



## Research Article

## STUDIES ON THE LIPID COMPOSITION OF LIVER (DIGESTIVE GLAND) OIL OF CUTTLEFISH

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Article Received on: 25/02/14 Revised on: 31/03/14 Approved for publication: 09/04/14

DOI: 10.7897/2230-8407.050465

## ABSTRACT

In the present investigation, the hooded cuttlefish *Sepia prashadi* were collected from Mudasalodai landing centre, east coast of Tamil Nadu, India. Collected animals were dissected for their liver (digestive gland) and the dried liver was ground well and the cuttlefish liver oil was extracted. The percentage of oil yield was noted as 32.45 %. The extracted liver oil was used for estimating total lipids, triglycerides, total cholesterol, High Density Lipoproteins cholesterol (HDL), Low Density Lipoproteins cholesterol (LDL), Very Low Density Lipoproteins cholesterol (VLDL) and free fatty acids. The fatty acid composition of the liver oil such as Saturated Fatty Acids (SFA), Mono Unsaturated Fatty acids (MUFA) and Poly Unsaturated Fatty acids (PUFA) was analyzed using GC- MIS. The results of the present study revealed that the cuttlefish (*S. prashadi*) liver oil was found to contain more MUFA and also contain considerable amount of triglycerides and cholesterol. The cuttlefish oil can be used as a bio enrichment of live feeds and the residual waste during oil production can be used as manure, poultry feed, bio diesel production, etc. Moreover production of oil from the animal wastes such as liver is an eco-friendly process and can reduce the environment pollution. The quality of oil can be improved to increase the PUFA content for human consumption. Apart from all the above advantages, cuttlefish oil can be used as an alternative to fish oil, as it is very easy and cheap to produce than the fish oils.

**Keywords:** Cephalopod, *Sepia prashadi*, Digestive Gland, Total Lipids, Triglycerides, Total Cholesterol, Free fatty acids.

## INTRODUCTION

The marine environment comprises complex ecosystem and many of the organisms are known to possess bioactive components as a common means of self-defense or for the protection of eggs and embryos from the predators. Some organisms derive chemistry from the dietary source, while others synthesize the compounds de novo<sup>1</sup>. The phylum mollusca is considered to be large assemblage of animals having diverse shapes, sizes, habits and occupy different habitats. Marine molluscs have received more attention because of their aesthetic and gastronomic appeals<sup>2</sup>. Among them cephalopods were given much attention, for organized exploitation of molluscan resources from Indian waters till recently<sup>3</sup>. Cephalopods are exclusively marine molluscs and there are about 660 species in the world oceans, which are diverse in form, size and nature<sup>4-7</sup>. Of these less than a hundred species are of commercial importance. Cuttlefishes, squids and octopods are the three major groups of cephalopods which belong to the highly evolved class Cephalopoda. There are about 80 species of cephalopods of commercial and scientific interest distributed in the Indian Seas<sup>8-10</sup>. They are regarded as key species in many marine ecosystems<sup>11,12</sup>. They represent an essential link in marine trophic chains and are eaten by many marine top predators, fish, birds and mammals<sup>13-16</sup>. Traditionally, viscera of cephalopods have been considered as waste and have been utilized only to a minor extent<sup>17</sup>. Nevertheless, cuttlefish viscera represent an important part of the cuttlefish mass (15–25 %), and thus their waste represents an important commercial loss. Moreover, the digestive gland, which corresponds to 15 % of viscera, contains about 10 % of lipids rich in poly-unsaturated fatty acids<sup>18</sup>. Despite evidence of limited lipid metabolism in cephalopods, lipid storage has been reported for the digestive gland, the only cephalopod organ consisting of more than a few percent lipids<sup>19-21</sup>. The

