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Extraction and Molecular Characterization of Collagen from Poultry Meat Processing by-Product (Chicken Skin)

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ABSTRACT

A study was conducted to utilize less value chicken skin, a by-product obtained during poultry meat processing to extract collagen and to determine type and level of collagen available in it. Collagen was extracted with 0.5M acetic acid containing 1% pepsin after pretreatment of samples to remove non collagenous protein and fat, using 0.1N NaOH and 20% ethanol. The extracted collagen was lyophilized and characterized. SDS-PAGE study revealed that collagen had molecular pattern with two α chain (α 1 and α 2) and one ß chain which is indicative of Type-I collagen. UV spectrum analysis of collagen was done and found that no contamination with non collagen protein. The amino acid analysis showed that glycine was the major component and less amount of lysine, isoleucine. FTIR study showed the characteristic amide band at 3305.19 cm^{-1}, 2922.52 cm^{-1}, 1633.98 cm^{-1}, 1549.08 cm^{-1}, 1238.07 cm^{-1} for amide A, B, I, II and III of collagen respectively. The micro architecture studies of collagen using Scanning Electron Microscopy confirmed that the collagen is fibrillar in structure. The result of our study clearly indicated that chicken skin has significant amount of Type-I collagen which can be extracted using acetic acid with pepsin method.

Key words: *Chicken skin, by-product utilization, collagen extraction, Type I collagen, value addition.*

INTRODUCTION

Collagen is the major structural protein present in vertebrates and constitutes about 30% of the total animal protein. They were widely distributed in the skin, bones, tendons, vascular system and intra muscular connective tissues where they contribute to the stability and structural integrity of the tissues and organs[7]. The structure and function of 28 distinct vertebrate collagen types were well understood and have been identified[20]. Among which type one collagen was the predominant which contributes 90% of the total collagen. The chicken skin is the one of the major by products that is generated during poultry meat processing process. Commercially the chicken skin are used to prepare animal meal for animal feed formulation where as a small proportion is incorporated in to meat emulsion or used as a source of fat mainly for soup preparation[5].

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Chicken skin contains 3% collagen[3] in which approximately 75% is type one collagen and 15% type III collagen[1]. Collagen products are mainly manufactured from skin and bones of animals. The most common raw material used in collagen products are porcine skin and bone. Due to objection against to use pork in collagen products, alternative resources gained tremendous attention from the researcher and poultry processing by-product might be used as alternative resource[4]. Avian collagen is considered as one of the alternative source for various skin care and medical application instead of conventional bovine and porcine collagen[13]. Hence, this study was conducted for utilization of chicken skin for collagen extraction and characterizes collagen to see the possibilities to use in product preparation.

MATERIALS AND METHODS

Preparation samples:

Chicken skin was collected from in and around vepery, chennai area. They were taken to the laboratory in the icebox and thoroughly washed with distilled water. The superficial adhesive fat from the chicken skin was manually removed, cut into small pieces and minced by using meat mincer.

Collagen Extraction:

The collagen from the pre weighed minced skin samples were extracted [18] with proper modification at 4°C. Fat and non collagenous protein were removed by soaking the minced sample for 24 hours in 20% ethanol and 0.1N NaOH by changing the solution at every 8 hours interval. The ratio of sample solution was maintained at 1:20 (w/v). Then the collagen from the samples was solubilized by soaking in 0.5M acetic acid containing 1% pepsin (1:3000) at 1:30 w/v ratio for 48 hours. Then the collagen was precipitated by addition of NaCl and collected by centrifugation at 4°C in refrigerator centrifuge. The extracted collagen was purified by dialysis followed by lyophilization and stored at refrigerator for further analysis.

Analytical Procedure:

SDS–PAGE was done using 8% stacking gel and a 5% resolving gel[12]. The amino acid profile of the extracted collagen was analyzed by HPLC**.** A 100 mg sample of extracted collagen was hydrolysed in 8 ml of 6M HCl at 110 °C for 22 h. The ultraviolet absorption spectra of the chicken skin and feet collagens were recorded by an Epoch ™ Microplate spectrophotometer. The collagen was dissolved in 0.5M acetic acid to obtain a concentration of 1 mg/ml. Prior to measurement, a base line was set with 0.5M acetic acid.

