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Original Article

EXTRACTION AND CHARACTERIZATION OF GELATIN: A FUNCTIONAL BIOPOLYMER

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ABSTRACT

Objective: Gelatin is widely used biopolymer in various industries due to its excellent biocompatibility, biodegradability properties. In the present study, gelatin was extracted from fish wastes, as an alternative source.

Methods: This biopolymer was extracted from the scales of freshwater fish, *Labeo rohita*. After extraction, the proximate analysis and physico-chemical analysis of the fish scale gelatin were carried out. This functional polymer was also characterized using different analytical methods, such as UV-vis spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) for the evaluation of crystalline and surface morphology, and fourier transform infrared spectroscopy (FTIR) for structural determination.

Results: The scales of *L. rohita* yield 24% (dry weight basis) of gelatin, indicating this fish species as potential source of gelatin. The proximate analysis determined was low moisture content (4.2%), ash (1.4%) and high protein (90%) content. The result of the study confirms the effectiveness of extraction method used.

Conclusion: The fish scales of *L. rohita* are found to be a sustainable and renewable source of gelatin with desirable functionalities and it is the best alternative for mammalian gelatin in food and other industries.

Keywords: Gelatin, Fish scale, Extraction, Characterization

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INTRODUCTION

In recent years, there has been an increasing interest for using biodegradable materials in packaging, agriculture, food, medicine etc. A variety of blends using biopolymers can be the alternative of currently used synthetic polymeric materials. The most common and potential biopolymers are starch, gelatin, chitosan, alginate, PLA, PHAs, etc. [1]. Among these, gelatin has increased global demand as it is an important functionally active biopolymer [2]. Gelatin is a naturally occurring macromolecular and biodegradable protein that is produced by the controlled partial hydrolysis of collagen synthesized from skins, white connective tissues and bones of animals which is composed of amino acid residues at different proportions and combinations [3]. Because of the favorable properties, such as high water solubility, non-toxicity, thermo-reversible sol–gel transition, high mechanical strength and elasticity in a dry state, moisturizing cause by binding a plenty of water, gelatin are widely used as raw material in photography, pharmaceutical and cosmetic industries, it is used as water-soluble capsules, coating materials for oral drugs, stabilizer of photo-sensitive reagents in photographic films, adsorbent for diluted chemicals and adhesive agents [4, 5]. In the food industry, gelatin is one of the water soluble polymers that can be used as an ingredient to improve the elasticity, consistency and stability of foods. They are utilized in confections (mainly for providing chewiness, texture, and foam stabilization), low-fat spreads (to provide creaminess, fat reduction and mouth feel), dairy (to provide stabilization and texturization), baked goods (to provide emulsification, gelling and stabilization) and meat products (to provide water-and fat-binding) [6]. There are two main types of gelatin. Type A, with isoionic point of 7 to 9, is derived using exclusively acid pretreatment. Type B, with isoionic point of 4 to 5, is the result of an alkaline pretreatment [7].

Generally, the sources of gelatin produced from bovine and pig skins and demineralized bones and hooves [8]. For several reasons there are still serious concern among the consumers to consume gelatin which produce from bovine and porcine bones and skins. This is because some problems such as religious matter, mad cow disease (Bovine Spongiform Encephalophy: BSE) and social reasons. Factors such as the outbreak of BSE and increasing demand for non-mammalian gelatin have revived the interest to discover the alternatives sources of gelatin from fish (marine and freshwater) and poultry as raw materials [9, 10]. The waste generated from the worldwide production and processing of shell-fish and fish scales is a serious problem of growing magnitude. This abundant waste may pose environmental hazard [11]. The use of this waste for renewable products such as biopolymers is a dual-purpose opportunity [12]. Therefore, the abundance sources of fish byproduct such as bones, scales and skin can be the great sources of gelatin. Fish scales and bones are more preferable in the extraction of gelatin because it yields large amount of gelatin due to high content of amino acids (proline) compared to fish skin [13]. Thus the aims of this investigation were to extract the gelatin (Type B) from scales of *L. rohita*, a fresh water fish, to characterize and to study some its physicochemical properties.

MATERIALS AND METHODS

Collection and preparation of materials

Scales of *L. rohita* with an average body weight of 100–200 g were collected at Guntur, Andhra Pradesh, India. The fish scales were removed with hands, packaged in zip locked polyethylene bags, iced, and quickly transported to the laboratory. The scales were treated with chilled water, washing it twice to clean before being dried, finally dried and kept at 5 °C until further use. All the reagents used in this work were of analytical grade and purchased from Hi-Media (Mumbai).

Extraction of gelatin

Gelatin-Type B was extracted by alkali treatment method [14]. Dried scales were initially stirred with 5% sodium chloride (NaCl) solution (1/10, w/v) for 30 min at room temperature. This step was repeated twice and the second step was completed by stirring with 0.4% sodium hydroxide (NaOH) (1/10, w/v) for 60 min to remove the non-collagenous proteins from the scales. Alkali solution was changed every 30 min and the third step proceeded using 10% isobutyl alcohol (1/4, w/v) to remove lipids from the scales. This step was repeated three times for 30 min in a digital linear shaker.

