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ORIGINAL ARTICLE

# Study of chemical properties and evaluation of collagen in mantle, epidermal connective tissue and tentacle of Indian Squid, *Loligo duvauceli* Orbigny

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**Abstract** The chemical composition and evaluation of Indian squid (*Loligo duvauceli*) mantle, epidermal connective tissue and tentacle is investigated in this current study. It is observed that squid mantle contains 22.2% total protein; 63.5% of the total protein is myofibrillar protein. The unique property of squid myofibrillar protein is its water solubility. Squid mantle contains 12.0% total collagen. Epidermal connective tissue has highest amounts of total collagen (17.8%). SDS-PAGE of total collagen identified high molecular weight α-, β- and γ- sub-chains. Amino acid profile analysis indicates that mantle and tentacle contain essential amino acids. Arginine forms a major portion of mantle collagen (272.5 g/100 g N). Isoleucine, glutamic acid and lysine are other amino acids that are found in significantly high amounts in the mantle. Sulphur containing cystine is deficit in mantle collagen. Papain digest of mantle and epidermal connective tissue is rich in uronic acid, while papain digest, collagenase digest and urea digest of epidermal connective tissue has significant amounts of sialic acid (25.2, 33.2 and 99.8 μmol /100 g, respectively). PAS staining of papain digest, collagenase digest and urea digest also identify the association of hexoses with low molecular weight collagen fragments. Histochemical sectioning also emphasized the localized distribution of collagen in epidermal and dermal region and very sparse fibres traverse the myotome bundles.

**Keywords** Collagen · Squid · PSC · SDS-PAGE · Histochemistry · Skin · Mantle · Tentacle · Glycoprotein

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## Introduction

Indian squid, *Loligo duvauceli* Orbigny, is one of the most common squids along the nertic waters from Mozambique to South China Sea and Philippines Sea to Taiwan. This Indo-Pacific species is most abundant squid in Indian waters and have become increasingly important as food; and is one of the most commonly consumed cephalopod around the world (Meiyappan et al. 1993). Until recently, considerable amounts of squid were considered a delicacy in East and Southeast Asia, and in the Mediterranean countries. Today, in many countries, the consumption of cephalopods is increasing essentially as chilled and frozen ready meals (Guerra and Rocha 1994; Jeyasekaran et al. 2010). It is eaten as raw and/ or in different processed forms. A number of value-added products such as, tubes, rings, stuffed and battered products, etc., developed from squid have always been in demand among South Eastern countries. Tentacles are also quite in demand for developing value added products.

Squid is an abundant source of protein; and the advantage of this cephalopod muscle comprises of its high processing output, low fat content, fine flavour and extremely white meat. Collagen, the major fraction of connective tissue protein, is greatly responsible for its typical texture. Nineteen variants of collagen (type I to type XIX) have been reported (Bailey et al. 1998). A few proteins resemble collagen and hence, the types of collagen have always been under controversy. It is widely distributed in skin, bones, cartilages, tendons, ligaments, blood vessels, teeth, cornea and other organs of vertebrates (Senaratne et al. 2006). Collagen finds immense use in all major fields such as, food, pharmaceutical and photographic industries (Liu et al. 2011; Regenstein and Zhou 2007). It also plays a noteworthy role in cosmetic, biomedical and leather industry

(Kittiphattanabawon et al. 2005). In food industry, it is used as clarifying agent (Liu et al. 2011); to develop edible casings for sausages, salami and snack sticks, and gelatine and denatured collagen, as stabilizers and thickeners (Hassan and Mathew 1996). Scientists report that skin collagen from various species such as, brown-banded bamboo shark (Kittiphattanabawon et al. 2010); bigeye snapper (Jongjareonrak et al. 2005), Nile perch (Muyonga et al. 2004), Baltic cod (Sadowska et al. 2003) and deep-sea red fish (Wang et al. 2007) could be a promising alternative resource for collagen.

The aim of the study is to research the chemical composition and evaluate the protein fractions of highly commercialized Indian squid, *L. duvauceli* Orbigny. Moreover, very little knowledge is available on the squid collagen; and hence, the emphasis of the study is on its chemical composition and distribution that determine the physico-chemical and functional properties of squid mantle, epidermal connective tissue and tentacle.

