Indian Journal of Geo Marine Sciences
Vol. 51 (05), May 2022, pp. 490-495
DOI: 10.56042/ijms.v51i05.65565

Valorization of discarded industrial processing wastes of Siboga squid (*Doryteuthiis sibogae*) for the extraction and physico-chemical characterization of gelatin

S R Radhika Rajasree*[a,b], L Aranganathan[a] & M Gopalakrishnan[a]

[a]Centre for Ocean Research (NIOT-SU Joint Initiative Research Centre), Sathyabama Institute of Science and Technology, Chennai, Tamilnadu – 600 119, India

[b]Department of Fish Processing Technology, Kerala University of Fisheries and Ocean Studies, Kochi, Kerala – 682 506, India

*[E-mail: radhikarajasree@kufos.ac.in]

Received 25 January 2022; revised 28 April 2022

A growing concern on the accumulation and disposal of fish processing discards and environmental degradation induces a profound attraction on the development of valuable byproducts for human purposes. Gelatin was isolated from Siboga squid (*Doryteuthiis sibogae*) skin through cold maturation process and its physico-chemical as well as structural properties *viz*. Water Holding Capacity (WHC), emulsifying capacity, emulsifying stability and viscosity were analyzed. The alkaline treatment of skin yielded 7.5 % gelatin powder. Extracted gelatin was characterized using UV-visible spectroscopy, ^{1}H NMR, FT-IR and Raman analyses. Based on FT-IR and Raman fingerprints, peak located at 1664 cm^{-1} (C=O stretch Amide I band) could be inferred that secondary structure of proteins as well as coiled structure of gelatin. Topographic analysis by AFM showed presence of aggregates of the polymer. The results suggest that the Siboga squid gelatin is an alternative source of gelatin and its potential applicability at industrial scale.

[**Keywords**: ^{1}H NMR, AFM, Siboga squid, gelatin, Waste valorization]

Introduction

Cephalopods including squids exploited from Indian waters are a predominant group of fishes earning foreign exchange. India exports frozen squid to over 60 countries predominantly to Japan, US, EU, UAE, Italy and France. In the year 2020-21, India has exported worth 158.78 USD million and nearly 90 % of fish caught is used for processing meant for export in the form of 'ready to eat', 'ready to cook' or 'ready to fry' products which generate huge amount of cephalopod processing waste. Gelatin, an important biopolymer has wider applications in food and pharma industries as a stabilizer, thickener and gelling agent[1]. Generally, gelatin is from the remnants of pig and cattle by alkaline or acidic treatment process[2]. However, due to the problems with respect to transmittable "bovine spongiform encephalopathy", new options are being sought for sources of collagen, mainly from industrial fisheries by-products[3,4]. Moreover, gelatin from porcine sources cannot be utilized in "Kosher and Halal foods" owing to religious reasons[5]. Fishery processing wastes are usually considered as cheap resources with insignificant market value and pose severe environmental threat if unattended. One third of so-called wastes comprising bones and skins are abundant sources of collagen, the gelatin precursor. Due to this, fish gelatin generated from fish processing wastes, has gathered considerable attention as a potential substitute of mammalian gelatin[5].

Several species of freshwater and marine fishes have been utilized for the extraction of gelatin[6-13]. Commercial importance of gelatin depends on their properties such as thermal stability, gelation, rheological parameters, which are having a pronounced effect on gelatin quality[14]. Siboga squid-*Doreyteuthiis sibogae* is an important fishery item for local and export use, constituting a lucrative fishery in terms of capacity and economic output along the South East Coast of India. It is a renewable natural resource capture from the Bay of Bengal. However, skin and tentacles generated as by-products shown to have no market value. The wastes generated from squid processing industry after filleting amounts to be around 75 % of the total fish catch weight[15] which can be converted into enriched compounds (collagen and gelatin). In this work, gelatin extraction from the skin of Siboga squid (*D. sibogae*) and their physico-chemical characteristics were evaluated.

