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Insights into the Structural and Thermal Properties of Pepsin-Soluble Collagen from Daggertooth pike conger *(Muraenesox cinereus)*

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ABSTRACT

Pepsin soluble collagen (PSC) is a type-1 collagen which is abundant in skin. The pepsin soluble collagen was successfully extracted from the skin of Daggertooth pike conger *(Muraenesox cinereus)* by using the conventional method of salt precipitation followed by dialysis. Pepsin-soluble collagen showed a yield of 10.12% on wet matter basis and 25.5% on dry matter basis. The triple helical structure of collagen was confirmed by Fourier Transform Infrared Spectroscopy (FTIR) and UV-Visible spectrophotometry. Isolated PSC was confirmed as type 1 collagen by Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). FTIR spectra of PSC suggested that the secondary structure of the triple helical collagen was intact even after pepsin digestion. Pepsin-soluble collagen was soluble in acidic pH with maximum solubility at pH 4. The solubility was found to be decreased with an increase in the concentration of sodium chloride with a minimum solubility at 4%. The present PSC isolate from eel fish exhibited comparatively high thermal stability of 36 °C. Morphological analysis by Scanning electron microscopy (SEM) revealed a porous structure and comparatively high thermal stability makes it a promising biomaterial.

Keywords: Type- 1 collagen, Hydroxyproline, FTIR spectra, Marine eel.

INTRODUCTION

Collagen serves as the fundamental building component of the human body with three polypeptide chains forms the triple helix structure [1]. Collagen can be extracted from a wide range of sources including animals (like calf, porcine, rodent/insect and marine/aquatic species), and synthetic sources (like recombinant human collagen- rhCOL) [2]. Inevitable functions of collagen include enhancement of blood clotting, angiogenesis and protective functions. It also has an essential role in providing elasticity, waterproofing capacity and strength to the skin [3,4,5]. Collagen has its application in various fields like the pharmaceutical industry, biomedical field, cosmetic industry and so on [6]. Collagen can be formulated as high-value drugs/supplements, hydrogels, sponges and biomaterials with the help of modern-day techniques. All of these applications point out the essentiality of collagen [7,8,9].

The fisheries and aquaculture have significantly increased as a result of the rapid urbanization and industrialization. This led to the global emergence of new eating habits. The amount of global fish waste has risen significantly as a result of the increased consumption. Almost two-thirds of processed fish including the bones, skin, and scales are being wasted creates environmental and financial problems by its improper disposal. Fish wastes must be properly disposed of and recycled in order to address this issue. Low-value fish waste, when appropriately managed, can be utilized to synthesize high-value products such as collagen, enzymes, and bioactive peptides [10,11].

Collagen is an essential valuable product that can be produced from fish. The production of collagen from fish has many advantages like its abundance, low immunogenicity and high metabolic compatibility. The important point to be noted is that it does not create the risk of zoonosis. So, fish can be considered as an influential source of collagen [12]. Daggertooth pike conger (*Muraenesox cinereus*), is a temperate, carnivorous eel species distributed predominantly along the coast of the Indo-west Pacific Ocean, China and Japan [13]. Outer skin of this fish is considered as waste product and is not generally edible. This skin is a potential low value waste material which can be used to obtain collagen, especially type-1 collagen which is plentiful in skin. Pepsin soluble collagen (PSC) is a type-1 collagen that can be extracted with the aid of pepsin. The transformative approach of producing high value products from low value material serves as a foundation of current study to isolate Pepsin soluble collagen from Daggertooth pike conger.

MATERIAL AND METHODS

Sample collection and initial treatment of fish skin

The Daggertooth pike conger (*Muraenesox cinereus*) was procured from a fish landing facilities in Mangalore, Karnataka, which is located along India's southern coast (12°55′02″ N, 74°51′21″ E). The skin was peeled off after washing. The residual meat was removed. The obtained skin was sliced into small pieces (1.5 ± 0.5 cm2) and rinsed with cold water. These samples were stored at -20°C until further analysis.

Isolation of collagen

Pepsin soluble collagen was extracted from eel skin by following the protocol of Nagai and Suzuki (2000) with slight modifications [14]. All the extraction techniques were carried out at 4 °C. The skin pieces were kept in 10% butyl alcohol solution for 48 h at a sample-to-alcohol ratio of 1:10 (w/v) for defatting the skin. Defatted skin was washed using ice-cold distilled water. The non-collagenous protein was removed by treating the defatted skin with 0.1 N NaOH solution at a sample-to-solution ratio (w/v) of 1:10 to remove non-collagenous proteins. Alkali treatment for three days by changing the solution every 24 h and the solution was stirred at eight-hour intervals. After three days of treatment, the samples were washed until neutral pH was obtained. Fish skin samples were added with 0.5 M acetic acid (acetic acid: sample (2:1)) and digested with 10% porcine pepsin at 4° C for 48 h. It was centrifuged at 20000 X g for 1 hour.