digestive gland in cephalopods is one of the few organs that consistently exhibit high levels of lipid<sup>22,23</sup>. Hence, the digestive gland of *Sepia prashadi* was chosen for the lipid analysis. In many marine animals, lipid deposits are used during reproductive maturation by providing an energy source for the production of vitellogenic oocytes<sup>24</sup> and as an energy source during periods of starvation<sup>25</sup>. They have a diverse range of biological functions in cell membranes as phospholipids, and as a major source of stored energy in adipose tissue as triacylglycerols. Triacylglycerols, phospholipids, cholesterol and their component fatty acids, transported between sites of synthesis in the liver and intestine to peripheral tissues, for utilization and storage in macromolecular complexes of lipid and protein called lipoproteins. Fish and fish products play an important role in human's life. Fish lipids are excellent sources of the essential polyunsaturated fatty acids (PUFAs) in both the omega-3 and omega-6 families of fatty acids. Omega-6 PUFAs are also derived from vegetable oil, whereas long chain omega-3 PUFAs, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) derives mainly from fish<sup>26</sup>. The fish oils constitute an important source of omega-3 polyunsaturated fatty acids (PUFA), mainly the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA). The omega-3 PUFA provides several benefits to the human health which is essential for the development and function of certain organs and for several biochemical and physiological responses of the organism<sup>27</sup>. Over the past two decades, an increasing interest in the health benefits of fish oil consumption has emerged<sup>28</sup>. In recent years, the significance of polyunsaturated fatty acids analysis has gained much attention because of their various biological activities in health and disease, especially the ω-3 and ω-6 fatty acids. These fatty acids play an important role in the prevention and treatment of cardiovascular diseases,

autoimmune diseases, eye sight and the improvement of learning ability<sup>29</sup>. DHA take part in the brain development and retina formation of the child during pregnancy<sup>30</sup>. Premature children fed with enriched DHA formulae reach visual sharpness faster than those with deficiencies<sup>31</sup>. The ω-3 PUFAs have shown to be useful in the treatment of mental disorders like schizophrenia<sup>32</sup> and in the combat against cancer<sup>33</sup>. Recent research has implicated dietary fish oils in the reduction of eicosanoids formed from n-6 polyunsaturated fatty acids (PUFAs) and amelioration of chronic diseases such as coronary heart disease, atherosclerosis and inflammation. A range of anti-inflammatory and immunomodulatory effects of the n-3 family of PUFAs, particularly those found in fish oils have been identified. Further, consumption of PUFAs has been reported to lower blood pressure<sup>34</sup>. Fatty acids have been found to differ from mollusk species and are influenced by taxonomic relations and environmental conditions, and also depend on nutrient habits and food availability and also physiological conditions. Marine organisms are a rich source of structurally novel and biologically active metabolites. So far chemically unique compounds of marine origin with different biological activity have been isolated and a number of them are under investigation<sup>35-38</sup>. Thus in order to isolate the bioactive compounds from marine organisms; it is necessary to do a biochemical analysis, which stands as a reason for the present study. The main aim and objectives of the present investigation was (i) Extraction of oil from the liver of *S. prashadi* (ii) estimation of the total lipid and triglyceride content in the liver oil. (iii) Determination of Total cholesterol, High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and Very Low Density Lipoproteins (VLDL) in the liver oil and (iv) determination of Free fatty acids and fatty acid profile in the liver oil.

## MATERIALS AND METHODS

### Study Area

In the present study, the hooked cephalopod *S. prashadi* were collected from the Mudasalodai landing centre along Parangipettai coast (Southeast coast of India, Tamil Nadu, India) located along the east coast, near Parangipettai (Lat. 11°29'N; Long. 79°46' E) which lies in between the mouth of the Vellar estuary and Killai backwaters (Figure 1). The five villages involved in fishing activities are bringing the catches to this landing centre.

### Dissection of liver from *S. prashadi*

The animals collected from Mudasalodai were brought to the laboratory in an ice box. The animals were identified by using the publications of<sup>39,40</sup> and they were dissected to remove the liver (digestive gland). The liver were cut into small pieces and blotted with the filter paper, to remove the water adhered on to it. Then they were dried in hot air oven at 60°C for overnight. The dried material was ground in a pestle and mortar to fine powder and used for further analysis.

### Liver oil extraction

2 g of powdered liver was extracted with methanol: chloroform (2:1) as described by<sup>41</sup> for overnight at 10°C. Then the sample was centrifuged at 2000 rpm for 20 minutes. After centrifugation the supernatant was concentrated in a rotary evaporator under reduced pressure. Concentrated extract was stored in dark brown bottle at -20°C for further use.