Samples were prepared for FTIR according to the method previously described[9]. 10 mg of collagen was mixed with approximately 100 mg of potassium bromide (KBr). All spectra were obtained from 4000 to 400 cm^{-1} at a data acquisition rate of 4 cm^{-1} per point. The SEM observations were made at 15 kV accelerating voltage with a high vacuum (HV) mode.

RESULTS AND DISCUSSION

Collagen yield, most probably depends on the proportion of fractions of different protein in the samples used for collagen extraction[10]. The yield of collagen from chicken skin in this study was 10-12 percent. The amino acid composition plays a major role in physical properties of collagen. The chicken skin collagen had approximately 31.25% of glycine as the major amino acid followed by histidine (20.24%), threonine (9.32%), arginine (8.18 %), serine (6.54%), alanine (4.98%), valine (4.39%), leucine (4.35%) glutamic acid (3.18%), methionine (2.82%), tyrosine (1.38%), phenylalanine (1.03%), aspartic acid (1.18%), lysine (0.82%) and isoleucine (0.36%). The analysis of amino acid composition of collagen from extracted

chicken skin revealed that, glycine is abundant in collagen, constitutes around 30 percent of the total amino acid content. However, the value may vary according to species and body parts[8].

The protein patterns of collagen on SDS-PAGE is shown figure 1. The collagen from chicken skin comprised of two different α-chains (α_1 and α_2) with slightly different mobility and one ß chain which indicated that the collagen from the skin, might be type I collagen[11]. Acetic acid was used as solvent for collagen extraction in which no fragments less than 116 kDa were observed in the electrophorogram and the molecular structures of collagen were well maintained during extraction process[21].

The ultra-violet spectra of the collagen (figure 2) in this study had high intensity absorbance ranging from 230 to 240 nm and no or less absorption peak at 280 nm. The result indicated that high efficiency of non-collagenous protein removal2.Collagen commonly has a low amount of tyrosine, which could absorb UV-light at 280 nm6,25.

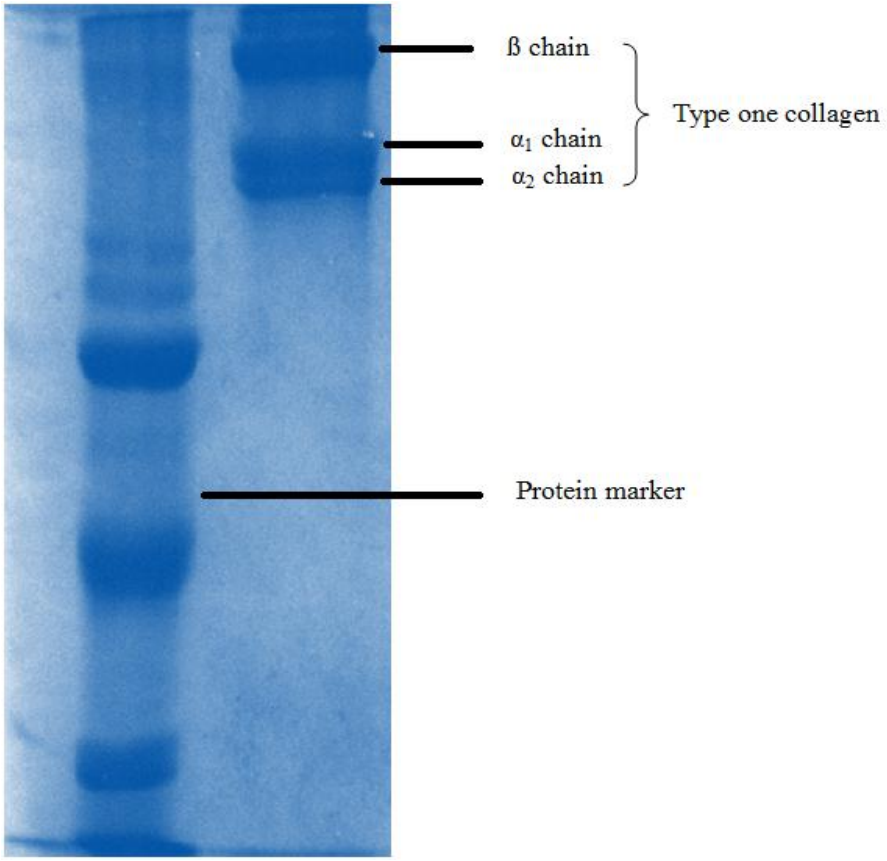
Fourier transform infrared spectroscopy of collagen of chicken skin (figure 3) showed characteristic peaks of amide A, B, I, II, III at 3305.19 cm^{-1}, 2922.52 cm^{-1}, 1633.98 cm^{-1}, 1549.08 cm^{-1} and 1238.07 cm^{-1} respectively. The absorption characteristics of amide 'A' is commonly associated with N-H stretching vibration this occurs in the wave range of 3400-3440 cm^{-1}. The absorption peak of Amide 'A' band for collagen from chicken skin found at 3305.19 cm^{-1}. This decrease in absorbance may be due to the N-H group which is involved with H-bond in peptide chain, the position starts to shift to lower frequencies[14,16].

