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EXTRACTION AND CHARACTERISATION OF TYPE I COLLAGEN FROM BROILER CHICKEN WASTE

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ABSTRACT

The objective of this research was to evaluate alternative source for collagen. Collagen is one of the major connective tissue to animal proteins and has wide biomedical application. The common source of collagen is from bovine and pig. The outbreak of diseases, such as bovine spongiform encephalopathy (BSE) and transmissible spongiform encephalopathy (TSE), has resulted in anxiety among users of collagen derived from these animals. Thus, there is need to find an alternative source of collagen. Broiler Chicken waste is one of the alternative sources because of its abundant collagen protein content. Acid- solubilized collagen (ASC) and pepsin-solubilized collagen (PSC) were isolated from the chicken waste, with the yields of 43.9% and 88.6% on the basis of dry weight, respectively. Total collagen protein was estimated for both ASC and PSC respectively, by Bradford assay and it was denoted for ASC as 36.7 mg/ml and PSC as 72.4 mg/ml. From FT-IR spectrum results it showed and confirmed of secondary structure of ASC and PSC. Molecular weight of ASC and PSC was analyzed and confirmed by SDS- PAGE analysis technique. The morphological analysis of collagen showed the fibular structure and interlinking property of collagen. Thus from the report it indicates that broiler chicken waste might be a new useful alternative source of collagen.

INTRODUCTION

Collagen is an abundant protein which is presented in major fraction of connective tissues such as tendon, skin, bone, the vascular system of animals. Type I collagen, which consists of two $\alpha 1$ polypeptide chains and one $\alpha 2$, is a prevalent protein in bone, skin, and tendon [1]. Collagen have been widely used in food industries as ingredients to improve the elasticity, consistency and stability of foods, making it of interest to the pharmaceutical, biomaterial- based packaging, and photographic industries. [2]. These collagens have been extracted from the skins of some vertebrate species such as pig and calf or bovine. However, the outbreaks of bovine spongiform encephalopathy (BSE) and transmissible spongiform encephalophathy (TSE) have resulted in unpleasant among users of collagen and collagen-derived products from these animals. Recently, alternatives of these animals as collagen sources are extremely desired.

A slaughterhouse waste from broiler chicken processing includes organic solid by-products such as bone, skin, feather and feet. Common methods for disposing these wastes are burning, incineration, compositing as fertilizer and animal feed. The disposal of chicken carcasses presents significant environmental, biological, and financial problems for the poultry industry. This waste also contains a large amount of the useful proteins such as keratin and collagen and thus broiler chicken waste was utilized as resources for collagen production.

MATERIALS AND METHODS

All the preparative procedures were performed at 20°C. The methods for extraction of collagen from broiler chicken waste consisted of the following steps; sample preparation, removing noncollagenous tissue, solublizing collagen, centrifuging and precipitating collagen, concentration measurement and characterizations of collagen.

Sample collection

Broiler chicken waste was obtained from Trichy local market and it was placed in bag full of ice. At the lab, the chicken organs were cleaned to remove blood strains and dirt using RO treated distilled water. After cleaning, it was cut into small pieces (0.5x 0.5 cm) using sterile knife and kept on ice prior to collagen extraction.

Removing noncollagenous tissue

In order to remove non collagenous substance and to make the tissue very loose, the waste was treated with 0.1 N of NaOH for 24 hours. Then, the solution was placed for gentle stirred. The solution was changed every 8 hours, and then washed with distal water [5].

