (Bio-Rad, CA, USA). Lane 1: skin; lane 2: neck; lane 3: shinbone; lane 4: breast bone; lane 5: wingtip; lane 6: shanks; lane 7: upper backbone; lane 8: lower backbone; lane 9: bone mixture. The c and g represented collagen and gelatin, respectively.

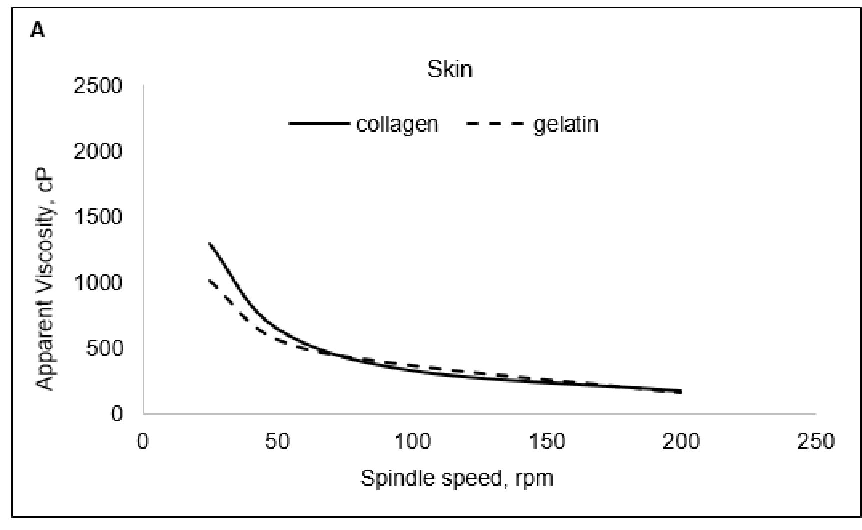
Apparent Viscosity and Gel Strength of Collagen/Gelatin Solutions

Figure 5 shows apparent viscosity varying with spindle speed in collagen and gelatin solutions of poultry skin and MDM samples. The viscosity decreased with the increasing speed that inducing shear force, thus indicating the shear-thinning behavior of collagen/gelatin solutions measured at RT. Li et al. [32] reported that viscosity decreased with the increasing shear rate in collagen solutions, indicating the shear-thinning phenomenon. Non-Newtonian shear thinning behavior of collagen and gelatin solutions could be explained through diminished entanglements of protein aggregates due to the shear force and decreased internal and intramolecular friction forces, facilitating the flow of the solutions [33]. Thus, the increasing shear rate gave rise to decreasing viscosity of collagen/gelatin solutions.

Since the amount of solvent used in the pre-treatment and extraction stages was high, the viscosity values obtained were low accordingly. In case skin sample the viscosities of collagen and gelatin were almost identical, however, in case MDM residue sample containing the bone mixture viscosity of gelatin was higher than that of collagen ($p<0.05$). This might be arisen from a difference in extraction procedures of them, suggesting increased inter-and intramolecular interactions of gelatin sub-units in the latter case. It should be noted that a lower content (mg/mL) of protein was determined in gelatin solutions than in collagen solutions of both samples as given in Figure 3. When the apparent viscosities of skin and MDM residue of the bone mixture were compared, no significant difference was observed in case of collagen solutions. However, in case of gelatin solutions, the apparent viscosity of the bone mix MDM residue was higher that that of the skin samples ($p<0.05$).

Gel strength is an important parameter of collagen/gelatin solutions. Although a few attempts, unfortunately, we could not measured the gel strength in regular procedures (Bloom test) in our study. Due to excessive use of solvent during extraction and no concentration step applied, the protein concentrations in the final collagen and gelatin solutions were low. Instead, we measured the hardness through the texture analyzer compression test to determine the gel strength of the collagen and gelatin solutions comparatively.

