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Research Article

MOLECULAR CHARACTERIZATION OF CHICKEN SHANK EXTRACTED COLLAGEN BY FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY

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ABSTRACT: Collagen is the most abundant protein found in bone, cartilage, tendon, skin and other connective tissues of animals and humans. Collagen is widely used in biomedical applications such as tissue engineering, wound healing and drug delivery systems owing to its biocompatibility, biodegradability and minimal immunogenicity. The collagen can be extracted by multiple extraction methods from low-value animal byproducts but the biomedical applicability mainly depends upon its structural integrity and purity which must be accessed before use. A total of 24, day-old broiler chicks were procured commercially and reared at Livestock Farm Complex, Faculty of Veterinary and Animal Sciences, RGSC, Banaras Hindu University, Mirzapur, Uttar Pradesh, India. The chicks were divided into three groups of 8 birds each viz. Group 1, Group 2 and Group 3 and were sacrificed for collagen extraction from their shank at 28, 35 and 42 days respectively. Chicken shank tissue was subjected to collagen extraction by alkali and acid treatment followed by enzymatic hydrolysis using pepsin enzyme. The lyophilized collagen samples were analyzed for different molecular vibrations and spectral analysis using a Fourier transform infrared (FT-IR) spectrometer in ATR mode. The FT-IR spectroscopy analysis of the extracted collagen from each of the study group indicated the characteristic molecular structure, as evident by the amide peaks in mid infrared region i.e. 4000-500 cm^{-1}. The study underlined the efficacy of extraction process by alkali and acid treatment followed by enzymatic hydrolysis using pepsin enzyme and suggested that chicken shank collagen extracted by this method retains normal molecular structure necessary for biomedical application. The FT-IR spectroscopy analysis of the chicken shank extracted collagen using alkali and acid treatments followed by enzymatic hydrolysis exhibited well-preserved triple-helical structure which can be potentially used as biomaterial.

Keywords: Collagen, Shank, Molecular characterization, FT-IR.

INTRODUCTION

Collagen is the most abundant protein of vertebrates and accounts for about 30% of the total protein [1]. A total of 28 collagen types have been identified based on the protein structure, amino acid sequence and molecular properties among which type I collagen is the most abundant protein of connective tissue [2, 3]. Collagen molecules form three polypeptide chains

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which bind together to form a triple helix structure primarily through hydrogen bonds. Each polypeptide chain contains about 1000 amino acid residues and has an average length of 300 nm and diameter of 1.4 nm. The amino acid sequence consists mainly of (Gly-X-Y)n, where glycine (Gly) makes up about 1/3 of the collagen peptide chain and X, Y are arbitrary amino acids other than glycine. Typically, the classical composition is proline (Pro) at the X-position and 4-hydroxyproline (Hyp) at the Y-position [4]. The various types of collagen found in nature are type I collagen predominantly present in skin, bones and connective tissues; type II and type III collagen found in cartilage and reticular fibers respectively.

The primary role of collagen is to maintain tissue integrity, support cellular structures and promote elasticity in the body. In addition, collagen has a crucial role in various biological functions of cells such as cell survival, proliferation and differentiation. Collagen possesses unique properties such as biocompatibility, biodegradability and low immunogenicity, making it suitable biomaterial for a wide range of biomedical applications [5, 6].

The collagen can be extracted from skin, bone and other organs of large animals, fish and poultry. The reports on chicken shank extracted collagen are meager as most of the studies on collagen extraction have been done on large animals owing to increased yield. The extraction of collagen from chicken low-value byproducts such as bone, cartilage, stomach, feathers, shank, skin, etc. are of great importance as it has no social taboos. The extracted collagen can be utilized in various industries including food, pharmaceuticals, cosmetics, and biomedical [7]. However, for potential usage in biomedical industrythe extracted collagen must retain normal molecular structure and should be free from impurities and deformities. Therefore, it is crucial to assess the molecular structure of the extracted collagen before its commercialization and application in biomedical fields.

