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Structural Properties of Gelatin Extracted from Marine Fish Gethar (*Sarda orientalis*)

Pranoti Kirdat[1],Padma Dandge[1]
pbd_biochem@unishivaji.ac.in;panukirdat@gmail.com

Department of Biochemistry,Shivaji University,Kolhapur,Maharashtra,India-416004

Abstract

The current developments in fish processing technology increases demand of fish processing by-products in various sectors. In present approach, gelatin was extracted by using 0.2 M lactic acid from waste material of *Sarda orientalis* (Gethar) and acid soluble collagen extracted from it. Both methods yielded 18.57±0.18% and 9±0.09% gelatin respectively. Proximate analysis of waste and gelatin revealed that it has good protein content. A structural property of extracted gelatin was studied by using UV visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction analysis (XRD). Current study revealed that fish waste gelatin can be considered as more suitable option to be used as a gelling agent in neutraceutical sector. The development of an effective method for extracting gelatin by chemical method using organic acid from *S.orientalis* fish waste will be beneficial to environment by reducing pollution caused due to waste.

Keywords:- Sarda orientalis, fish waste, gelatin, collagen, lactic acid, neutraceutical.

1. Introduction

Gelatin is one of the broadly used biopolymer (polypeptide) in food pharmaceutical and cosmetic sectors. Due to distinct physical characteristic, gelatin is utilized both as food additive and functional food in the food industries. Gelatin was employed as a thickener, stabilizer, adhesive, emulsifier in biodegradable films as well as gelling, foaming and microencapsulation agent in various food products like confectionary, jelly, milk, yoghurt, ice cream, cheese, and canned foods. Gelatin possesses some bioactive properties which includes antimicrobial, antioxidant and also showed antihypertensive activity by inhibiting angiotensin converting enzyme (ACE) (Atma and Ramdhani 2017).

Gelatin exhibits several functional properties in food processing and formulations. They are classified into two classes: first one is correlated with gelling process and another one is related to the surface behavior of gelatin. The degree of collagen conversion into gelatin associated with the strength of pre-treatment and extraction method as a function of pH, temperature, and extraction period. Depending on the acid or alkaline pre-treatment process, two categories of gelatin were obtained, called as type-A (isoelectric point at pH ~8–9) and type-B (isoelectric point at pH ~4–5), respectively (Sousa et al.2017).

During last few years, the neurological disorders like Bovine Spongiform Encephalopathy (BSE) have been identified in several countries and it may be exported from cow meat and meat products. On the other hand, consumption of pork meat has ethical issues in some religions. Therefore, there is need to find out new sources of gelatin like fish wastes which is generated in more amount during fish processing by industries and local markets. About 75% waste was produced after fish processing and it was major environmental pollutant. Skin and bone part of waste contains higher amount of collagen protein. The major distinguishing feature between fish and mammalian gelatin is the quantity of imino acids proline and hydroxyproline, which balances the ordered configuration during gel formation of gelatin. Due to less amount of these imino acids gelatin possess low gelling and melting temperature (Tavakolipour 2011).

The generation of gelatin from fish waste mainly from fish skin has much importance due to its characteristic properties and qualities. Fish and fish products were accepted by all religions, so there are no ethical issues. It imparts a solution to the implementation of vast amounts of fish wastes generated by fish processing industries. The total world fisheries accounts about 141.6 million tons (FAO, 2006) and it was increases day by day. Thus, there is need to convert, this enormous quantity of fish waste into beneficial products such as fish gelatin (Herpandi et al.2011).

The extraction of gelatin from waste material involves acid or alkaline treatment to cleave the collagen crosslinks which was followed by extraction with hot water. Heat treatment is important to destabilize the triple helix confirmation of collagen to transform its helical configuration into a coiled structure, resulting in a gelatinous nature when the matter comes to cooling temperature (Dincer et al.2015).

In current approach, gelatin was extracted from waste material of Gethar (*Sarda orientalis*), also called as striped bonito. It is tuna like fish of length 102 cm. It belongs to species of marine perciform fish and family Scombridae. It is distributed through the Indo-Pacific and East Pacific region and occurs at depths from 1 to 167 meters from sea surface. Thus, gelatin was extracted by using two methods and studied for its structural as well as functional properties.