Hardness values of collagen and gelatin extracted from the skin and MDM samples were given in Table 3. According to the results, the 1st and 2nd cycle values of hardness were quite low due to low protein concentration. However, they were quite close in each respective cycle in both, showing good viscoelastic properties of the tested protein solutions with low structural deformation. Moreover, hardness obtained in skin gelatin was the highest amont the others ($p<0.05$). When compared to viscosity findings, interestingly, the hardness of skin gelatin (~21.75 g) was higher than that of MDM gelatin (~17.75 g). In a previous study, fish bone gelatin hardness (~201.48 g) was reported as almost ten times higher than ours based on hardness measurements [34]. But, the protein concentration of the gelatin solutions in that study was higher correspondingly as well.



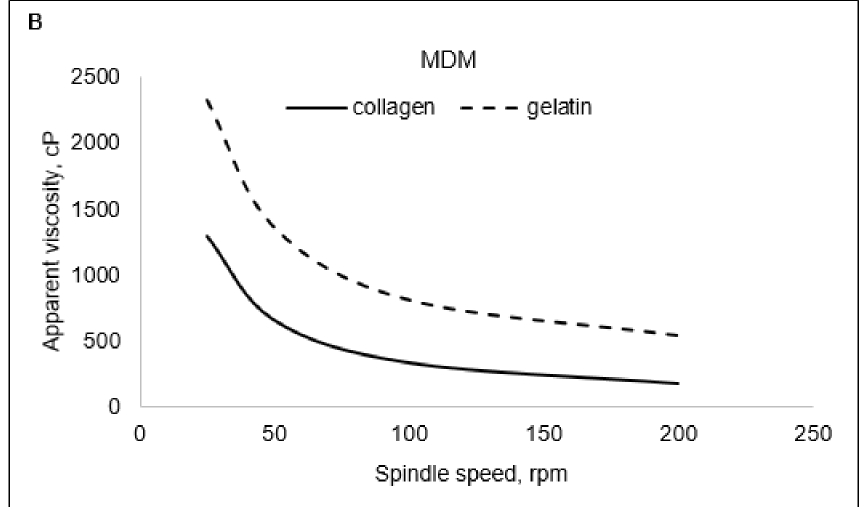


Figure 5. Apparent viscosity of extracted collagen/gelatin varying with spindle speed for the skin sample (A) and the bone mix MDM residue sample (B). Spindle rates were 25, 50, 100 and 200 rpm.

Treatment of protein samples with NaOH during extraction procedure could affect gel strength due to revealing α-chain subunits with the degradation of the collagen molecule, and thus preventing the formation of proper helical structures [21]. A low amount of use of NaOH in a limited treatment period could improve gel strength which could be desirable for various applications of the resultant collagen or gelatin product.

Table 3. The hardness of the extracted collagen/gelatin from skin and mixed MDM residue samples

Parameters	Skin		Bone mix MDM residue	
	collagen	gelatin	collagen	gelatin
Hardness (g), 1st cycle	17.25±0.75^{A}	21.75±1.75^{A}	17.00±0.50^{A}	17.75±0.75^{A}
Hardness work done (mJ), 1st cycle	0.91±0.03^{AB}	1.13±0.05^{A}	0.87±0.02^{B}	0.94±0.06^{AB}
Hardness (g), 2nd cycle	17.00±0.00^{B}	21.5±0.50^{A}	16.50±0.00^{B}	17.25±0.75^{B}
Hardness work done (mJ), 2nd cycle	0.81±0.04^{B}	1.03±0.04^{A}	0.65±0.04^{B}	0.82±0.00^{B}

Different letters in the same raw represent significant difference ($p<0.05$)

