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Extraction and characterization of chicken feet soluble collagen

Cunshan Zhou [a], Yanhua Li [a], Xiaojie Yu [a], Hua Yang [b], Haile Ma [a, *], Abu ElGasim A. Yagoub [c], Yu Cheng [a], Jiali Hu [a], Phyllis Naa Yarley Otu [a, d]

[a] School of Food and Biological Engineering, Jiangsu Provincial Key Laboratory for Physical Processing of Agricultural Products, Jiangsu University, No. 301 Xuefu Road, Zhenjiang, 212013, China
[b] College of Biological and Environmental Sciences, Zhejiang Provincial Top Key Discipline of Biological Engineering, Zhejiang Wanli University, No. 8 South Qian Hu Road, Ningbo, 315100, China
[c] Faculty of Agriculture, University of Zalingie, P.O. Box 6, Zalingie, Sudan
[d] Science Laboratory Technology, Accra Polytechnic, P.O. Box 561, Accra, Ghana

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Acetic acid (PubChem CID: 176)
Bromophenol blue (PubChem CID: 8272)
Hydrochloric acid (PubChem CID: 313)
Potassium dihydrogen phosphate (PubChem CID: 516951)
Sodium hydroxide (PubChem CID: 14798)
SDS (PubChem CID: 3423265)
Sodium chloride (PubChem CID: 5234)
Tris hydrochloride (PubChem CID: 93573)
Urea (PubChem CID: 1176)

ABSTRACT

Sodium chloride-soluble collagen (SSC), acetic acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) were extracted from the skin of chicken feet and then characterized. PSC, ASC and SSC showed the yields of 49.10%, 14.49% and 1.13% (Based on lyophilized dry weight), respectively. PSC, ASC and SSC were characterized as type I collagen, containing α_1 and α_2 chains as well as β and γ-chains. Circular dichroism (CD) and Fourier transform infrared (FTIR) spectra of PSC, ASC and SSC were similar, suggesting that they maintained their intact triple helical structure. PSC, ASC and SSC contained Gly as the major amino acid with high contents of Glu, Ala, Pro and Hyp. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) images of PSC, ASC and SSC revealed that their surface topography were similar. Dynamic elastic behavior in PSC, ASC and SSC was detected. PSC showed the largest elasticity. Temperature sweeps test indicated that PSC had the highest denaturation temperature, followed by ASC, and then by SSC. Proline hydroxylation of PSC was higher (45.8%) than that of ASC, and SSC and accordingly PSC showed the highest thermal stability. PSC showed the highest degree of hydrolysis compared to ASC and SSC.

1. Introduction

Traditionally, collagen is extracted and characterized from by-products of mammal and marine animals (Li, Mu, Cai, & Lin, 2009). However, China is currently one of the leading countries in production of poultry. Chicken skin, a byproduct derived from chicken meat processing, is highly underutilized, constituting huge cost for waste disposal and danger to the environment (Feddern et al., 2010). Several attempts have previously been made at developing novel chicken skin based products in order to diversify its utilization and reduce waste (Onuh, Girgih, Aluko, & Aliani, 2014). However, an area of research that is yet to be explored is the development of chicken skin based products with functional and health promoting values. Chicken feet are mainly used to produce animal feed and low-end meat products. Extraction and characterization of collagen from chicken feet could effectively increase its economic value-added and hence increase its comprehensive utilization.

Type I collagen is the most abundant collagen in the organisms and is mostly found in skin, tendon and ligament. It is characterized by a unique right-handed triple helical structure with polypeptide chains of a G-X-Y amino acids repeating sequence, with X and Y

* Corresponding author.
E-mail address: mhl@ujs.edu.cn (H. Ma).

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often represent hydroxyproline and proline (Nalinanon, Benjakul, Hideki Kishimura, & Osako, 2011). Type I collagen has a wide scope of applications, particularly in pharmaceutical, food and biomedical industries (Yamauchi, & Sricholpech, 2012; Zhang, Olsen, Grossi, & Otte, 2013).

Investigations on collagens extracted from the chicken feet are scanty. There is growing interest in extraction of collagen from chicken by-products by aid of acids (Cheng, Hsu, Chang, Lin, & Sakata, 2009). However, no studies on hydrolysis and rheological properties of collagens extracted by sodium chloride, acetic acid and pepsin from the chicken feet skin are found in the literature (Liu, Lin, & Chen, 2001). Accordingly, the objective of this paper was to prepare and characterize sodium chloride-, acetic acid- and pepsin-soluble collagens from the chicken feet skin. This paper can provide basis for production of collagens, with potential commercial applications.

2. Materials and methods

2.1. Materials

Frozen chicken feet were purchased from a local market in Zhenjiang, Jiangsu province, China. They were transported to the Food Science laboratory, Jiangsu University within 30 min, and then immediately frozen at −20 °C and kept for further use. All chemicals used were of analytical grade.

2.2. Extraction of collagen

2.2.1. Pretreatment of chicken feet

The frozen chicken feet were first thawed at 4 °C and then a skin was removed manually and cut into small pieces (1 × 0.5 cm). The pieces were homogenized for 5 min at speed of 10,000 rpm using a homogenizer (Nengu, NSR-I, China) placed in an ice-water bath. The homogenate was soaked in a solution of 20% (w/v) NaCl in 0.05 M Tris-HCl (pH 7.5) at a ratio of 1:20 (w/v). The mixture was centrifuged at 10,000*g* for 20 min by a centrifuge (Avanti J-26XP, Beckman Coulter,USA) and the precipitate was washed repeatedly with distilled water to remove fats and bubbles. The pretreated skin sample was lyophilized and kept in a desiccator until use. The extraction process is shown in Fig. 1 (Miller & Kent Rhodes, 1982).

