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Extraction and a Study on the Characterization of Collagen from Fish Scales

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ABSTRACT

Collagen, a well-known protein regarded as one of the most useful biomaterials and has wide biomedical applications due to its biocompatibility and biological characteristics. It is the principal structural protein present in the skin, tendon and bones of the vertebrate body. Different types of vertebrate collagens have been identified of which the dominant collagen is Type – I. The present study focussed on the extraction of collagen from the scales of fish collected as biowaste. The extracted collagen characterised by UV-VISIBLE, FTIR and SDS PAGE techniques to confirm it as Type-I.

Keywords: Fish scales, collagen, UV-Visible, FTIR, SDS PAGE.

1. INTRODUCTION

Collagen is regarded as one of the most useful biomaterial and structural protein in the eukaryotes. It is the most abundant protein in vertebrates and constitutes about 30% of the total protein present in the skin, tendon, bones etc,[1,2] Collagen has a vital role in biomedical applications as wound dressing material, vitreous implant ,drug carriers etc. Among the various types, Type-I collagen is commonly found in mamanalian tissues. However, due to outbreak of Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephlopathy (TSE), Fort and Mouth Disease (FMD) in pigs and cattle, use of collagen from animal sources have been limited[3,4,5]. Type –I collagen has also been extracted from fish tissues such as skin, bone and fins. Several papers have reported on the characteristics and possible isolation procedures of skin, bone and scale collagen from various fish species[6,7,8].

Fish scales are biocomposites of highly ordered type-I collagen fibres and hydroxyapatite $Ca_{10}(OH)_2(PO_4)_6$ [9]. The present study focussed on the extraction of collagen

from fish scales of biowastes. During the processes of fish, a great amount of fish scales are dumped, which is a great waste because the scales of fish contain a large amount of collagen. We expect to obtain a protein with physiochemical parameters suitable for cosmetic, pharmaceutical and biomedical applications. Therefore in the present study, we have extracted collagen from fish scales which were collected as biowaste and characterised the extracted collagen as type-I.

2. MATERIALS AND METHODS

2.1 Materials

All the chemicals bought were of Analytical grade from Hi-media and used as such.

2.2 METHODS

EXTRACTION OF COLLAGEN

The extraction of collagen from fish scales was performed using a modified method[7]. Fresh fish scales were collected as biowastes from local market. The scales were washed thoroughly with distilled water to remove sand and other foreign bodies and later exposed under sunlight.200 g of dried fish scales were soaked in 10% sulphuric acid solution for 24 h. The fish scales were then minced with an industrial lab blender and the resultant fine paste was subjected to centrifugation (12,000 rpm) at 4ºC for 20mins. This supernatant was collected and its pH was adjusted to 7 using calcium hydroxide solution. The supernatant solution was further centrifuged at 10,000 rpm for 15 minutes to remove calcium sulphate salts. The supernatant solution containing collagen (60% solids) was stored at 4ºC for further potential application.

Fig-1 Fish scales

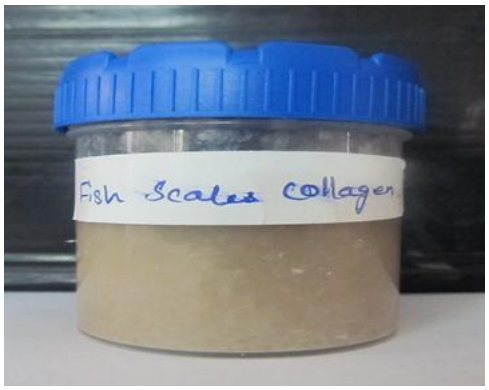


Fig- 2 Fish scale collagen

3. CHARACTERIZATION

The extracted fish scale collagen were analysed by UV –visible spectrophotometer of the model SHIMADZU 1650 PC and FT-IR spectroscopy using IR affinity 1, model of SHIMADZU IR 1650 PC.

UV-Visible spectroscopy

The UV-VISIBLE spectrum shows a maximum absorption at 231nm, closer to the absorption of Carp fish scale collagen which was in accordance with the characteristic absorption of collagen[8,9].