Materials and Methods

Pretreatment of squid skin

Fresh skins of Siboga squid were obtained from the fish market from local areas of Chennai, Tamil Nadu, India. The samples were transferred to the laboratory in chilled condition, with a skin to ice ratio of 1:3 (w/w). The skins were cleaned with distilled water and drained well on a mesh screen. They were chopped into smaller pieces and stored in poly ethylene covers at -20 °C before the experiment

Gelatin extraction

For gelatin extraction, 100 g of squid skin was soaked in 0.8 % NaCl in the ratio of 1:6 (w/v)[16]. The whole mixture was agitated using a magnetic stirrer (100 rpm) continuously for 15 min at 26 °C. The sample was then washed with tap water and rinsed well to remove the salt content. Further the sample was mixed with 0.2 M NaOH for 30 min at 4 °C for non-collagenous protein removal. The same process was done thrice. The sample after treated with alkali was rinsed thoroughly with ice cold distilled water to reduce NaOH content until pH 7 was obtained. Further, the sample was soaked in 0.05 M CH_3COOH for 3 – 4 h and washed repeatedly with distilled water. The swollen sample was soaked in distilled water in the ratio of 1:4 (w/v) for half day with continuous stirring for gelatin extraction. Using a double layered cheese cloth, the solution is filtered and the filtrate was kept for 48 h at 4 °C. The freeze-dried sample was lyophilized to obtain dry powder of gelatin. Physico-chemical properties analysis such as water holding capacity[17], Emulsifying Capacity (EC%), emulsifying stability, viscosity etc. were carried out.

UV-visible spectroscopic analysis

The Gelatin in solution form (0.5 %) (w/v) was heated at 60 °C for half an hour, then cooled at 25 °C and finally filtered with a Whatman No. 4 filter paper. The filtrate was recorded with a UV-vis spectrophotometer (Shimadzu UV-vis 1800) at the wave range of 200 – 800 nm.

Nuclear magnetic resonance (^{1}HNMR): With a Bruker Advance III 500 MHz nuclear magnetic resonance spectrometer, ^{1}H NMR spectra of gelatin were acquired. For the determination, approximately 1 mg of gelatin and 0.5 ml of a 1% (v/v) DMSO was dissolved in NMR tubes.

FT-IR and Raman spectroscopy

Dried gelatin powder (2 mg) and KBr (250 mg) was hydraulic pressed after mixing to make pellets. The spectra were recorded in a wavelength range of 4,000 – 400 cm^{-1} using FT-IR (Shimadzu IRAffinity-1S). For Raman spectra, gelatin was examined on FT-Raman spectrophotometer (BRUKER RFS 27) with excitation from 5000 – 50 cm^{-1}.

Atomic force microscopic analysis

The solution of gelatin (1 %) was heated at 50 ºC for 20 min and mixed well with a vortex mixer. The solution was diluted to 0.1 % with deionized water and filtered. A drop of the gelatin was mounted on the cover slip and dried for 1 h at room temperature. Topographic analysis of gelatin using tapping mode (NT-MDT model) was done to observe the microscopic structure of the particles.

Results and Discussion

The gelatin yield isolated from squid wastes was 7.5 % on a dry weight basis. These results from fishes such as megrims (7.4 %), soles (7.3 %), cods (7.2 %), hakes (6.5 %) were found to be very much comparable[2]. Gelatin yield varies with species and age of the fish. Besides, the parameters *viz*. temperature, extraction time and pH, pre-treatment conditions, the innate properties and the method of preservation of the raw material etc. determines the degree of conversion of collagen into gelatin[19,20].

Analysis of phyisco-chemical properties

Water holding capacity

Dried gelatin powder showed WHC of 1.56 %, shows that that the prepared product was able to retain 0.015 gm of water per gram of gelatin. The water holding capacity of gelatin is mainly due to its it primary chemical structure hydrophilic and hydrophobic balance as well as particle size.

Emulsifying capacity and stability

The EC of prepared gelatin at 60 °C was 20.1 % and emulsifying stability was calculated to be 84.6 %. It was reported that gelatin extracted at 45 °C shown an emulsifying stability of 32.5 %, and emulsifying capacity (55.6 %), but the emulsifying stability of gelatin extracted from *Doryteuthiis sibogae* was comparatively higher[21].

Viscosity

Viscosity is an important parameter for the commercial property of gelatin[22]. The viscosity of gelatin was measured to be 0.616 cP. The viscosity of gelatin was comparatively low of Pacu fish skin gelatin in which the viscosity was reported to be 6.23 cP at 40 °C[23].