### Estimation of total lipid

Total lipid was estimated by following the methodology of<sup>42</sup>. To 400 mg of sample in a test tube, 5 ml of chloroform: methanol (2:1) mixture was added and incubated overnight at room temperature. After incubation, the mixture was filtered using Whatmann No.1 filter paper. The filtrate was collected in a 10 ml pre-weighed beaker which was then kept on a hot plate to evaporate the chloroform: methanol mixture completely. The beaker with the dried residue was weighed and the percentage of total lipid content was calculated.

### Extraction of lipid

Lipid was extracted from the liver of *S. prashadi* using chloroform-methanol mixture (CHCl<sub>3</sub>: CH<sub>3</sub>OH) (2:1 v/v) and quantified gravimetrically<sup>42</sup>. The dried lipid, thus extracted was dissolved in 5 ml of CHCl<sub>3</sub>: CH<sub>3</sub>OH mixture (2:1 v/v) and transferred into a centrifuge tube. 2 ml of 0.1M potassium chloride was added, shaken well and centrifuged. The upper aqueous layer containing gangliosides was discarded. Then CHCl<sub>3</sub>- CH<sub>3</sub>OH – potassium chloride mixture (1:10:10 v/v) was added to the precipitate and centrifuged. This washing was repeated thrice and each time, the upper layer was discarded. The lower layer was made up to 5 ml and used for the estimation of triglycerides, total cholesterol, High Density Lipoproteins, Low Density Lipoproteins, Very Low Density Lipoproteins, free fatty acids, and fatty acid profile.

### Estimation of Triglycerides

The estimation was done using GPO kit by the following procedure. 1 ml of enzyme reagent was added to 10 µl of sample. The mixture was shaken well and incubated at 37°C for 10 minutes. The OD reading was taken at 546 nm using a spectrophotometer (UV-1800, SHIMADZU).

$$\text{Concentration of Triglycerides (mg/dl)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times 200$$

### Estimation of cholesterol

The estimation was done using GPO kit by the following procedure. 1 ml of enzyme reagent was added to 10 µl of sample. The mixture was shaken well and incubated at 47°C for 5 minutes. The OD reading was noted at 505 nm using a spectrophotometer (UV-1800, SHIMADZU).

$$\text{Concentration of Cholesterol (mg/dl)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times 200$$

### Estimation of HDL cholesterol

The estimation was done using GPO kit by the following procedure. 1 ml of enzyme reagent was added to 10 µl of sample. The mixture was shaken well and incubated at 37°C for 5 minutes. The OD reading was noted at 505 nm using a spectrophotometer (UV-1800, SHIMADZU). LDL and VLDL content in the sample was calculated by using the values of total Cholesterol and HDL cholesterol content of the sample.

$$\text{Concentration of HDL Cholesterol (mg/dl)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times 100 \times 1.1$$

### Estimation of free fatty acids

Free fatty acids in the liver oil were estimated by the method of<sup>43</sup>. Free fatty acids were extracted with chloroform-heptane-methanol mixture to eliminate interference from

phospholipids and the extract was shaken with a high density copper reagent at pH 8.1. The copper soaps remained in the upper organic layer from which an aliquot was removed and copper content was determined calorimetrically by treating with diphenyl carbazide. The free fatty acids were expressed as mg/dl or mg/g wet tissue.

### Estimation of fatty acids

#### Preparation of fatty acid methyl esters (FAME)

The fatty acids in the extracted liver oil of *S. prashadi* were analyzed by using the GC- MIS. The extracted oil was esterified by following the method of<sup>44</sup>. The solvent content in the oil sample was evaporated. About 5 ml of BF<sub>3</sub> methanol was added to the oil and maintained in boiling water bath for 5 minutes. The mixture was cooled and about 6 ml of saturated NaCl solution was added. The above contents were transferred to a separating funnel and extracted three times with petroleum ether. The extract was washed with water for 3 times. The final extract was filtered through anhydrous Na<sub>2</sub>SO<sub>4</sub> and allowed to evaporate. Then the sample was made up to 1 ml with petroleum ether. The resulting sample was used for the analysis of fatty acid profile.

