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Importance of deep sea squid *Mastigoteuthis flammea* in healthcare as a rich source of ω -3 polyunsaturated fatty acids, especially eicosapentaenoic and docosahexaenoic acids

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Abstract
Though globally squid is considered as a delicious seafood item, the biochemical composition of deep sea squid species especially *Mastigoteuthis flammea* available in Arabian Sea is scanty. In the present study, we have investigated the proximate composition, amino acid composition and fatty acid profiling of *M. flammea* caught from Arabian Sea. The moisture, crude protein, crude lipid and ash contents of the squid were 60.20 g/100g, 23.13g/100 g, 17.05g/100 g, and 1.61g/100 g respectively. The squid species was found to contain the essential and non-essential amino acids in a balanced proportion, which is an essential criterion for attenuating protein deficiency related malnutrition disorders. Interestingly, its body oil was found to comprised of healthcare important n-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in considerable quantities. The results of the present study suggest that the deep sea squid *M. flammea* can be considered as a potential resource of nutrients required for the normal maintenance of human health.

Keywords: ω-3 polyunsaturated fatty acids, Deep sea squid, *Mastigoteuthis flammea*, EPA, DHA

1. Introduction
Deep sea squid fishery in India has been in a low pace in its potentiality because of weak demand on molluscan sea food among Indian population. The world wide acceptance of squid is mainly because of its taste and the presence of fatty acids, Cholesterol, vitamin E and minerals [1, 2]. World trade of cephalopods has increased significantly during the last decades [3].Cephalopods are morphologically diverse, and nutrient rich predatory mollusks, and some of these animals are among the most metabolically active poikilotherms known [4]. They are exciting marine organisms due to their commercial, nutritional and ecological importance [3]. Nowadays the demand of fin fish is increasing because of their health importance and are having good palatability as well. In countries like Japan, Spain, Korea and Italy squid is much appreciated and utilized in various forms of food mainly as a source of protein [5].
Squid is a rich source of macromolecules and micronutrients such as proteins, lipids, amino acids, vitamins which are essentially required for the normal maintenance of human health. Squid meat is very rich in proteins and all the essential amino acids and a rich source of lysine in particular which is essential for growth [6, 7]. The amount of health beneficial fatty acids and amino acids are in a considerable amount in mollusks especially in Squid species which is a common item in the daily diet of foreign population.

Fig 1: *Mastigoteuthis flammea* Chun, 1908

As a marine resource, squids are nutritionally precious regarding their concentrations in eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) expressed as having, antiarrhythmic, and hypotriacylglycerolemic, antithrombotic, antiatherogenic and anti-inflammatory properties [8]. Subsequently, EPA and DHA are considered as protective agents against coronary heart diseases (CHD) [9]. Seafood is rich in long chains of ω-3 [10, 11] with a recognized importance in the prevention of several of the modern health disorders [12]. However, people in India prefer consumption of fin fishes as compared to shell fishes. Especially the nutritional awareness aspects on presence of healthcare important ω-3 polyunsaturated fatty acids in squid is relatively low due to non-availability of database on the biochemical composition of squid species available in Indian waters. In this present study, proximate, fatty acid and amino acid profiling of deep sea squid *M. flammea* (Fig.1) caught from Arabian Sea was studied to understand whether the available deep sea squid can be a rich source of health important nutrients or not.

2. Materials and Methods

The squid sample for the present study was collected from Arabian Sea and identified with the help of FAO and Fish base online sites. The squid sample was minced as whole (gut and pen removed) and used for further analysis.

2.1 Determination of proximate composition

In this study, moisture content was detected by drying the samples in a hot air oven at 105 °C. The total protein and crude fat have been analyzed by Micro-Kjeldahl method and Soxhlet extraction procedure respectively [13]. The total nitrogen was multiplied by 6.25 to get the crude protein in the squid meat.

The determination of fatty acid profile of lipid extracted from squid was carried out in Thermo Trace Gas chromatograph equipped with flame ionization detector and Varian FFAP column (25m 0.32mm 0.3µm #CP 7485) using N_2 as carrier gas. In GC, oven temperature was initially held at 110 ^{0}C for 4 min and then set to increase up to 240 ^{0}C at a rate of 2.7 ^{0}C min^{-1}, held at 240 ^{0}C for 5 min. Ash content of the squid sample was determined by placing the sample for 12 h in a furnace at 525 °C [13].

