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Nutritional composition of liver (Digestive gland) from thondi squid (*Sepioteuthis lessoniana*)

Meivelu Moovendhan[1,2]*, Annian Shanmugam[1], & Vairamani Shanmugam

[1]Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India.

[2]Bioengineering and Drug Design Laboratory, Department of Biotechnology, Bhupat Jyoti Mehta School of Biosciences, Indian Institute of Technology, Madras, Chennai, Tamil Nadu, India.

*[E-mail: moovendhan85@gmail.com]

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The present study was undertaken to investigate the nutritional composition such as protein, lipid, carbohydrate, and ash of *Sepioteuthis lessoniana* liver. The liver was collected from the fish landing centre and the proximate composition total protein, carbohydrate and lipid content were found to be 31.16 ± 0.28%, 3.9 ± 0.05% and 21.06 ± 0.11%, respectively. The moisture and ash content of the *S. lessoniana* liver were recorded as 52.1 ± 0.17 % and 8.03 ± 0.05 %, respectively. The heavy metal content was estimated by acid digestion method and was found to be as follows: Cd-0.020 ppm, Cu-2.723 ppm, Mn-1.121 ppm, Ni-0.109 ppm, Pb-0.190 ppm, and Zn - 0.522 ppm. The present study concluded that the *S. lessoniana* liver (non-edible part) is suitable for human consumption, and is good for aquaculture and poultry feed formulations.

[**Keywords:** Proximate composition; Liver; Squid; Heavy metals.]

Introduction

Cephalopods are considered delicacy in foreign markets and its high quality is the norm especially in international trade. It is a remarkable feature that almost all the cephalopods processed are exported. Cephalopods have good nutritive value with the amino acid content almost similar to fish[1]. About 70% of the cephalopod body is edible (mantle, arms, tentacles and fin) and as such most importantly used as human food. In India, the entire catch is exported mostly in the frozen form with very little being consumed in the domestic market. The viscera of squids is a good poultry feed, and a good source of manure and is served as fishmeal.

Cephalopods don't have a well developed liver, called "Liver-cum-Digestive gland" or "hepatopancreas"[2]. The liver (digestive gland) attracts extraordinary consideration, since the role of the liver in the total metabolism of squid (as well as molluscs in general) seems to be specifically essential. The cephalopod liver differs from other tissues by having more amounts of basic biochemical substances (Protein, lipids and glycogen)[3]. Several reports were found regarding the biochemical composition and weight of the squid liver: *S. oualaniensis* liver reported 1.5-7.5% times more dry weight, 2 times additional glycogen, 1.2-1.4% times more protein and 3.6-7.5% times more lipid, than muscular tissues[2]. The weight of the liver in *S. oualaniensis* is more than 4% of total body mass[4,5] and is discarded during processing. The cephalopods store and utilize the lipid as a source of energy. The current discrimination for cephalopods is that lipids can't be digested rapidly. However, in cephalopods more lipids are constantly present in the digestive gland involved in digestion and storage[3]. Hence in the present study, it was planned to study the proximate composition such as protein, lipid, carbohydrate, moisture and ash content apart from heavy metals and elements such as carbon, hydrogen and nitrogen in the liver (digestive gland) of squid, *S. lessoniana*.

Materials and Methods

Collection and processing of liver sample

Squid liver was collected from fresh specimens of *S. lessoniana*, at the processing plant "Nila Sea Foods", Thondi (Lat. 9° 44' N; Long. 079° 02' E) (Ramanathapuram), Tamil Nadu, India. They were brought to the laboratory in ice. After thoroughly washed in distilled water to remove the ink and adhered particles, they were dried in incubator at 35 °C and used for evaluation of physico-chemical and biochemical studies. All the biochemical and analysis of heavy metals was done in triplicate and the standard deviation was calculated.

Estimation of total protein

The protein content of *S. lessoniana* liver was estimated by following the method described by Lowry [6] using Bovine Serum Albumin (BSA) as a standard.

Estimation of total lipid

The total lipid content of *S. lessoniana* liver was quantified gravimetrically after extracting the lipid using chloroform: methanol (2:1) folch[7].

Estimation of carbohydrate

The total carbohydrate content of *S. lessoniana* liver was estimated calorimetrically by the phenol-sulfuric acid method described by Dubois[8]. D-Glucose was used as a standard.