The amide 'B' peak of collagen from chicken skin found to be 2922.52 cm^{-1} which is related to asymmetrical stretch of CH_2[7]. Similar absorption between collagen suggested that collagen complex with hydrogen bonding between free N-H stretches attaches with hydrogen in polypeptide chain.

The peaks of amides 'I' and 'II' of chicken skin collagen was 1633.98 cm^{-1}, 1549.08 cm^{-1} respectively. It was found that the amide 'I' band, with characteristic frequency in the range from 1600 to 1700 cm^{-1} was mainly associated with the stretching vibrations of the carbonyl groups (C=O bond) along the polypeptide backbone[19] and was the sensitive marker of the peptide secondary structure[24].

In addition, the absorption peaks around 1451–1450 cm^{-1} were also found. This considerably corresponded to pyrrolidine ring vibration of proline and hydroxyproline[17]. The band in the spectrum between 1200 and 1300 cm^{-1} are unique "fingerprint" of collagen molecular conformation attributed to particular tripeptide (Gly-Pro-Hyp)n [9]. Moreover, the N-H stretching band (3300 cm^{-1}) of denatured collagen (gelatin) was not detected. These results implied the collagen still preserved as native conformation during purification process. The absorption of amide 'III' for collagen at 1238.07 cm^{-1} wavelength indicates the existence of helical structure [15].

In the present study, the SEM images (figure 4) of chicken skin collagen at low magnification showed fibrillar like structure and further at high magnification, the collagen to be regular and uniform with net working of porous and honey-comb like structures on the surface[20,23]. The pore size, porosity and surface areas are widely recognized as important parameters for a collagen based biomaterial[22]. Generally, uniform and regular network structure of collagen as drug carrier is propitious, for well proportioned distribution of drugs[26].Based on the finding of this study collagen which could be extracted efficiently, chicken skin collagen can be suited to preparation of collagen based products.



Electrophoresis (SDS-PAGE)