Fourier Transform Infrared (FT-IR) spectroscopy is widely employed to analyze the molecular structure and purity of the collagen. The FT-IR spectra provide information on the functional groups present in the collagen, which is critical for verifying its structural integrity and identifying any contaminants. Keeping the above facts in consideration, the present research work is planned to analyze molecular structure of extracted chicken shank collagen by FT-IR spectroscopy.

MATERIALS AND METHODS

Sample Collection

A total of 24, day-old broiler chicks were procured commercially from Varanasi, Uttar Pradesh and reared at Livestock Farm Complex, Faculty of Veterinary and Animal Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, Uttar Pradesh under standard management conditions. The study was approved by Institutional Animal Ethics Committee (IAEC) via No. IAEC/RGSC-BHU/2023-24/180. The broiler birds were divided into three study groups of 8 birds each viz. Group 1, Group 2 and Group 3 and sacrificed for extraction of collagen from shank at 28, 35 and 42 days respectively as most commercial broilers reach slaughter weight between 28-42 days of age.

Processing of collected shank for collagen extraction

The collected chicken shanks were properly cleaned, trimmed, peeled and deboned followed by mincing and grinding. Thereafter the grinded material was subjected to different dry rendering temperatures for removal of fat.

Extraction of collagen

Collected chicken shanks were subjected to extraction process by alkali and acid treatments followed by enzymatic hydrolysis as described by earlier researchers [8]. The major steps involved in the extraction process involved

I. Pre-treatments of shank tissue for collagen extraction

a) Alkali pre-treatment

The rendered shank tissue was soaked in 0.1 mol/L NaOH solution for 6 hours in a 1:10 ratio (w/v) at 4°C and 165 rpm in orbital shaker incubator (REMI RIS-24 Plus TFT) with replacement of NaOH solution at every 2 hours interval. Thereafter, washing was done with double distilled water (DDW) till neutralization.

b) Chemical de-fatting

The alkali pre-treated shank tissue was further treated with 10% butyl alcohol in 1:10 ratio (w/v) for 12 hours at 4°C and 165 rpm in orbital shaker incubator. The 10% butyl alcohol solution was replaced at every 6 hours interval followed by washing with DDW.

c) Demineralization

The chemically defatted shank tissue was further treated with 0.1 N HCl in 1:6 ratio (w/v) for 24 hours at 4°C and 165 rpm in orbital shaker incubator for removal of inorganic constituents and promotion of shank swelling. Thereafter, washing was done thrice with DDW.

II. Collagen extraction from chicken shank tissue

The pre-treated shank tissue was subjected for collagen extraction using pepsin in acetic acid under following steps:

a) Acetic acid treatment/Hydrolysis

The pre-treated shank tissue was processed in a 1:6 ratio (w/v) of 0.5 mol/L acetic acid solution for 48 hours at 4°C and 165 rpm in orbital shaker incubator.

b) Enzymatic Hydrolysis

The collagen tissue obtained after acetic acid treatment was hydrolyzed in a 1:6 ratio (w/v) of 0.5 mol/L acetic acid solution containing 0.1% pepsin (activity of 1,000.0 U/mg) (w/v) for 24 hours at 4°C and 165 rpm in orbital shaker incubator.

III. Precipitation and Dialysis of extracted collagen

The hydrolyzed, soluble collagen was filtered by a double layered nylon plastic strainer and precipitated with the help of 2.6 mol/L NaCl solution in Tris (hydroxymethyl) aminomethane hydrochloride (0.05 M) at a pH of 7.0. The precipitated collagen was sedimented at 12,000 rpm for 15 minutes at 4°C using centrifugation machine (Eppendorf centrifuge 5810R, Germany). The precipitated collagen was dialysed against 20 volume of 0.1 M acetic acid for 12 hours, followed by DDW for another 24 hours with the change of dialysis solution at every 6 hours. Dialysis was undertaken using a dialysis membrane (HIMEDIA, Dialysis membrane-50) at 4°C and 175 rpm in orbital shaker incubator. The dialyzed shank collagen was finally lyophilized (-40ºC, 100 mbar pressure, 16 hours) in a freeze dryer (MAC, MSW-137) and stored in a sealed container under room temperature.