Salting out and dialysis

Collagen from the viscous solution was salted out using sodium chloride to a final concentration of 0.9 M. The NaCl was added to a 0.05 M Tris-HCl solution to precipitate collagen to a final concentration of 2.3 M (pH 7.5). The resultant precipitate from the reaction was then centrifuged at 20,000 X g for 20 min at 4°C. The pellet was dissolved in 10 volumes of 0.5 M acetic acid, dialysed against 10 volumes of 0.1 M acetic acid for a day, and then rinsed with distilled water until the pH was neutral. The pellet was then lyophilised using an Operon FDU 7003 lyophiliser.

Proximate composition

A small portion of freeze dried fish skin was given for the proximate analysis to determine the moisture on wet matter basis and crude protein, crude fat, and total ash on dry matter basis. This was analysed by AOAC (2006) method [15].

Yield of extracted Pepsin soluble collagen

The yield of PSC was determined by measuring the percentage weight of freeze-dried collagen to the weight of the skin on both dry and wet bases.

$$\text{Collagen (\%)} = \frac{\text{Weight of lyophilised collagen in grams}}{\text{Weight of skin in grams (dry weight)}} \times 100$$

$$\text{Collagen (\%)} = \frac{\text{Weight of lyophilised collagen in grams}}{\text{Weight of skin in grams (wet weight)}} \times 100$$

Estimation of the concentration of the collagen

The amount of PSC was estimated by Lowry's method (Lowry et al., 1951) with the aid of a total protein kit, Micro Lowry and Peterson′s Modification (Sigma Aldrich) [16].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed using vertical gel electrophoresis device (Biorad, USA) following the method of Laemmli (1970) so as to determine protein patterns of PSC [17]. A wide range protein marker (10-245 kDa) from Sigma Aldrich and SDS-PAGE kit by HiMedia, India was used to perform SDS-PAGE. The collagen standard (calf skin collagen) purchased from Sigma Aldrich was diluted as per the manufacturer's instructions. The gel was initially run at a constant voltage of 50 V/cm till the dye front crossed the stacking gel. The voltage was then increased to 100 V/cm till the dye front reached the end of the gel. The gel was then transferred to distilled water, viewed and photographed in the ChemiDocTM MP imaging system (Biorad, USA).

UV- Visible spectroscopic analysis

UV-VIS spectrophotometer was used to measure the UV spectrum of collagen samples (Lamda 25TM, PerkinElmer, USA). One milligram of lyophilised collagen was dissolved in 100 mL of 0.02 M sodium acetate buffer that contain 2 M urea (pH 4.8). The solution was then poured into a quartz cuvette with 1 mm path length with an interval of 1nm and a scan speed of 2 nm/s, a spectrum between 200 and 400 nm was analysed.

Attenuated total reflectance- Fourier transform infrared spectroscopy (FTIR)

Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR) was used to analyse PSC (Perkin Elmer Private Limited, Singapore). At a resolution of 4 cm^{-1} per point, 32 scans were performed to collect the spectra in the 400–4000 cm^{-1} range. Software SCAN ANALYSE was used to analysis the FTIR spectrum.

Scanning electron microscopy (SEM)

The morphological features of the isolated PSC were observed under a scanning electron microscope (TESCAN VEGA-3 LMU, Czech Republic). PSC sample was mounted on a sample holder and fixed. The sample holder was used to prepare 20-s glow-discharged carbon support adhesive films (tape) having a thickness of 30 nm. The collagen sample was placed over adhesive film and sputter coated with gold ion. The surface morphology of the collagen was examined by introducing the coated sample into the specimen chamber.

Analysis of denaturation temperature (T_d) by Circular dichroism spectroscopy and Thermal denaturation curve

The thermal stability of PSC was analysed using Circular dichroism (CD) spectroscopy as well as using Ostwald's viscometer. CD spectra of PSC were recorded using JASCO circular dichroism spectroscopy. The PSC extract was diluted with 5 mL of 0.1 M glycolic acid for analysis. 1 ml of diluted sample was loaded into the quartz cell having a path length of 1 cm. Molar ellipticity (θ) at 220 nm was determined by heating the samples at 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 32°C, 34°C, 36°C, 38°C, 40°C, 42°C and 45°C. The scans were taken in a range of 190-250 nm under a nitrogen atmosphere at a scan speed of 2 cm/min. Each spectrum was obtained at three runs at each temperature. The melting curve of PSC was computed by measuring molar ellipticity (θ) at 220 nm. CD spectra were expressed as mean residual ellipticity and expressed as deg cm^2 $dmol^{-1}$. The denaturation temperature of the collagen was determined by measuring 221 (θ221). It was measured as a function of temperature.