Estimation of moisture

The moisture content of *S. lessoniana* liver was analyzed as described in AOAC[9]. The percentage of moisture content was calculated using the formula,

$$\text{Percentage of moisture} = \frac{\text{Wet weight of liver}}{\text{Dry weight of liver}} \times 100$$

Estimation of ash content

The ash content was determined by burning the pre-weighed sample (2g of liver (dry weight)) in a muffle furnace (Subra Scientific, Pondicherry) at 560 ^{o}C (AOAC, 1995)[10] for 5 hr. The final residue was weighed and the percentage was calculated using the formula,

$$\text{Percentage of Ash} = \frac{\text{Dry weight of liver}}{\text{Amount of Ash}} \times 100$$

Heavy metal analysis

Three grams of dried and powdered liver sample was taken in 100 ml teflon vials and digested overnight with 7 ml of pure nitric acid and 3 ml of hydrogen peroxide on a hot plate (High Performance Microwave Labstation, Milestone, USA). The digested content was transferred to acid-washed polypropylene bottle and made up to 25 ml with double distilled water and subjected to various metal analyses in inductively coupled plasma mass spectrometry (ICP) (Perkin-Elmer Analyst 300) following the AOAC method AOAC[9].

C, H & N analysis

The composition of carbon, hydrogen and nitrogen in *S. lessoniana* liver was done in the Perklin Elmer 2400 series II elemental analyzer.

Results

Proximate composition

The proximate composition (total protein, lipid, carbohydrate, moisture and ash content) of *S. lessoniana* liver is given in Table 1. The average total protein, carbohydrate and lipid content were found to be 31.16 ± 0.28%, 3.9 ± 0.05% and 21.06 ± 0.11%, respectively. The moisture and ash content of the *S. lessoniana* liver were recorded as 52.1 ± 0.17 % and 8.03 ± 0.05 %, respectively.

Heavy metal content

The content of the heavy metals such as cadmium, copper, manganese, nickel, lead and zinc of *S. lessoniana* liver is presented in Figure 1. The amount of heavy metals reported in the liver tissue is as follows: Cd-0.020 ppm, Cu-2.723 ppm, Mn-1.121 ppm, Ni-0.109 ppm, Pb-0.190 ppm and Zn-0.522 ppm.

C, H & N analysis

The percentage of carbon, hydrogen and nitrogen present in *S. lessoniana* liver is given in Table 2.

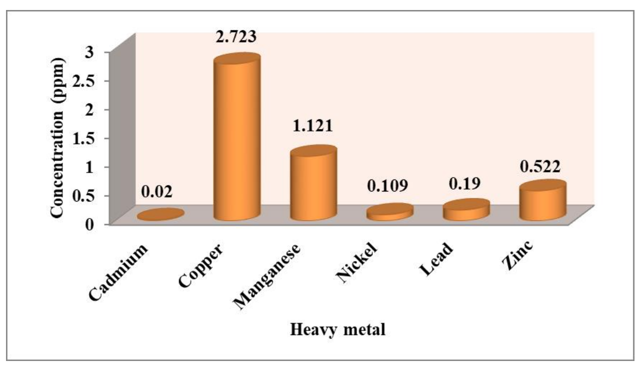

Fig. 1 — Heavy metal content in *S. lessoniana* liver

Table 1 — Proximate composition of *S. lessoniana* liver

Sample	Total Protein (%)	Total Carbohydrate (%)	Total Lipid (%)	Moisture (%)	Ash (%)
S. lessoniana liver	31.16 ± 0.28	3.9 ± 0.05	21.06 ± 0.11	52.1 ± 0.17	8.03 ± 0.05

Table 2— Percentage of carbon, hydrogen and nitrogen in the *S. lessoniana* liver

Sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)
S. lessoniana liver	32.68 ± 0.03	11.06 ± 0.11	5.10 ± 0.005

Discussion

According to Anon[11], the cephalopod production was 1,12,762 tonnes and the waste was calculated as 50,742 tonnes equal to 45% of the total production. Squids represent a major fishery resource widely distributed throughout the oceans of the world[12] representing 73% of the cephalopod world catches. According to the Ministry of Agriculture, Government of India, the total cephalopod catches (particularly squids and cuttlefishes in Tamil Nadu) during 2007-2012 were as follows: 2007-11,646 and 9,581 tonnes; 2008–11,813 and 6,116 tonnes; 2009–12,561 and 6,819 tonnes; 2010–12,781 and 6,923 tonnes; 2011–12,835 and 6,572 tonnes; and 2012–14,388 and 10,616 tonnes, respectively (Fisheries Statistics, 2014).

Especially in cephalopods, the edible parts such as mantle, arms, tentacles and fins contribute 60-80% and non-edible visceral parts contribute 15-25% among which the digestive gland (liver) shares 15% (of the viscera)[13]. The four major biochemical constituents of both the edible and non-edible portions in cephalopods are: Water, proteins, lipids and ash (minerals), which are referred as proximate composition[14]. Different factors like environmental parameters such as temperature, salinity, pressure and availability of food etc. have profound influence on the biochemical composition[15].