### RESULTS

In the present study, oil was extracted from the liver (digestive gland) of cuttlefish, *S. prashadi*. The extracted oil was used further for the analysis of Total lipids, Triglycerides, Total Cholesterol, HDL Cholesterol, LDL Cholesterol, VLDL Cholesterol and Free Fatty acids apart from the fatty acid profile and the results are as follows:

#### Yield of oil

The yield of oil extracted from the liver of *S. prashadi* was found to be 32.45 %.

#### Lipid composition of liver oil

The total lipid content in the extracted oil of *S. prashadi* was found to be 2.31 mg/g. Similarly triglycerides estimated in the extracted oil were reported as 256.81mg/dl. The total cholesterol level in the oil was found to be 212.85 mg/dl. HDL, LDL and VLDL cholesterol level in the oil were calculated as 54.10 mg/dl, 107.38 mg /dl and 51.36 mg/dl respectively. The free fatty acid content of *S. prashadi* liver oil was determined as 12.73 mg/dl. The fatty acid profile of the liver oil is given in Table 1.

### DISCUSSION

In the present study, oil was extracted from the liver (digestive gland) of the cuttlefish *S. prashadi* and its lipid content, triglycerides, total cholesterol, HDL, LDL, VLDL, free fatty acids and fatty acid profile were determined. Marine environment possess the vast depot of lipids. The varying lipid content stored by marine organisms is also considered to reflect differing requirements for energy storage during times of reduced food availability.<sup>45,46</sup> reported that the total lipid content in some squid species such as *Slosarczykovia circumantarctica*, as 11.0 % and *Todarodes filippovae* 8.8– 10.1 % (w/w). Saify ZS et al (2000) found that the lipid content in the liver of Hammer-headed Shark *Eusphyra blochii* (66.19 %) was higher than that of shark *Carcharhinus bleekeri* (39.94 %).<sup>47</sup> Similarly,<sup>48</sup> reported that oil extracted from the liver of ray fish *Himantura bleekeri* confirmed that the liver is relatively rich in lipids which implies 54 % of the fresh matter containing lipids. In

accordance with previous studies, lipid content of other elasmobranch livers was ranging from 23 to 67 %<sup>49, 50-53</sup> reported that the lipid content of liver of *Dasyatis brevis* and *Gymnura marmorata* as 50.80 % and 51.50 % of the total lipids respectively. The lipid content of seawater species ranged from 1.01 ± 0.12 -12.4 ± 0.45 % for blue fish to sea bream<sup>54,55</sup> demonstrated that the total lipid content was about 32 mg/g (w/v) in wild sea bass and 37.5 mg/g in farmed sea bass. The total lipid content of *S. prashadi* liver (2.3075 mg/g) was lower than that of the squid from Macquarie Island (26.8 ± 12.9 %) and Heard Island as (41.7 ± 8.5 %); whereas several squid species represented 62-84 % of the total lipids<sup>22,54</sup>. The total lipid content in squids and cuttlefish varies by 1-2 % on wet weight basis<sup>56</sup>. Triglycerides are the main component of lipid depository in animal and vegetal cells. Dietary fats are stored as triglycerides in the body by attaching fats to a sugar molecule which is an important part of the cholesterol profile. However, when levels of triglyceride in the blood become too high, this can be a reason for heart disease in humans. Triglycerides have a close relationship with HDL cholesterol, and many of the factors that affect lowering HDL also affect the elevation of triglycerides.<sup>57</sup> found that the mantle of *Eledone moschata* (octopus) was rich in proportion of triglycerides (33 % of total lipids), which were found to contain a substantial proportion of n-3 polyunsaturated fatty acids. In contrast, *Sepia officinalis* and *Todarodes sagittatus* (squid) were found to contain low or minimal amounts of triglycerides (10 % and 1.4 % of total lipids, respectively) and only trace amounts of n-3 poly unsaturated fatty acids. The triglyceride content of liver oil of *S. prashadi* was 256.81 mg/dl. The amount of triglycerides in oil of fish *Dasyatis brevis* and *Gymnura marmorata* were calculated as 759 and 744 mg/g of oil<sup>53</sup>. The lungfish, a lean fish yields high liver oil content (above 50 %) and the fat is in the form of triacylglycerol (50-80 %) in the liver. Cod (*Gadiformes* order) liver oil contains 60–70 % of triglycerides<sup>58,59</sup> and the above results are very much similar to that of the present study where triglycerides level is more in all cases. Thus it is clear that the liver oil of *S. prashadi* contains maximum triglyceride content as in other fishes. Francois Pouletier de la Salle, (1769) was the very first to identify LDL cholesterol in solid form in gallstones. But, in 1815, chemist Eugene Chevreul named the compound as “cholesterine”. Marine invertebrate meals and oils (i.e., squid, clam, crab) are all excellent sources of cholesterol<sup>60</sup>. Rosa R et al (2007) reported highest cholesterol content of 1.61 ± 0.01 % in the digestive gland of *Todarodes sagittatus*, the lowest, in *Loligo vulgaris* (0.42 ± 0.02 %), followed by *Architeuthis sp.* (0.60 ± 0.02 %).<sup>61</sup> Whatever it may be the form (free or esterified), the cholesterol was predominant and the other compounds were present in very low concentrations which is confirmed through the previous study on the liver oil of *Dasyatis bleekeri* where cholesterol was found at the level of 68 %; while 22-dehydrocholesterol, campesterol and sitosterol were detected in low amounts.<sup>49</sup> Sunarya et al (1996) reported the cholesterol content of dogfish (*Squalus acanthias*) as 1.15 g/ 100 g of oil. These results were supported by<sup>63</sup> where the liver oil of dogfish in Japan contains 1.03 % of sterols (which is 90 % cholesterol).<sup>62</sup> Shen C et al (2007) found that the total cholesterol in cuttlefish (*Sepiella meindroni*) oil was about 1.39 mg/100 g of oil, much lower than that in fish muscle<sup>64</sup>. Similarly,<sup>65</sup> reported the total cholesterol content as 196.7 mg/100 g in cuttlefish (*Loligo duvancelii*), 365.0 mg/100 g in cod, 267.1 mg/100 g in sole and 294.9 mg/100 g in hake.