Using Ostwald's type viscometer viscosity was measured to calculate the denaturation temperature of PSC in terms of fractional viscosity. The method employed was according to Kimura et al. (1988) with slight modifications [18].

Test for solubility

The effect of different NaCl concentrations and pH on the functional property of PSC was determined by protocol of Montero (1991) with slight modifications [19].

Effect of pH variations on solubility

Pepsin soluble collagen was dissolved in 0.5 M acetic acid to a final concentration of 3 mg/mL. The mixture was kept at 4 ℃ for 3 h and was stirred continuously for complete mixing. From this solution, eight milliliter aliquots of collagen were taken and transferred to a 15 mL centrifuge tube. The

pH of the aliquots was adjusted with 6 N HCl / 6 N NaOH as required. The final pH of these aliquots ranged from 1-10. The volumes of each aliquot were made up to 10 mL by adding distilled water with the same pH as that of collagen solution. Aliquots were again stirred at 4℃ for one hour and centrifuged at 8500 X g for 20 min. Supernatants after centrifugation were collected and the protein content in each supernatant was estimated by Lowry's method. Bovine serum albumin was used as the standard for the estimation. Absorbance at 660 nm corresponding to each supernatant was taken and plotted in a solubility graph with pH on X-axis and absorbance at 660 nm on Y-axis to obtain pH giving the highest solubility.

Effect of different concentrations of NaCl on solubility

The PSC was dissolved in 0.5 M acetic acid to a final concentration of 3 mg/mL. Aliquots of the mixture were prepared in 10 mL of NaCl at various concentrations to a final concentration of 0, 10, 20, 30, 40, 50, and 60 g/L. All of these aliquots were stirred continuously for one hour followed by centrifugation at 8500 × g for 20 min. Supernatants obtained from each aliquots were used to determine the protein concentration by Lowry's method using bovine serum albumin as standard. Absorbance at 660 nm corresponding to each supernatant was taken and plotted in a solubility graph with a concentration of NaCl on the X-axis and absorbance on the Y-axis to obtain the concentration of NaCl giving the highest solubility.

Amino acid analysis

1 ml 6 N HCl was added to 25 mg of the sample. Then the sample was placed in a water bath at 100 ° C for 24 h. After 24 h the sample was taken out and allowed to cool down to room temperature. About 20 µL was injected after derivatisation using FMOC and OPA. The analysis was performed in Agilent 1260 series HPLC system (Agilent technologies, Palo Alto, CA, USA)

Hydroxyproline content estimation

Hydroxyproline concentration in PSC was determined with the help of a method by Woessner (1961) with slight modifications. The absorbance was measured at 560 nm [20].

RESULTS

Proximate analysis

As per the result of proximate analysis the eel skin were containing 68.73% moisture content. The result of other parameters like crude fat, moisture, total ash and protein is given in the Table1.

Yield of collagen

PSC was isolated from Daggertooth pike conger (Fig.1). The yield of PSC from the fish skin in the current study was around 10.21% on wet matter basis and 25.5% on dry matter basis.

Total protein in PSC

Protein content of lyophilized collagen was analysed using Lowry's Method (GeNei ™) with suitable modifications. The standard curve (Fig.2A) was used to compute the protein concentration. The estimated protein concentration of PSC was found to be 83.76 µg/ mL of sample.

SDS-PAGE Analysis

The electrophoretic pattern of PSC revealed 4 bands including 2 α chains (α 1 and α 2) and their subunit β against standard calf skin collagen (Fig. 2B)

UV absorption spectrum

The UV absorption spectrum of PSC in a range of 200-400 nm have showed a maximum absorption at 221 nm (Fig. 3), suggesting the presence of freely accessible C=O, -COOH and -CONH2 in polypeptide chains.

FTIR Spectra

The FTIR analysis showed transmission peaks at various intensities. FTIR spectrum obtained from PSC is depicted below (Fig. 4). The spectra of PSC revealed the presence of amide A band at wave number 3296.43 cm^{-1} which is due to N-H stretching of proteins. An amide B CH_2 asymmetric stretching was also displayed in the spectrum which is clearly visible at 2925.21 cm^{-1}. Similarly, amide I and amide II peaks were visible at 1637.73 cm^{-1} and 1545.99 cm^{-1} which were due to C=O stretching and N-H bending of proteins respectively. A CH_3 asymmetric stretch was visible at 1451.50 cm^{-1}. At 1238.76 cm^{-1} amide III stretch was revealed which symbolizes the presence of β sheet of protein. PSC isolate from conger eel showed a peak around 1440 cm^{-1} and 1451.50 cm^{-1}. The bands observed at respective wave numbers are given in the table 2.