EXTRACTION OF COLLAGEN

Acid solubilized collagen (ASC)

The non collagenous removed matter was soaked in 0.5 M acetic acid with a sample per solution ratio of 1: 30 (W/V) for 2 days with a gentle stirring and minced well. This acetic acid suspension was filtered by using cheese cloth to remove the bone residue. In order to precipitate the solublized collagen, solution was neutralized using 1N NaOH solution. The resultant precipitate was collected by centrifugation at 10,000 x g for 15 minutes at 10°C. The pellet was collected and then dissolved in 0.5 M acetic acid. The solution obtained was dialyzed with 0.1M acetic acid in a dialysis membrane for 12 hours. Dialysate was freeze dried and referred to as acid- solubilized collagen (ASC). [7]

Pepsin solubilized collagen (PSC)

Collagen could be solubilized by pepsin treatment. Chicken waste was treated with crystalline pepsin (conc. 0.1 mg/ml) for a period of 2 days[8]. After digestion, the solution was further precipitated by addition of 1N NaOH. The resultant precipitate was collected by centrifugation at 10,000 x g for 15 minutes at 10°C and re- dissolved in 0.5 M acetic acid [9]. The solution was dialyzed and freeze-dried in the same manner with ASC preparation. Dry matter was referred to as Pepsin- solubilized collagen (PSC) [10].

Collagen protein estimation

Estimation of collagen protein from broiler chicken waste was determined by Bradford method with BSA used as standard.[11] [12].

Fourier Transform –Infra Red Spectrum Analysis (FT-IR)

FT-IR spectra were obtained for both Acid solubilized collagen and Pepsin solubilized collagen using a Bruker infrared spectrophotometer (Bruker Instruments, Billerica, MA) from 4000 to 400 cm−1at data acquisition rate of 2 cm_1 per point [13]. FT-IR spectra were obtained from discs containing 2mg sample in approximately 100 mg potassium bromide (KBr). Triplicate samples of collagen were analyzed and spectra shows effective peaked [14].

SDS-PAGE of collagen from broiler chicken waste

Protein pattern of collagen samples was analyzed using SDS-PAGE according to the method of Laemmli [15] with slight modification. Using the discontinuous Tris-HCl/ glycine buffer system with 7.5% resolving gel and 5% stacking gel .A volume of 10-20 µl of sample buffer, containing 200 µg of sample was loaded into slab gel. A constant current of 25 mA was used. After electrophoresis, the gel was stained with Coomassie brilliant blue R-250 and destained with 7.5% acetic acid and 5% methanol.

Morphology of collagen under SEM

The lyophilized collagen samples were visualized by SEM. Morphological of collagen under SEM analysis was undertaken using a QUANTA-200, S3400Sem (FEI company, USA) at an accelerating voltage of 15KV.The ASC and PSC were coated with platinum for morphological observations [16].

Determination of Denaturation Temperature

The denaturation temperature (Td) is measured from changes in viscosity, where Ubbelohde viscometer can be used. The viscometer containing 5 ml of 0.03% collagen in 0.1 M acetic acid was immersed in a water bath was used for viscosity measurements [17]. Thermal determination curve is obtained by measuring the solution viscosity at seven stepwise-raised temperatures from 10°C to 60°C, each temperature being maintained for 30 min.

RESULT AND DISCUSSION

Isolation of ASC and PSC from chicken waste

Collagen from broiler chicken waste were isolated into acid-solubilized collagen (ASC) and pepsin- solubilized collagen (PSC) with yield of 43.9 % and 88.6 % (dry weight basis), respectively. The result suggested that the covalently cross- linking at the telopeptide region of collagen molecules were not solubilized by acid extraction. These cross-linking molecules generally caused the decrease in solubility of collagen. With the pepsin digestion, the cross-linked molecules at the telopeptide region were cleaved without damaging integrity of the triple helix. From the result, the high content of PSC fraction was in broiler chicken waste accordance with those reported in extraction on Yezo Sika Deer, fish, bovine and pig.

FT-IR analysis

Fourier transform infrared (FTIR) spectroscopy has been used to study changes in the secondary structure of collagen. It has been shown to include prominent absorption at 3466 cm_1 and 3340 cm_1. Amide bands were observed at 15413 and 1238 cm–1, it showed N-H bending vibrations and C-H stretching, respectively. From the FT-IR results, absorption of ASC and PSC shows the spectral data which are indicative of collagen secondary structure.

