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Isolation, characterization and valorizable applications of fish scale collagen in food and agriculture industries

Prashant K. Bhagwat [a], Padma B. Dandge [b,*]

[a] Department of Microbiology, Shivaji University, Kolhapur 416004, India
[b] Department of Biochemistry, Shivaji University, Kolhapur 416004, India

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ABSTRACT

Collagen has enormous applications in food, biomedical and pharmaceutical industries; but its high cost severely limits its use. Fish processing waste is a promising and cost efficacious source of collagen, which otherwise stated as an earnest environmental pollutant. Carp (*Cyprinus carpio*) is one of the main species of freshwater fish consumed in India. Acid-soluble collagen (ASC) was isolated from scales of *Cyprinus carpio*. Scales were demineralized by EDTA treatment and ASC was extracted from the demineralized scales. The yield of scale ASC was 9.79% (on the wet weight basis). SDS–PAGE and FTIR corroborated the isolated protein as collagen. Denaturation temperature (Td) of isolated collagen was found to be 37 °C. Considering bioactive properties of collagen, a milk based food product – paneer was developed by incorporation of the extracted collagen. Composed paneer was found to be acceptable with good sensorial and textural attributes. Same scales were further treated enzymatically and the released metabolites were tested for their competency to promote the plant growth. Released metabolites showed excellent plant growth promotion and hence could be successfully employed as an economic source of nitrogen fertilizers for plants.

1. Introduction

Fishing industry is one of the established food sectors which supply ample amount of food to deliberately growing population. Fish continues to be one of the most-traded food commodities worldwide. India's worldwide share in fishery industry is increasing day by day. India stands at 7th position worldwide in case of fish captures from marine sources and it is at 2nd position in fish captures from inland sources. Interestingly inland fish production has been growing with a great speed showing necessity of fish as a food source in noncoastal regions of India (FAO, 2014).

A huge amount of fish production also tends to produce nearly equal amount of waste as that of final product. Processing of fish involves stunning, grading, slime removal, deheading, washing, scaling, gutting, cutting of fins, meat bone separation and steaks and fillets. During these steps significant amount of waste (20–80% depending upon the level of processing and type of fish) is generated (Ghaly et al., 2013). Very large quantity of waste produced should be properly disposed, but a good care cannot be taken for all the waste produced. Currently waste is disposed in a number of ways, polluting our environment in a very rigid manner. Landfilling and incineration are some of the methods which are used many times, but they are not fruitful as they are costly as well as require a good maintenance (Kim and Venkatesan, 2014). Moreover using these techniques waste having important biomolecules are not utilized properly and world's current scenario does not allow us to do so, hence we should try to use and isolate the important constituent present in those wastes in a technological way.

Collagen is very important biomolecule and is most abundant protein in mammals representing nearly 30% of total proteins in the animal body. (Pati et al., 2010) Enormous health benefits of collagen have led to the establishment of collagen supplements industry. Food and pharmaceutical industries all over the world are witnessing an increasing demand for collagen. The collagen may advance the function of skin dermis and epidermis by increasing the water absorption ability of the outermost skin layer. Hydration of skin tissue is directly allied to smoothness and reduces wrinkling (King'ori, 2011). Collagen supplement can boost up lean muscle gain, decrease recovery time, reconstruct damaged joint structure and improve cardiovascular performances. Therefore, collagen is in demand within the sports nutrition field (Hashim et al., 2015). Among various types, type I collagen has been extensively used as biomaterial for the development of tissue engineering constructs and wound dressing systems due to its low antigenic and high direct cell adhesion properties (Pati et al., 2010).

* Corresponding author.
E-mail address: pbd_biochem@unishivaji.ac.in (P.B. Dandge).

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Mammalian collagens (porcine and bovine) are the most popular and widely used but are facing problems due to their allergic reactions as well risk of transmissible diseases like bovine spongiform encephalopathy (mad cow disease), ovine and caprine scrapie, and other zoonoses (Lynn et al., 2004; Pati et al., 2012). The major protein constituent of seafood processing waste like skin, bone, swim bladder, and scales resembles, in many ways, the more widely studied collagen of mammals (Eastoe, 1957; Giraud-Guille et al., 2000). As fish collagen reportedly possesses similar characteristics to porcine collagen, and may, thus, be considered as an alternative to mammalian collagen (Kim and Venkatesan, 2014; Pati et al., 2012).

So current study aims for (i) Isolation and characterization of collagen from the scales of *Cyprinus carpio*. (ii) Use of extracted collagen in paneer production. (iii) Further recovery of collagen from the same scales by enzymatic treatment. (iv) Use of enzymatically solubilized collagen extracts for plant growth promotion studies.