Morphological analysis with SEM

Fibrilar and porous three dimensional structures were clearly visible under scanning electron microphotography with a higher magnification as given in Fig.5. The fibril width range was variable in different regions of the image. The maximum and minimum collagen fibrillar width observed was 2.83 µm and 1.05 µm respectively. The collagen bundles are interconnected by collagen fibrils forming interconnected crosslinks in between the molecules. In the image represented below the structure of PSC was tubular in nature with nodular form.

Analysis of thermal stability

At a temperature range of 25–45°C and a wavelength range of 190–300 nm, the CD measurement of the pepsin-soluble collagen was detected. The obtained CD spectra are displayed below (Fig. 6). A cross over point is noted at a wavelength of 214 nm, with a rotatory maximum and minimum at 224 nm and 208 nm respectively. The collagen triple helix was still intact up to 36 °C, beyond that, it started to get denatured.

The same result was reproduced in the analysis of Td using Ostwald viscometer. The thermal denaturation curve of PSC is shown below (Fig. 7). The T_d value obtained can be regarded as the temperature at which triple helix get deformed. According to thermal denaturation curve, PSC has T_d value of 36 ℃.

Solubility of collagen

The maximum solubility of PSC was observable at pH 4. The minimal solubility was achieved at 4% of NaCl, which was then followed by a drop in solubility. The salting out effect is the cause of the decreased solubility at low NaCl concentrations. The Fig. 8 A and B illustrates relative solubility of PSC at different PH and NaCl concentrations.

Amino acid analysis

Amino acid analysis by HPLC showed the presence of amino acids like proline, cysteine, leucine, isoleucine, phenyl alanine, tryptophan, methionine, alanine, aspartic acid, glutamic acid, histidine, glycine and arginine in the PSC.

Hydroxyproline content

A standard curve was plotted using standard hydroxyproline to determine the amount of hydroxyproline in the extracted PSC (Fig. 9). The concentration of hydroxyproline in 1 mg collagen was found to be 71.72 μg.

DISCUSSION

The extracellular matrix of various connective tissues primarily consists of collagen, a structural protein with a triple helix formed by three polypeptide chains. Collagen finds extensive applications in tissue regeneration, reconstructive surgery, wound healing, bone grafting, and the pharmaceutical industry. Due to the risk of disease outbreaks, marine-derived collagen is preferred over mammalian-derived collagen.

The isolation of pepsin-soluble collagen (PSC) from daggertooth pike conger was carried out with a similar process of collagen extraction employed for Nile tilapia skin [21]. The proximate composition analysis revealed a moisture content of 68.73%, crude protein content of 30.24%, crude fat of 12.17% and total ash of 2.16%. Total ash content in the conger eel was comparatively lower when compared to other marine and fresh water fishes might be due to absence of scales and spines on eel skin [22].

PSC extracted from the eel has a protein content of about 8.3% (83.76 mg/mL). The higher protein concentration was due to high amino acid and high triple helix content of collagen [23]. Conger eel revealed an electrophoretic pattern of 2 α (α1, α2), and β chains. The eel fish's electrophoretic pattern resembled with that of type 1 collagen in calf skin. The result obtained was similar to the electrophoretic pattern from *Evenchelys macrura* [22]. The bands in PSC also included β chains (dimers) which participate in the crosslinking of collagen [24].

UV-visible spectroscopy is one of the methods that are used to characterise collagen. UV absorption spectrum of the PSC was measured at wave length ranges from 190-400 nm. In current study PSC have shown a maximum peak of absorption at 221 nm. The UV absorption of PSC from *Evenchelys macrura* showed a maximum absorption at 228 nm. Collagen triple helix normally shows a maximum absorption around 230 nm due to the presence of freely accessible C=O, -COOH, CONH2 groups in the polypeptides chains of collagen [22]. The isolated collagen did not showed peak at 280 nm which indicates the absence of aromatic amino acids in the triple helix. This supports the potency of alkaline treatment to remove non-collagenous proteins [25]. Also concentration of the aromatic amino acids is reported to be low in fish collagen [24].

FTIR spectroscopy is a valuable tool for analyzing macromolecules, especially proteins, providing insights into their dynamics, environment, and structure [26]. In the case of conger eel collagen, the FTIR spectrum revealed characteristic bands such as Amide A (3296.43 cm^{-1}) indicating N-H stretching vibrations, and Amide B (2925.21 cm^{-1}) associated with CH2 asymmetric stretching vibrations. Amide I (1637.73 cm^{-1}) serves as a marker for secondary structure and its unchanged wavenumber post-pepsin hydrolysis suggests no structural alterations. However, a lower shift implies increased hydrogen bonding, strengthening the triple helix structure. The Amide II band (1545.99 cm^{-1}) reflects NH bond deformation and CN elongation, with a lower frequency shift indicating more NH groups involved in hydrogen bonding. Amide III (1440 cm^{-1}) is related to NH bond bending and stretching, revealing the presence of proline and hydroxyproline amino acids in the collagen triple helix. PSC isolate from conger eel showed a peak around 1440 cm^{-1} due to the vibration of pyrrolidine ring of in the collagen. Intensity of the peak shows the number of proline and hydroxyproline amino acids. Conger eel showed a peak at 1451.50 cm^{-1} is an indicative of the iminoacids proline and hydroxyproline in collagen triple helix [27,28,29,24,30].

