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Full Length Research Paper

Extraction, structural and physical characterization of type I collagen from the outer skin of *Sepiella inermis* (Orbigny, 1848)

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The acid soluble collagen (ASC) and pepsin soluble collagen (PSC) were extracted from the outer skin of *Sepiella inermis* and further characterized partially. The yield of ASC was low (0.58% on dry weight basis); whereas the yield of PSC was comparatively more (16.23% on dry weight basis). The protein content in ASC and PSC was calculated as 20.24 and 69.56%, respectively (on dry weight basis). The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel profile showed two bands for ASC and PSC with corresponding molecular weight of 86 and 67 kDa and 86, 63 and 58 kDa respectively. The differential scanning calorimetry (DSC) results showed that ASC withstand up to 75.93°C whereas the PSC withstand up to 75.05°C. The fourier transform infrared spectroscopy (FT-IR) spectrum of both ASC and PSC recorded 11 and 13 peaks, respectively. The fine structure of both ASC and PSC was also studied using scanning electron microscopy (SEM).

Key words: *Sepiella inermis,* acid soluble collagen (ASC), pepsin soluble collagen (PSC), differential scanning calorimetry (DSC), fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy **(**SEM).

INTRODUCTION

The collagen from different tissues varies considerably in chain composition, amino acid composition and physicochemical characteristics thereby, contributing to the specific functional requirements of the tissues. The structure and functions of as much as 28 distinct vertebrate collagen types are well understood and have been identified (Gordon and Hahn, 2010). Collagen plays an important role in the formation of tissues and organs, and is involved in the biological functions of a cell, such as cell proliferation, survival and differentiation. Owing to its excellent biological properties, the use of collagen in biomedical applications has been rapidly growing and widely expanding to bioengineering areas. The characteristic feature of a typical collagen molecule, tropo collagen, is its long, stiff, triple-stranded helix, in which three collagen polypeptide chains are wound around one another in the form of a rope-like super helix. These peptides are extremely rich in proline and glycine, both of which are important for the formation of the collagen-specific helical structure (Lehninger, 1987; Alberts et al., 1994; Rossler et al., 1995; Zubay, 1998). The collagens from different tissues vary considerably in chain composition, amino acid composition and physico-chemical characteristics thereby, contributing to the specific functional requirements of the tissues (Bateman et al., 1996). Though the basic biochemistry of both

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Abbreviations: ASC, Acid soluble collagen; **PSC,** pepsin soluble collagen; **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **FT-IR,** fourier transform infrared spectroscopy; **SEM,** scanning electron microscopy; **DSC,** differential scanning calorimetry; **UV-vis,** ultra-violet visible.

invertebrate and vertebrate collagen appears to be similar, the collagen is a family of fibrous proteins comprising more than 12 different types. The major collagenous component of vertebrate muscles is type I collagen and some minor components such as types III, IV and V collagen are also present (McClain, 1974; Epstein and Munderloh, 1975; Kimura et al., 1976; Duance et al., 1977; Mayne and Zettergen, 1980).

Collagen not only plays an important role in the development of muscle tissue but also has been utilized as a raw material for food, cosmetics, pharmaceuticals and experimental reagents. So far, the main sources of collagen are limited to those of land-based animals, such as the skin and bone of bovine and porcine. However, the outbreak of bovine spongiform encephalopathy (BSE) and the foot and mouth disease (FMD) crisis has resulted in anxiety amongst users of collagen and collagen-derived products of land animal origins (Helcke, 2000).

A close relationship has recently been reported between texture and collagen content from the muscles of many aquatic species, suggesting the importance of this protein in association with physical properties of marine food (Hatae et al., 1986; Sato et al., 1983; Olaechea et al., 1993; Mizuta et al., 1994). Thanonkaew et al. (2006) isolated the collagen from cuttlefish (*Sepia pharaonis*) muscle and analyzed its chemical composition and thermal property. Mingyan et al. (2009) isolated the acid soluble collagen (ASC) and pepsin soluble collagen (PSC) from skin of squid (*Ommastrephes bartrami*) and the structural and physico chemical properties were studied through fourier transform infrared spectroscopy (FT-IR), sodium dodecyl sulfate (SDS), denaturation temperature, amino acid composition and differential scanning calorimetry (DSC). Palpandi et al. (2010) extracted collagen from the mangrove archeaogastropod *Nerita* (*Dostia*) *crepidularia* which was structurally characterized through FT-IR. Shanmugam et al. (2011) prepared ASC and PSC from the outer skin of cuttlefish *S. pharaonis* which was characterized through FT-IR and ultra-violet visible (UV-vis) spectrum. In the present study, an attempt has been made to isolate and characterize the type - I collagen from the outer skin of the cuttlefish, *Sepiella inermis*.

MATERIALS AND METHODS

The animals (*S. inermis*), weighing 70 to 110g, captured during the post monsoon season were purchased in the landing centre at Thondi, Tamil Nadu, India. They were brought to the laboratory within 4 h in an ice box with dry ice and thoroughly washed with distilled water. The outer skin was removed manually, cut into small pieces and stored in polythene bags at -85°C until used. The procedure of Nagai et al. (2001) was followed for the extraction of ASC and PSC from the skin of *S. inermis*.

Extraction of acid soluble collagen (ASC)

All the preparative procedures were performed at 4°C. The skin was extracted with 0.1 M NaOH to remove non-collagenous proteins for three days, then washed with distilled water and freeze-dried. The freeze-dried skin was extracted with 0.5 M acetic acid for three days, and the extract was centrifuged at 50,000 X g for 1 h. The residue was re-extracted with the same solution for two days, and the extract was centrifuged under the same conditions. Each solution was mixed and salted out by adding 0.8 M NaCl, followed by the precipitation of the collagen by the addition of 2.3 M NaCl at a neutral pH (adjusted with 0.05 M Tris HCl, pH 7.5). The resultant precipitate was obtained by centrifugation at 50,000 X g for 1 h and dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid for 24 h followed by distilled water for 24h at 4°C and then freeze-dried which is nothing but ASC.

Extraction of pepsin soluble collagen (PSC)

The residue from the acetic acid extraction was suspended in 0.5 M acetic acid and was digested with 10% (w/v) pepsin (Sigma, USA) at 4°C for 48 h. The pepsin-solubilized collagen was centrifuged at 50,000 X g for 1 h and the supernatant was dialyzed against 0.02 M Na_2HPO_4 (pH 7.2) for three days at 4°C with change of solution once per day. The precipitate obtained by (4°C) centrifugation at 50,000 X g for 1 h was dissolved in 0.5 M acetic acid and salted out by adding 0.8 M NaCl followed by precipitation of collagen by further addition of 2.3 M NaCl [adjusted with 0.05 M Tris HCl (pH 7.5)]. The resultant precipitate was obtained by centrifugation at 50,000 X g for 1 h and dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid for 24 h followed by distilled water for 24 h and then freeze-dried. The resultant freeze-dried substance is the PSC.

