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CHARCTERIZATION OF GELATIN ISOLATED FROM MARINE SOURCES

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Abstract:

Gelatin has unique functional properties. Hence it is widely used in pharmaceutical, food, cosmetic, and photographic applications. Gelatin is a mixture of peptides and proteins. It is produced by partial hydrolysis of collagen extracted from the connective tissues, skins and bones of animals such as cattle, chicken, pigs, and fish. In this study, gelatin was extracted from skin, arms and visceral organ of marine source (Octpus areolatus) using alkaline pretreatment method and their phsico- chemical properties were characterized. FTIR confirmed the presence of characteristics bonds of gelatin.

Keywords: Gelatin, marine source, Octpus areolatus, alkaline pretreatment, FTIR.

Introduction:

Gelatin is one of the most popularly used as bio polymers. It has wide range of applications in various fields [1]. In food industry, gelatin is used for providing texture, chewiness, foam stabilization and emulsification. It is used in low-fat spreads to provide creaminess and fat reduction. It is also used in dairy industries to provide stabilization and texturization. [2,3]. Gelatin, is low in calories hence used to reduce carbohydrate levels in foods formulated for diabetic patients [4]. In the pharmaceutical companies, gelatin is used as matrix for implants, in intravenous infusions and in injectable drug delivery systems [5,6,7]. It is also used in production of live attenuated viral vaccines for immunization against rabies, diphtheria, measles, Japanese encephalitis, mumps. It is used as stabilizing agent for tetanus toxin [6]. Gelatin is widely used for the manufacture of hard and soft capsules, plasma expanders, and in wound healing matrix.

The global demand for gelatin has been increasing over the years. Recent reports indicated that annual production of gelatin is nearly 326,000 tons. Porcine gelatin accounts for the highest levels of production (46%), followed by gelatin derived from bovine hides (29.4%), bones (23.1%), and other sources (1.5%) [8].

However, gelatin has a wide range of useful applications; there is a major limitation in its usage mainly due to religious sentiments. Both Judaism and Islam forbid the consumption of any pork-related products, whereas Hindus do not consume cow-related products [9]. In addition, there is increasing concern among researchers about gelatin derived from animal tissues are capable of transmitting pathogenic vectors such as prions and outbreak of BSE [10].

Hence there is a need to develop alternative source to mammal-derived gelatin. Recently there has been increasing interest in gelatin derived from fish and poultry. Commercial production from poultry skin and bones is limited by low yields. Therefore, fish gelatin is a better alternative to mammalian gelatin, especially in physical properties such as a lower melting point which results in faster dissolution in the mouth [11, 12].

Fish used for human consumption accounts for 78% of the total fish catch in many countries, leaving about 21% for non-food uses [13]. Its Processing leads to the generation of a large biomass of fish waste (skin, bones, and fins), which is generally discarded (~7.3 million tons/year) [14].

Consequently, research has been initiated to investigate an increased utilization of collagenous fish waste for the production of gelatin [15]. The following research aims at exploring marine source such as *Octopus areolatus* for the production of commercial protein.

Material and Methods:

Procedure for gelatin extraction

1. Preparation of Material

The samples were cleaned according to protocol in Yang et al., 2007. Three different parts ie. skin, visceral organs and arms were thawed at 4°C for about 20 h and then cut into small pieces both in length and width of about 1–2 cm. Then washed with tap water (1:6 w/v) for 10 min. Washing was repeated two more times. Then it was drained using four layers of cheesecloth for 5 min to remove the liquid. The samples were stored in deep freezer. [16-17]

2. Pretreatment

All procedures were performed at 4°C. For alkaline pretreatment, 30 gm of each sample skin, arms and viscera parts was weighed separately and added into the flask and then treated with different concentration on NaOH (1:6w/v) (0-1M for 0-90mins). Then the samples were drained using cheese cloth and rinsed with tap water. The above procedure was repeated two times. Weight of sample after pretreatment was taken.[18-20]

3.Gelatin extraction

Pretreated samples were kept in flask. Deionised water was added and flask was covered with aluminum foils and parafilms. The flask was heated at 50°C in hot water bath for 3hrs. Then the gelatin solution was filtered through four layers of cheese cloth prior to characterization work.[21-24].

Properties of gelatin:

Yield of gelatin (%)

Yield of gelatin was calculated on dry weight basis. The formula used

$$\frac{\text{Weight of freeze dried gelatin}}{\text{Wet weight of fresh skin}} \times 100$$

pH

pH of the gelatin solution was determined by using pH meter.

Yield of protein

The soluble protein concentration was determined by biuret method using spectrophotometer at 540nm with bovine serum albumin as a standard. Yield of protein was calculated using the formula. [25]

$$YP\,(\%) = \frac{(\text{protein concentration (g/ml)} \times \text{volume of extract})}{(\text{Wet skin after pretreatment} \times \text{wt. of sample})} \times 100$$

AFM determination

Gelatin solution is disrupted using a Vortex mixer to break the agglomerates due to storage at lower temperature and to obtain a homogeneous mixture. Small volume (about 20 µl) was pipetted rapidly onto a piece of freshly cleaved silicon wafers.

The silicon surface was then air dried by spin coating such that a thin uniform layer is formed. AFM was used to determine the nanostructure of gelatin in non-contact mode. [26-27]

FT-IR

FTIR spectra were obtained from discs containing 2mg sample in approx 100mg KBr. All spectra were obtained using Perkin Elmer infrared spectrophotometer from 600-4000cm^{-1}.

Results and discussion:

Table 1: Properties of gelatin from marine source.

	Alkaline skin	Alkaline arms	Alkaline visceral
pH	10	13.3	13.1
Yield of gelatin(%) (dry basis)	20	24.46	22

Leaching of collagen in the skin and degradation of gelatin during extraction are the probable reasons for lower yield. The pH was found to be 4.7, 4.9, 5.2 in acidic treatment of skin, arms, visceral organs respectively.

Table -2: Yield of protein of the gelatin extracts.

Yield of protein %	
	Alkaline Pretreatment
Skin	23.96
Arms	27.57
Visera	9.8

AFM image for alkaline Pretreatment for skin

a) 3D image b) 2D image

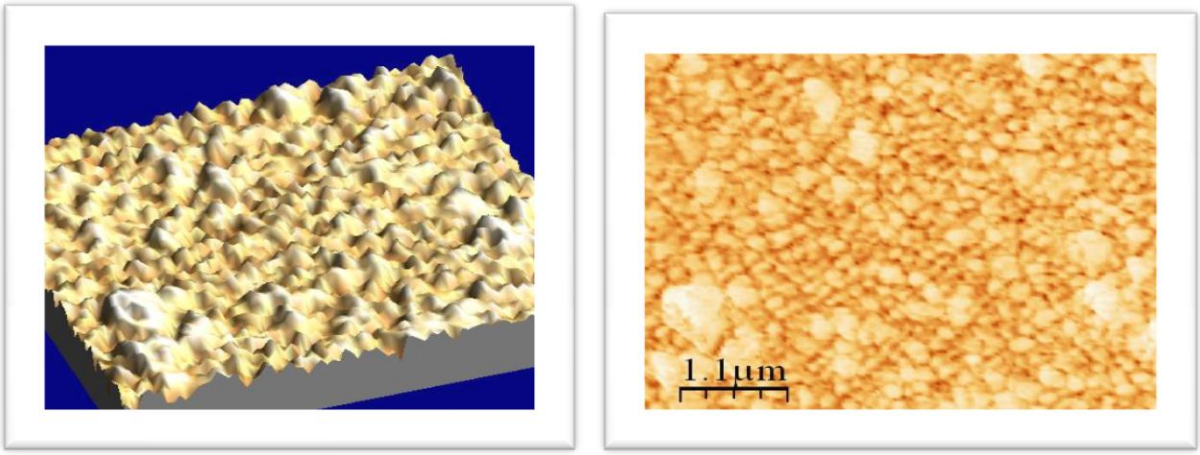
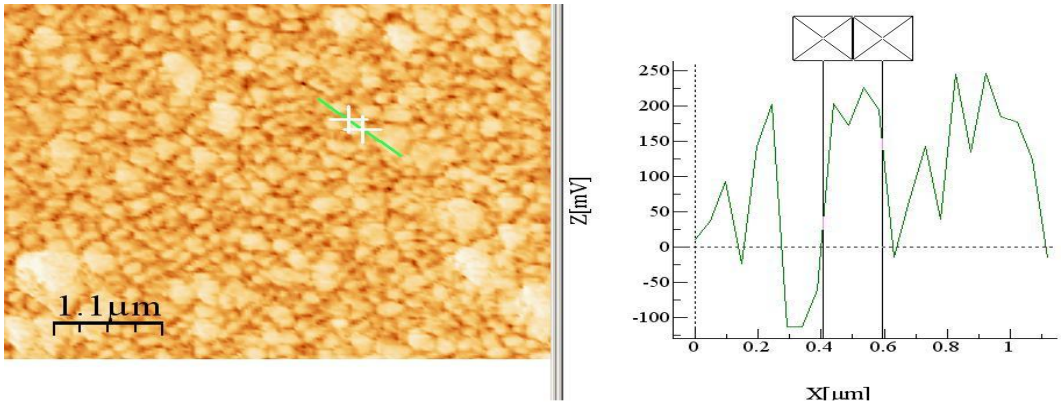
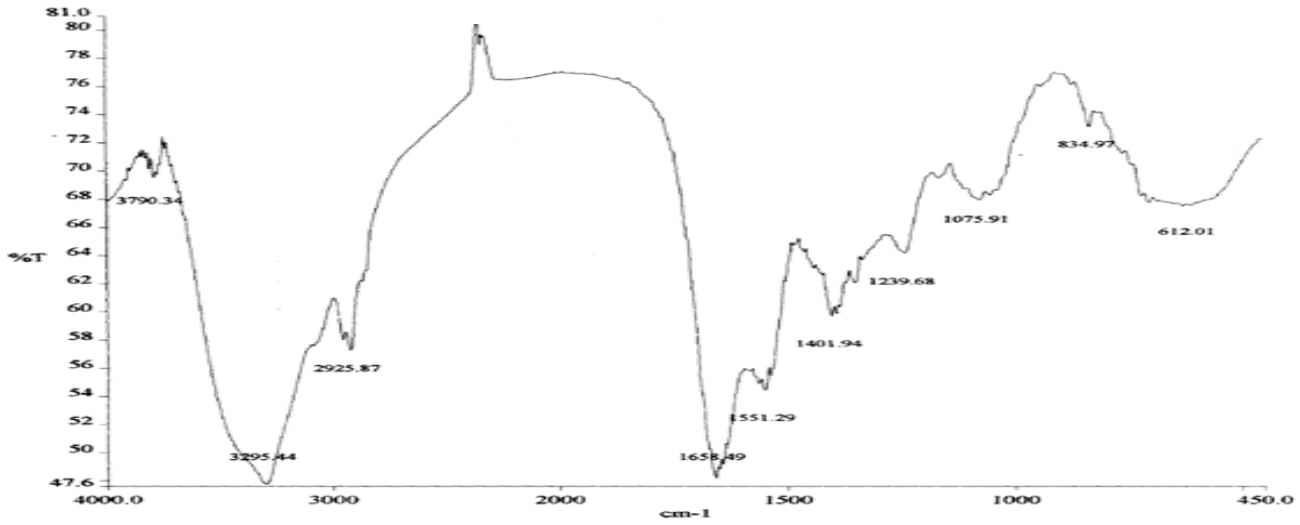


Fig 1



Nano size particles were obtained. The size of the particles was 0.2µm.

FT-IR Spectra of alkaline pretreated skin



Bonds	**WAVELENGTH (Cm^{-1})**
Amide I N-H bending	1658.49
Amid II C-N	1551.29
N-H stretching	3295.44
C-H stretching and bending	2925.87
OH stretching	3295.44
OH bending	1401.94
C=O stretching	1658.48

Presence of these bonds confirms the presence of gelatin in the sample.

Conclusion:

Gelatin was isolated and purified from marine source *Octopus areolatus.* Further characterization of samples showed that marine gelatin can effectively substitute procine gelatin

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