## Materials and methods

### Sample preparation

Indian squid (*L. duvauceli* Orbigny), collected from the last haul of catch were caught along the south-west coast of India by commercial fishing boats. The selected samples measured 15–20 cms in length. These samples were wrapped in thin-film polythene bags and chilled using ice; and were brought to the laboratory within 8 h of catch. The edible portions of the squid were separated into mantle and tentacle portions. The mantle was cleaned, de-skinned and eviscerated. For the tentacles, the eyes were removed and small quantity of skin was retained. The tentacle portion is generally consumed with the skin attached. The epidermal connective tissue, carefully removed from the mantle, was collected for analysis. The proximate composition analyses were carried out in fresh samples. All analyses were done in triplicates; and at temperature not higher than 4°C.

### Proximate composition

Squid mantle were determined for moisture, protein, lipid and ash according to AOAC method (1999).

### Protein fractionation and evaluation

Protein fractionation in squid mantle was carried according to Hashimoto et al. (1979) and pepsin soluble collagen was extracted using Mizuta et al. (1994). Homogenized mantle tissue (10 g) with 0.05 M phosphate buffer (pH 7.5) was centrifuged using refrigerated centrifuge (MB-20 Superspeed Refrigerated Centrifuge) and, to the supernatant collected, trichloroacetic acid was added to precipitate out the sarcoplasmic protein fraction (SP). The original residue was homogenized with 0.6 M NaCl-phosphate buffer (pH 7.5) and supernatant obtained after centrifugation was myofibrillar protein fraction (MY). The residue was once again subjected to overnight exhaustive extraction at room temperature with 0.1 N NaOH, and alkali soluble protein fraction (ASF) was collected. Finally, the washed precipitate was measured as total collagen (TC) (Raman and Mathew 2005). The nitrogen content in the supernatants and residue collected were determined using micro-kjeldahl method (AOAC 1999).

### SDS-PAGE

Electrophoresis was performed by the method of Laemmli (1970) on a Large Vertical Model Electrophoreses unit (Genei, Bangalore, India) at 200 V, 30 mA and 15°C. Qualitative fractionation of sarcoplasmic, myofibrillar and alkaline soluble proteins were done in 10% resolving gel and pepsin soluble collagen at 7% gel strength using discontinuous Tris–HCl/glycine buffer system. Stacking gel strength was 4%. The samples were dissolved in sample buffer in 1:4 ratios and boiled for 4 min at 95°C. 10 µl of each sample were loaded in the gel. Protein bands were stained with Coomassie Brilliant Blue R-250. De-staining was performed in an aqueous solution of 5% methanol and 7.5% acetic acid and samples were stored in a solution of 10% glycerol and 7.5% acetic acid.

The standard was a broad range molecular mass calibration kit (Genei, Bangalore, India) consisting of: Myosin (205 kD), Phosphorylase b (97 kD), Bovine Serum Albumin (66 kD), Ovalalbumen (43 kD), Carbonic anhydrase (30 kD), Soyabean trypsin inhibitor (20.1 kD), Lysozyme (14.3 kD), Aprotinin (6.5 kD) and Insulin (3 kD).

### Squid mantle, epidermal connective tissue and tentacle collagen extraction and evaluation

Collagen was extracted from squid mantle, epidermal connective tissue and tentacle, according to the methodology of Kolodziejska et al. (1999). The tissue homogenized with hydrogen peroxide was centrifuged and the residue obtained was homogenized with 0.1 N sodium hydroxide for 24 h. It was finally washed with 1% hydrogen peroxide (containing 0.01 M sodium chloride) at 5: 1 ratio (residue: solution) for 48 h. The experiments were carried at 20°C.