Estimation of total protein

The total protein content in ASC and PSC was estimated by Lowry et al (1951) using bovine serum albumin (BSA) as a standard.

Physical properties of collagen

Molecular weight determination

The gel separation (sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE) (10%) was performed following the protocol described by Sambrook and Russell (2001). The bands were observed under gel documentation system and the molecular weight was compared not only with the molecular marker but also with standard collagen (Sigma, USA). Finally, the molecular weight of collagen was determined by using total lab (version 1.11).

Fourier transform- infra red spectral analysis (FT-IR)

FT-IR spectroscopy of solid samples of standard (Human placenta, Sigma, USA), ASC and PSC from *S. inermis* were relied on a Bio-Rad FT-IR – 40 models, USA. Sample (10 mg) was mixed with 100 mg of dried potassium bromide (KBr), and compressed further to prepare as a salt disc (10 mm in diameter) for reading the spectrum.

UV-vis spectral analysis

The collagen sample was dissolved in 0.5 M acetic acid and UV-vis absorption spectra were recorded in a Shimadzu spectro-photometer UV -1800 according to the method of Yan et al. (2008).

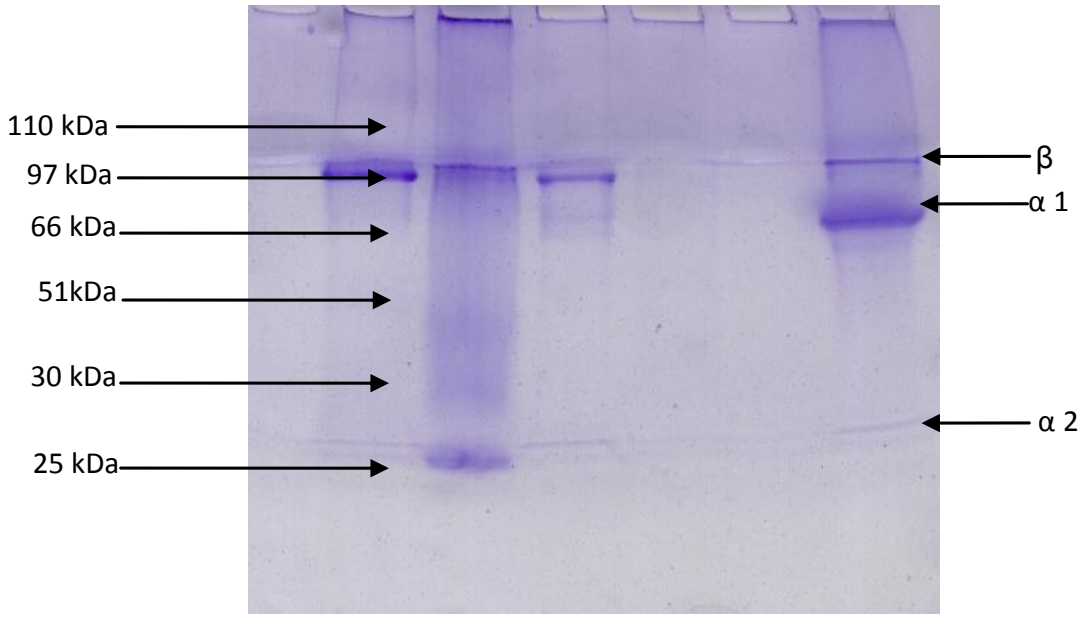

Figure 1. SDS-PAGE gel profile of *S. inermis* (lane 1, standard collagen; lane 2, protein marker; lane 3, ASC; lane 4, PSC).

Scanning electron microscopy (SEM)

SEM was used to examine the microstructure of the collagen of *S. inermis*. The collagen sample was cut using a punch and fixed to an adhesive carbon stub. Imaging was carried out using a tabletop SEM (Hitachi High-Technologies Corp., Japan) operated at 15 kV.

DSC measurement

Differential scanning calorimetry (DSC 200 F3 NETZSC H) determined the shrinkage temperature (Ts) of collagen of *S. inermis* matrices indicating the resistance against thermal denaturation. 25 g of *S. inermis* collagen sample was taken and thermatically sealed in aluminium pans. Heating was carried out at a rate of 2°C/min. in the temperature range of 20 to 200°C with an empty aluminium pan as the reference probe. Ts were determined as the onset value of the occurring endothermic peak and the value of shrinkage enthalpy (Hs) was calculated with respect to the mass of collagen matrices.

RESULTS

In the present study, both ASC and PSC were extracted from *S. inermis* skin and further partially characterized. The details of their yield, protein content, molecular weight, FT-IR spectral analysis, UV-vis spectroscopy, SEM and DSC are given below.

Yield of collagen (ASC and PSC)

The yield of ASC was low (0.58% on dry weight basis) and it was grayish; whereas the yield of PSC was comparatively more (16.23% on dry weight basis) and it was pinkish and fibre-like.

Total protein content

The protein content in ASC and PSC was calculated as 20.24 and 69.56% (on dry weight basis), respectively.

Physical properties of collagen

Molecular weight determination

The SDS-PAGE gel profile and the pixel positions of the bands obtained for the standard collagen, protein marker and ASC and PSC from *S. inermis* is depicted in Figure 1. The gel obtained through SDS - PAGE showed two bands in lane 1 representing standard collagen (Human placenta, Sigma, USA) with a molecular weight of 92 and 71 kDa, respectively and the protein marker in lane 2 recorded six bands with the molecular weight of 130, 100, 80, 60, 40 and 20 kDa, in that order; whereas the ASC in lane 3 depicted two bands with a molecular weight of 86 and 67 kDa and the PSC in lane 4 showed three bands with a molecular weight of 86, 63 and 58 kDa, respectively.

FT- IR spectral analysis of collagen

The FT-IR spectrum of standard collagen showed 17

major peaks (Figure 2); whereas the FT- IR spectrum of both ASC and PSC depicted 11 and 13 peaks (Figures 3 and 4), respectively. The peak details and their corresponding chemical structures are given in Table 1.

UV –vis spectrum

As can be seen from the UV-vis spectra (Figures 5 and 6), the distinct absorbance of ASC and PSC were obtained between 220 to 230 nm.

Scanning electron microscopy (SEM)

The SEM analysis of *S. inermis* collagen in low magnification shows highly porous collagen, interconnected with scaffolds and their surface is rough and uneven. At high magnification, there is a significant difference between ASC microstructure and PSC; but, the collagen of PSC in high magnification appeared regular and uniform net work with porous and honey-comb like structures with a size of pores on the surface ranging from few to ten micrometres (Figures 7 and 8).