2.Experimental Methodology

2.1. Chemicals

Pure gelatin, lactic acid and SDS-PAGE chemicals were purchased from Himedia, India and SRL, India. Different chemicals and reagents utilized for this study were of analytical grade.

2.2. Collection and cleaning of fish waste

For collagen and gelatin extraction fish waste like skin, scales, eyeballs, fins and tail of fish *Sarda orientalis* (Gethar) were collected from Gadre fish industries and fish market Ratanagiri (MS, India). The waste material of fish was collected by maintaining suitable conditions. It was washed with cold tap water and scrubbed with knife to bring out flesh. The cold demineralized water was used for final wash and cut into small pieces for further extraction.

2.3. Proximate analysis of fish waste

The standard methods approved by AOAC (1980) was carried out to determine proximate composition of fish waste. The content of moisture, fat, ash and protein was evaluated on wet weight (WWB) and dry weight basis (DWB).Also proximate analysis of gelatin was carried out after extraction.

2.4. Pre-treatment of waste

Pre-treatment was carried out according to procedure of Kumar and Nazeer (2013) with slight modifications. The whole process was conducted at 4ºC. Non-collagenous proteins were removed by soaking the waste pieces (5 gm) in 0.1 M NaOH (1:30 w/v) for 24 hr. Treated waste was washed with cold demineralized D/W till pH 7 and subjected to defatting by 10% butyl alcohol (1:30 w/v) for 48 hr. After defatting, material was washed repeatedly with cold demineralized water till neutral pH and it was subjected to collagen extraction by 0.2 M lactic acid for 40 h.

2.5. Extraction of collagen

Collagen was extracted from waste material of fish by using procedure of Bhuimbar et.al. (2019) with minor changes. Extraction method was optimized with respect to acid type and concentration. It was found that, 0.2 M lactic acid gives maximum yield of collagen within 40 hr. Extracted collagen was characterized and tested for its functional properties. The pure collagen was used for gelatin formation by thermal hydrolysis.

2.6. Gelatin extraction

Partial hydrolysis of collagen by acid, alkali or thermal treatment causes formation of gelatin. One method utilizes extracted collagen for gelatin preparation while in another method pre-treated waste material was directly used for gelatin extraction. The extraction was carried out according to following two methods.

2.6.1. By collagen hydrolysis

Method given by Du et.al. (2014) was slightly modified for formation of gelatin from collagen. Extracted collagen (2 gm) were mixed distilled water with 1:3 (w/v) ratio after that gelatin was formed by thermal treatment at 60ºC-80ºC for 7 hr. The gelatin which was soluble in water was recovered by centrifugation at 5000 rpm for 15 min at 20ºC.

2.6.1. From waste

Gelatin was extracted directly from pre-treated waste by the method of Tinrat and Asna (2017) with slight modifications. The 5 gm pre-treated fish waste material was mixed in a ratio of 1:10 (w/v) with 0.2 M lactic acid and placed under continuous stirring for 2 hr. The acid treated waste was washed thoroughly with distilled water till neutral pH. The final extraction was carried out with distilled water in a ratio of 1:10 (w/v) and kept for continuous stirring at 80ºC-100ºC for 1.5 hr. The viscous solution obtained after extraction was centrifuged at 5000 rpm for 10 min, to remove impurities and it was filtered through muslin cloth. The resultant filtrate was dried using hot air oven at 60ºC for 48 hr. Powder form of fish gelatin was stored for further applicatory studies.

2.7. Yield of collagen and gelatin

Yield of collagen extracted from fish waste was calculated by following formula;

$$\text{Yield}(\%) = \frac{\text{weight of freeze dried collagen (g)}}{\text{weight of initial skin (g)}} \times 100$$

Yield of gelatin extracted by two methods were calculated by following formula;

$$\text{Yield}(\%) = \frac{\text{weight of dried gelatin (g)}}{\text{weight of dried collagen (g)}} \times 100$$

$$\text{Yield}(\%) = \frac{\text{weight of dried gelatin (g)}}{\text{weight of dried waste (g)}} \times 100$$ ----------(Bordignon et al.2019)

2.8. Structural characterization of gelatin

Extracted gelatin was characterized by UV visible spectroscopy (Shimadzu UV-1800 Japan) between 200-400 nm and characteristic peak was observed. The Fourier transform infrared spectroscopy (FTIR) was carried out to determine different functional groups attached to gelatin. FTIR study was carried out on the instrument Affinity-1S Shimadzu model. X-ray diffraction pattern gives information about the distribution and orientation of gelatin. X-ray diffraction analysis of extracted gelatin was done by Bruker AXS analytical instrument (Germany). The samples were subjected to Cu Ka radiation at 40 kV voltage and current of 40 mA with scanning range 10°-80° (2θ).