CONCLUSION

The need for collagen and gelatin to be used in food and non-food applications is increasing day by day worldwide. On the other hand, due to insufficient available resources, there are strong efforts in securing of the sustainable alternatives and optimization of production conditions based on raw material sources. A quite high amount of poultry processing by-products and wastes are formed annually in the world. Processing of these by-products/wastes is important for the production of high value-added alternative products, beneficial for the economy and required for preventing environmental pollution. In this study, skin and bone residues, which are by-products/wastes of poultry processing, were used to extract collagen/gelatin. Our results indicated that they were extracted successfully, especially from the mixed MDM residue, which is the final waste of the

poultry process. Gelatin production with high added value from these wastes has importance in terms of efficient use of resources. It is also necessary to develop recovery methods in which the protein content is best preserved during the separation of irrelevant components and impurities in the pre-treatment and extraction steps involved. Due to its considerable collagen/gelatin content, MDM residue of the bone mixture is technologically suitable for using with alternative purposes because it is produced in large quantities in slaughterhouses and meat processing plants and thus, it can be served as a sustainable resource for industry in different applications.

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REFERENCES

[1] Brinckmann, J. (2005). Collagens at a glance. In: Collagen: Primer in structure, processing and assembly. Brinckmann, J., Notbohm, H. and Müller, P.K. (eds) Springer, NY, pp. 1-6.

[2] Ergel, J., Bachinger, H.P. (2005). Structure, stability and folding of the collagen triple helix. In: Collagen: Primer in structure, processing and assembly. Brinckmann, J., Notbohm, H. and Müller, P.K. (eds) Springer, NY, pp. 23-24.

[3] Johns, P., Courts, A. (1977). Relationship between Collagen and Gelatin. In: The Science and Technology of Gelatin, Ward AG, Courts A (eds), Academic Press, USA, PP. 138-168.

[4] Hinterwaldner, R. (1977). Technology of Gelatin Manufacture. In: The Science and Technology of Gelatin, Ward AG, Courts A (eds), Academic Press, USA, pp. 315-361.

[5] Erge, A. Zorba, Ö. (2016). Jelatin ve fizikokimyasal özellikleri. *Akademik Gıda*, 14(4), 431-440.

[6] Ersus Bilek, S., Bayram, S.K. (2015). Kolajen hidrolizatının fonksiyonel bir bileşen olarak gıda endüstrisinde kullanılması. *Akademik Gıda*, 13(4), 327-334.

[7] Ledward, D.A. (2000). Handbook of hydrocolloids, Capter-4, Edited by G. O. Philips and P. A. Williams. UK. Woodhead Publishing in Food Science and Technology, 450p.

[8] Boran, G. (2011). Bir gıda katkısı olarak jelatin: yapısı, özellikleri, üretimi, kullanımı ve kalitesi. *Gıda*, 36(2), 97-104.

[9] Nur Hanani, Z.A., Ross, Y.H., Kerry, J.P. (2014). Use and application of gelatin as potential biodegradable packaging materials for food products. *International Journal of Biological Macromolecules*, 71, 94-102.

[10] Duconseille, A., Astruc, T., Quintana, N., Meersman, F., Sante-Lhoutellier, V. (2015). Gelatin structure and composition linked to hard capsule dissolution: A review. *Food Hydrocolloids*, 43, 360-376.

[11] Ockerman, H.W., Hansen, C.L. (1988). Animal By-Product Processing. Ellis Horwood Ltd., Chichester England, 366p.

[12] Petersen, B.R., Yates, J.R. (1977). Gelatin Extraction. United States Patent No:4,064,008.

[13] Boran, G., Lawless, H.T., Regenstein, J.M. (2010). The effect of extraction conditions on the sensory and instrumental characteristics of fish gelatin gels. *Journal of Food Science*, 75(9), S469-S476.

[14] Boran, G., Regenstein, J.M. (2009). Optimization of gelatin extraction from silver carp skin. *Journal of Food Science*, 74(8), E432-E441

[15] Cho, S.H, Jahncke, M.L., Chin, K.B. Eun, J.B. (2006). The effect of processing conditions on the properties of gelatin from skate (*Raja kenojei*) skins. *Food Hydrocolloids*, 20, 810-816.