The morphology analysis of PSC was done with the help of scanning electron microscopy. PSC under SEM exhibited fibrillar and porous morphology. This porous nature of the freeze dried collagen makes it as an ideal biomaterial in various biomedical applications like wound healing because freeze dried collagen sponges are directly applied to the wound area. Well defined pores with the interconnected pore walls observed in conger eel PSC is suggestive to use this collagen as a biomaterial in tissue engineering, cell seeding and delivery of drug molecules [31,32]

Circular dichroism spectroscopy effectively discerns molecular order and protein structural changes [33]. In the scanning range of 190-250 nm, PSC exhibited a cross-over point at 214 nm, with a rotatory maximum and minimum at 224 nm and 208 nm, mirroring observations *in Evenchelys macrura* PSC [22]. The collagen triple helix in conger eel PSC remained intact up to 36°C, beyond which denaturation commenced. The CD spectrum of native collagen revealed a polyproline II-like helical structure, with positive and negative peaks at 221 nm and 198 nm, respectively, and a crossover point at 213 nm [35,36]. Until 34°C, PSC maintained consistent thermal stability, indicated by an intact triple helix at 36°C. Above 36°C, denaturation started, which have reflected the disappearance of the positive peak, and a red shift in the negative peak. The denaturation temperature (36°C), determined *via* relative viscosity, aligns with the CD spectroscopic analysis. Elevated denaturation temperature is attributed to increased cross linkages in PSC. Pyrrolidine rings of proline and hydroxyproline, along with hydrogen bonding through the hydroxyl group of hydroxyproline, contribute to its thermal stability. Compared to freshwater fish northern pike (Td of 27°C), marine conger eel exhibited a higher denaturation temperature, suggesting a greater hydroxyproline content [38].

Solubility testing showed maximum solubility at pH 4 The solubility of the collagen protein was improved by the repulsive forces between chains when the pH was not equal to pI because the net charge of the protein molecules was greater than zero. The overall net charge of the protein molecules near zero and precipitation occurred when the pH was close to or equal to pI. Isoelectric point of collagen ranges from 6 to 9 [39]. Fish collagen typically found in the skin, scales, swimming bladder, cartilage, and bones dissolves in a pH range of 1-6, 1-5, 1-4, 1-5, and 1-4, respectively [40]. Solubility of PSC isolated from conger eel in different NaCl concentration showed a minimum solubility at 4% of NaCl (40 g/L). As the concentration of NaCl increases solubility was found to be decreased [41]. Salt ions interact weakly with the charged groups on the surface of proteins at lower concentrations of NaCl. Therefore, the charge and solubility were unaffected by low salt concentrations. Contrarily, the solubility of PSC dramatically decreased when NaCl was present in large amounts. The significant drop in solubility may be due to salting out phenomenon of collagen. The hydrophobic interaction between protein chains was strengthened at high salt concentrations. The water was also more tightly bonded with salt at the same time. This lead to the collagen precipitation [42].

The amino acid profiling of PSC from Daggertooth pike conger showed the presence of 13 amino acids, which are glycine, proline, cysteine, leucine, isoleucine, phenyl alanine, tryptophan, methionine, alanine aspartic acid, glutamic acid, histidine, and arginine. The formation of the collagen triple helix requires the presence of non-proteogenic amino acid hydroxyproline [44]. By creating an interchain hydrogen bonding through the hydroxyl group, hydroxyproline helps to stabilise the triple helical structure of collagen [45]. Hydroxyproline content in 1mg of collagen in Dagger tooth pike conger was found to be 7.1% (71.72 mg/g). The result was similar to the hydroxyproline content obtained from PSC of *Trachinotus blochii*

skin - 73.7 mg/g (Cao et al., 2019) and *Hypophthalmichthys nobilis* bone - 73.8 and skin-73.2mg/g [46,47].

Table 1: Proximate composition of pepsin soluble collagen from conger eel.

Dry matter (%)		Wet matter (%)	
Crude protein	30.24%	Moisture content	68.73%
Ether extract/ crude fat	12.17%		
Total ash	2.16%		

Table 2: Wave numbers and corresponding bands of PSC from FTIR spectra.