Differential scanning calorimetry

DSC has been used extensively as a sensitive technique to quantify the addition of covalent cross-links and reductions in triple-helical content. When hydrated collagen is heated, the crystalline triple helix is transformed into amorphous randomly coiled peptide chains that results in shrinkage of the collagen fibre formed. The thermal denaturation profile of *S. inermis* collagen has provided useful clues to the thermal stability of collagen. The denaturation temperature (*Td*) of *S. inermis* collagen was calculated from the thermal denaturation curve (Figure 9). The result shows that the *S. inermis* ASC withstands up to 75.93°C; whereas the PSC withstands up to 75.05°C (Figure 10).

DISCUSSION

Collagen is abundant in most invertebrates as well as vertebrates (Adams, 1978). It makes up about one fourth of the content in multicellular animals (Bailey, 1968). Several reports on invertebrate collagen emphasized its morphological and functional characteristics (Gosline, 1971). In the squid *Loligo*, it participates in the propulsion by pumping water in and out via alternate contraction of antagonistic circular and radical muscle in the mantle (Ward and Wainwright, 1972). In multicellular organisms, the extra cellular matrix (ECM) is composed mainly of collagens and proteoglycans.

In Mollusca, the presence of collagen molecules has been demonstrated for several years, in particular, mussel byssus collagen from bivalves, squid skin collagen, or abalone *Haliotis* muscle collagen (Gosline and Shadwick, 1983; Kimura and Tanaka, 1983; Olaecha et al., 1993; Qin and Waite, 1995; Deming, 1999; Yoneda et al., 1999). Nagai et al. (2001) isolated about 2% of ASC and 35% of PSC from the skin of *S. lycidas* on dry weight basis. In the present study, the yield of ASC (0.58%) and PSC (16.23%) on dry weight basis was low when compared to the result of the above. The low levels of collagen content may be due to the denaturation of protein during the process of methodology and difference in environmental temperature (Rigo et al., 2002). However, the yield of the present study is more than that of *Perna viridis* (the yield of PSC and guanidine hydrochloride-soluble collagen (GSC) from whole body tissue was found to be 0.33 and 0.01%, respectively) (Amit Kumar, 2008).

Sivakumar, and Chandrakasan (1998) extracted 60% of PSC and only 12% of ASC from the cartilage of *Salvia officinalis* on wet weight basis; whereas, in the same animal, Sivakumar et al. (2003) estimated the yield of ASC and PSC as 5.52 ± 1.3 and 27.6 ± 3.07 mg/g from the cranial cartilage and cornea, respectively. In *Octopus vulgaris*, Mizuta et al. (2003) extracted 1.4 and 1.9% of collagen from the arm and mantle muscles and the protein content was reported as 9.1% and 14.0%, respectively.

Nagai (2004) found very much less yield of ASC from the diamondback squid (*Thysanoteuthis rhombus*) skin that is, about 1.3% only on dry weight basis. On the contrary, PSC was perfectly solubilized from the residue from the acetic acid extraction, and was effectively purified by differential salt precipitation. The yield of PSC was very high, about 35.6% on dry weight basis.

In the present study, the collagen was extracted from the dried skin of *S. inermis* and expressed on dry weight basis. However, the collagen content in many animals, on wet weight basis, reported higher values. ASC and PSC were isolated from brown stripe red snapper skin with yields of 9.0 and 4.7% (wet weight basis), respectively (Jongjareonrak et al., 2005). Swatschek et al. (2002) described the purification and characterization of a novel heterotrimeric collagen from cuttlefish cartilage that bears close similarity to the vertebrate cartilage minor collagens, type V and XI, as well as to the corneal collagen of the same animal. Some studies on collagen reveal that collagen represents the chief structural protein accounting for approximately 30% of all vertebrate body protein. The major impediment in the dissociation of collagen type I from tissue is the presence of covalent cross-links between molecules.

Sadowska et al. (2003) extracted collagen from the skin of baltic cod (*Gadus morhua*) and on an average, it was found to be 21.5% (on wet weight basis) and 71.2% (on dry weight basis). From the skin of Japanese Sea bass, Chub mackerel and Bullhead shark, the yield of collagens was reported to be very high and the values were about 51.4, 49.8 and 50.1% respectively based on lyophilized

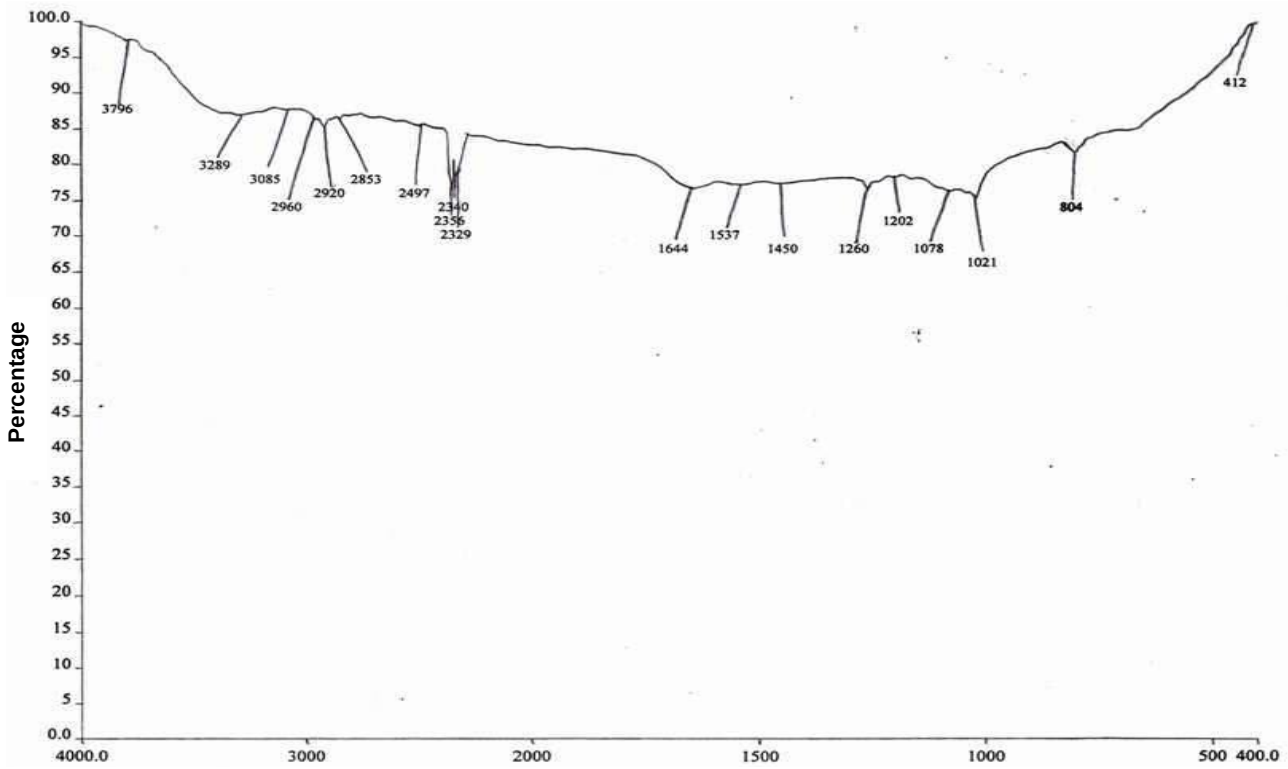

Figure 2. Showing the FT - IR spectrum of standard collagen.