Fourier transform infrared (FT-IR) spectroscopy

The lyophilized collagen samples extracted from Group 1, Group 2 and Group 3 were analyzed for different molecular vibrations and spectral analysis using a Fourier transform infrared (FT-IR) spectrometer (BRUKER ALPHA II, FT-IR spectrometer) in ATR mode. The FT-IR analysis was performed in the Department of Physics, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

RESULTS AND DISCUSSION

Macroscopic observation

Grossly, the shank extracted collagen from each of the study groups viz. Group 1, Group 2 and Group 3 revealed white colour with slightly spongy and paper like consistency with no morphological difference. Similar findings were recorded earlier in the chicken feet collagen extracted using salt, acetic acid and pepsin [9].

Fourier transform infrared (FT-IR) spectroscopy

The FTIR spectroscopy analysis of the extracted collagen from the shank samples of all age groups were acquired over the range of 4000 to 500 cm^{-1} (mid infrared region) at a resolution of 2 cm^{-1}.

In shank collagen extracted from Group 1, amide A band peak was observed at 3285 cm^{-1} (N–H stretching), and amide B band was found near 2923 cm^{-1} and 2847 cm^{-1} (asymmetric -CH_2 stretching). The amide I band region was observed at 1741 cm^{-1} (C=O stretching) whereas the amide II band region was observed at 1541 cm^{-1} (N-H bending and C-N stretching) and amide III band peak was appeared at 1229 cm^{-1}, showing vibrations of C–N stretching and N–H bending (Figure 1, Table 1).

Table 1. Fourier transform infrared (FT-IR) spectra peak locations with assignments for shank collagen extracted from Group 1.

Peak positions in wavenumber (cm^{-1})	Assignments
3285	N-H Stretching
2923	C-H Stretching
2847	C-H Stretching
1741	C=O Stretching
1621	C=C Stretching
1541	N-O Stretching
1453	C-H Bending
1328	O-H Bending
1229	C-N Stretching
1151	C-O Stretching
1066	C-O Stretching
958	C=C Bending
865	C=C Bending

In shank collagen extracted from Group 2, amide A band peak (N–H stretching) was detected at 3302 cm^{-1}, and amide B band peak (-CH_2 stretching) appeared at 2920 cm^{-1} and 2845 cm^{-1}. The amide I (C=O stretching), amide II (N-H bending and C-N stretching) and amide III (C–N stretching and N–H bending) band peaks was found near 1739 cm^{-1}, 1531 cm^{-1} and 1235 cm^{-1} respectively (Figure 2, Table 2).

In shank collagen extracted from Group 3, amide A band peak (N–H stretching) was detected at 3280 cm^{-1} whereas amide B band peak (-CH_2 stretching) appeared at 2920 cm^{-1} and 2852 cm^{-1}. The amide I band peak (C=O stretching) was found near 1744 cm^{-1}, amide II

(N-H bending and C-N stretching) was seen at 1531 cm^{-1} and the amide III band peak (C–N stretching and N–H bending) was seen around at 1404 cm^{-1} and 1233 cm^{-1} (Figure 3, Table 3).

Table 2: Fourier transform infrared (FT-IR) spectra peak locations with assignments for shank collagen extracted from Group 2.