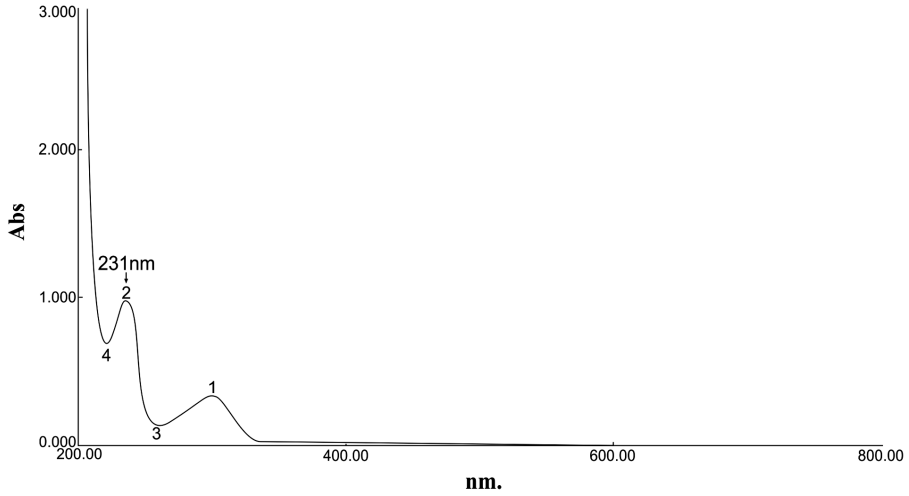

Fig. 1 — UV-vis absorption spectra of Gelatin

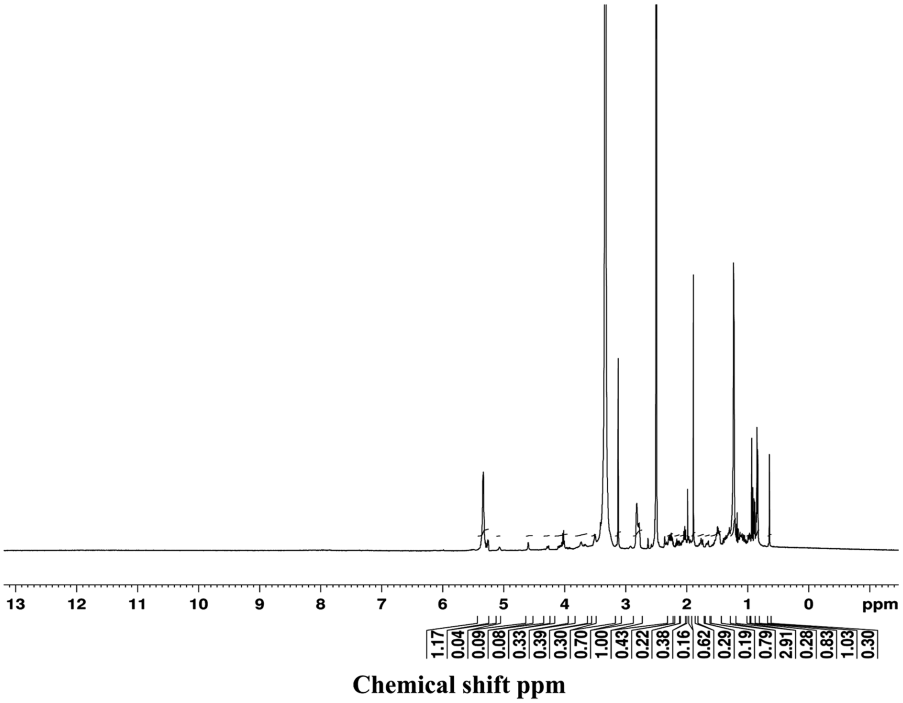

Fig. 2 — Nuclear magnetic resonance (^{1}H NMR) of gelatin from *D. sibogae*

Protein estimation

The total protein present in the gelatin was estimated to be 4.8 mg/ml as per Lowry's method.

Spectroscopic and microscopic properties

The gelatin solution UV-vis absorption spectrum was depicted in Figure 1. Absorption peak spotted at 231 nm wavelength showed the occurrence of peptide bonds in the gelatin polypeptide chains[24].