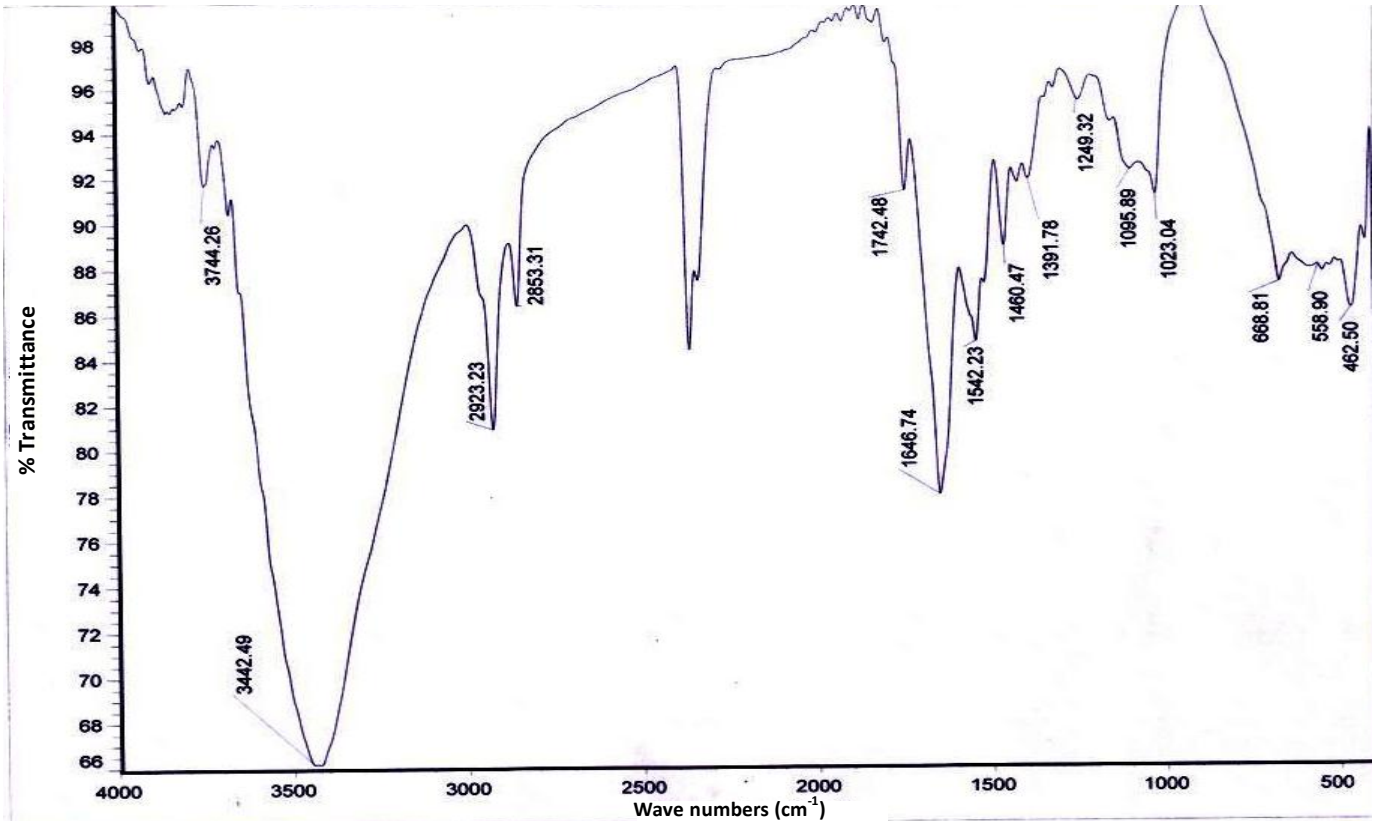

Figure 3. Showing the FT - IR spectrum of ASC of *S. inermis*.