The nutritive value of food is determined by the proportions in which essential amino acids are present in the composition of total amino acids in proteins[16]. Krzynowek and Murphy[26] in an NOAA report, pointed out the protein content of the squid *Nototodarus sloanii* as 19.57% in mantle, 16.7% in fins, 19.7% in arms and tentacles, 19.2% in whole edible portion and 18.5% in whole squid tissue and in *Sepioteuthis bilineata* as 19.4% in mantle, 14.7% in fins, 15.4% in arms and tentacles, 19.1% in whole edible portion, and 17.6% in entire squid. Okuzumi and Fujii[17] reported the crude protein content in the jumbo flying squid (*Dosidicus gigas*) as 11.0% in mantle, 11.6% in fins, 7.3% in liver and 11.6% in arms in large-sized animals. Vairamani[18] found out the total protein content of 35% in head, 36.36% in mantle and tentacles and 45.35% in arms in *S. inermis*. Murthy[19] found out the crude protein content as 3.15% in squid and 63.88% in fish processing waste (Viscera).

Sugiyama[20] quantified the total protein content of 20% in the squid (*T. pacificus*) viscera. The protein content of 16.91, 18.6, 14.83 and 12.21% was estimated in the arms (viscera) of *S. officinalis, L. vulgaris, O. vulgaris* and *E. Moschata,* respectively from Mediterranean Sea[21]. Likewise, Sambasivam[22] estimated the total protein content of 45.85-62.67% in arm, 54.50-62.96% in mantle, 17.45-18.77% in digestive gland, and 53.98-74.56% in gonad in different size groups of octopus (*Octopus dollfusi*). In all the above findings, in edible and non-edible parts of different species of cephalopods, the total protein content was found varying from 6.20 to 63.88%. Whereas, in the present study, the protein content of the squid *S. lessoniana* liver was found to be 31.16 ± 0.28%. Suresh[23] documented the protein content of the squid liver as 47.5% and also pointed out the cost of the squid liver powder as Rs. 26,000/tonne in India for the preparation of feed in aquaculture. Similarly, Salim Uddin[24] analyzed the proximate composition of raw and deoiled squid viscera and mentioned the protein content as 45.76 and 71.12%, respectively. On comparison, the *S. lessoniana* liver (non-edible part) showed moderate range of protein (31.16 ± 0.28%), and so the liver can also be utilized as protein supplement wherever possible.

Generally, the carbohydrate content of the fishery products is low and does not represent as a main source of food for human consumption. The glucide content of the fishery products is around 1% for fish, 1% for crustaceans and 1 to 8% for shellfish in general[25]. In the present study, the carbohydrate content of the squid *S. lessoniana* liver was estimated as 3.9%. Sambasivam[22] estimated the carbohydrate content to be 2.24-3.13 % in arm, 2.37-3.38% in mantle, 1.76-1.84% in digestive gland and 1.26-1.48% in gonad in different size groups of octopus *O. dollfusi*. Krzynowek and Murphy[26], in an NOAA report, documented the different levels of carbohydrate content in edible and non-edible parts (Viscera) such as mantle, fins, arms and tentacles and whole body of the squid *N. sloanii* as 0.20, 0.19, 1.4 and 0.23, respectively. Vairamani[18] reported the carbohydrate level of squid *S. inermis* as 3.31% in tentacles and 5.63% in mantle. Similarly, Jayalakshmi[16] quantified the carbohydrate content of the non-edible parts of the cuttlefish *S. pharonis* as 15.56% in head, 23.42% in arm, 24.29% in tentacles and 21.92% in mantle. Trivedi and Sarvaiya[27] estimated the carbohydrate content of squid, *L. duvauceli* and found it as 0.05% of wet tissue; whereas Dious[28] quantified 0.20 to 0.45% of carbohydrate content in *S. inermis*. But, the cod liver showed only 1% of carbohydrate content (National Food Institute, 2009). Krzynowek and Murphy[26] reported the absence of carbohydrate in Bluefish (*Pomotomas sallatrix).* Most of the studies reported the least amount of carbohydrate content in the viscera. But, the present study reported comparatively more carbohydrate content in *S. lessoniana* liver. From the forgoing account, it could be concluded that carbohydrate content differs from species to species, and different body parts and stages of growth.

Dious[28] reported the lipid content of 5.5% to 11.7% in *S. inermis* from Parangipettai; whereas Vairamani[8] recorded it as 6.88% in the same species from Thondi. In Rainbow trout *Oncorhynchus mykiss* liver, the total lipid content was reported to be varying month-wise and was found to be 22.3 ± 1.13% in the 18 month-old fish[29]. Sasaki and Ohori[30] reported the lipid content of 41.8% in the squid *G. borealis,* 37.3% in *M. robusta* liver, 47.4% in the liver of *T. pacificus* and 54.1% in *O. bartrani* liver. Sambasivam[22] estimated the total lipid content in the non-edible parts such as arm, mantle, digestive gland and gonad of different size groups of *O. dollfusi* as 6.46–9.03 %, 8.66–10.61%, 35.27–42.614% and 8.82–11.26%, respectively. In the present investigation, the total lipid content of the *S. lessoniana* liver (non-edible part) was recorded as 21%. When compared to the above studies, it is seen that a good amount of lipid is present in *S. lessoniana* liver and further it is also observed that the total lipid content of the edible and non-edible parts of cephalopods differ in terms of size and influence of parameters even in the same species itself[18, 22].