2.2.2. Extraction of salt-soluble collagen (SSC)

The salt-soluble collagen (SSC) was prepared by soaking the pretreated chicken feet skin in a salt solution (0.45 M NaCl in 0.05 M Tris-HCl, pH 7.5) at a ratio of 1:80 (w/v). Extraction mixture was homogenized for 5 min at speed of 10,000 rpm, using the homogenizer placed in the ice-water bath, and then left to stand for 48 h. At the end of extraction process, the mixture was centrifuged at 17,000*g* for 30 min. The supernatant (SSC) and residue were kept for further processing.

2.2.3. Extraction of acid-soluble collagen (ASC)

The acid soluble collagen from the skin of chicken feet was isolated following the method of Nalinanon, Benjakul, Visessanguan, and Kishimura (2008) with some modifications. Undissolved matter from salt soluble collagen extraction was soaked in 0.5 M (w/v) acetic acid at a ratio of 1:80 (w/v). The mixture was homogenized for 5 min as before and then left to stand for 48 h. The mixture was centrifuged at 17,000*g* for 30 min. The supernatant (ASC) and residue were kept for further processing.

2.2.4. Extraction of pepsin-soluble collagen (PSC)

The pepsin-soluble collagen was extracted according to Nalinanon, Benjakul, Visessanguan, and Kishimura (2007). The residual pellet from acid extraction was soaked in 0.5 M acetic acid (pH 2) containing 0.1% (w/v) pepsin (extracted from porcine gastric mucosa, EC 3.4.23.1; 4500 units/mg protein, Sigma-Aldrich Co., USA) at a ratio of 1:80 (w/v). The mixture was homogenized for 5 min as before and then left to stand for 48 h. The homogenate was centrifuged at 17,000*g* for 30 min. The supernatant (PSC) was kept for further processing.

2.2.5. Purification of collagen

Collagens extracted with the aid of sodium chloride, acetic acid, and pepsin (supernatants of SSC, ASC and PSC) were purified by salting-out overnight in 0.9 M NaCl (supernatant of SSC was acidified in 0.01 M HCl before salting-out). At the end of salting-out, the supernatant was centrifuged at 2500*g* for 30 min. The precipitate was dissolved in 1.0 M NaCl (prepared in 0.05 M Tris-HCl, pH 7.5). The solution was centrifuged at 2500*g* for 30 min and the pellet was removed. The resulting supernatant was salted-out overnight in 2.4 M NaCl and then centrifuged as before. The resulting pellet was dissolved in 0.5 M acetic acid and subsequently dialyzed in 0.1 M acetic acid for 24 h and in distilled water for 48 h. The purified SSC, ASC and PSC were lyophilized and stored in a desiccator until use. All operations were carried out at 4 °C.

2.2.6. Determination of hydroxyproline content

The hydroxyproline content of lyophilized SSC, ASC and PSC were determined according to the method of Bergman and Loxley (1963) with a slight modification. A predetermined weight of collagen sample was hydrolyzed with 6 M HCl at 110 °C for 24 h in an oil bath. The hydrolysate was clarified with activated carbon and filtered through Whatman No. 4 filter paper. The filtrate was neutralized with 10 M and 1 M NaOH to obtain a pH of 6.0–6.5. The neutralized sample (0.1 mL) was transferred into a test tube and isopropanol (0.2 mL) was added and mixed well. An aliquot of 0.1 mL of oxidant solution [mixture of 7% (w/v) chlororamine T and acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v)] was added and mixed thoroughly. Then 1.3 mL of Ehrlich's reagent solution [mixture of 2 g of *p*-dimethylamino-benzaldehyde in 3 mL 60% (v/v) perchloric acid (w/v) and isopropanol at a ratio of 3:13 (v/v)] were added. The mixture was agitated and heated at 60 °C for 25 min in a water bath and then cooled for 2–3 min in running water. The solution was diluted to 5 mL with isopropanol. Absorbance was read against water at 558 nm. A hydroxyproline standard solution, with concentrations ranging from 10 to 60 ppm, was used. Hydroxyproline content was calculated and expressed as mg/g of sample. The conversion factor for calculating the collagen content from hydroxyproline of chicken feet skin was 7.7 (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005).

2.3. Characterization of collagen

2.3.1. Analysis of amino acids

The amino acids content was determined by hydrolyzing a sample in 6 M HCl under vacuum at 110 °C for 24 h. The hydrolysate was dried by a vacuum concentrator at 60 °C and then dissolved in a citrate buffer (pH 2.2). The amino acid content was measured by an automatic amino acid analyzer (Sykam S-433 D, Germany).

2.3.2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of SSC, ASC and PSC were determined according to the method of LaemmLi (1970). Electrophoresis gels used were 12% separating gel and 5% stacking gel. The lyophilized collagen was dissolved in 0.1 M Tris-HCl containing 1.0% (w/v) SDS and 3 M urea, pH 6.8, at a ratio of 0.1% (w/v). The resulting solution was mixed