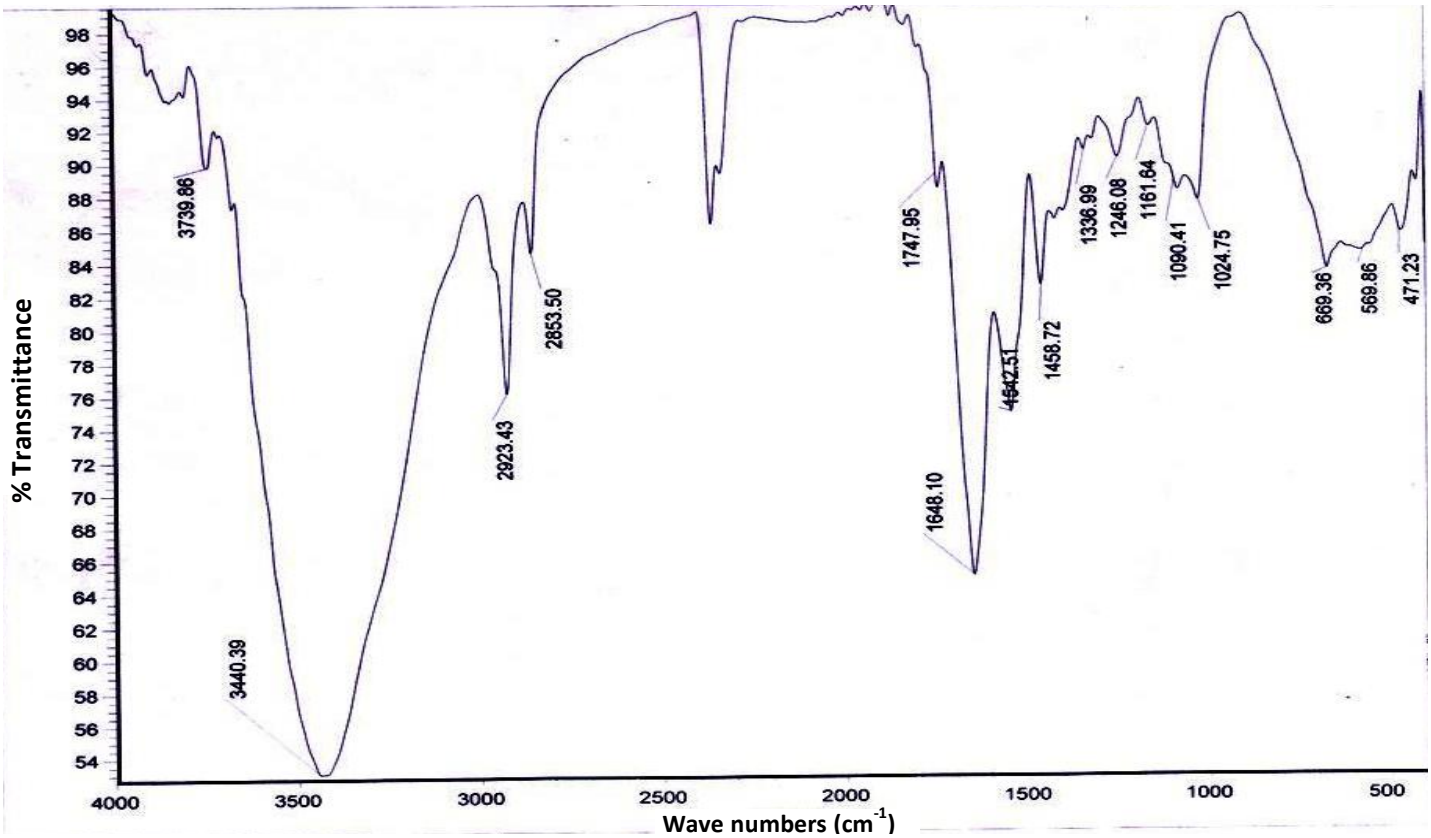

Figure 4. Showing the FT - IR spectrum of PSC of *S. inermis* UV –vis spectrum.

Table 1. Fourier transform - infra red spectral peak location and assignment for standard collagen, ASC and PSC.

Region	Standard	ASC	PSC	Assignment
Amide A	3289	3442	3440	NH Stretch coupled with hydrogen bond.
Amide B	2920	2923	2923	CH_2 asymmetrical stretch.
	2853	2853	2853	CH_2 asymmetrical stretch.
Amide I	1644	1646	1648	C=O Stretch/Hydrogen bond coupled with CN stretch.
Amide II	1537	1542	1542	NH bend coupled with CN stretch
	1450	1460	1458	CH_2 bend.
	-	-	-	COO – Symmetrical stretch.
	-	1391	1336	CH_2 wagging of Proline.
Amide III	1260	1249	1246	NH bend coupled with CN stretch.
	1078	1095	1090	C-O stretch.
	1021	1023	1024	C-O stretch.
	804	-	-	Skeletal stretch.
	-	668	669	Skeletal stretch.

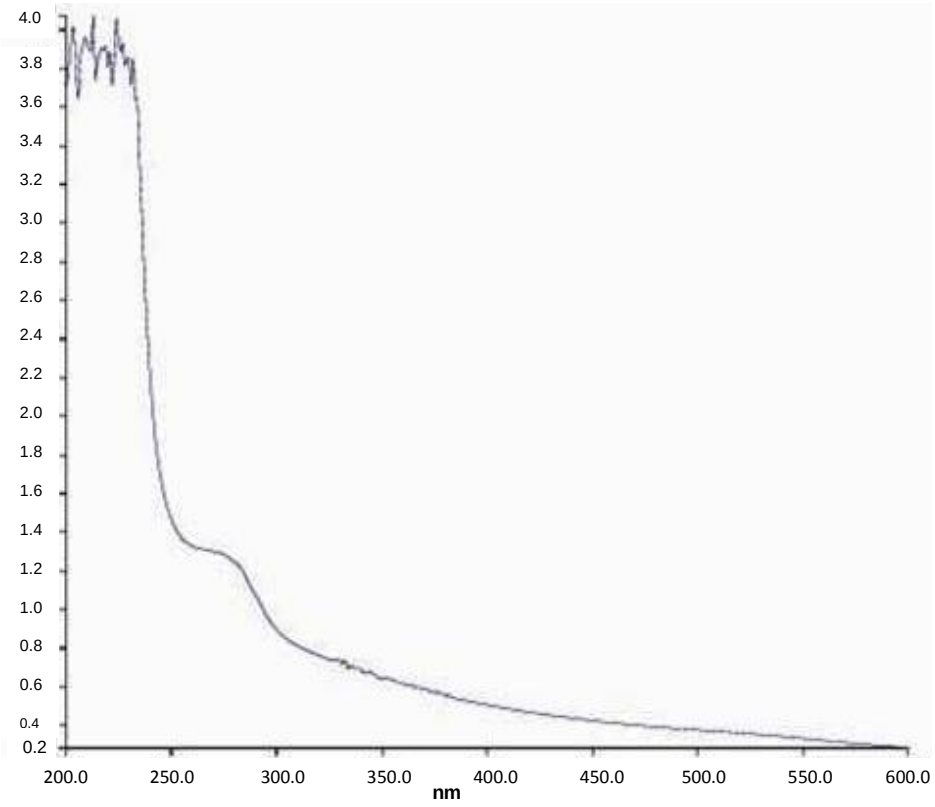

Figure 5. UV –vis spectra of ASC of *S. inermis.*

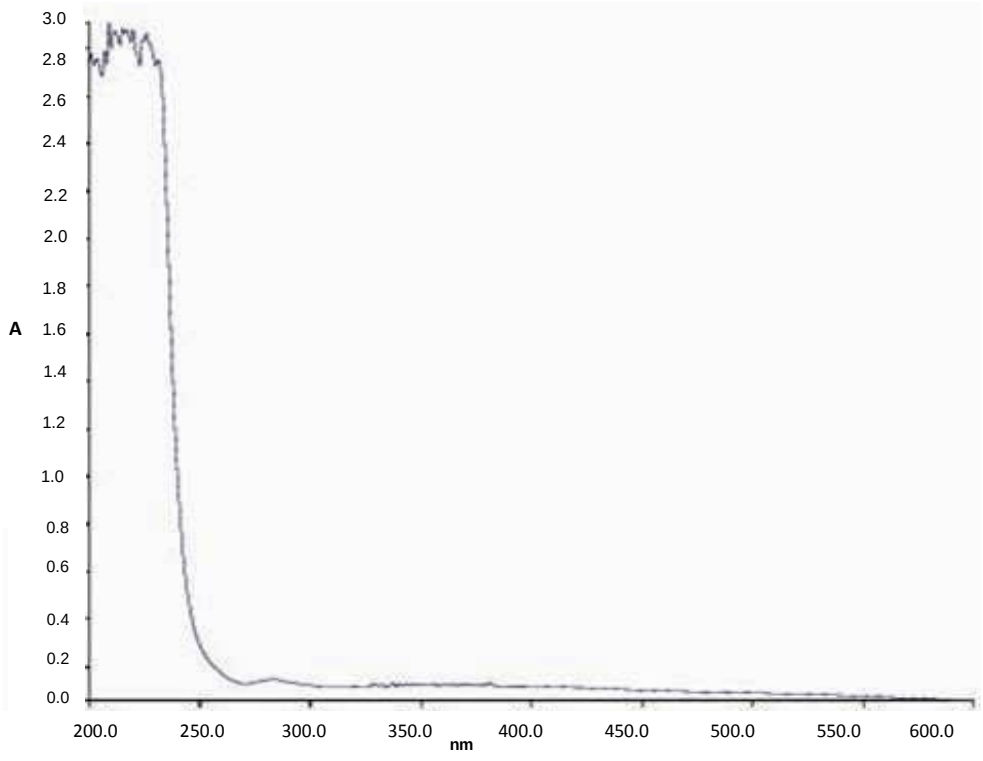

Figure 6. UV –vis spectra of PSC of *S. inermis*.