### Amino acid profile and chemical composition of collagen

Amino acids were estimated according to the procedure of Ishida et al. (1981). Mantle, epidermal connective tissue and tentacle collagen were hydrolysed in 6 N HCl and digested

overnight at 110°C; and the hydrolysates were flash evaporated, and the residue was dissolved in citric acid buffer solution for analysis of amino acids, using Shimadzu HPLC LC-10 AS Amino Acid Profiling System (Shimadzu Corporation, Japan) using ISC-07/S/Na column (19×5 cm) packed with styrene divinyl benzene copolymerized with sulfinic group. The analysis were done with non-switching flow method and fluorescence detection after post-column derivatization of O-phthaldehyde. Tryptophan content was estimated by the methodology of Sastry and Tummuru (1985). Hydroxyproline was measured in the collagenous residue. The extracted collagen from mantle, epidermal connective tissue and skin was hydrolyzed with 6 N HCl at 105°C for 24 h (AOAC 1999). Hydroxyproline oxidized with choramine-T to pyrrole and red-purple colour developed after addition of 4-dimethylaminobenzaldehyde, was measured spectrophotometrically at 560 nm (Hitachi U2800 UV–VIS Spectrophotometer), according to Kolar (1990). For *Loligo duvauceli*, the conversion factor for hydroxyproline to collagen was 14.1 and that of nitrogen to protein was 6.25 (Sadowska and Sikorski 1987).

Acetone dry defatted powder of collagen was prepared according to Mathew et al. (1982). An aliquot was digested with papain, and the uronic acid content was estimated by the sulphuric acid-carbazole method (Bitter and Muir 1962). Another aliquot of acetone dry powder was digested using collagenase for estimating collagenase solubilized glycoprotein and glycosaminoglycans and the residue obtained was further digested with 6 M urea (Mathew and Kurup 1984). The digest was estimated for uronic acid (Bitter and Muir 1962), hexose (Dubois et al. 1956), fucose (Dische and Sheltes 1948) and sialic acid (Warren 1959). The qualitative fractionation of glycoprotein localized in collagen using SDS-PAGE in tube gel was performed at 7% resolving gel strength (Laemmli 1970). Staining was done using Periodic Acid Schiff's (PAS) reagent in 5% acetic acid at room temperature.

### Histochemistry and histological sectioning

Squid tissue measuring 2 $cm^2$ were cut into 0.5 cm blocks and fixed overnight in Bouin's fixative, followed by dehydration in serially diluted ethanol (70–96%), absolute alcohol and acetone. The samples were then embedded in paraffin wax with ceresin (congealing point 60°C) and cut into thin sections of 8 μm using rotary microtome (SIPCON SP 1120 Rotary Microtome, India). Double staining using Weigert's Haematoxylin stain and Van Geison's stain were performed. The myofibrils and collagen were stained yellow and red, respectively, which were then photographed using Nikon Eclipse E-200 compound light microscopes fitted with Nikon DN 100 Digital camera (Ando et al. 1999).

### Statistical evaluation

All results were subjected to analysis of variance (ANOVA) and are given as mean ± S.D. from triplicate determinations. Tukey's multiple comparison tests were performed to calculate the significant difference between means. Minitab software (ver. 14.0 for Windows) was used for statistical analyses.

## Results and discussion

The chemical composition and protein fractions in the mantle of *L. duvauceli* Orbigny are shown in Table 1. Suyama and Kobayashi (1980) analyzed eight species of squid and observed that moisture varied from 75–80%, crude protein 16–21% and ash from 1–2% with 1% crude fat. The chemical compositions of cephalopods are dependent on species, growth stage, habitat, season and anatomical region of the cephalopod (Kreuzer 1984; Sinanoglou and Miniadis-Meimaroglou 1998; Ozogul et al. 2008). Statistical evaluation imply significant amounts of moisture ($P<0.005$) and protein ($P<0.01$). The results are in accordance with the findings of Shimizu and Simidu (1960); Hassan and Mathew (1996) and Raman and Mathew (2005). Myofibrillar protein is significantly high among other protein fractions ($P<0.005$). Total collagen was observed to be high in squid and possibly the properties of squid mantle are influenced by its collagen content. Statistical evidences also indicate high amounts of collagen in squid mantle ($P<0.05$).