[16] Anonymous, (2016). Grand view research, Gelatin market size expected to reach 4.08 billion by 2024. retrieved from http//www.grandvewresearch.com/presss-releaseglobal -gelatin-market

[17] Anonim, (2018). http://insanvehayat.com/9-maddede-turkiyede-jelatin-gercegi., Erişim Tarihi, 02.04.2021

[18] Almeida, P.F., Lannes, S.C.D.S. (2013). Extraction and physicochemical characterization of gelatin from chicken by-product. *Journal of Food Process Engineering*, 36(6), 824-833.

[19] Cansu, Ü., Boran, G. (2015). Optimization of a multi-step procedure for isolation of chicken bone collagen. *Korean Journal for Food Science of Animal Resources*, 35(4), 431.

[20] Arunmozhivarman, K., Abraham, R., Rao, A., Parthiban, M. (2017). Extraction and molecular characterization of collagen from poultry meat processing by-product (chicken skin). *International Journal Pure Applied Biosciences*, 5(5), 1085-1091.

[21] Munasinghe, K.A., Schwarz, J. G., Nyame, A.K. (2014). Chicken collagen from law market value by-products as an alternate source. *Journal of Food Processing*, Article ID 298295.

[22] Erge, A., Zorba, Ö. (2018). Optimization of gelatin extraction from chicken mechanically deboned meat residue using alkaline pre-treatment. *LWT*, 97, 205-212.

[23] Association of Official American Chemists International (AOAC) (2000). Official Methods of Analysis, 17th Edition, William, H. (ed) Washington, DC, USA.

[24] [23] Association of Official American Chemists International (AOAC) (1990). Official Methods of Analysis, 15th Edition, Washington, DC, USA.

[25] Bradford M.M. (1976). A rapid sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.

[26] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259), 680-685.

[27] Erge, A. (2016). Tavuk Kesimhane Artıklarından Jelatin Üretim Koşullarının Araştırılması ve Optimizasyonu. Doktora Tezi. Abant İzzet Baysal Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Bolu, 130s.

[28] Hou, H., Li, B., Zhao, X., Zhang, Z., Li, P. (2011). Optimization of enzymatic hydrolysis of Alaska pollock frame for preparing protein hydrolysates with low-bitterness. *LWT-Food Science and Technology*, 44(2), 421-428.

[29] Henry, W.E., Bratzler, L.J., Luecke, R.W., (1963). Physical and chemical relationships of pork carcasses. *Journal of Animal Science,* 22(3), 613-616.

[30] Ferraro, V., Gaillard-Martinie, B., Sayd, T., Chambon, C., Anton, M., Santé-Lhoutellier, V. (2017). Collagen type I from bovine bone. Effect of animal age, bone anatomy and drying methodology on extraction yield, self-assembly, thermal behaviour and electrokinetic potential. *International Journal of Biological Macromolecules*, *97*, 55-66.

[31] Zhang, Z., Guoying, L., Bi Shi, S., (2006). Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. *Journal of the Society of Leather Technologists and Chemists*, 90, 23-27.

[32] Bigi, A., Cojazzi, G., Panzavolta, S., Rubini, K., Roveri, N. (2001). Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. *Biomaterials*, 22(8), 763-768.

[33] Li, Y., Qiao, C., Shi, L., Jiang, Q., Li, T. (2014). Viscosity of collagen solutions: influence of concentration, temperature, adsorption, and role of intermolecular interactions. *Journal of Macromolecular Science, Part B*, 53(5), 893-901.

[34] Yang, H., Duan, L., Li, Q., Tian, Z., Li, G. (2018). Experimental and modeling investigation on the rheological behavior of collagen solution as a function of acetic acid concentration. *Journal of The Mechanical Behavior of Biomedical Materials, 77,* 125-134.

[35] Sanaei, A.V., Mahmoodani, F., See, S. F., Yusop, S. M., Babji, A.S. (2013). Optimization of gelatin extraction and physico-chemical properties of catfish (*Clarias gariepinus*) bone gelatin. *International Food Research Journal*, 20(1), 423.