Generally, moisture and lipid content in fish fillets are inversely related and their sum is approximately 80%[31]. In most of the previous studies, the moisture content was estimated in edible and non-edible parts (arms, tentacles and ovary) of different species of cephalopods, i.e., 79% and 80.7% in fins and 76.9% and 80.9% in arms and tentacles in *Nototodarns sloanii* and *Sepioleulhis bilineala,* respectively[26]; 81.02, 78.51, 83.41 and 84.64% in the mantle of *S. officinalis*, *L. vulgaris, O. vulgaris* and *E. Moschata,* respectively[21]; 87, 87 and 82% in the mantle of *Illex illecebrosus*, *Loligo opalescens* and *Loligo pealei,* respectively[26] ; 68-80% in edible part of *L. duvauceli*[32]; 64.8 to 76% in ovary of *I. argentines*; and 79.55% in mantle of *S. officinalis*[33]. At the same time, 52.5% and 40.5% of moisture was reported in the liver of the shark *C. sorrah*[34] and squid[35], respectively. From the above, it could be noticed that the moisture content in edible parts was found more, irrespective of species than that of the liver including the present study where the moisture content in *S. lessoniana* liver was calculated as 52.1 ± 0.17%.

Ash is the remaining inorganic residue, after the removal of water and organic matter by heating in the presence of oxidizing agents, that is equal to the total amount of minerals within a food[36]. Anto'nio[37] reported the ash content of *S. officianalis* mantle tissue (fresh and ice stored) as 1.39 ± 0.03% and 0.52 ± 0.01%, respectively. Ozogul[21] quantified the ash content of different cephalopods such as *S. officianalis, L. Vulgaris O. vulgaris* and *E. moschata* as 1.12, 1.49, 1.17 and 1.35%, respectively. Whereas Shakir[37] documented the ash content of the five squid species such as *L. duvauceli, D. gigas, S. inermis, S. pharonis* and *S. prashadi* as 1.31, 1.42, 1.59, 1.75 and 1.44%, respectively. In another study, the liver of ray fishes such as *D. pastinaca, D. violacea* and *R. marginata* recorded the ash content of 0.45 ± 0.03%, 0.51 ± 0.01% and 0.89 ± 0.04%, respectively. In cod liver the ash content was found to be 6%[38]. But, in the present study, 8.1% of ash was recorded which is quite higher than that of all the studies mentioned above, since liver is said to be the storage organ.

Generally all metals are toxic in nature if present in high concentration and lethal even at very low concentrations too. Some trace metals play a vital role in the biological process, but when at higher level, they may be toxic to the biota also[39]. High concentrations of toxic heavy metals, especially cadmium and lead in squids (*Loligo sp.*) and squid products were frequently observed in India and Thailand. Miramand and Bentley[40], in *Eledone cirrhosa* and *Sepia officinalis,* reported that the digestive gland accumulated more than 80% of silver, cadmium, cobalt, copper, iron, lead, and zinc. In the present study, results of the heavy metal concentration of *S. lessoniana* liver reported the order of: Cu (2.723) > Mn (1.121) > Zn (0.522) > Pb (0.190) > Ni (0.109) > Cd (0.020). Whereas, Murthy[19] reported cadmium, nickel, lead, copper and mercury in commercial squid meal as 2.54-14.11, 1.11-5.61, 0.46-1.77, and 28.55-67.55 ppm, not detected, respectively. On comparison, the concentration of heavy metals in *S. lessoniana* liver is found very less.

Tavakoli and Yoshida[41] estimated the carbon, hydrogen and nitrogen content in the squid (*T. pacificus*) waste as 58.86, 9.625 and 8.84%, respectively. Whereas, Clarke[42] reported lower levels of carbon, hydrogen and nitrogen in the male and female digestive glands of *Illex argentines,* i.e., carbon content as 5.9% in male and 43.3% in female, nitrogen content as 5.9% in male and 12.2% in female. In the present study, the element (C, H and N) content of *S. lessoniana* liver showed 32.71, 11.00 and 5.10%. When compared to the above findings, the *S. lessoniana* liver showed higher carbon content than any other elements.

Conclusion

With respect to all the above, it is clear that *S. lessoniana* liver (non-edible part) is suitable for human consumption and is good for aquaculture and poultry feed formulations. Further, the high content of HUFA and good cholesterol makes it much suitable for oil extraction for human consumption.

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