^{1}H NMR spectrum of gelatin showed the presence of numerous peaks of amino acids that form the peptide. The presence of different amino acids observed in NMR peaks has been represented in Figure 2. The peak values recorded along with protons and amino acids are enclosed in closed brackets – (0.90 ppm: -γ-& δ-CH_3: Val, Leu, Lle); (1.22 ppm: Tre: -γ -CH_3); (2.5 ppm: Pro β-, γ - δ-

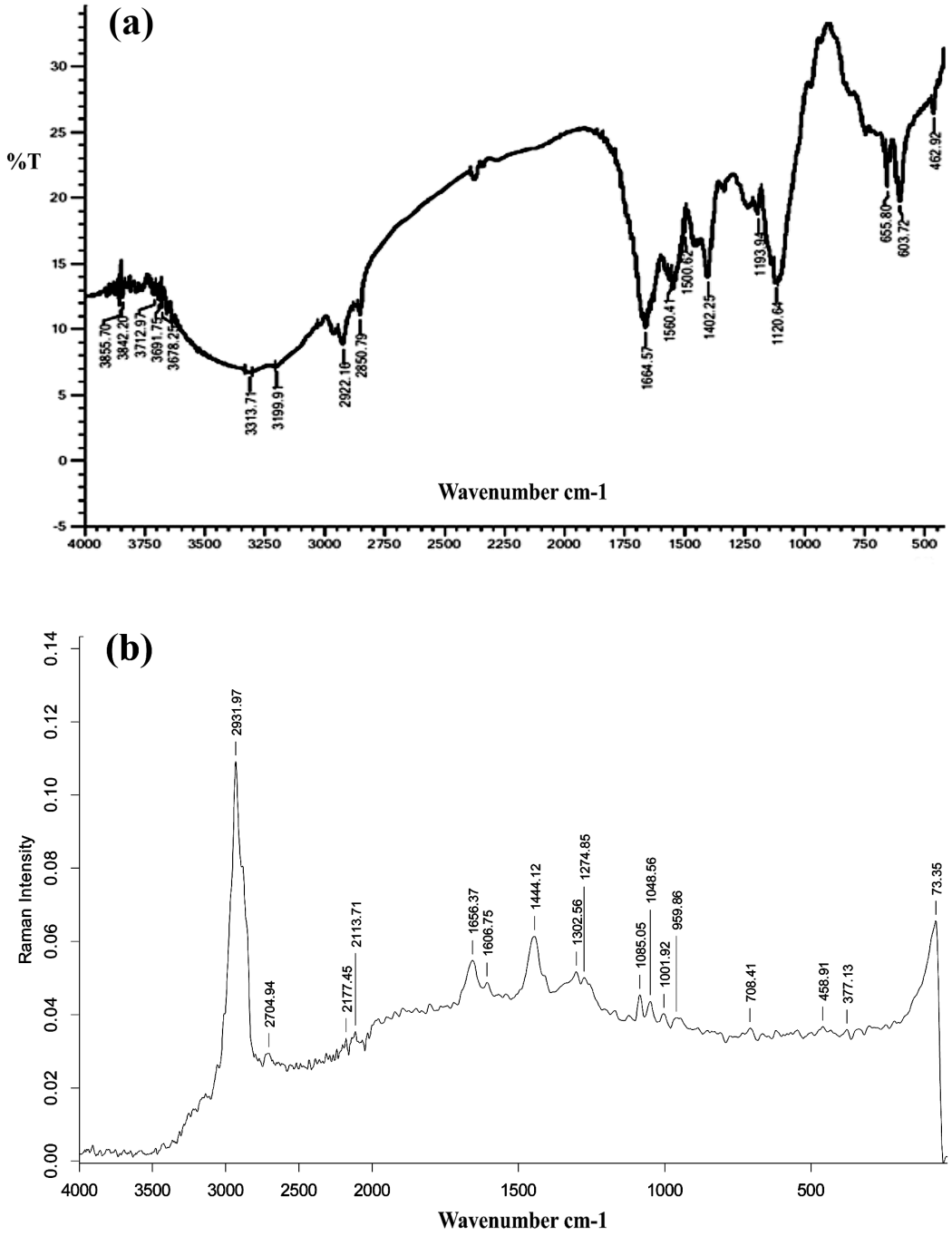

Fig. 3 — a) FT-IR spectra of gelatin isolated from *D. sibogae*, and b) Raman spectra of gelatin isolated from *D. sibogae*