Wavenumber	Bands	Significance
3296.13 cm^{-1}	Amide A	N-H stretching
2925.21 cm^{-1}	Amide-B	CH2 asymmetric stretching
1637.73 cm^{-1}	Amide-I	C=O stretching
1545.99 cm^{-1}	Amide-II	N-H deformation
1238.76 cm^{-1}	Amide-III	N-H bending
1440 cm^{-1}	-	Vibration of pyrrolidine ring
1451.50 cm^{-1}	-	Imino acids

Figure 1: Extraction of pepsin soluble collagen from conger eel. A) Fresh Daggertooth pike conger, B) Peeled eel skin after cleaning for extraction C) Isolated PSC after lyophilization.

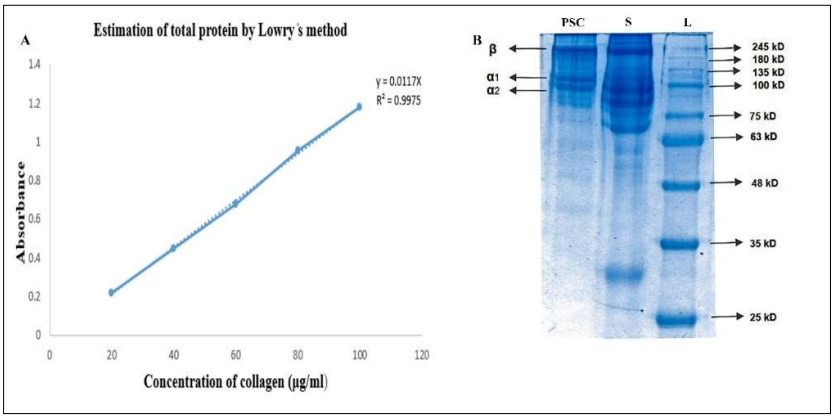

Figure 2: A) Graph of of total protein from the isolated PSC B) SDS-PAGE analysis of PSC – from left to right. Lane -1 PSC with 2α and β bands, Lane-2 Standard type -1 calf collagen, Lane-3 high molecular weight range protein ladder (25-245 KD)

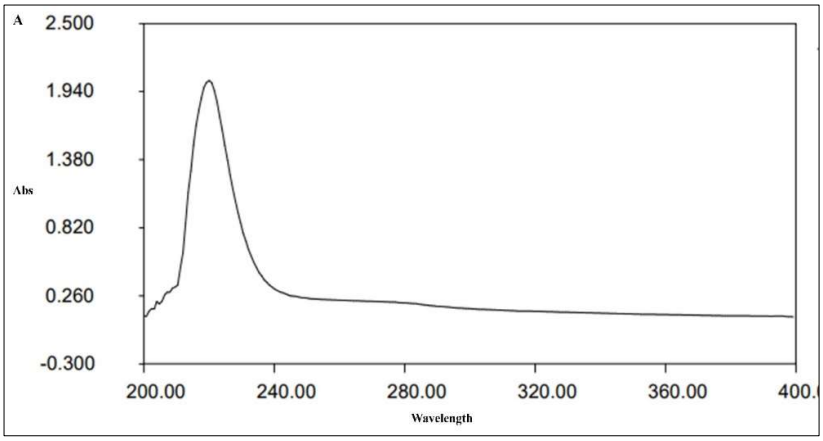

Figure 3: UV–visible spectra of PSC in a range of 200-400 nm.

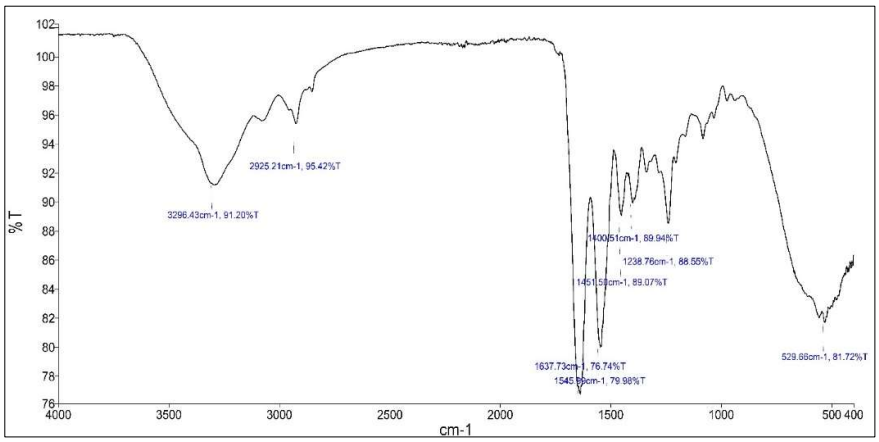

Figure 4: FTIR spectra of PSC.