Peak positions in wavenumber (cm^{-1})	Assignments
3302	N-H Stretching
2920	C-H Stretching
2845	C-H Stretching
1739	C=O Stretching
1636	C=C Stretching
1531	N-O Stretching
1450	C-H Bending
1325	O-H Bending
1235	C-N Stretching
1149	C-O Stretching
1017	C-O Stretching
875	C=C Bending

The FT-IR spectra of shank extracted collagen from Group 1, Group 2 and Group 3 showed the preservation of characteristic molecular structure of collagen as indicated by the occurrence of distinct amide bands. The Amide bands A, B, I, II and III in our study confirms the proper extraction of collagen from chicken feet, which was similar to the previous findings [9-12]. In a previous study [10] the amide A band (N–H stretching) was found near 3412 cm^{-1} and 3338 cm^{-1} whereas the Amide B band region (-CH_2 asymmetrical stretching) was observed near 2925 cm^{-1}. In addition, Amide I band (C=O stretching), Amide II band (N-H bending and C-N stretching) and Amide III bands (C–N stretching and N–H bending) were appeared around 1745 cm^{-1}, 1550 - 1600 cm^{-1} and 1220 - 1320 cm^{-1} to 1451-1450 cm^{-1} respectively. The shifting of absorption peak towards lower wavenumbers (cm^{-1}) for amide I and amide II in our study denotes the existence of more and stronger hydrogen bonds in the collagen structure which further suggests a more stable and robust triple-helical conformation.

The collagen extracted from Group 1, Group 2 and Group 3 revealed no significant molecular differences suggesting collagen from the different age group included in the present study mainly represents type I collagen with high degree of structural integrity.

The FT-IR spectrum analysis was used as a quantitative method for confirmation of retention of the triple helix structure in extracted collagen. The confirmation was derived from the earlier described method [13, 14] based on the ratio of the peak intensities of amide III and 1450 cm^{-1} (amide III/1450 cm^{-1}), which should be equal to 1 for well-preserved collagen. The present study showed that the ratio of amide III/1450 cm^{-1} for Group 1, Group 2 and Group 3 was found as 0.84, 0.85 and 0.85 - 0.96 respectively which indicated that extracted collagen from all the study groups maintained well-preserved triple-helical

Table 3: Fourier transform infrared (FT-IR) spectra peak locations with assignments for shank collagen extracted from Group 3.

Peak positions in wavenumber (cm^{-1})	Assignments
3280	N-H Stretching
2920	C-H Stretching
2852	C-H Stretching
1744	C=O Stretching
1629	C=C Stretching
1531	N-O Stretching
1453	C-H Bending
1404	C-N Stretching (Amide III band)
1338	O-H Bending
1233	C-N Stretching (Amide III band)
1149	C-O Stretching
1070	C-O Stretching
873	C=C Bending
709	C=C Bending
581	C-I Stretching

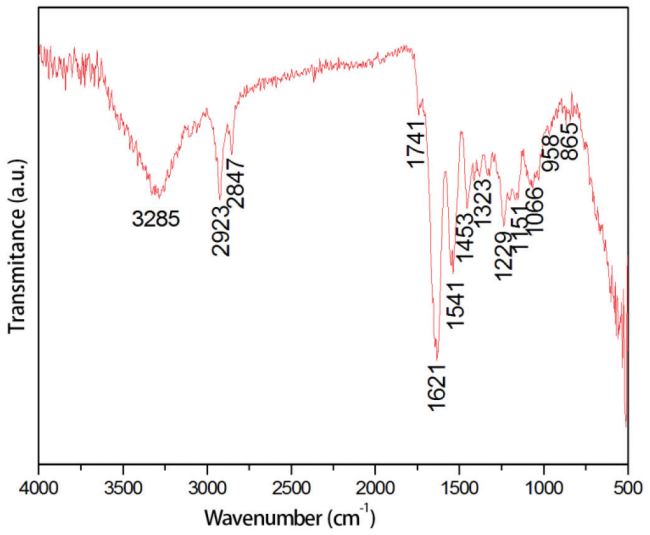

Fig. 1. Fourier transform infrared (FT-IR) spectra of shank collagen extracted from Group 1.