The final step was demineralization with 0.5 N ethylenediamine tetraacetic acid (EDTA) solutions at an inherent pH of 7.66 for four different time periods of shaking: 12 h, 2 h, 2 h, and 1 h. In each step, scales were recovered by filtering through a sieve and washed with distilled water to remove any residual matter. Recovered scales were soaked in 0.05 M acetic acid solution for 3 h. After filtering, 1/3 (w/v) water was added and heated at 60 °C overnight in an oven. This was filtered and the filtrate (dried thin films) was dried in plastic trays at room temperature using air conditioning overnight (set at 18 °C, flow temperature was 10±2 °C). Dried thin films were ground using a grinder and gelatin powder was obtained.

Physiochemical properties

After gelatin extraction, quality factors were determined according to national and international standards.

Gelatin yield

The yield of gelatin was calculated based on dry weight of fish scale [15] by using the following equation:

$$\text{Yield of gelatin (\%)} = \frac{\text{Weight of dried gelatin (g)}}{\text{Dry weight of fish scale (g)}} \times 100$$

Proximate analysis

The moisture, ash, total protein contents and fat contents of the gelatin were determined according to the standard methods. Conversion factors were 5.51 was used for calculating the protein content of the gelatin [16, 17].

Determination of gelatin pH

The pH of gelatin solution was determined by preparing 1% (w/v) gelatin solution in distilled water and cool to 25 °C in a water bath and pH was measured.

Determination melting temperature

The method for melting point measurement was described by Choi and Regenstein [18]. The sample (1 g) was heated and stirred using a spatula and repeated three times.

Characterization of biopolymer

UV-vis spectroscopy analysis

The UV-vis absorption spectrum of gelatin was recorded using a UV-vis double-beam spectrophotometer (UV 8500 II, Techcomp) in the range of 200–400 nm.

Fourier transform infrared (FTIR) spectroscopy analysis

Fourier transform infrared (FTIR) (Jasco-FTIR 4100 type A, Japan) spectrum was recorded to detect the chemical and structural nature of gelatin powder. 2 mg of gelatin powder were mixed with 100 mg of potassium bromide [19] and placed on the crystal cell of the FTIR spectrophotometer. Measurement was performed at 4000–400 cm^{-1} at room temperature and automatic signals were collected from 32 scans at a resolution of 4 $cm^{-1.}$

X-Ray diffraction analysis of gelatin

Further characterization of gelatin powder was done using X-ray diffraction technique. It was carried out in an X-ray diffractometer (X'Pert Pro A Analytical) operated at 45 kV voltage and 40 mA current. The pattern was recorded by Cu Kα radiation in a θ–2θ configuration.

Scanning electron microscopy (SEM) analysis

The surface morphology of gelatin was analyzed by scanning electron microscope (FEI Quanta 200 SEM). The surface of dried gelatin was coated with gold in vacuum using sputter coater, and was photographed.

Statistical analysis

All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences. Values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Yield of gelatin

The yield of extracted light yellow color gelatin was 24% based on dry weight basis from fish scales. This result was higher than that reported by Jamilah and Harvinder [10], with the yield of extracted gelatin of red tilapia and black tilapia of 7.81% and 5.39%, respectively.

Physico-chemical and proximate composition of gelatin

The proximate compositions of extracted gelatins are tabulated in table 1. The moisture content of the fish scale gelatin was 4.2% which is much below the prescribed limit of 15% [20] for edible gelatin. The sample gave a low ash content of 1.4% that well below the recommended maximum of 2.6% [21]. The extracted gelatin showed high protein (90%) content as protein is the main constituent of gelatin. The gelatin was found to be low lipid content (1.05%). pH value of gelatin extracted by an alkali pretreatment method from fish scale found to be 4.35 which may be depend on the extraction method, because it has been already reported that alkali pretreatment results in Type B gelatin with pH in the range of 4-5 [8]. The melting temperature of the extracted Type B gelatin was 27 °C which significantly affects its gelling properties.

Characterization of biopolymer

To identify the amino acids contributing to the specific of gelatin, UV-vis spectrophotometer was used to characterize in the range of wavelength 200–400 nm. Chromophore groups which give absorption at 210-240 nm indicate the presence of characteristic peptide bond fragments from each of the gelatin [22]. Fig. 1 shows the characteristic UV-vis spectrum of gelatin at 224 nm, confirms the distinctive structure of gelatin.

The application of gelatin depends on the chemical stability without undergoing degradation like partial oxidation. FTIR measurement was made to identify the possible biomolecules, bonds responsible for the structural and functional stabilization of the gelatin extracted from the fish scales. Proteins are comprised of amino acids joined together by amide bonds. The polypeptide and protein repeat units give rise to nine characteristic infrared (IR) absorption bands, namely; amide A, B, and I-VII [23]. Amide bands represent different vibrational modes of the peptide bond. The absorption bands of gelatin in the IR spectra are situated in the amide band region; amide-I represents C=O stretching/hydrogen bonding couple with COO, amide-II represents bending vibration of N-H groups and stretching vibrations of C-N groups, Amide-III is related to the vibrations in plane of C-N and N-H groups of bound amide [24]. Fig. 2 shows the peaks of the gelatin at 3433 cm^{-1} attributed to the presence of hydrogen bond water and amide-A, 1630 cm^{-1} peaks corresponds to the occurrence of amide-I, at 1565 cm^{-1} is indicating amide-II, band at 1240 cm^{-1} indicates the amide-III, peaks ranges from 1460 cm^{-1} to 1380 cm^{-1} were attributed to the symmetric and asymmetric bending vibrations of methyl group [25-27].