CH_2); (3.2 ppm: Arg, His δ-CH_2, β-CH_2). These peak values are in accordance with the results of Uriarte-Montoya *et al.*[25]. The peak at 2.8 ppm denotes Arg, Hys with proton type δ –CH_2; β-CH_2 according the results of Rodin & Izmailova[26]. Other peaks such as (3.3 ppm Pro: δ-CH_2) (3.4 ppm: Thre: -γ -CH_3) were also identified. The band observed in the spectrum at δ = 2.5 denotes the occurrence of water molecules that act as stabilizing agents by establishing hydrogen bonds between adjacent chains[27].

FT-IR spectra of gelatin extracted from Siboga squid are depicted in Figure 3(a). Peaks located at 3313 cm^{-1} (OH of alcohols), 2922 cm^{-1} (Alkyl-CH Stretch), 1664 cm^{-1} (C=O stretch Amide I band), 1560 cm^{-1} (C–C stretch (in–ring) Aromatics), 1120 cm^{-1} (C–N stretch aliphatic amines) represents different

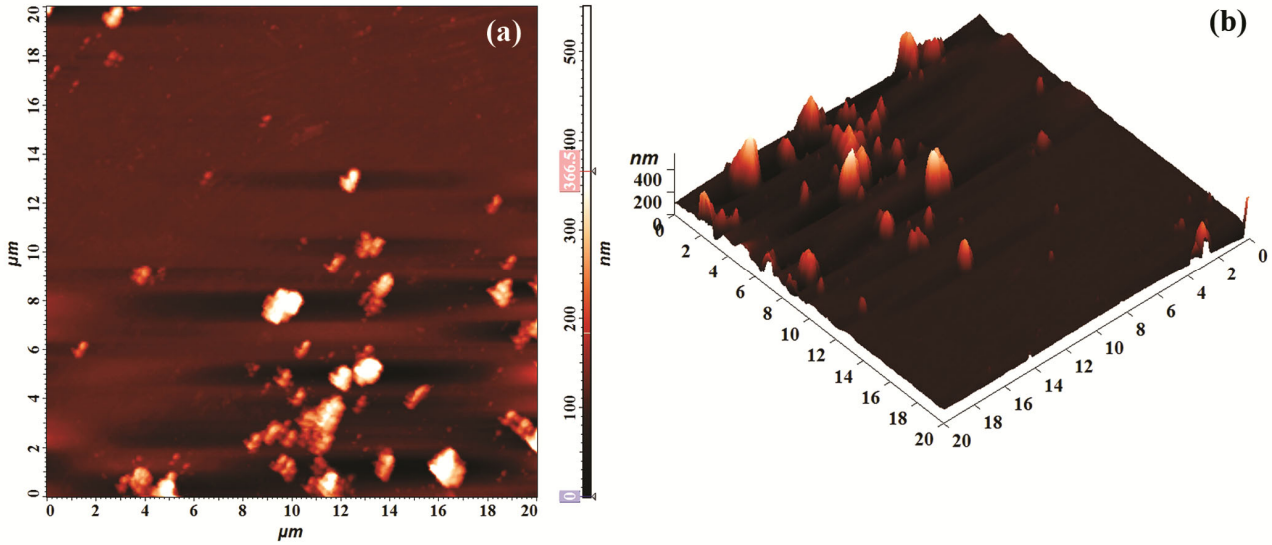

Fig. 4 — Atomic force microscopic view of polymeric structures in gelatin

functional groups present in gelatin. Similarly, Raman spectra were also recorded for gelatin (Fig. 3b). An intense spectrum is pointed at 2931 cm^{-1} (Asymmetric stretch of CH_2), 1656 cm^{-1} (Amide I), 1444 cm^{-1} (Amide II) and 1085 cm^{-1} (C-O stretching). In the present work, amide I band was recorded in the 1664 cm^{-1} and 1656 cm^{-1} in both spectral analyses. It could be inferred that the amide I band observed between 1700 and 1600 cm^{-1} indicates the secondary structure of proteins as well as coil structure of gelatin[28]. Raman peaks identified as amide in gelatin are in accordance with the results of Bruce *et al.*[29].