Fig. 1: Molecular pattern of chicken skin collagen on SDS- Polyacrylamide Gel

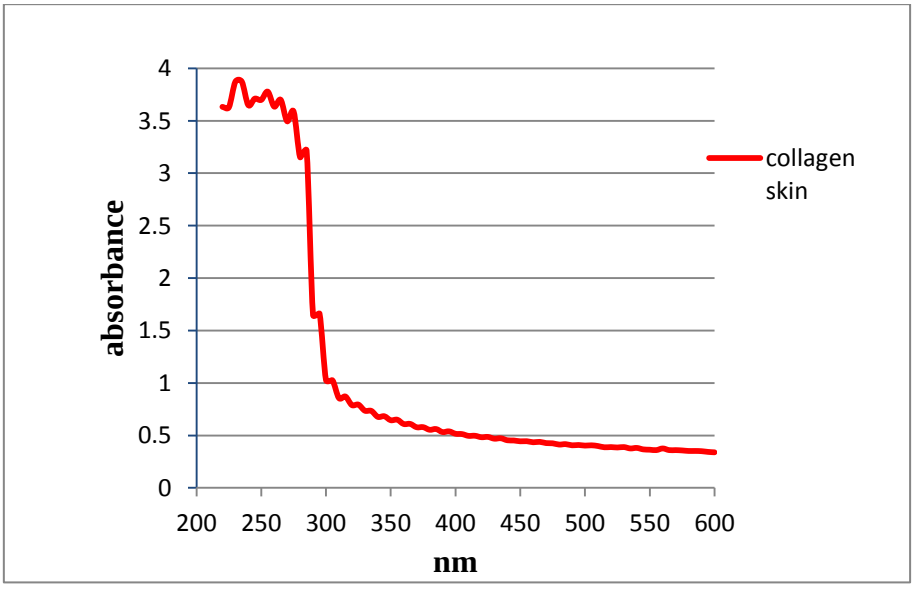


Fig. 2: UV spectrum analysis of collagen

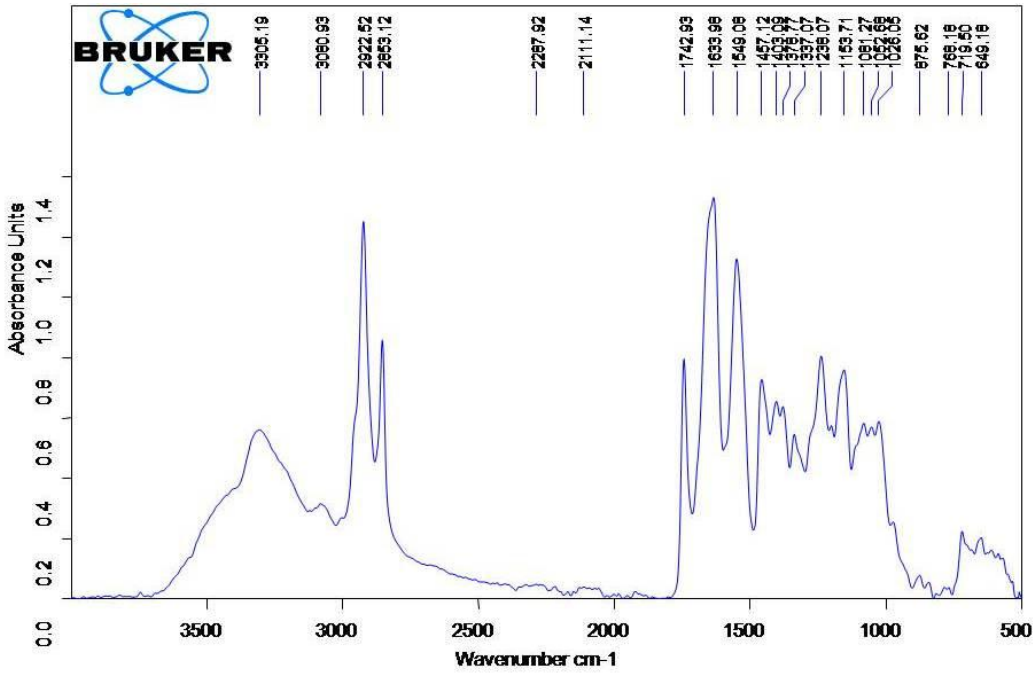
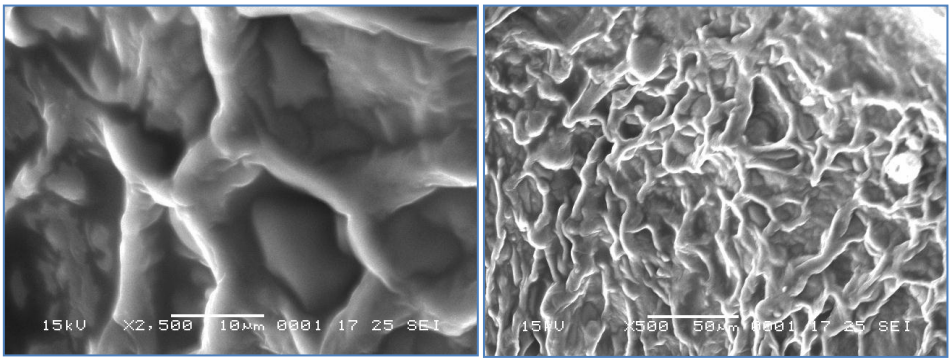


Fig. 3: FTIR spectra of collagen from chicken skin



2500 X (High magnification) 500 X (Low magnification)

Fig. 4: Scanning Electron Microscopy image of collagen from skin

CONCLUSION

The present study concluded that chicken skin can be effectively utilized to extract Type I collagen and the process can be optimized by inclusion of pepsin during extraction. Proper collection and utilization of these wastes also prevent dumping of wastes in to environment and thereby reduce environmental hazards. The extracted collagen can be used to develop biomaterial like collagen sheet and it can be used for various clinical conditions (e.g wound healing), which require further investigation.

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