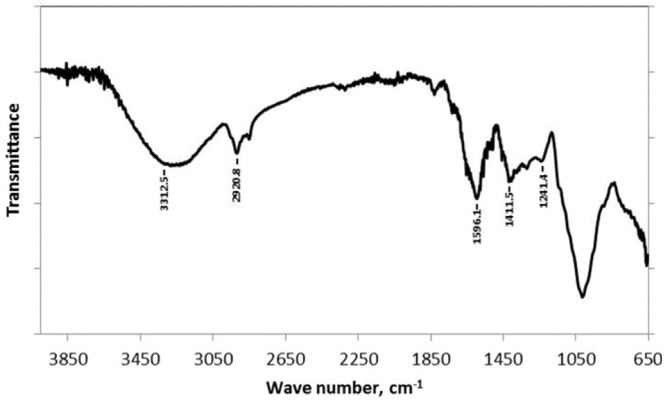

Fig. 1. FTIR analysis of fish scale collagen of *Cyprinus carpio*.

2. Materials and methods

2.1. Collagen extraction from fish scales

Extraction of collagen from fish scales was carried out in two steps. First step was used for demineralization of calcium from fish scales using EDTA and subsequently acid soluble collagen (ASC) was isolated by dilute acetic acid treatment. Both processes were performed at the temperature no higher than 4 °C to reduce temperature induced defragmentation of collagen.

2.1.1. Demineralization of fish scales

Fish scales of *Cyprinus carpio* were collected from local market of Kolhapur, (MS) India. These scales were thoroughly washed five times with distilled water. Then for removal of unwanted surface proteins, scales were treated in a system containing 1.0 M NaCl, 0.05 M Tris HCl, 20.0 mM EDTA at pH 7.5 for 48 h. It was followed by the demineralization process, in which scales were treated with 0.5 M EDTA solution at pH 7.4 for 48 h. The demineralized fish scales were washed thrice with distilled water and used further.

2.1.2. Isolation of collagen

Demineralized fish scales were treated with 0.5 M acetic acid solution at pH 2.5 for 48 h and the insoluble part of the scales were filtered out. Filtrate having soluble collagen is then recovered by salting out technique using NaCl to a final concentration of 0.9 M and kept undisturbed for 24 h. After incubation it was centrifuged at 8000 rpm for 20 min and precipitate was again solubilized in 0.5 M acetic acid. Both the processes of salting out and centrifugation were repeated twice for better purification of collagen. This purified collagen was then dialyzed against 0.1 M acetic acid and distilled water for 24 h each and freeze-dried (Pati et al., 2010).

2.2. Characterization of the extracted collagen

2.2.1. FTIR analysis

Sample of collagen was mixed with approximately 5 times of vacuum dried KBr and pressed into pellets by hydraulic press. Infrared spectra were obtained in the range between 4000 and 650 cm^{-1} using an infrared spectrophotometer (Model – Jasco FTIR-410).

2.2.2. SDS–PAGE

SDS–PAGE was performed by following the method of Laemmli (Laemmli, 1970) with 8% separating gel and 4% stacking gel. Extracted collagen sample was dissolved to 4 mg/ml in sample buffer, type I collagen from calf skin was parallely run for comparison. About 50 μl of sample was loaded per well. The gels were stained using 0.1% (w/v) Coomassie brilliant blue R250 dissolved in methanol-water-acetic acid (5:4:1, v/v/v, respectively) and destained with distilled water-methanol-acetic acid (20:3:2).

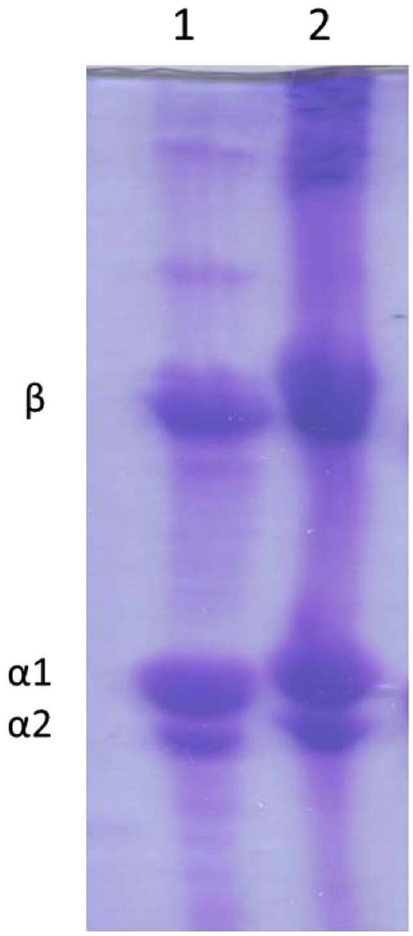

Fig. 2. SDS-PAGE pattern of acid soluble collagen from scales of *Cyprinus carpio* (ASC). Lane 1: ASC; lane 2: Type I collagen from calf skin.

2.2.3. Temperature induced change in viscosity

The denaturation of collagen was determined from temperature induced viscosity change using an Ostwald's viscometer. Solutions of 0.1% (w/v) collagen were prepared in 0.1 M acetic acid at 10 °C. The solutions were loaded to viscometer and incubated at 10 °C for 30 min through submerging in water bath. The temperature was raised stepwise to 50 °C and incubated for 10 min at each temperature. Fractional viscosity was calculated for each temperature as follows: