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Functional properties of gelatin obtained from croaker fish (*Johnius sp*) skin by rapid method of extraction

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Abstract
Gelatin from croaker fish (*Johnius sp*) skin was prepared by using rapid method of extraction. The functional and physical properties of extracted gelatin were assessed. The UV absorption spectra showed a peak in the wavelength of 220 nm and a small hump between 275-280 nm. The functional properties of gelatin assessed included foaming and emulsion properties, and water holding capacity. The foaming capacity and stability were directly proportional to gelatin concentration used while that of emulsion properties was inversely proportional to the concentration of gelatin. The water holding capacity of gelatin was found to be 2.46 ml/gm which was higher than commercial porcine gelatin.

Keywords: Croaker fish gelatin, UV absorption spectra, functional properties

1. Introduction
Gelatin is a functional biopolymer and high molecular weight polypeptide derived by denaturation of collagen. Gelatin has a wide range of applications in food, pharmaceutical, cosmetic and photographic industries as stabilizing, thickening and gelling agent due to its surface active properties [1, 2]. Fish used as human food accounts for 78% of the total fish catch, leaving about 21% for non-food uses [3]. The fish processing waste comprises of skins, bones, fins, air bladder and visceral mass. Utilizing the fish waste for the production of high value compounds such as gelatin and hydrolysates could add value to processing and food industry, and also minimizes the environmental pollution [4]. The amount of gelatin used in the world wide food industry is increasing annually. The estimated world usage of gelatin is 200,000 metric tons/year from beef and pork skin and bones [5].
The skin and bone of bovine and porcine are the major sources for gelatin production. Due religious restrictions, fish gelatin has gained attention as an alternative source to mammalian gelatin. Several studies reported the extraction and characterization of fish gelatin, such as Tiger-toothed croaker and Pink perch [6]; Splendid squid [1]; Fresh water fish [7] swim bladder [8]; Clown featherback [2]. At present the method of extraction uses the acid and alkali treatment and time consuming. Hence, there is a need to develop a rapid method of extraction of gelatin without compromising the desired properties. The quality of gelatin depends on its physicochemical properties, raw material used and extraction method [8]. In any commercial operation, the properties of gelatin should match the expected requirements. The objective of present investigation is to prepare gelatin from croaker fish (*Johnius sp*) skin by rapid method of extraction and assess the functional properties of obtained gelatin.

2. Materials and Methods
2.1 Fish
The Croaker fish (*Johnius sp*) were procured from landing centre Mangalore, West coast of India, in iced condition and brought to the laboratory. The fishes were washed in water, beheaded, gutted and filleted manually. The cleaned skins were washed and stored at -20˚C and used for gelatin extraction within fifteen days. All the chemicals used were of either analytical grade reagent (AR) or guaranteed grade reagent (GR).

2.2 Proximate Composition
Proximate composition of raw material and extracted gelatin were analyzed by measuring moisture, ash, protein and fat contents according to AOAC official methods [9]. A factor of 5.55 was used to convert nitrogen value to protein.

2.3 Gelatin extraction

The gelatin extraction was carried out by the method as described by Kolodziejska *et al*. 2008 [10] with slight modification. The frozen skins were thawed, and cut into small pieces of 1 cm^3 and pre-treated with 0.75 M (Sodium chloride) NaCl solution for 10 min at 4˚C. The ratio of fish skin to NaCl solution was 1:6 (w/v). The NaCl treatment and washing with water was repeated twice. The temperature of 85˚C for the duration of 60 min was used for the extraction of gelatin. The ratio of skin to water was 1:6 (w/v). The samples were cooled and subjected to centrifugation at 10,000 x g at 25˚C for 15 min, using a refrigerated centrifuge (Sorvall Legand XTR centrifuge, Thermo Fisher Scientific, New Hampshere, USA) to separate the insoluble material. The supernatant was filtered using Whatmann filter paper No.1 (Whatman Plc, Maidstone, Kent, UK). The clear filtrate was freeze- dried to obtain gelatin.

2.4 UV absorption spectra

The gelatin solution 0.5% (w/v) was prepared and filtered using a Whatmann filter paper No 4. The ultraviolet absorption spectra of gelatin solution were recorded in the wavelength range of 200-350 nm using using double beam spectrophotometer (UV-VIS spectrophotometer, LaboMed, Inc., Los Angeles, CA, USA). A plot of absorbance vs wavelength was obtained to get absorption spectra.

2.5 Measurement of Turbidity

The turbidity of gelatin solution was determined by the following method as described by Fernández-Díaz *et al*., 2001 [11]. A 6.67 % (w/v) of gelatin solution was prepared by dissolving dry gelatin in distilled water at 40 for 20 min. The turbidity of gelatin solution was measured at 360 nm using double beam spectrophotometer (UV-VIS spectrophotometer, LaboMed, Inc., Los Angeles, CA, USA).

2.6 Foaming properties

The foaming properties were determined according to the method as described by Sathe and Salunkhe, 1981 [12]. The foaming properties were determined according to the method of (Sathe and Salunkhe, 1981). Different concentrations (0.5%, 1% and 2%) of gelatin solution (20 ml) were whipped at a speed of 13,500 rpm using Ultra Turrax homogenizer (Ultra Turrax, T 25, Janke & Kunkel GMBH & Co., KG Staufen, Germany) to incorporate the air for 2 min at room temperature. The whipped sample was immediately transferred into a 100 ml measuring cylinder and volume was recorded. The foaming capacity was calculated using the following formula:

$$\text{Foaming capacity} = \frac{(\text{Volume after whipping}) - (\text{volume before whipping})}{\text{Volume before whipping}} \times 100$$

The whipped sample was allowed to stand at 20 °C for 30 min and the volume of whipped sample was then recorded. Foam stability was calculated as follows:

$$\text{Foaming stability} = \frac{(\text{Volume after standing}) - (\text{volume before whipping})}{\text{Volume before whipping}} \times 100$$

2.7 Emulsion properties

The emulsion properties were determined according to the method as described by Pearce and Kinsella, 1978 [13]. Different concentrations (0.5%, 1% and 2 %) of gelatin solution (30 ml) and refined sunflower oil (10 ml) was mixed and homogenized in the Ultra Turrax homogenizer at 20,500 rpm for 1 min. Aliquot of the emulsion (50 µl) was pipetted out from the bottom of the container at 0 and after 10 min of homogenization and mixed with 5 ml of 0.1 % sodium dodecyl sulphate (SDS) solution. The absorbance of the solution was measured at 500 nm using double beam spectrophotometer (UV-VIS spectrophotometer, LaboMed, Inc., Los Angeles, CA, USA). The absorbance was measured immediately 0 min (A_0) and after 10 min (A_{10}) of emulsion formation. The readings were used to calculate the emulsion activity index (EAI) and emulsion stability index (ESI) as follows:

EAI = 2T ΦC

Where, T - turbidity (T = 2.303A_{500} / l; A_{500} is absorbance at 500 nm; l is path length); ϕ - oil volume fraction (0.25); C - Gelatin concentration.

$$\text{EAI } (m^2/g) = \frac{2 \times 2.303 \times \text{Abs}_{500nm}}{0.25 \times \text{gelatin weight (g)}}$$

$$\text{ESI (min)} = \frac{A_0 \times \Delta t}{\Delta A}$$

Where, ΔA = Absorbance at 0 min (A_0) – Absorbance after 10 min (A_{10}) and Δt = 10 min

2.8 Water Holding Capacity

Water holding capacity was determined according to the method as described by Diniz & Martin, 1997 [14]. A 0.5 gm of gelatin was dissolved in 20 ml of water and stirred using magnetic stirrer for 1 min. The homogenous solution was then transferred to centrifuge tube and allowed to stand at room temperature for 6 hours, and then centrifuged at 2800 x g for 30 min. The supernatant was filtered through a Whatman No.1 filter paper and volume recovered was measured. The difference between initial volumes to the volume of the supernatant was determined and expressed as ml of water absorbed per gram of gelatin sample.

$$\text{WHC (ml/gm)} = \frac{(\text{Initial volume of distilled water} - \text{Volume of supernatant})}{\text{One gram of gelatin}}$$

2.9 Statistical Analysis

One-way ANOVA was used to analyze the data Keppel, 1973 [15]. Significant difference between the means of triplicates were determined by Duncans multiple comparison test using statistical software IBM SPSS.2

3. Results and Discussion

3.1 Proximate composition

The proximate composition of raw material and extracted gelatin are given in Table 1 & 2. The protein and fat content of extracted gelatin were found to be 89.9% and 0.97%, respectively. The moisture and ash content of gelatin obtained from croaker skin was 9.67% and 1.98%, which was within the recommended limit prescribed for edible gelatin [16]. The obtained results indicated that there was an efficient removal of fat from the processed skin.

Table 1: Proximate composition of croaker fish skin

Skin	Composition (%)
Crude protein	16.11±0.10
Moisture	72.56±0.87
Fat	1.69±0.18
Ash	1.76±0.36

Mean ± Standard deviation values from triplicates

Table 2: Proximate composition of croaker skin gelatin

Gelatin	Composition (%)
Crude protein	89.99±0.60
Moisture	9.67±0.30
Fat	0.97±0.10
Ash	1.98±0.25

Mean ± Standard deviation values from triplicates

3.2 UV absorption spectra

The UV absorption spectra of gelatin are given in Fig.1. The spectra showed higher absorption in the wavelength region of 220-240 nm indicating the presence of non-aromatic amino acids of the gelatin structure [17]. Absorption in the wavelength region of 260 - 280 nm may be due to a few aromatic residues like phenylalanine, tyrosine and tryptophan [18; 19].

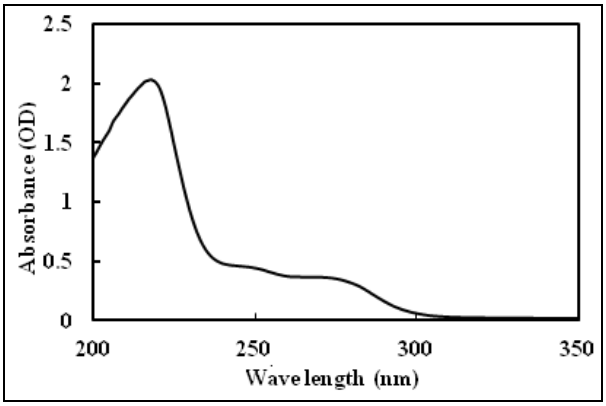

Fig 1: UV absorption spectra

3.3 Measurement of turbidity

Turbidity of gelatin solution extracted from croaker skin was found to be (1.93) given in Table 3. The turbidity of croaker skin gelatin was much higher than the commercially available porcine gelatin (0.26). It was found that that the turbidty mainly depends on the raw material, extraction and filtration process whereas, the dark colour of gelatin solution may be due to inorganic, protein and muco-substance contaminants which mainly affect turbidity [11, 20, 21].

Table 3: Turbidity & Water holding capacity of extracted gelatin

Turbidity (Abs at 360 nm)	Porcine gelatin	Fish gelatin
Water holding capacity(mg/ml)	0.26±0.08	1.93±0.04
	1.83±0.76	2.46±0.56

Mean ± Standard deviation values from triplicates

3.4 Foaming properties

Foaming capacity and stability of extracted gelatin at different concentrations given in Fig.2. Foaming capacity at different concentrations 0.5%, 1%, and 2% were found to be in the range of 53.3 – 77.7%. Foaming capacity of 0.5% solution differs significantly from 1% and 2% solutions (P<0.05). However, there was no significant difference between 1% and 2% solutions. Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air-water interface [22]. The foaming capacity and stability of croaker skin gelatin increased with increasing concentrations (P<0.05). Foams with higher concentration of proteins were denser and more stable because of an increase in the thickness of interfacial films [23, 24].

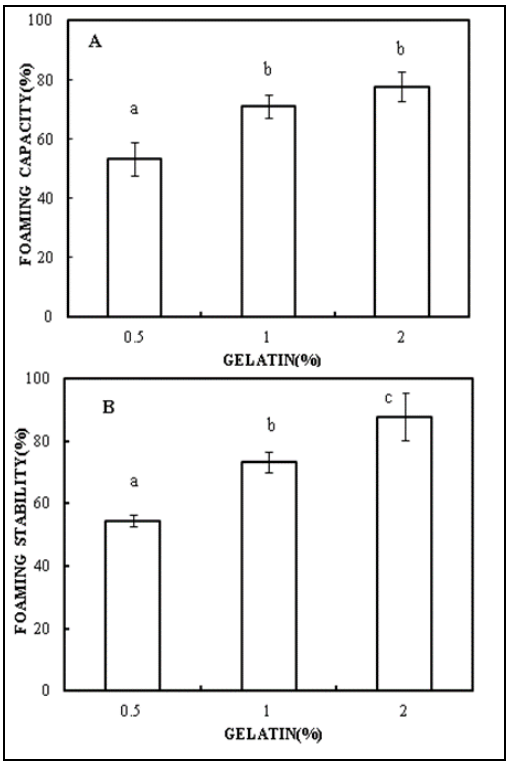

Fig 2(A). Foaming capacity **(B)** Foaming stability

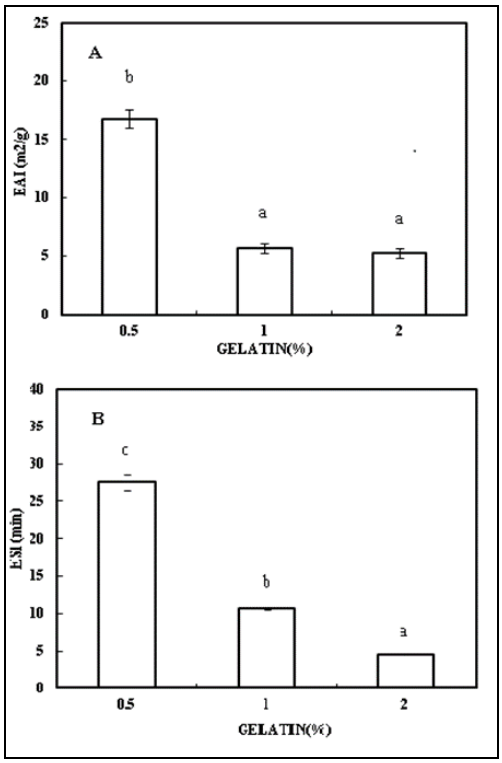

Fig 3: (A) Emulsion activity index (B) Emulsion stability index

(Different small letters on the error bars indicate the significant difference (P<0.05)

Foaming stability of gelatin solutions differ significantly (P<0.05) with gelatin concentration. Foaming stability increased rapidly with gelatin concentration. Foaming stability depends on various parameters, such as the rate of attaining equilibrium surface tension, bulk and surface

viscosities, steric stabilization, and electrical repulsion between the two sides of the foam lamella [25]. The factors that influence the foaming properties include source of protein [26], protein surface properties [22] and the extent of protein-protein interaction within the matrix [27].

3.5 Emulsion properties

Emulsion activity index (EAI) and emulsion stability index (ESI) of extracted gelatin at different concentrations 0.5%, 1%, and 2% are given in Fig.3. EAI of croaker skin gelatin at concentrations of 0.5%, 1%, and 2% were 16.7±0.77 m2/g, 5.67±0.39 m2/g and 5.30±0.41 m2/g, respectively. EAI of 0.5% solution was significantly higher than 1% and 2% solutions (P<0.05).

ESI of croaker skin gelatin at 0.5%, 1%, and 2% were 27.5±1.07, 10.6±0.16 and 4.60±0.04 showing significant differences (P<0.05). The EAI and ESI of croaker skin gelatin decreased with increasing concentrations, similar results were obtained for gelatin from splendid squid and unicorn leather jacket [1, 24]. The surface hydrophobicity of proteins contributes to their affinity for the oil phase [23]. Furthermore, the emulsion properties of gelatin is influenced by several factors like intrinsic properties of protein [28], methods of preparation [5], and solubility of protein [29].

3.6 Water holding capacity

The water holding capacity of croaker fish skin gelatin was found to be 2.46 ml/gm which is comparably higher than commercial porcine gelatin 1.83 ml/gm given in Table 3. The water holding capacity of tiger-toothed croaker and skin Pink perch skin was found to be 4.50 ml/gm and 2.36 ml/gm, repectively [6]. The water holding capacity is an important part of functional properties of proteins in food system, which refers to the ability of protein to imbibe water and retain it against a gravitational force within protein matrix [6]. It was found that the water-holding capacity is mostly depend on the amount of hydrophilic amino acids, protein structure and the size of free space where the water is retained in the protein structure and the existence of molecules that contribute with charges and allow dipole-dipole interactions [30, 31, 32].

4. Conclusion

The gelatin obtained from the skin of croaker fish skin by rapid method has exhibited good physical and functional properties, varied with protein concentration. The extracted gelatin contains protein as the major component with less fat and ash contents. The procedure has a potential for commercial exploitation as a simple approach in small-scale extraction of gelatin from abundant fishery resources. Thus, the croaker skin is a good source to produce gelatin with desirable functional and physical properties, and comparable to commercially available mammalian gelatins, which could find the application in food and pharmaceutical industry.

5. Acknowledgment

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