Qualitative fractionation of mantle protein fractions by SDS-PAGE showing sarcoplasmic protein, myofibillar protein, alkaline soluble protein and pepsin soluble collagen are in Fig. 1. Sarcoplasmic proteins are low molecular weight proteins (~30 kD) but possibly due to the water soluble nature of squid myofibrillar protein, it is being extracted out along with the sarcoplasmic protein fractions and additional bands appear on the top of the gel. The myofibrillar

**Table 1** Chemical composition and different protein fractions of *L. duvauceli* Orbigny

| Proximate composition (%) | Mantle tissue |
|---|---|
| Moisture | 74.6±0.30[c] |
| Fat | 1.1±0.02[a] |
| Protein | 22.2±0.12[b] |
| Ash | 1.2±0.03[a] |
| Protein fractions (% of total protein) | |
| Sarcoplasmic protein | 13.9±0.19[b] |
| Myofibrillar protein | 63.5±0.70[c] |
| Alkaline soluble protein | 13.2±0.32[b] |
| Total collagen | 12.0±0.07[a] |

Values are given as mean ± S.D. from triplicate determinations

Different superscripts indicate significant differences, a- ($P<0.05$), b- ($P<0.01$) and c- ($P<0.005$)

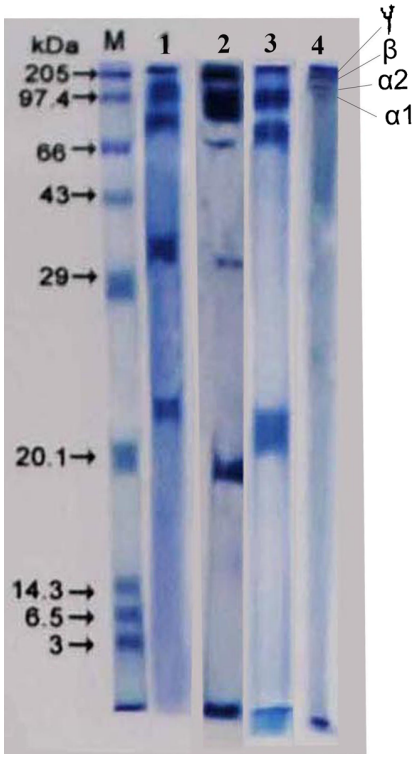


**Fig. 1** SDS-PAGE patterns of sarcoplasmic protiens (1), myofibrillar protiens (2), alkaline soluble protiens (3) and pepsin soluble collagen (4) from squid mantle (M), Broad range protien marker

protein contained myosin heavy chains (192 kD), α-actinin (97.4 kD), tropomyosin and troponin with the molecular weight closer to 29 kD. These results are in accordance with Perzanowska and Smialowska (1981). Presence of paramyosin (205 kD) is an important feature of squid myofibrillar proteins (Woods 1969). Myosin heavy chains and paramyosin because of their heavy molecular size probably did not separate into distinctive bands. Alkaline soluble proteins were extracted out by exhaustive treatment with sodium hydroxide and possibly some of the protein molecules were disintegrated and denatured forming low molecular weight fractions that got leached out. Three thick bands were observed approximately at 22 kD, 70 kD and 130 kD, respectively that could be the denatured proteins. On the top of the gel, a thick band was observed that could be due to the denatured protein that could not traverse through the gel. Total collagen in squid mantle resembles the amount of collagen found in sharks. The protein bands obtained could be classified as α-chains and β-chain, the major components, with α-chains comprising of $\alpha_1$ and $\alpha_2$ components that differ in their densities, as could be seen from difference in their mobility rates. Based on their density and mobility rates, it can be suggested that they are type I collagen. A great amount of β- and γ-chain were also observed and the results were in agreement with earlier findings (Kittiphattanabawon et al. 2005). Fu et al. (2008) reported two types of collagen, type I and type V in pepsin digested and differential salt precipitated samples of *L. japonica*. Zhang et al. (2007) reported that pepsin soluble collagen from the skin of grass carp contained $\alpha_1$-chain and $\alpha_2$-chain. Mizuta et al. (1996) suggested that at least two distinct molecular species of collagen were present in the skin, tunic and mantle muscle of the squids *L. vulgaris* and *Sepia officinalis*. From the electrophoresis pattern, it is difficult to say, if the collagen contained $\alpha_3$-chain. It could not be separated under the present conditions possibly because of its similar migration rates as $\alpha_1$-chain (Kimura 1992).