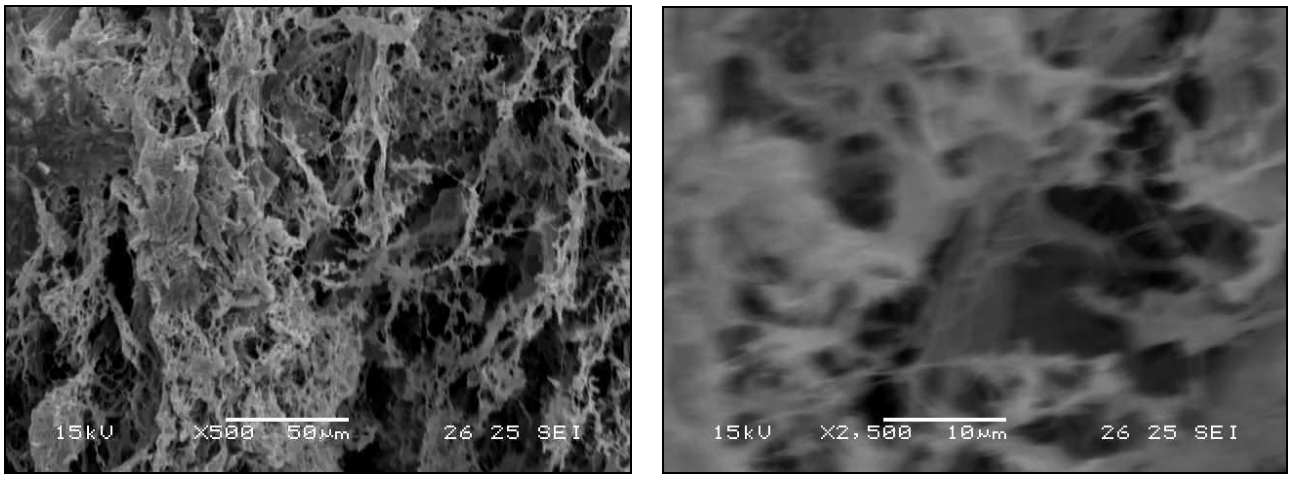

Figure 7. SEM photographs of ASC.

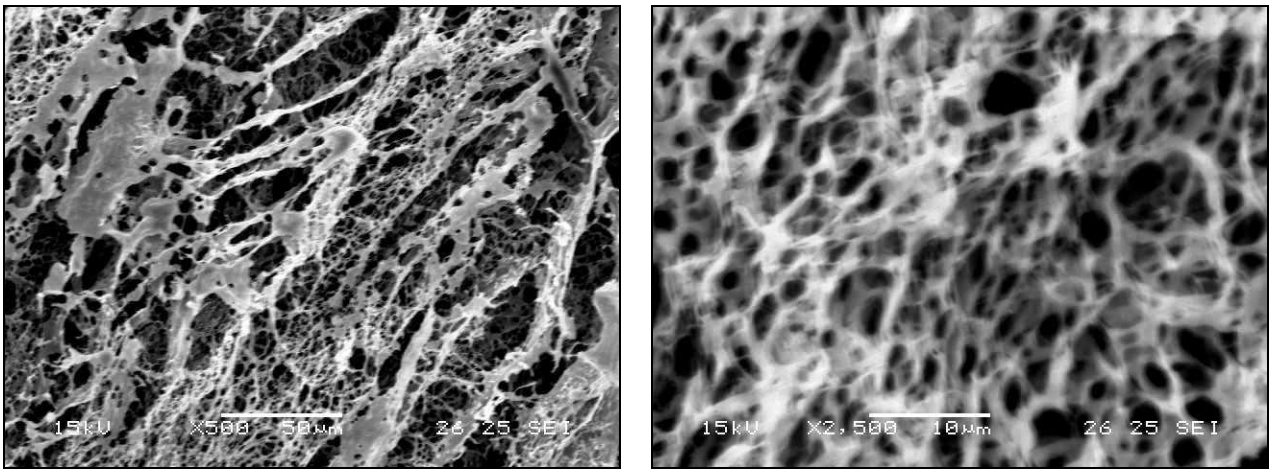

Figure 8. SEM photographs of PSC.

dry weight (Nagai and Suzuki, 2000). Senaratne et al. (2006) accounted the total collagen as 54.3% on the basis of lyophilized dry weight compared to other vertebrates. The collagen content from the skin of skate (*Raja kenojei*) was found to be estimated as 8.9 and 35.6% of wet and dry tissue, respectively (Hwang et al., 2007). Zeng et al. (2009) reported the yield of ASC from the skin of *Octopus niloticus* as 39.4% on the basis of dry weight. The yield of ASC and PSC from brown-banded bamboo shark (*Chiloscyllium punctatum*) was calculated as 9.38 and 8.86% (on wet weight basis) respectively (Kittiphattanabawon et al., 2010). Nevertheless, in the present study the yield of ASC and PSC was found to be low (0.58 and 16.23%) on dry weight basis, respectively.

While reporting the collagen content in arm and mantle of *O vulgaris*, Mizuta et al. (2003) found lesser collagen content (1.4 and 1.9%) in arm and mantle, respectively on wet weight basis) and lesser protein content (9.1 ad 14%, respectively) when compared to the present study in *S. inermis* (0.58 and 16.23%) and (20.24 and 69.56%) on dry weight basis of yield and protein in ASC and PSC, respectively). The high yield of total protein in *S. inermis* may be due to the reason that the skin contains more protein when compared to arm and mantle of *O. vulgaris* as observed Takema and Kimura (1982) in the case of the minor collagen type in the octopus arm muscle.