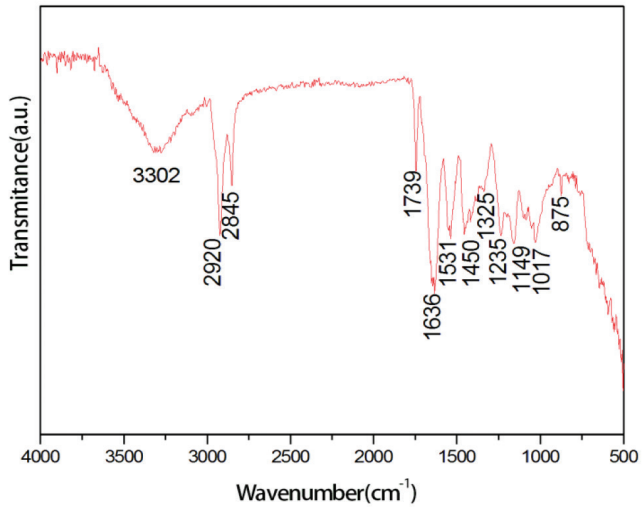

Fig. 2. Fourier transform infrared (FT-IR) spectra of shank collagen extracted from Group 2.

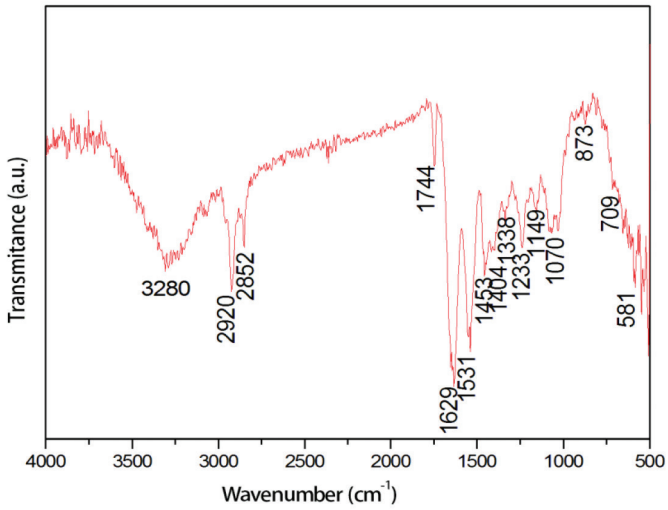

Fig. 3. Fourier transform infrared (FT-IR) spectra of shank collagen extracted from Group 3.

structure. In some studies [12], researchers found that amide III defines integrity of helix structure in collagen, with a ratio value close to 1.0 in our study we might say that the method of collagen extraction used in this study provides well preserved biomedical grade collagen from chicken feet. The triple helix structure is responsible for the biological and mechanical function of collagen and plays a crucial role in forming higher order structures by virtue of its inherent amino acid-amino acid interactions [15]. Therefore, the structural integrity of the extracted collagen is crucial for its commercialization as biomaterial.

The result of this study showed the extraction process by alkali and acid treatments followed by enzymatic hydrolysis provides collagen with higher structural integrity, with minimal contamination from other biological materials. This confirms the effectiveness of the isolation process and supports the potential use of this shank collagen in biomedical applications where structural integrity and purity are crucial.

CONCLUSION

The FT-IR spectroscopy analysis of the chicken shank extracted collagen using alkali and acid treatments followed by enzymatic hydrolysis exhibited well-preserved helical structure. The study further underlined the advantages of extraction process and suggested that chicken shank collagen extracted by this method retains normal molecular structure necessary for biomedical application. In addition, molecular characterization of the collagen extracted by other available methods may be done to strengthen the existing information on collagen extraction for biomedical use.

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ETHICS APPROVAL

The experimental work was approved by the Institutional Animal Ethics Committee (IAEC) with IAEC No. IAEC/RGSC-BHU/2023-24/180.