SDS-PAGE of collagen from broiler chicken waste

SDS-PAGE using 7.5% gel, the acid-solubilized collagen and pepsin-solubilized collagens from broiler chicken waste was investigated. As a result, two distinct α chains corresponding to $\alpha 1$ and $\alpha 2$ were detected for their different positions in mobility (**Figure 1**). Small amounts of β- and γ-chains were detected in this collagen. When comparing the proportion of high MW components between PSC and ASC the intra- and inter-molecular cross-links of collagens were richer in PSC than in ASC.

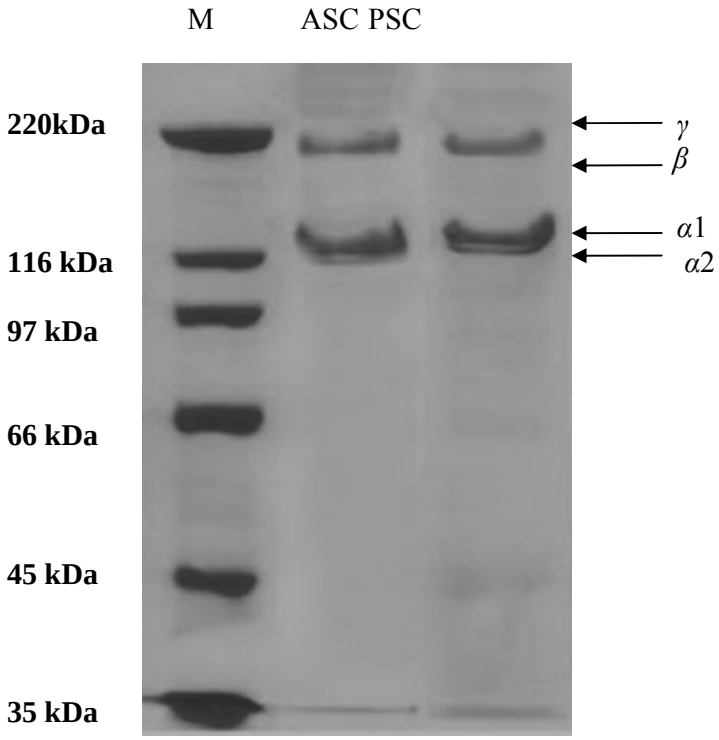

FIGURE. 1 SDSPAGE pattern of collagen from the broiler chicken waste; Lane 1: M-Protein marker; Lane 2: ASC-Acid –Soluble collagen; Lane 3: PSC-Pepsin – Soluble collagen

Morphology of collagen under SEM

After analytical tests, both ASC and PSC lyophilized collagen samples were visualized SEM images of both groups revealed porous collagen structure with interconnected network as shown in Figure2 and 3**.** We noted that both collagen scaffolds had a high proportion of the pore diameters in the range of 30 to 50 µm and 50 to 100 µm after the diameters of the selected pores were sorted. Only a small portion of the pore size was smaller than 30 or exceeded 100 µm.

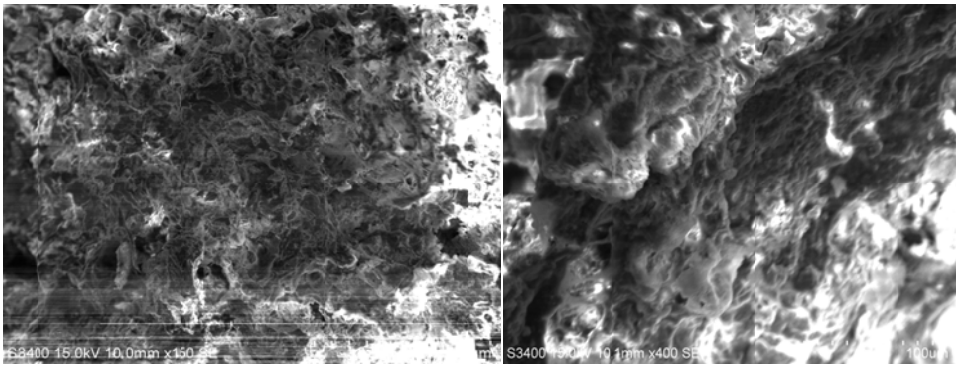

FIGURE. 2 and FIGURE. 3 SEM of the fibrillar collagen of Acid Solubilized Collagen (ASC) and pepsin solubilized collagen (PSC) from broiler chicken waste.