The minimal molecular mass to elicit hypertensive activity in collagen hydrolysates appeared to be between 900 and 1,900 Da. In particular, the molecular weight distribution, which is 1,000 Da, has been shown to be determinant (Byun and Kim, 2001). Molecular weight distribution of fish skin collagen hydrolysates ranged from 300 to 1,500 Da, and most of peptides were 1,200 Da (Jian-Xin and Zheng, 2009). In the present investigation, the molecular weight of ASC was seen lying between 67 and 86 kDa, represented by two bands in the SDS-PAGE

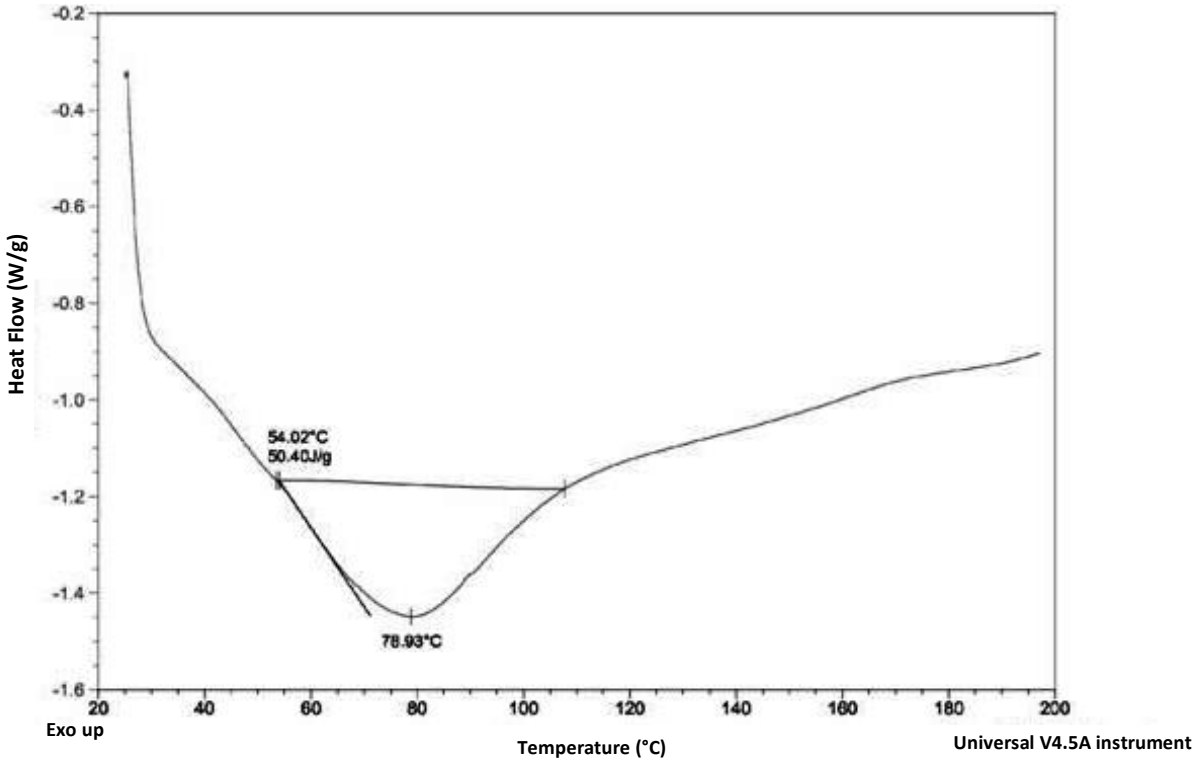

Figure 9. DSC of ASC.

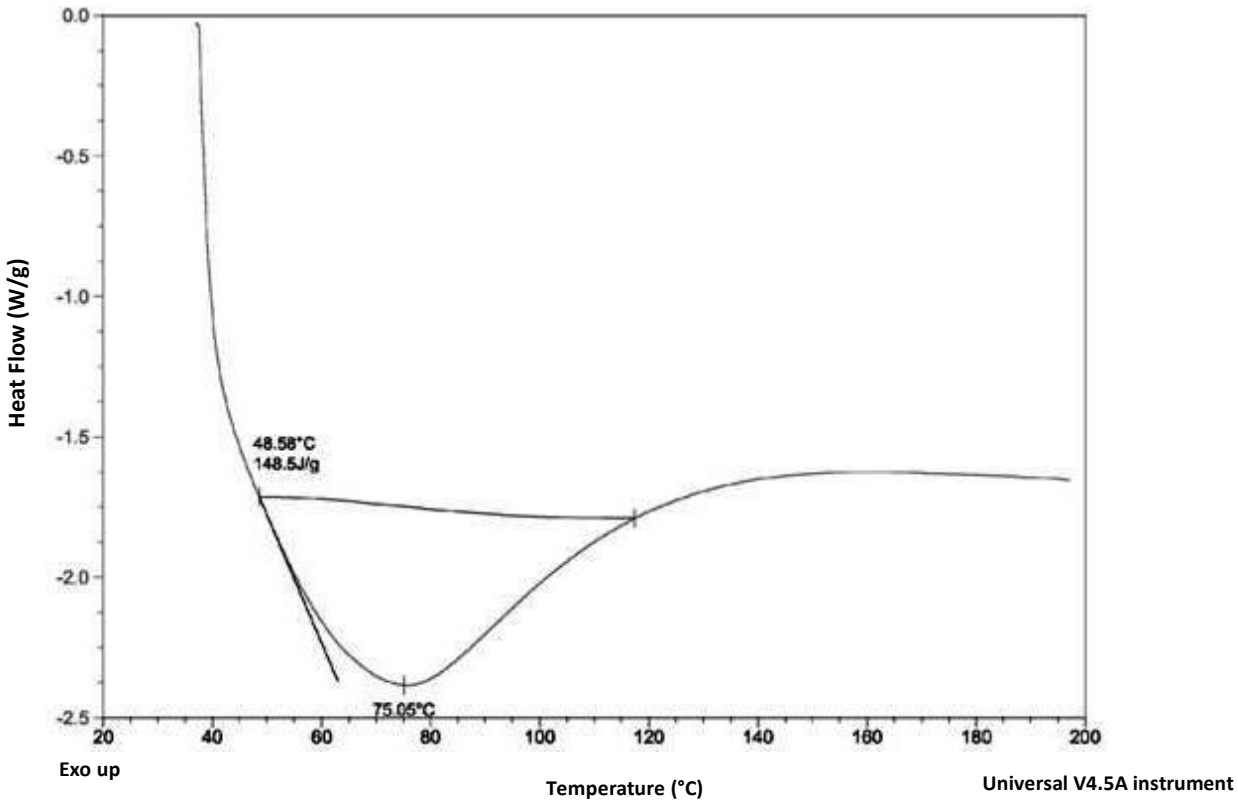

Figure 10. DSC of PSC.

gel profile and PSC was found ranging from 58 to 86 kDa represented by three bands in the SDS-PAGE gel profile.