The assessment of hydroxyproline and the collagen content in mantle, epidermal connective tissue and tentacle, are shown in Table 2. Hydroxyproline and collagen content were significantly high in epidermal connective tissue ($P<0.05$) than mantle and tentacle. The mantle collagen calculated from hydroxyproline was comparable with that obtained during fractionation. The aspartic acid content observed in this experiment includes hydroxyproline as its imino group is converted to amino. The stability of collagen is dependent on the number of imino acid residues (Ikoma et al. 2003) and it plays a key role in stabilizing the triple helical structure of collagen (Ramachandran 1988). The molecular structure of collagen is preserved by the restrictions on the modifications of the secondary structure of the polypeptide chain, imposed by pyrrolidine rings of proline and hydroxyproline. The hydrogen bonding ability of the hydroxyl group of hydroxyproline also maintains it partially. Nagai et al. (2008) reported that lower the imino acid content lower denaturation temperature than their hydroxylation.

Table 3 show the amino acid profile of mantle, epidermal connective tissue and tentacle collagen. Relatively high amounts of isoleucine and glutamic acid were observed in mantle and tentacle. Arginine was also observed to be relatively high in mantle. Glutamic acid was not detected in epidermal connective tissue. Tryptophan was not detected in tissues probably because it was available in low amounts and was devoid of epidermal layer (Lapa-Guimarães et al. 2005; Fox and Vevers 1960). Ommochrome is the principle pigment material in epidermis, produced from tryptophan as starting material. Serine, glutamic acid, proline, glycine,

**Table 2** Hydroxyproline content (g/100 g) and collagen (% of total protein) in squid mantle, epidermal connective tissue and tentacle

| Tissue | Hydroxyproline | Collagen |
|---|---|---|
| Mantle | $0.14\pm0.048^{a}$ | $12.1\pm0.10^{a}$ |
| Epidermal connective tissue | $0.20\pm0.060^{b}$ | $17.8\pm0.36^{b}$ |
| Tentacle | $0.15\pm0.055^{a}$ | $12.8\pm0.21^{a}$ |

Values are given as mean ± S.D. from triplicate determinations.

Different superscripts indicate significant differences, a- ($P<0.05$) and b- ($P<0.01$).

**Table 3** Amino acid composition in squid mantle, epidermal connective tissue and tentacle collagen (g/ 100 gN)

| Amino acids | Mantle | Epidermal connective tissue | Tentacle |
|---|---|---|---|
| Aspartic acid | 42.0±0.06$^{c\ C}$ | 10.5±0.02$^{a\ B}$ | 23.3±0.14$^{b\ C}$ |
| Threonine | 17.0±0.26$^{b\ B}$ | 17.9±0.08$^{b\ C}$ | 10.0±0.13$^{a\ B}$ |
| Serine | 15.1±0.07$^{b\ B}$ | ND | 9.3±0.17$^{a\ B}$ |
| Glutamic acid | 95.3±0.36$^{b\ D}$ | ND | 46.6±0.69$^{a\ D}$ |
| Proline | 6.9±0.36$^{b\ A}$ | ND | 3.9±0.24$^{a\ A}$ |
| Glycine | 18.7±0.47$^{b\ B}$ | ND | 19.1±0.07$^{b\ C}$ |
| Alanine | 30.0±0.12$^{b\ B}$ | ND | 18.2±0.42$^{a\ C}$ |
| Cystine | ND | 20.3±0.25$^{b\ C}$ | 2.0±0.02$^{a\ A}$ |
| Valine | 20.1±0.03$^{b\ B}$ | ND | 11.0±0.08$^{a\ B}$ |
| Methionine | 34.2±0.39 | 17.8±0.09$^{a\ C}$ | 19.2±0.08$^{a\ C}$ |
| Isoleucine | 174.2±0.21$^{c\ E}$ | 34.6±0.20$^{a\ D}$ | 57.1±0.33$^{b\ E}$ |
| Leucine | 24.8±0.19$^{c\ B}$ | 10.8±0.19$^{a\ B}$ | 19.2±0.10$^{b\ C}$ |
| Tyrosine | 20.1±0.25$^{c\ B}$ | 9.7±0.14$^{a\ B}$ | 13.0±0.13$^{b\ C}$ |
| Phenylalanine | 1.0±0.03$^{a\ A}$ | ND | 1.5±0.07$^{a\ A}$ |
| Histidine | 37.0±0.30$^{c\ C}$ | 5.6±0.15$^{a\ B}$ | 22.2±0.38$^{b\ C}$ |
| Lysine | 80.7±0.32$^{b\ D}$ | 42.2±0.29$^{a\ D}$ | 41.1±0.33$^{a\ D}$ |
| Arginine | 272.5±0.47$^{c\ E}$ | 0.50±0.06$^{a\ A}$ | 1.2±0.07$^{b\ A}$ |
| Tryptophan | ND | ND | ND |

Values are given as mean ± S.D. from triplicate determinations.