Table 1: Fatty acid profile of the cuttlefish *S. prashadi*

Fatty acid	Common name	%
C10:0	Caproic or Decyclic acid	0.06
C12:0	Lauric acid	0.15
C13:0	Tridecyclic acid	0.20
C14:0	Myristic acid	6.91
C15:0	Pentadecyclic acid	2.80
C17:0	Margaric acid	5.32
C19:0	Nonadecyclic acid	1.46
C20:0	Arachidic acid	1.79
<b>Σ Of SFAs</b>		<b>18.69</b>
C14:1 $\omega$ -5	Myristoleic acid	0.04
C16:1 $\omega$ -5	Palmitoleic acid	0.29
C16:1 $\omega$ -7	Palmitoleic acid	8.92
C16:1 $\omega$ -9	Palmitoleic acid	0.53
C17:1 $\omega$ -8	Cendic acid	1.55
C18:1 $\omega$ -5	Oleic acid	0.37
C18:1 $\omega$ -7	Vaccenic acid	6.98
C18:1 $\omega$ -9	Oleic acid	12.84
C19:1 $\omega$ -9	Nonadecanoic acid	0.71
C20:1 $\omega$ -9	Eicosenoic acid	8.31
<b>Σ Of MUFAs</b>		<b>40.54</b>
C18:2 $\omega$ -6	Linoleic acid	2.02
C18:3 $\omega$ -6	Rumelenic acid	1.46
<b>Σ Of PUFAs</b>		<b>3.48</b>
C13:1	-	0.05
C14:0 Iso	-	0.15
C14:0 Iso 3OH	-	0.05
C15:0 Iso	-	0.79
C15:0 Anteiso	-	0.31
C15:1 Iso G	-	0.08
C16:0 Iso	-	0.67
C16:0 Anteiso	-	0.16
C16:0 N alcohol	-	0.76
C16:0 10 methyl	-	0.43
C16:1 $\omega$ -7 alcohol	-	0.11
C17:0 Iso	-	1.34
C17:0 cyclo	-	0.49
C17:0 Anteiso	-	0.69
C17:1 Iso	-	3.18
C18:0 Iso	-	0.92
C18:1 Iso H	-	2.73
C18:1 2 OH	-	0.25
TBSA 10 Me 18:0	-	0.19
11 methyl C18:1 $\omega$ -7	-	0.48
C19:1 Iso I	-	17.83
C20:0 Iso	-	0.40
<b>Σ Of Branched</b>		<b>31.17</b>
<b>Unknown and Others</b>		<b>5.23</b>
<b>Total</b>		<b>100</b>