Atomic force microscopy

Morphological examination of gelatin under AFM showed a topographic image of gelatin through alkaline pre-treatment. Gelatin appeared as aggregates (polymeric attachments; Fig. 4). Similar observation of aggregates structures was reported in gelatin extracted from catfish (*Ictalurus punctatus*)[30]. The appearance of non-uniform shaped aggregates of gelatin was due to the collagen polypeptide chains breakdown of into smaller pieces obtained by alkaline extraction. The appearance of polymeric structures in different shapes are influenced by the pH of the pre-treatment of the gelatin between acid and alkaline solutions. The pre-treatments have clear influence on the primary structure of the polypeptides by hydrolysis of collagen at different locations of amino acid.

Conclusion

Gelatin from the skin of Siboga squid isolated by an alkaline-acidic extraction method had various characteristics and functional properties. Spectral studies conducted by Fourier infrared, Raman and NMR spectroscopy revealed amide linkages of the protein. Atomic force microscopic observations clearly depicted the polymeric structures of gelatin. Based on results of the findings, skin of *Doreyteuthiis sibogae* could help as substitute raw material for extraction of gelatin. The study also underlines the efficiency of the conversion of marine disposal to valuable products to achieve the sustainability and circular economy. Further studies are necessary to evaluate their biological behaviour over time.

Acknowledgements

The authors express sincere thanks to Sathyabama University for facilitating the research work. The authors also acknowledge SAIF, Indian Institute of Technology, Madras (IITM) for characterizing gelatin samples using proton NMR and Raman Spectroscopic facilities.

Conflict of Interest

The corresponding author, on behalf of all authors, declares that in the research work there is no conflict of interest.

Ethical Statement

The endangered and live organisms are not used in this study.

Author Contributions

The authors confirm the contribution of the paper as follows: SRRR - Study conception and general design;

LA - Analysis and results interpretation; and MG - Draft manuscript preparation. All the authors declare that they reviewed and approved the final version.

References

1 Regenstein J M & Zhou P, Collagen and gelatin from marine by-products, In: *Maximising the Value of Marine By-Products*, edited by *F Shahidi*, (Woodhead Publishing Limited), 2007, pp. 279–303.

2 Gómez-Guillé M C, Turnay J, Fernández-Díaz M D, Ulmo N, Lizarbe M A, *et al*., Structural and physical properties of gelatin extracted from different marine species, *Food Hydrocoll*, 16 (2002) 25–34.

3 Mizuta S, Hwang J & Yoshinak R, Molecular species of collagen from wing muscle of skate (*Raja kenojei*), *Food Chem*, 76 (2002) 53-58.

4 Shen X R, Kurihara H & Takahashi K, Characterization of molecular species of collagen in scallop mantle, *Food Chem*, 102 (2007) 1187-1191.

5 Kittiphattanabawon P, Benjakul S, Visessanguan W & Shahidi F, Isolation and characterization of collagen from the cartilages of brown banded bamboo shark (*Chiloscyllium punctatum*) and blacktip shark (*Carcharhinus limbatus*), *LWT – Food Sci Technol*, 43 (2010) 792–800.

6 Boran G, Mulvaney S J & Regenstein J M, Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources, *J Food Sc*, 75 (2010) 565-571.

7 Jellouli K, Balti R, Bougatef A, Hmidet N, Barkia A, *et al*., Chemical composition and characteristics of skin gelatin from grey triggerfish (*Balistes capriscus*), *LWT-Food Sci Technol*, 44 (2011) 1965-1970.

8 Uriarte-Montoya M H, Santacruz-Ortega H, Cinco-Moroyoqui F J, Rouzaud-Sández O, Plascencia-Jatomea M, *et al*., Giant squid skin gelatin: chemical composition and biophysical characterization, *Food Res Int*, 44 (2011) 3243–3249.

9 Nagarajan M, Benjakul S, Prodpran T, Songtipya P & Kishimura H, Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures, *Food Hydrocoll*, 29 (2012) 389-397.