3. Result and discussion

3.1. Proximate composition of fish waste and gelatin

Proximate composition like moisture, protein, lipid and ash content of fish waste and gelatin extracted from it were tabulated in table no.1. The pre-treatment carried out during extraction abolish some cross linked components present on waste and used to eliminate impurities as well unwanted materials (Ward & Courts 1977). In proximate composition analysis of fish waste, 60.03±0.54 % moisture, 42.35±0.21 % protein, 7.85±0.63 % lipid and 1.34±0.78 % ash was observed. Muyonga et al. (2004) described that, increase in collagen content of material causes maximum production of gelatin from waste.

The protein content of gelatin was much higher than waste gelatin. Other contents like moisture, lipid as well as ash was found to be lower than waste. Extracted gelatin contains 15.42±0.38 % moisture, 88.87±0.08 % protein, 1.67 ±0.32 % lipid and 1.05±0.27 % ash. Similar results were obtained for gelatin extracted from catfish skin (Ardekani et al.2013), Calf skin, Carp scale and Pork skin(Dincer et al.2016).

Table 1: Proximate analysis of fish waste and gelatin

Samples	**Moisture (%)**	**Protein (%)**	**Lipid (%)**	**Ash (%)**
Fish waste	60.03±0.54	42.35±0.21	7.85±0.63	1.34±0.78
Gelatin	15.42±0.38	88.87±0.08	1.67± 0.32	1.05±0.27

Values were means ± standard deviation from triplicate determinations.

3.2.Yield of collagen and gelatin

The lower degree of cross links present in collagen molecule gives minimum yield (16).Thus, 26% yield (1.3 gm) was obtained from 5 gm fish waste by acid extraction method. Extraction of gelatin from pre-treated waste material of fish by using combination of both acid and heat gives economically viable yield and also produce gelatin with high gel strength (17).Yield of gelatin by collagen hydrolysis was found to be 9±0.09% and from waste material about 18.57±0.18% gelatin was obtained. The results were closely related to gelatin extracted from shark skin (18.65%) (Aberoumand 2011), tilapia skin (12.24%) (Boulahsen et al.2018), as well as skin and bone of *P. hypophthalmus* (17.29% and 14.16%.) (Chavan et al.2018).

3.3.Structural properties of gelatin

3.3.1.UV visible spectral analysis

UV visible spectroscopic analysis of extracted gelatin was carried out in the range of 200-400 nm. Chromophore groups present in gelatin exhibited characteristic absorption peak in UV analysis (Hermanto et al.2013). In figure 1, gelatin extracted from Gethar showed UV absorption peak at 280 nm which approves the distinguishing structure of gelatin.