The result of the present study is also comparable with that of the total cholesterol content in *S. prashadi* (212.81 mg/dl). High-density lipoprotein, or HDL, is known as the "good" cholesterol. The results regarding the HDL cholesterol concentration of liver oil from *S. prashadi* was 54.10 mg/dl. LDL Cholesterol is the main sterol, a combination of steroid and alcohol, synthesized by animals, but little quantities are also produced in plants and fungi. LDL cholesterol is often called "bad" cholesterol. Of the various lipoproteins, LDL particles have the highest proportion of cholesterol and transport approximately 70 % of plasma total cholesterol. The LDL and VLDL content in *S. prashadi* was noted as 107.38 mg/dl and 51.36 mg/dl respectively. The FFA value is one of the most important factors to check the lipid quality. The lower FFA content showed higher quality and lower further oxidation. The maximum limit for FFA content was reported as 7 % in fish oil by<sup>66</sup>. But the lipid samples from *Euthynnus affinis* liver exceeded this limit. The FFA content of *E. affinis* waste lipids was 7.28  $\pm$  0.03 %. In comparison between different parts of *E. affinis* waste lipid, intestine and liver oil

has the highest FFA content than head oil<sup>67</sup>. According to results of Phillips KL *et al* (2001), FFA were moderately high, comprising of 11.7  $\pm$  6.9 % of total lipids in Macquarie Island squid and 5.4  $\pm$  1.2 % in Heard Island squid<sup>22</sup>. Similarly high FFA concentrations in the digestive gland have been reported for several other squid species and the lantern shark; *Etmopterus granulosus* contained 8 % of free fatty acids<sup>68-71</sup>. Fatty acids are "carboxylic acids (or "organic acid"), often with long aliphatic tails (long chains), either saturated or unsaturated.<sup>72,73</sup> reported that the seafood contains n-3 poly unsaturated fatty acids (PUFA) mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Human nutritionists have focused their attention on the numerous health benefits of maintaining sufficient levels of long-chain PUFAs in our diets<sup>74,75</sup> found that SFA was the most dominant fatty acid (45.8 %) in Aji-Aji fish (*Seriola nigrofasciata*). High level of MUFA (84 %) was obtained in the liver oil of *Centroscymnus plunketi*, but the SFA content was between 11 -26 % and PUFA content of 1- 13 %.<sup>76</sup> These results were similar to the results obtained in the present study animal *S. prashadi*, were the MUFA content was much higher (40.54 %) than the other values such as SFA (18.69 %) and PUFA (3.48 %). Nichols PD *et al* (1998) noted that the high concentrations of DHA found in some fish and invertebrate oils e.g. Gould's squid (*Notodorus gouldi*) 28 %, shrimp *Acanthephyra* sp. 24 % and spook fish *Winteria telescope* 20 % are as high as some of the oils currently marketed as sources of this fatty acid<sup>77</sup>. Pethybridge H *et al* (2010) found that MUFA content was dominated in fishes and squid (MUFAs 43  $\pm$  11 %, and 40  $\pm$  10 %, respectively), whereas crustaceans recorded higher polyunsaturated fatty acid (PUFA, mean 40  $\pm$  13 %). Only the wary fish, *Scopelosaurus* sp., had higher SFA level (37.5 %). Species particularly rich in MUFAs included pencil smelt, *Nansenias* p. (61  $\pm$  3 %); viper fish, *Chauliodus sloani* (57 %); rudder fish, *Tubbia tasmanica* (56 %); and red bait, *Emmelichthys nitidus* (55 %)<sup>46</sup>. Pethybridge H *et al* (2010) compared the eicosapentaenoic acid (EPA) content in cephalopods with the results of<sup>78</sup> and found that the cephalopods were rich in (6-15 %) and relatively low in arachidonic acid, C20:4 $\omega$ -6 (1-3%)<sup>46,79</sup> found that the percentage composition of fatty acids in the lipid classes of wing head shark's (*Eusphyra blochii*) liver and found that the SFA ranged from 56 % to 70.12 %. Palmitic acid was predominant and its composition ranged from 36.63 % to 46.97 % while stearic acid ranged from 9.34 % to 17.49 %. Chedoloh R *et al* (2011) noted that marine fishes such as *Megala spiscordyla* (hard tails cad) and *Parastromateus niger* (black pomfret) had more than 67 % of unsaturated fatty acids. Among unsaturated fatty acids, monoenoic was the major fatty acid in *E. blochii*. Oleic acid ranged from 11.10 to 26.45 %. The dienoic and trienoic were minor constituents. Polyunsaturated fatty acids (PUFA) ranged from 4.25 % to 15.21 % in which EPA was present from 0.41 % to 1.65 % and DHA ranged from 0.24 % to 3.07 %.<sup>80</sup> Ozogul Y (2012) reported that the major fatty acids found in *Sepia officinalis* were palmitic acid (C16:0), stearic acid (C18:0), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3).<sup>82</sup> found that in 15 Brazilian fishes, the n-3 PUFA fatty acid content was about 43.0- 45.0 % of the total fatty acids, mainly DHA.<sup>81</sup> The SFA, MUFA and PUFA percentage of *Sufflamrn capistratus* liver oil was found to be 36.32 %, 40.61 % and 23.07 % respectively<sup>83,84</sup> determined the PUFA in salmon shark (*Lamna ditropis*) as 40- 45 % of the total fatty acid composition and MUFA and SFA content