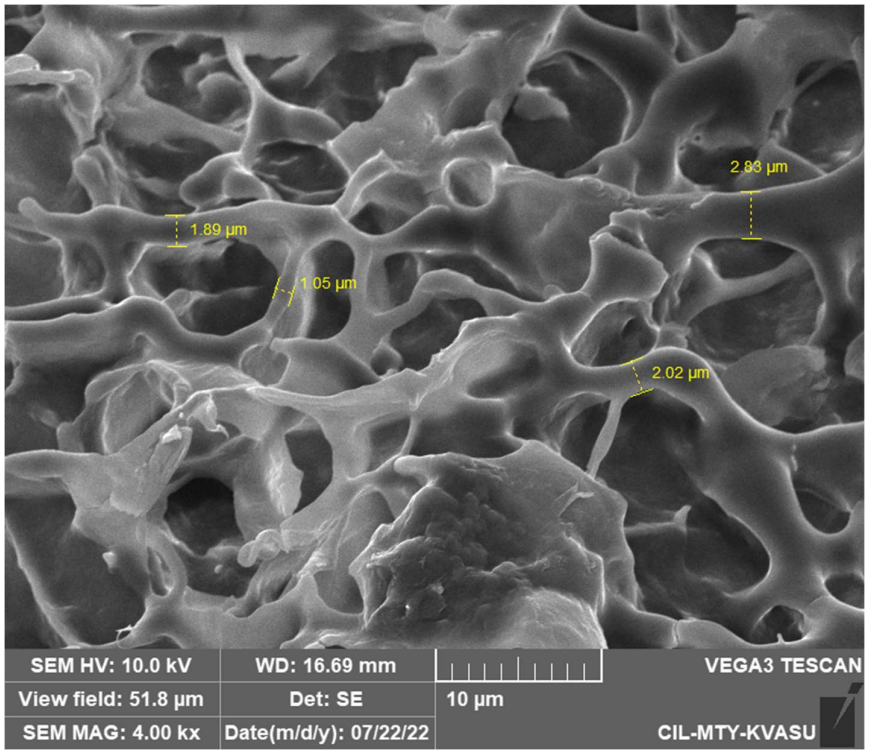

Figure 5: Scanning electron microphotograph of PSC.

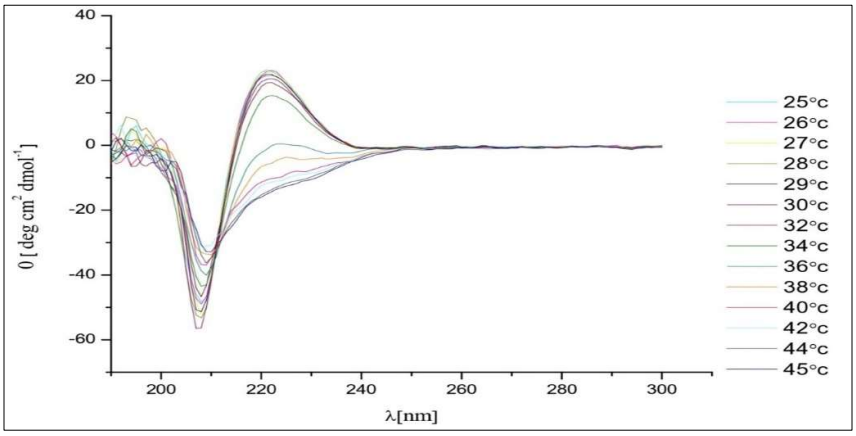

Figure 6: Circular dichroism spectra of pepsin soluble collagen in a range of 190-300 nm.

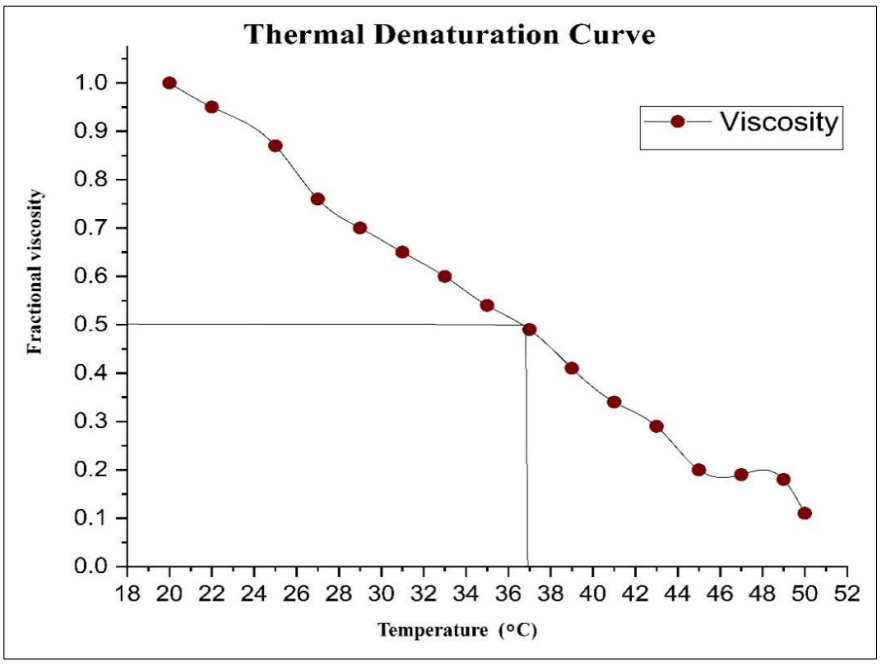

Figure 6- Thermal denaturation curve of PSC.