Table 1: Proximate and physico-chemical analysis of gelatin

Factors	Fish scale gelatin
Moisture	4.2%
Protein	90%
Ash	1.4%
Lipid	1.05%
pH	4.35
Melting temperature	27 °C

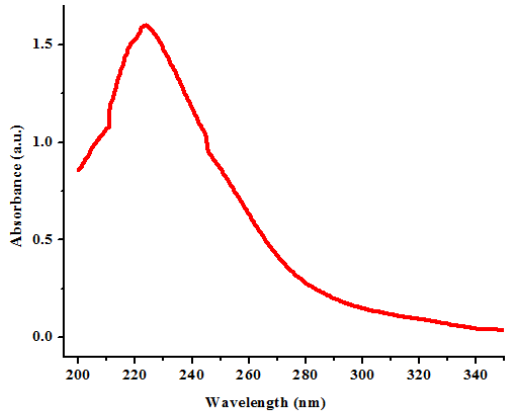

Fig. 1: UV-vis absorption spectrum of fish scale gelatin

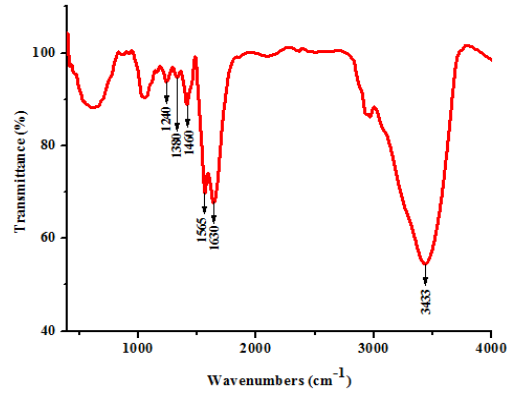

Fig. 2: FTIR spectrum of gelatin

Porosity characterization is based on the presence of open pores which are related to properties such as permeability and surface area of the porous structure. The microstructure obtained by SEM for the gelatin showed that polymers have an array of hollow cells that resembles spherical particle structures (fig. 3). Since a higher density of a scaffold usually leads to higher mechanical strength while a high porosity provides a favorable biological environment, a balance between the porosity and density for a scaffold must be established for the specific application [28]. The diffractogram pattern acquired on the pure gelatin powder were typical of a partially crystalline gelatin with a sharp peak with low intensity located at 2θ = ~7° and a broad peak located at 2θ = ~19°, shown in fig. 4. These characteristic peaks are usually assigned to the triple-helical crystalline structure in gelatin [29, 30].

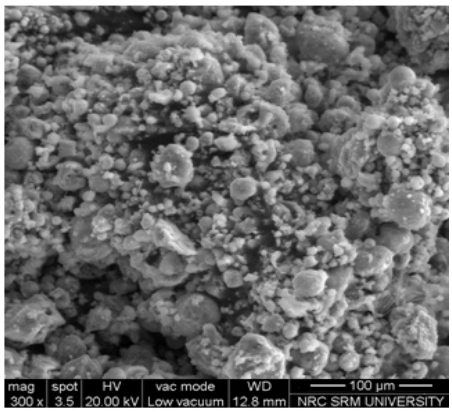

Fig. 3: SEM micrograph of gelatin extracted from fish scale

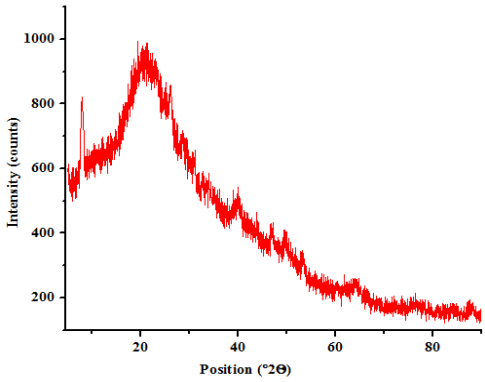

Fig. 4: XRD pattern of fish scale gelatin

CONCLUSION

This study revealed the potential of *L. rohita* scale as raw material for gelatin production, giving relatively high yield. Gelatin was characterized using UV-vis, FTIR, SEM and XRD methods. Morphological investigation showed that this polymer exhibit micro-porous morphology, FTIR spectrum indicates chemical bond formation of gelatin whereas XRD analysis revealed the crystalline structure of it. It also shows high protein content (90%) with low moisture (4.2%) and ash (1.4%) content. In brief, fish scale is a cost-effective and environmentally friendly source of gelatin which can be used in various industrial applications.

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CONFLICT OF INTERESTS

Declared none

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