10 Ahmad M & Benjakul S, Characteristics of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as influenced by acid pretreatment and extraction time, *Food Hydrocoll*, 25 (2011) 381-388.

11 Kaewruang P, Benjakul S & Prodpran T, Molecular and functional properties of gelatin from the skin of unicorn leatherjacket as affected by extracting temperatures, *Food Chem*, 138 (2013) 1431–1437.

12 Jridi M, Nasri R, Lassoued I, Souissi N, Mbarek A, *et al*., Chemical and biophysical properties of gelatins extracted from alkali-pretreated skin of cuttlefish (*Sepia officinalis*) using pepsin, *Food Res Int*, 54 (2013) 1680–1687.

13 Ktari N, Jridi M, Nasri R, Lassoued I, Ayed H B, *et al*., Characteristics and functional properties of gelatin from zebra blenny (*Salaria basilisca*) skin, *LWT - Food Sci Technol*, 58 (2014) 602-608.

14 Aewsiri T, Benjakul S & Visessanguan W, Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide, *Food Chem*, 115 (2009) 243-249.

15 Shahidi F, *Maximising the value of marine by-products*, (CRC press, Taylor & Francis group), (2006) 1-532.

16 Jongjareonrak A, Rawdkuen S, Chaijan M, Benjakul S, Osako K, *et al*., Chemical compositions and characterization of skin gelatin form farmed giant catfish (*Pangasaianodon gigas)*, *LWT-Food Sci Technol*, 43 (2010) 161-165.

17 McConnell A A, Eastwood M A & Mitchell W D, Physical characteristics of vegetable foodstuffs that could influence bowel function, *J Sci Food Agric*, 25 (1974) 1457–1464.

18 Lowry O H, Rosebrough N J, Farr A L & Randall R J, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 193 (1951) 265-275.

19 Johnston-Banks F A, *Gelatin*, In: *Food gels*, (Elsevier Applied Science, London), 1990, pp. 233–289.

20 Karim A A & Bhat R, Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins, *Food Hydrocoll*, 23 (2009) 563- 576.

21 Killekar V C, Koli J M, Sharangdhar S T & Metar Y S, Functional properties of gelatin extracted from skin of Black king fish (*Ranchycentron canadus*), *Ind J Fundam & Appl Life Sci*, (2012) 106-116.

22 Ward A G & Courts A, *The Science and technology of gelatin*, (London New York: Academic Press), 1977, pp. 1-564.

23 Sahoo R, Dhanapal K, Reddy G V S, Balasubramanian A & Sravani K, Study on the functional properties of gelatin extracted from the skin of the fish Pacu (*Piaractus Brachypomus*), *Int J Innov Sci Eng Technol*, 2 (2015) 218-232.

24 Chandra M V & Shamasundar B A, Rheological properties of gelatin prepared from the swim bladders of freshwater fish *Catla catla, Food Hydrocoll*, 48 (2015) 47-54.

25 Uriarte-Montoya M H, Santacruz-Ortega H, Cinco-Moroyoqui F J, Rouzaud-Sández O, Plascencia-Jatomea M, *et al*., Giant squid skin gelatin: chemical composition and biophysical characterization, *Food Res Int*, 44 (2011) 3243–3249.

26 Rodin V V & Izmailova V N, NMR method in the study of the interfacial adsorption layer of gelatin, *Colloids Surf A Physicochem Eng Asp*, 106 (1996) 95-102.

27 Fullerton G D, Nes E, Amurao M, Rahal A, Krasnosselskaia L, *et al*., An NMR method to characterize multiple water compartments on mammalian collagen, *Cell Biol Int*, 30 (2006) 66–73.

28 Yakimets I, Wellner N, Smith A C, Wilson R H, Farhat I, *et al*., Mechanical properties with respect to water content of gelatin films in glassy state, *Polymer*, 46 (2005) 12577–12585.

29 Bruce G, Frushour & Koenig J L, Raman scattering of collagen, gelatin, and elastin, *Biopolymers*, 14 (1975) 379–391.

30 Wang Y, Yang H & Regenstein J M, Characterization of fish gelatin at nanoscale using atomic force microscopy, *Food Biophysics*, 3 (2008) 269-272.