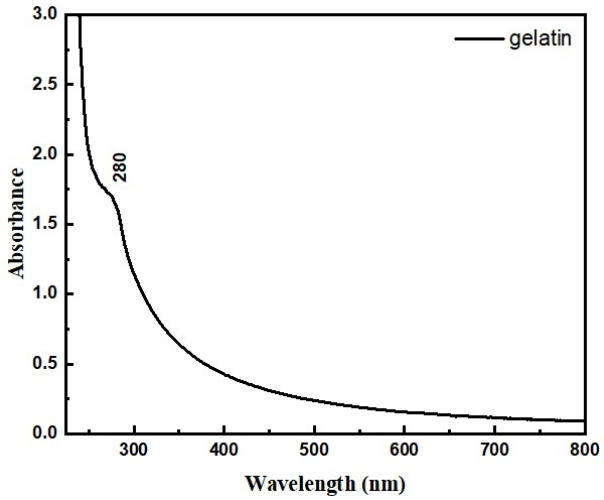

Fig. 1:UV visible spectroscopy analysis of gelatin

3.3.2. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was used to analyse different functional groups attached to gelatin during extraction process was given in figure 2. The stretching at 3300 cm-1 was due to N-H bond (amide-A band) and it's a characteristic pattern of gelatin (Silva et al.2014). Peaks present at 1384 cm-1 and 1448 cm were assigned due to methyl group which exhibit symmetric and asymmetric bending vibrations (Das 2017).The stretching occurred at 1211 cm-1 expresses C-N and N-H in-plane bending while bending at 1625 cm-1 occurred due to C=O (Mureithi et al.2017).The peak region present at 1034 cm-1 and 1116 cm-1 corresponding to Amide I, II and III (Arsyanti et al.2018).The similar spectrum of FTIR was exhibited in catfish skin gelatin (Sai-Ut et al.2012).

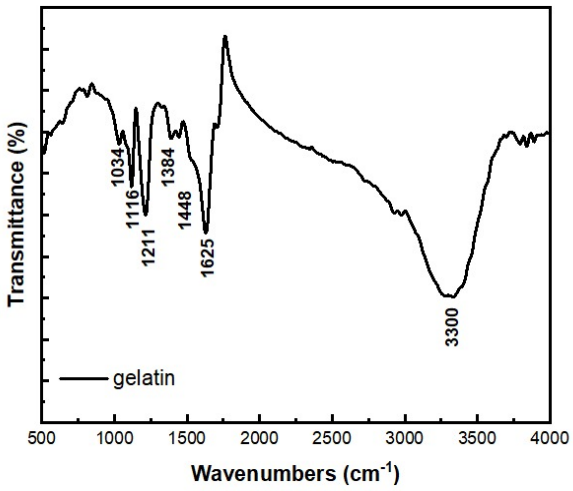

Fig 2:FTIR analysis of gelatin

3.3.3. X-ray diffraction studies

The X-ray diffraction pattern of pure gelatin was given in figure 3.The partially crystalline nature of powder was observed in diffratogram with a sharp intensity peak at 2θ = ~20°.These characteristic peak was occurred due to triple helical crystalline nature of gelatin (Das et al.2017). Also similar diffratogram were given by Yakimets et al.(2005) and Pena et al. (2010).

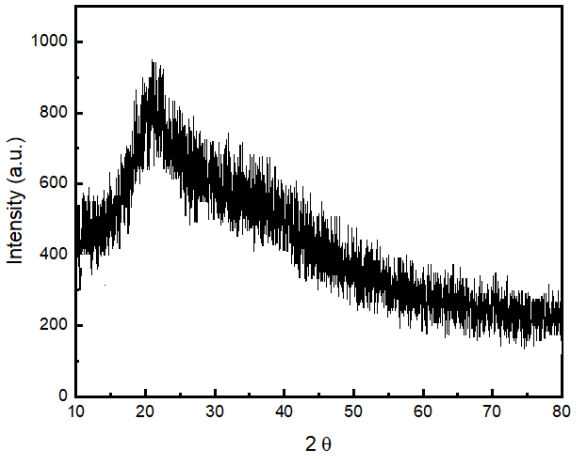

Fig 3:XRD analysis of gelatin

4. Conclusion

Gelatin is one of the widely accepted biopolymer derived from fibrous protein collagen. Gelatin can be easily extracted from fish waste which is major waste produced during fish processing. In current approach, gelatin was extracted from marine fish Gethar (*Sarda orientalis*).The gelatin was extracted by using collagen hydrolysis method as well as acid pre-tratement method followed by extraction with distilled water. Yield of gelatin by collagen hydrolysis was 9±0.09%while acid pre-treatment method yields 18.57±0.18%.The extracted gelatin was studied for its structural properties by using UV-spectroscopy, FTIR spectroscopy as well as by XRD analysis. The biopolymer exhibited micro porous morphology and showed characteristic peak at 280 nm. Various functional groups attached to gelatin were given by FTIR analysis. XRD analysis revealed partially crystalline nature of extracted gelatin. Thus, gelatin extracted from waste material of marine fish Gethar exhibited good structural properties and eco-friendly source of gelatin which can be utilized for various foods, pharmaceutical industrial applications.

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6.Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

7.Authors contribution

Pranoti Kirdat :- Data curation;Writing- original draft preparation;Methodology.
Dr.(Mrs.).Padma Dandge :- Supervision, Resources; Conceptualization.

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