Different lowercase superscripts indicate significant differences between the samples (a- $P<0.05$, b-$P<0.01$, c-$P<0.005$, d- $P<0.001$, e-$P<0.0005$).

Different uppercase superscripts indicate significant differences between amino acids (A- $P<0.05$, B- $P<0.01$, C-$P<0.005$, D- $P<0.001$, E-$P<0.0005$).

*ND* Not detected

alanine, valine and phenylalanine were not detected in epidermal connective tissue. Proline in the mantle was observed to be around 7% and in tentacle 4% while epidermis was found to be devoid of proline. Quite a good quantity of serine and threonine were observed in all the samples. All eighteen amino acids were observed to be present in tentacle. Mantle lacked cystine but it was present in epidermis and tentacle. Mantle and tentacle were found to contain essential amino acids. Statistical analysis of the amino acids between samples and within a sample shows significant variation. Statistical observation within a sample showed that mantle contained significantly high amounts of arginine and isoleucine ($P<0.0005$) followed by glutamic acid and lysine ($P<0.001$). Lysine and isoleucine were observed to significantly high in epidermal connective tissue ($P<0.001$). And tentacle was observed to contain statistically significant amounts of isoleucine ($P<0.0005$) followed by glutamic acid and lysine ($P<0.001$). Statistical evaluation indicated that tentacle and mantle collagen were significantly rich in amino acids compared to epidermal connective tissue. Arginine that was significantly rich in

**Table 4** Uronic acid, sialic acid and total hexose in the mantle, epidermal connective tissue and tentacle

| Digest collected | Uronic acid (%) | | | Total hexose (%) | | | Fucose (%) | | | Sialic acid (μmol / 100 g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mantle | Epidermal connective tissue | Tentacle | Mantle | Epidermal connective tissue | Tentacle | Mantle | Epidermal connective tissue | Tentacle | Mantle | Epidermal connective tissue | Tentacle |
| Papain | 0.09±0.009$^{c}$ | 0.10±0.010$^{c}$ | 0.05±0.001$^{b}$ | 3.4±0.05$^{b}$ | 3.0±0.12$^{b}$ | 15.4±0.05$^{d}$ | ND | ND | ND | 14.1±0.04$^{b}$ | 25.2±0.04$^{c}$ | 14.3±0.22$^{b}$ |
| Collagenase | 0.04±0.001$^{a}$ | 0.06±0.001$^{b}$ | 0.05±0.001$^{b}$ | 0.6±0.060$^{a}$ | 8.1±0.16$^{c}$ | 1.0±0.059$^{a}$ | ND | ND | ND | 12.2±0.06$^{b}$ | 33.2±0.05$^{c}$ | 8.3±0.24$^{b}$ |
| Urea | 0.05±0.001$^{b}$ | 0.05±0.003$^{b}$ | 0.04±0.002$^{a}$ | 0.4±0.010$^{a}$ | 0.58±0.030$^{a}$ | 1.3±0.16$^{a}$ | ND | ND | ND | 5.0±0.61$^{a}$ | 99.8±0.37$^{d}$ | 25.2±0.30$^{c}$ |

Values are given as mean ± S.D. from triplicate determinations.

Different lowercase superscripts indicate significant differences (a- $P<0.05$, b-$P<0.01$, c-$P<0.005$, d- $P<0.001$)

*ND* Not detected.

mantle was less significant in epidermal connective tissue and tentacle ($P<0.05$).

Uronic acid, total hexose, fucose and sialic acid content in mantle, epidermal connective tissue and tentacle are shown in Table 4. Uronic acid content in the papain digest of mantle and epidermal connective tissue showed a similar trend while it was slightly lower in tentacle; and was statistically significant ($P<0.005$). The amount of total hexoses and uronic acid in squid epidermal connective tissue were in accordance with earlier results (Moczar and Moczar 1976).