as 35-40 % and 20 % respectively. The myristic acid (C14:0) was found to be in highest concentration (6.91 %) in the SFA of cuttlefish *S. prashadi* liver oil. Heptadecanoic acid (C17:0) and Pentadecanoic acid (C15:0) (2.80 %) came next in their concentration. These results were very similar to the results presented by<sup>64</sup> in cuttlefish *S. meindroni* liver oil. The same results were obtained in most common marine fish oils<sup>52,85</sup>. Unsaturated fatty acids such as MUFAs were predominant in the liver oil of *S. prashadi*. Oleic acid (C18:1) was the MUFA present in the highest concentration (12.84 %) which was followed by Eicosanoic acid (C20:1). These results were much higher than in the Malaysian marine fish of 0.2- 2.34 %<sup>65</sup>.

## CONCLUSION

A number of squid and cuttlefish processing plants now operate along the coast of India and they give out an enormous amount of wastes (mainly composed of the visceral organs like liver (digestive gland), ink sac, gonads and the nidamental gland complex) leading to environmental pollution. The main advantage of lipid from animal waste is that it is much cheaper when compared to the lipid extracted from flesh. This lipid is considered as highly nutritive and has drawn the attention of the nutritionists as a good source for human consumption as well as industrial use. In this sense, the financial benefits can be obtained and environmental pollution is certainly decreased. Cuttlefish oil can be used as an immunostimulant and it can suppress the inflammatory response as well as inhibit the platelet aggregation in rats. Live feed organisms such as rotifers, *Artemia naupli* etc. can be enriched with cuttlefish liver oil for the successful aquaculture. Further it can be stated that the oil from the liver of cuttlefish may be used for various purposes such as manure (residual waste during oil extraction), bio enrichment, biodiesel production, poultry feed, etc. The information reported in the present study may be valuable for the pharmaceutical and food industries in the selection of cuttlefish oil and marine fish oils for the chemical studies.

## ACKNOWLEDGEMENT

The first author is grateful to Dr. V. Ramasubramanian, Assistant professor, Department of Zoology, Bharathiar University for his valuable guidance, encouragement and creative suggestions. The first author is also grateful to Dr. Vairamani, Assistant Professor, Dr. Shanmugam, Professor, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University for their immense support and unrestrained help leading to the completion of the present work.

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**Cite this article as:**

Kiruthika Ravi Muthukrishnan, Venkatachalam Ramasubramanian, Shanmugam Vairamani and Annaian Shanmugam. Studies on the lipid composition of liver (digestive gland) oil of cuttlefish *Sepia prashadi* (Winckworth, 1936). Int. Res. J. Pharm. 2014; 5(4): 306-312 <http://dx.doi.org/10.7897/2230-8407.050465>

Source of support: Nil, Conflict of interest: None Declared