In the present study, the ASC of *S. inermis* skin showed the characteristic peaks of amide A (3442 cm^{-1}), amide B (2923 cm^{-1}), amide I (1646 cm^{-1}), amide II (1542 cm^{-1}) amide III (1249 cm^{-1}) bands; whereas the PSC showed the characteristics peaks of amide A (3440 cm^{-1}), amide B (2923 cm^{-1}), amide I (1648 cm^{-1}), amide II (1542 cm^{-1}) and amide III (1246 cm^{-1}) bands. However, at the same time, the collagen standard showed a slight shift in the amide A (3289 cm^{-1}), Amide B (2920 cm^{-1}), amide I (1644 cm^{-1}), amide II (1537 cm^{-1}) and amide III (1260 cm^{-1}). The band patterning of PSC of *S. inermis* showed a slight shift in the amides A, B, I, II and III bands of the standard collagen. Amide A band at 3442 cm^{-1} in ASC and 3440 cm^{-1} in PSC of *S. inermis* is related to N-H stretching vibrations. Amide B band in ASC and PSC (2923 cm^{-1}) of *S. inermis* is associated with stretching of CH_2 asymmetrical stretch. Amide I (1646 cm^{-1}) in ASC and (1648 cm^{-1}) in PSC is associated with C=O stretch/hydrogen bond coupled with CN stretch. Amide II (1542 cm^{-1}) in ASC and PSC is associated with NH bend coupled with CN stretch. Amide III (1249 cm^{-1}) in ASC and (1246 cm^{-1}) in PSC is associated with NH bend coupled with CN stretch. Amide III (1320 to 1220 cm^{-1}) is related to CN stretching and NH and it is involved with the triple helical structure of collagen (Jakobsen et al., 1983; Surewicz and Mantsch 1988; Muyonga et al., 2004).

The skin collagen of young nile perch showed the amide bands of A, B, I, II and III at the wavelengths of 3434, 2924, 1650, 1542 and 1235 cm^{-1}, respectively and that of the adult nile perch skin collagen were at 3458, 2926, 1654, 1555 and 1238 cm^{-1}, respectively (Muyonga et al., 2004). Correspondingly the collagen from *S. inermis* also recorded the bands meant for amide regions of A, B, I, II, III at 3442, 2923, 1646, 1542 and 1249 cm^{-1} in ASC and 3440, 2923, 1648, 1542 and 1246 cm^{-1} in PSC, respectively.

The amide A band is associated with the N-H stretching frequency. According to Doyle et al. (1975), a free N-H stretching vibration occurs in the range of 3400 to 3440 cm^{-1}, and when the NH group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequencies. When the NH group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequencies. Amide B band of both collagens was observed at 2921 to 2925 cm^{-1}, in agreement with that reported by Nagai et al. (2001). The amide A band of skin collagen of *Sebastes mentella* was at 3425 cm^{-1}, while those of scale and bone were at 3296 and 3300 cm^{-1}, respectively, which indicate that more NH groups of scale and bone were involved in hydrogen bonding than in skin (Wang et al., 2008). Comparing to this, in the present investigation also the amide A band of ASC and PSC were located at 3442 and 3440 cm^{-1}, respectively. It also indicates the involvement of more NH groups in hydrogen bonding of ASC and PSC.

The peaks of amides I and II of PSC (1655 and 1548 cm^{-1}, respectively) were at a higher frequency than those of ASC (1644 cm^{-1}). These indicated that PSC had a higher degree of molecular order than ASC, since the shift of these peaks to higher frequencies was associated with an increase in the molecular order (Payne and Veis, 1988). It was found that the amide I band, with characteristic frequencies in the range from 1600 to 1700 cm^{-1} was mainly associated with the stretching vibrations of the carbonyl groups (C=O bond) along the polypeptide backbone (Payne and Veis, 1988) and was a sensitive marker of the peptide secondary structure (Surewicz and Mantsch, 1988).

The amide I band position was observed at 1644 and 1655 cm^{-1} (ASC and PSC, respectively), which is the absorption band of C=O stretching and is associated with the secondary structure of the protein. The absorption between the 1241 and 1240 cm^{-1} (amide III) and 1458 cm^{-1} (ASC and PSC respectively) wavelength demonstrated the existence of helical structure (Liu et al., 2007). The amide I peak underwent a decrease in absorbance, followed by a broadening accompanied by the appearance of additional shoulders when collagen was heated at higher temperature (Bryan et al., 2007). Due to the similarity in the amplitude, both collagens were most likely not denatured during the extraction. This was reconfirmed by the ratio of ~1 between amide III and 1454 cm^{-1} band of both collagens. Ratio of ~1 revealed the triple-helical structure of collagen. The amide II of both collagens appeared at 1538 to 1541 cm^{-1}, resulting from N–H bending vibration coupled with CN stretching vibration (Krimm and Bandekar, 1986). Thus, both ASC and PSC showed a similar secondary structure.

Generally, tyrosine and phenylalanine are sensitive chromophores and absorb UV light at 283 nm and 251 nm (Liu and Liu, 2006), where ASC and PSC have no evident absorbance. Therefore, the ASC and PSC from *S. inermis* skin well support the property of collagen that there is absorbance at 220 to 230 nm, with little or no absorbance near 280 nm as also observed by Yan et al. (2008) in *Theragre chalcogramma* and Shanmugam et al. (2011) in *S. pharaonis.*

The surface morphology of the collagen and the gelatin membrane is greatly different from each other. There are obvious fibril networks with a rough membranous structure for the collagen membrane. In contrast, the gelatin membrane without fibril networks appeared only as a smooth structure on the surface (Zhang et al., 2005). In the present study also, the SEM analysis of *S. inermis* collagen in low magnification shows highly porous collagen, interconnected with scaffolds and their surface is rough and uneven. At high magnification, there is a significant difference between in the microstructure of ASC and PSC; but, the collagen of PSC in high magnification appeared regular and uniform net work with porous and honey-comb like structures with a size of

pores on the surface ranging from few to ten micrometres.

In the study of thermo-physical properties of the collagen by DSC, the peak temperature of the collagen was reported to be 75.93°C for ASC and 75.05°C for PSC; but at the same time the peak temperature of the collagen membrane was about 59°C (Zhang et al., 2005). The difference may be due to the thermal behavior of collagen, which is related to the moisture content, the thermal history (that is, preparative conditions), type/species of fish and season and region of capture.

Conclusion

Cuttlefishes have thick skin, but this (skin) is treated as waste at home, in the fish shops, processing units, seafood industries and refrigerated factories. If this waste is dumped as such it would cause pollution and offensive odour. If substantial amounts of collagen could be obtained from this waste, it would provide alternatives to mammalian collagen in food, cosmetics and biomedical materials. Although there is a drift to decrease the waste in the world, the quantity produced is increasing year by year. Recently, there has been a lot of interest in investigating possible means of making more effective use of under utilized resources and industrial wastes. Hence, the collagen from the outer skin of *S. inermis* (ASC and PSC) were extracted and characterized. The result of the present study reveals the existence of helical arrangements of collagen and so it may be concluded that the skin throwing as waste from the seafood plants may become an additional and alternative possible non-conventional source for industries and pharmaceutical industries.

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