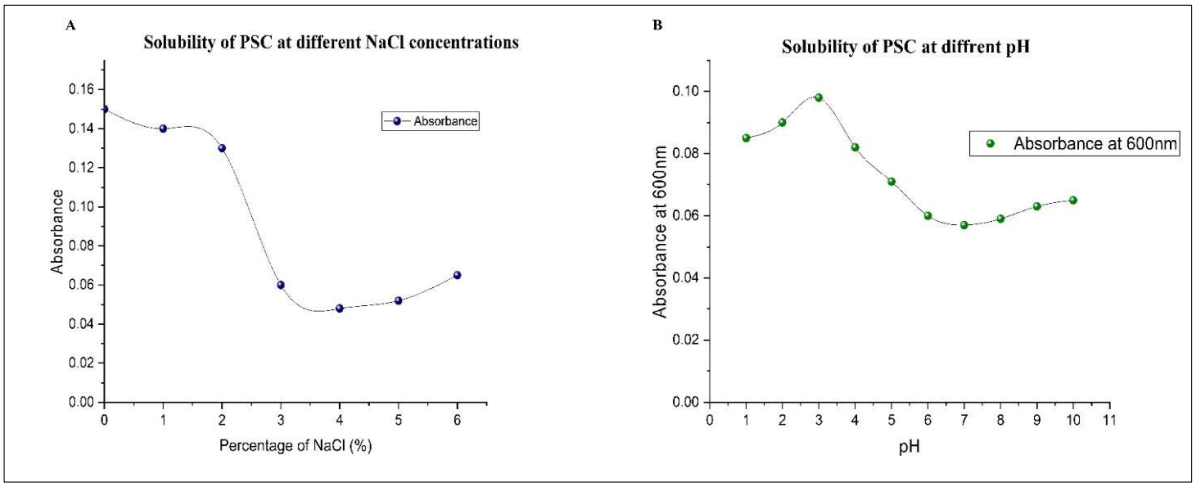

Figure 8: Graphical representation of A) Solubility of PSC at different NaCl concentrations, B) Solubility of PSC at different pH.

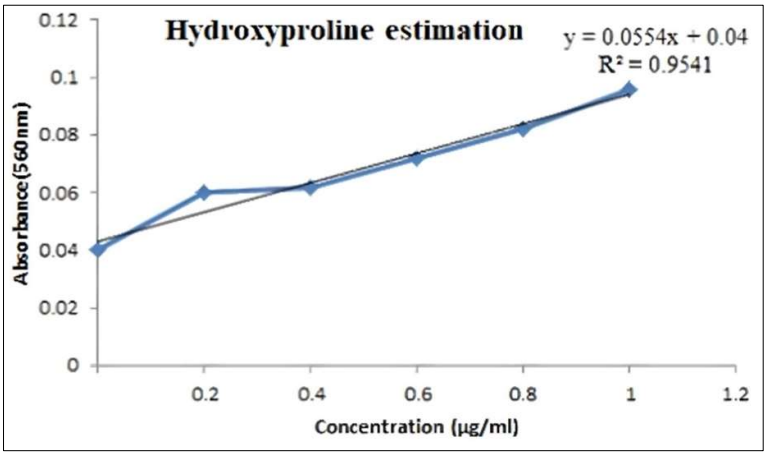

Figure 9: The standard curve of hydroxyproline estimation.

CONCLUSION

PSC was isolated from marine eel Daggertooth pike conger with a yield of 10.12% on wet matter basis and 25.5% on dry matter basis. FTIR spectroscopy, UV-vis spectroscopy and SDS-PAGE analysis confirmed the presence of type 1 collagen with a triple helical structure intact with 2 α and a β band. The solubility of PSC was observed to be highest at pH 4, and the solubility was found to be decreased with an increasing concentration of NaCl. Additionally, the isolated PSC exhibited high thermal stability up to 36 °C. Morphological analysis using SEM visualization revealed a porous structure of isolated PSC. The study revealed that the extracted PSC from low value material conger eel skin as a high-value product with all the viable structural properties of collagen. The findings of the research suggest that PSC from eel fish holds promising pharmaceutical and biomaterial applications, especially due to its porosity and high denaturation temperature similar to mammalian collagen.

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Conflict of interest

The authors declare that they have no conflict of interest.

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