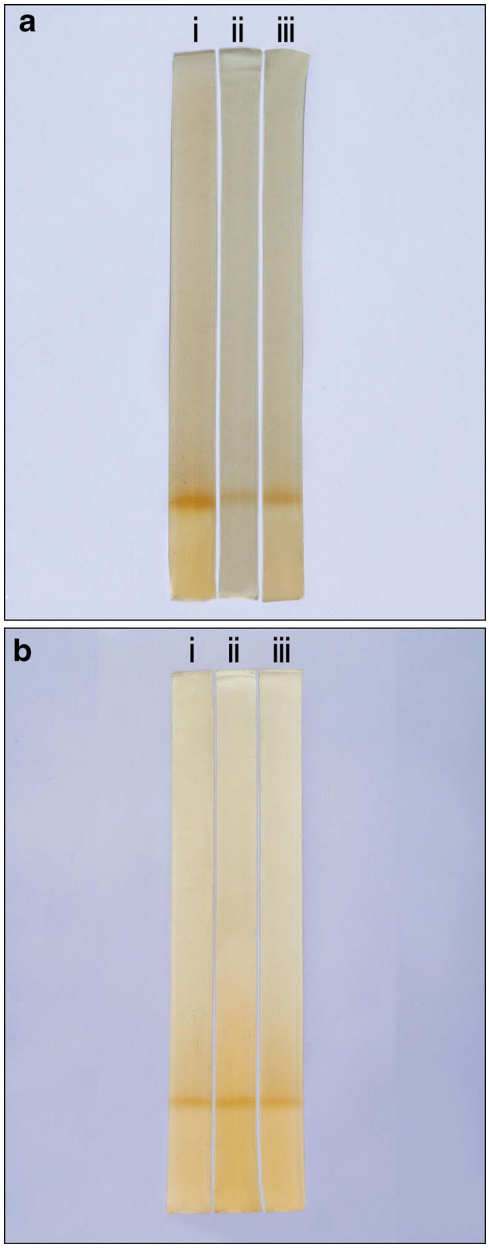


**Fig. 2** Tube gels showing glycoprotiens stained with PAS. (**a**) Papain digest, (**b**) Collagenase digest (*i*) Epidermal connective tissue, (*ii*) tentacle and (*iii*) mantle

Fucose was not detected in the samples. Uronic acid was primarily in the form of glucuronic acid. Urea digest of epidermal connective tissue ($P<0.001$) showed significantly higher sialic acid (99.8 μmol/ 100 g) content than papain and collagenase digest ($P<0.005$). Compared to mantle and tentacle, sialic acid was comparatively high in epidermal connective tissue. Urea digest of tentacle also showed significant amounts of sialic acid ($P<0.005$). Total hexoses were high in papain digest of tentacle (15.4 %) followed by collagenase digest of epidermal connective tissue (8.1%) and papain digest of mantle (3.4%). Ri et al. (2007) reported a greater amount of uronic acid than squid samples in the major and minor fractions of scallop mantle collagen. The results indicate the localized distribution of glycoproteins as