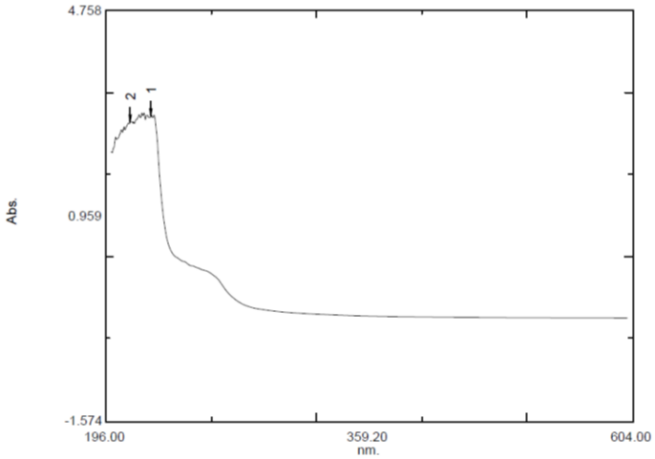
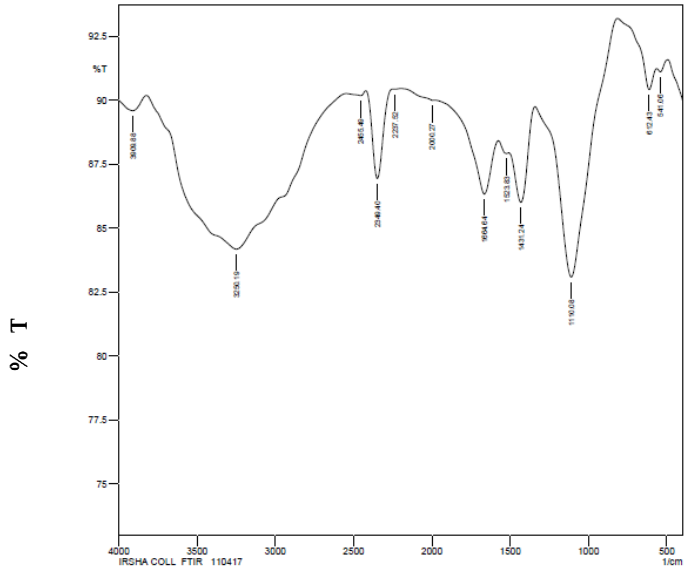


Fig- 3. UV-VISIBLE spectrum of fish scale collagen

FTIR Spectroscopy

The FTIR spectrum of extracted collagen is given in Fig- 4 . The spectrum shows the characteristic absorption bands 1664 cm^{-1}, 1523 cm^{-1}, 1431cm^{-1} for amide –I, amide-II and amide-III respectively. Also the band at 1110 cm^{-1} is due to the hydroxyl group of collagen.



Wavenumber (cm^{-1})

Fig-4 FTIR spectrum of fish scale collagen

SDS - PAGE METHOD

Electrophoresis is the process of migration of charged molecules through solutions in an applied electric field. Basically electrophoretic technique has been developed to separate macromolecules on the basis of their molecular weight. The rate of migration of molecule in an electric field is inversely proportional to the molecular size, shape, viscosity and temperature of the medium and directly proportional to the voltage and charge of the molecule.

Proteins could be resolved electrophoretically in a semi solid matrix strictly on the basis of molecular weight. Under these conditions, the mobility of the molecules would be inversely proportional to their molecular weight. SDS PAGE technique is exceedingly useful in analyzing and resolving complex protein mixtures.

SDS is an anionic detergent which denatures protein molecules without breaking peptide bonds. In PAGE, proteins are uniformly negatively charged by an ionic detergent SDS. SDS molecules bind and wrap around them by strong hydrophobic interactions and it has been estimated that 1.4g of SDS bins per gram of protein.

Polyacrylamide gel is formed by the polymerization of the monomer molecule acryl amide cross linked with N-N'-Methylene Bis acryl amide. Ammonium per sulphate is used to initiate the polymerization reaction by generating free radicals and catalyst acting as an oxygen scavenger TEMED is required to start the polymerization reaction.

Denaturation of proteins is performed by heating in a buffer containing a soluble sulphydryl reducing agent 2-mercapto ethanol and SDS. Mercaptoethanol reduces all the disulphide bonds of cysteine residues of free sulphydryl groups and heating with SDS that disrupts all intra and intermolecular protein interactions. This treatment to yield individual polypeptide chain which carries an excess negative charge induced by binding of the detergent SDS. The denatured proteins can be resolved electrophoretically on the basis of size in a buffered polyacrylamide gel.

Procedure

SDS-PAGE method was carried with the following reagents: Resolving gel 10%, Stacking gel 5%, Coomassie staining solution, Detaining solution[10].