Denaturation Temperature of collagen

The denaturation temperature of acid solubilized collagen and pepsin solubilized collagen was observed from figure 4 & 5 as 31°C and 33°C. According to [17] results that pepsin cleaved the telopeptide region containing the intermolecular cross- linked without damaging the integrity of triple helix. As a result, triple helix structure was still predominant in both ASC and PSC, resulting in the similar thermal characteristic between both fractions. However the denaturation temperature of ASC and PSC from broiler chicken waste was lower than mammalian collagen (41°C).

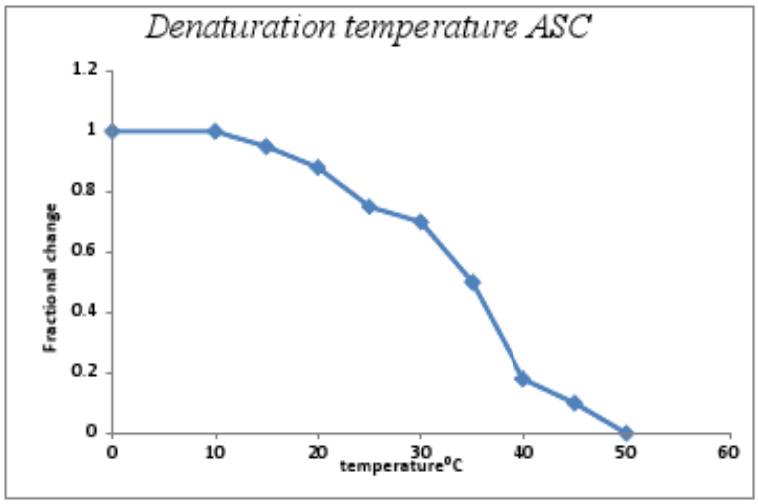

FIGURE. 4 Thermal Denaturation of Acid solubilized collagen

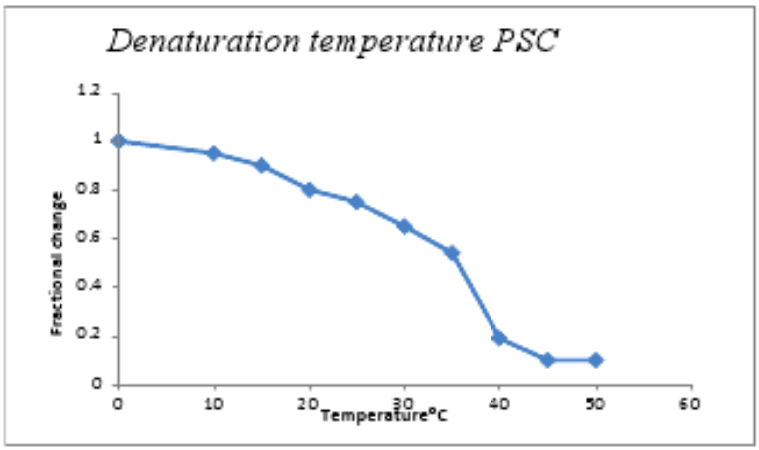

FIGURE. 5 Thermal Denaturation of Pepsin solubilized collagen

CONCLUSION

In this study, collagen type 1 was extracted and characterized from broiler chicken waste and high yield was observed in pepsin solubilized collagen (PSC) 88.6 %. From the FT-IR spectrum

analysis the secondary structure of collagen was predicted. Molecular weight characterization of collagen from broiler chicken waste was determined by SDS-PAGE analysis. The sharp bands showed up two distinct α chains corresponding to $\alpha 1$ and $\alpha 2$, then small amounts of β- and γ-chains were detected. From the result it shows promising evolution of the extraction process and providing alternative source for collagen, where a growing trend of exploiting non bovine source like chicken-skin is observed.

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