REFERENCES

1. Gelse K, Pöschl E, Aigner T. Collagens--structure, function, and biosynthesis. Adv Drug Deliv Rev. 2003; 55(12): 1531-1546. https://doi.org/10.1016/j.addr.2003.08.002.

2. Srinivasan S, Durairaj B. Collagen isolation and characterization from *Sardinella longiceps*. J Adv Vet Anim Res. 2021; 8(4): 679-686. https://doi.org/10.5455/javar.2021.h560.

3. Espinales C, Romero-Peña M, Calderón G, Vergara K, Cáceres PJ, Castillo P. Collagen, protein hydrolysates and chitin from by-products of fish and shellfish: An overview.

Heliyon. 2023; 9(4): e14937. https://doi.org/10.1016/j.heliyon.2023.e14937.

4. Campos LD, Santos Junior VA, Pimentel JD, Carregã GLF, Cazarin CBB. Collagen supplementation in skin and orthopedic diseases: A review of the literature. Heliyon. 2023; 9(4): e14961. https://doi.org/10.1016/j.heliyon.2023.e14961.

5. Lim YS, Ok YJ, Hwang SY, Kwak JY, Yoon S. Marine Collagen as A Promising Biomaterial for Biomedical Applications. Mar Drugs. 2019; 17(8): 467. https://doi.org/10.3390/md17080467.

6. Sorushanova A, Delgado LM, Wu Z, Shologu N, Kshirsagar A *et al*. The Collagen Suprafamily: From Biosynthesis to Advanced Biomaterial Development. Adv Mater. 2019; 31(1): e1801651. https://doi.org/10.1002/adma.201801651.

7. Gojkovic Z, Marova I, Matouskova P, Obruca S, Miloslav P. Use of ultrasonic spectroscopy and viscosimetry for the characterization of chicken skin collagen in comparison with collagens from other animal tissues. Prep Biochem Biotechnol. 2014; 44(8): 761-71. https://doi.org/10.1080/10826068.2013.867869.

8. Rana J, Keshri OS, Rahman CKF, Kumar V, Patel SK *et al*. Extraction and evaluation of collagen as biomaterial from chicken shank. Indian J Anim Res. 2024; (Online first article). http://dx.doi.org/10.18805/IJAR.B-5502.

9. Zhou C, Li Y, Yu X, Yang H, Ma H et al. Extraction and characterization of chicken feet soluble collagen. LWT - Food Science and Technology. 2016; 74: 145-153. https://doi.org/10.1016/j.lwt.2016.07.024.

10. Hashim P, Ridzwan MM, Bakar J. Isolation and characterization of collagen from chicken feet. International Journal of Bioengineering and Life Sciences. 2014; 8(3): 250-254.

11. Potti RB, Fahad MO. Extraction and characterization of collagen from broiler chicken feet (gallus gallus domesticus) - biomolecules from poultry waste. J Pure Appl Microbiol. 2017; 11(1): 315-322.

12. Suparno O, Prasetyo NB. Isolation of collagen from chicken feet with hydro-extraction method and its physico-chemical characterisation. In IOP Conference Series: Earth and Environmental Science. 2019; 335(1): 012018.

13. Doyle BB, Bendit EG, Blout ER. Infrared spectroscopy of collagen and collagen-like polypeptides. Biopolymers. 1975; 14(5): 937-957. https://doi.org/10.1002/bip.1975.360140505.

14. Gao LL, Wang ZY, Zheng LI, Zhang CX, Zhang DQ. The characterization of acid and pepsin soluble collagen from ovine bones (Ujumuqin sheep). J Integr Agric. 2018; 17(3): 704-711. https://doi.org/10.1016/S2095-3119(17)61751-9.

15. Fernandes LL, Resende CX, Tavares DS, Soares GA, Castro LO et al. Cytocompatibility of chitosan and collagen-chitosan scaffolds for tissue engineering. Polímeros. 2011; 21(1): 1-6.

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