Two glass plates are assembled like sandwich. APS and TEMED is mixed without the formation of bubbles. The gel solution poured between the glass plates and covered with resolving gel with 50% Isopropanol and 0.1% SDS solution. When polymerisation is completed, a clear line will appear between the gel surface. Stacking gel is poured over it and allowed to polymerize for at least 30 minutes. Then the gel was placed into the electrophoresis tank, which was already filled with IX Tris –glycine SDS buffer. Then the sample and protein ladder is loaded (10-300 kd) with probes and voltage was set at 50v for 30minutes and increased upto 150v.

In general, after staining with Coomassie brilliant blue, the SDS PAGE gel will exhibit protein band. In sample of crude collagen, clear visible protein bands of 107kD, 117kD and 146kD were observed compared with protein marker. In which 107kD and 117kD are partial digest of collagen protein.

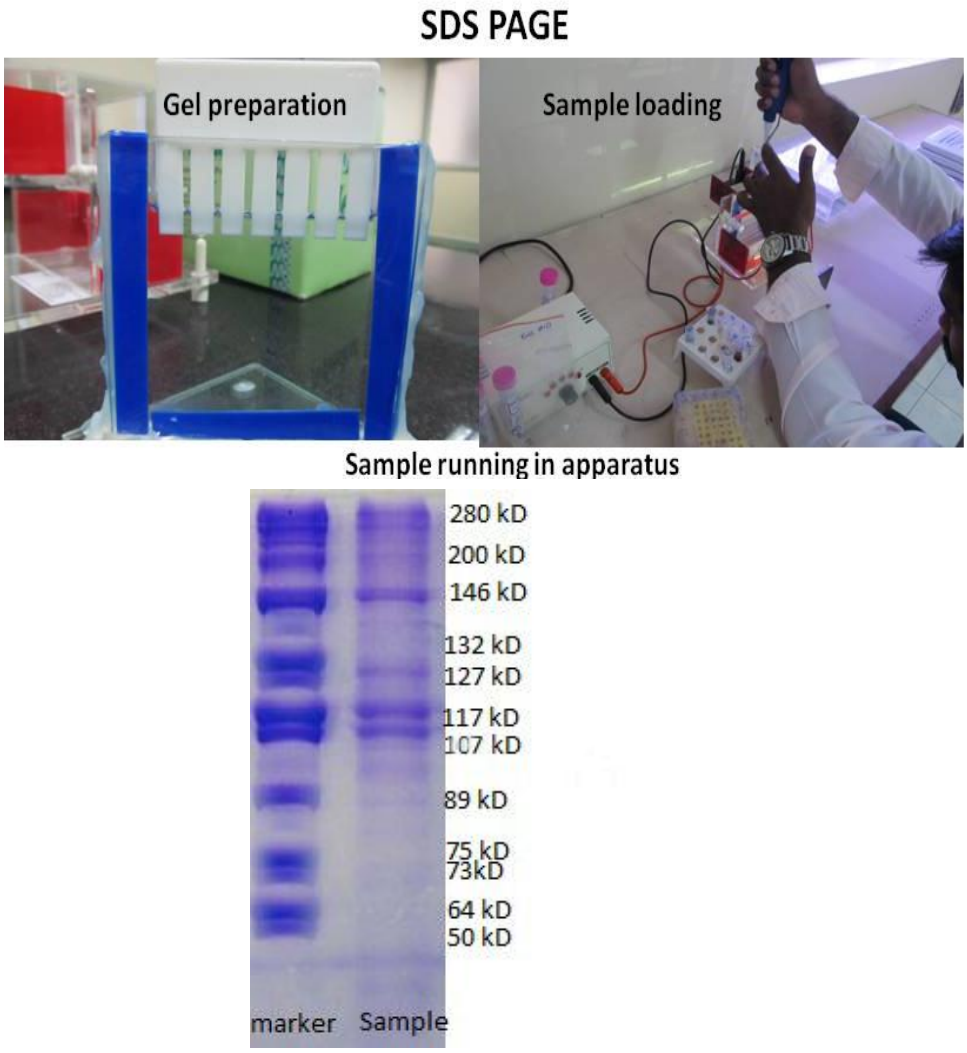


Fig- 5 SDS-PAGE Electrophoresis image

3. CONCLUSION

Fish scales collected as biowaste contain a rich amount of collagen. The authors extracted successfully type I collagen from fish scales. The UV and IR spectrum studies and SDS electrophoresis studies proved the extracted collagen as type- I. Collagen as a biomaterial has widespread applications in numerous fields such as pharmaceutical, medical, biomedical, food industry, cosmetics etc. Further its biological characteristics can be explored for other potential applications.

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