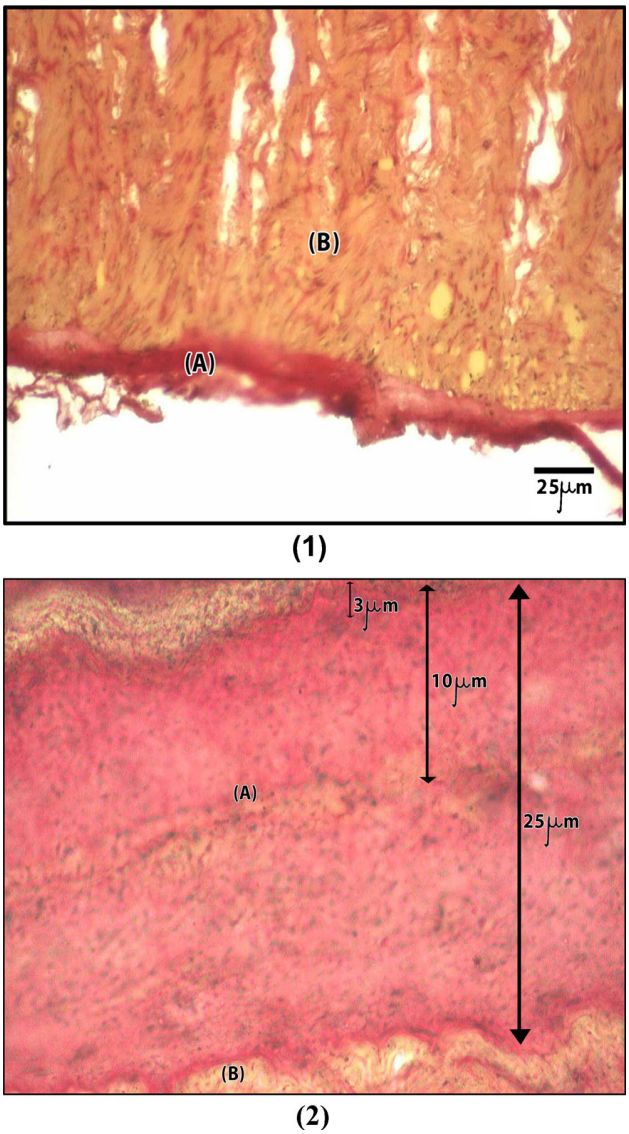


**Fig. 3** Histography of fresh sample of squid, *Loligo duvaucelli* showing collagen structure (**a**)-Collagen, (**b**)- Myofibrillar protein. Longitudinal section of (1) mantle with dermal layer (2) dermal collagen

part of the collagen structure, probably in the central core of the helical structure of collagen as they were easily extractable using collagenase enzymes. There are a few reports about the distribution of glycoproteins and glycosaminoglycans in different tissues in teleosts. Presence of sialic acid is an indication of glycoproteins. Glycoprotein and glycosaminoglycan might be an integral part of squid tissue and these tissues could be used as potential source for isolating these bioactive compounds. Suzuki et al. (1968) demonstrated that the squid cartilage is unique in producing chondroitin sulfate containing acetylgalactosamine 4, 6-disulfate residue. As glycosaminoglycans are hypolipidemic agents, these tissue could be used as readily available resources for isolating these compounds. Moreover, these will be an excellent means of utilizing unutilized resources such as squid epidermis. Figure 2 shows the PAS stained papain and collagenase digested glycoproteins from mantle, epidermis connective tissue and mantle confirming that papain digest and collagenase digest contained glycoproteins. The sugars might be attached to low molecular weight proteins. The glycoprotein fraction recovered from papain-digest and collagenase digest had similar electrophoretic pattern. The polyacrylamide gel electrophoretic pattern of the glycoprotein fractions of the three tissues exhibited differences in the band mobility. This is in agreement with the reports on charged heterogeneity for the glycoproteins interacting with collagen (IUPAC 1986). This charged heterogeneity could be due to difference in sialic acid (N-acetylneuraminic acid) content of the glycans as reported earlier (Page 1971). It is observed that to have high sugar content indicate the presence of glycoproteins that are of great significance in medicines.

Histological section of squid mantle that highlights the structural proteins such as myofibrillar protein and collagen in mantle section and tunics are shown in Fig. 3. Section (1) indicates that the greatest quantity of collagen is restricted to the outer sheath (dermal and epidermal region); and some sparse fibres traversed the muscles that are arranged in a network-like fashion. This cross-linking and particular dispersion might explain the solubility of squid collagen by enzyme-acid solution. Section (2) implies that tunic (25 μm) is a dense mass of collagen. The outer tunic of the skin tissue could be divided into three layers, namely epidermal connective tissue, chromatophore layer and dermal connective tissue; a layer of dermal connective tissue was also observed at the inner tunic of the skin tissue. These unique arrangements of collagen fibres play a significant role in demarcating the squid texture from other fishery products.

Squid mantle contains a good amount of collagen especially localized to dermal region. The dermal region is also rich in uronic acid, sialic acid and hexoses, making it a good source of glycosaminoglycans. Histochemical analysis also emphasizes the dense localization of collagen in epidermal connective tissue.

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