The amino acid composition of the studied squid species was carried out by using HPLC system with Shodex pak p-4219 No. P207074 column. The amino acid sample was prepared by overnight digestion of 200mg squid sample in 6N HCl in sealed test tubes at 110 ^{0}C [13]. Then the sample filtered through what man filter paper and the HCl in sample which was used to digest the sample is washed out by flash evaporator. The evaporated sample was washed thoroughly with distilled water to remove the remaining tinge of acid. Washed three times with distilled water to remove the acid content completely from the sample. After complete evaporation of water content, the sample is made up to 1 ml with Buffer A in a vial and then used for injection in HPLC.

2.2 Statistical analysis

Statistical analysis for triplicate values has been done and the results are expressed as mean ± S.D.

3. Results and Discussion

3.1 Proximate composition of *M. flammea*

The proximate composition of the studied squid species reveals that it is a good source of protein, lipid and minerals too and the values are represented as 'g%' per100 g meat. The moisture content of *M. flammea* is 60.20±1.6% and the protein content is also in a considerable amount (23.13±1.8%). The lipid content detected in a very good amount as 17.05±1.2% and ash content is 1.61±0.3%. The study shows that moisture content in *M. flammea* is low and the lipid content is relatively high as it is common in deep sea fishes and the amount of moisture and fat will be inversely proportional to each other too.

Table 1: Proximate composition of *M. flammea* (%)

Parameters	*M. flammea*
Moisture	60.20±1.6
Protein	23.13±1.8
Lipid	17.05±1.2
Ash	1.61±0.3

*Mean values ± S.D of determinations for triplicate samples

3.2 Fatty acid profile of *M. flammea*

The results of the present investigation have shown that the squid species *M. flammea* is rich in both the ω -3 and ω -6 polyunsaturated fatty acids, which have major physiological functions in the maintenance of normal cellular homeostasis and regulation of rhythmic metabolic activities. The present finding is in accordance with an earlier reported study [10], which indicated that squid species tend to accumulate n-3 PUFA in their muscle tissue, particularly of long-chain n-3 PUFA such as EPA and DHA, which are well known for their antagonist actions against cholesterol mediated adverse effects. Interestingly, the predominant n-3 PUFA present in *M. flammea* is DHA, which plays vital functions in the development of brain and retina of fetus during pregnancy.

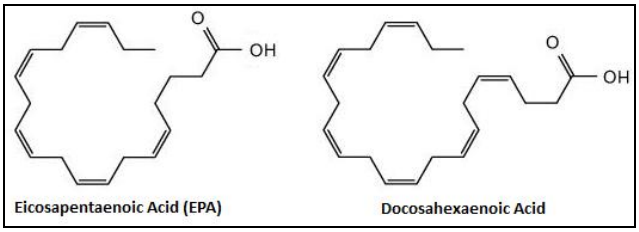

Fig 2: Structure of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)

The studied squid species also contains significant concentration of Eicosapentaenoic acid **(**EPA), another important ω -3 PUFA, essentially required for the regulation of neuronal and cardiovascular functions. ω -3 PUFA are well known to exert neuroprotective properties and to represent potential nutritional and therapeutic interventions in the treatment for variety of neurodegenerative and neurological diseases and disorders such as Alzheimer's Disease, Brain Cancer, Degenerative Nerve Diseases, Epilepsy, Genetic Brain Disorders Encephalitis, Head and Brain Malformations, Hydrocephalus, Stroke, Parkinson's Disease, Multiple Sclerosis, Amyotrophic Lateral Sclerosis etc. Other potential beneficial actions of EPA and DHA include regulation of inflammation, transcription, and cell membrane properties.

It is noticed that the other fatty acids like palmitic acid, stearic acid and eicosenoic acid form lesser part in composition of fatty acids as compared to ω -3.Nutritional lipid quality indexes such as total ω-3/ω-6, DHA and EPA consumption of the selected squid species are advisable in a balanced diet.

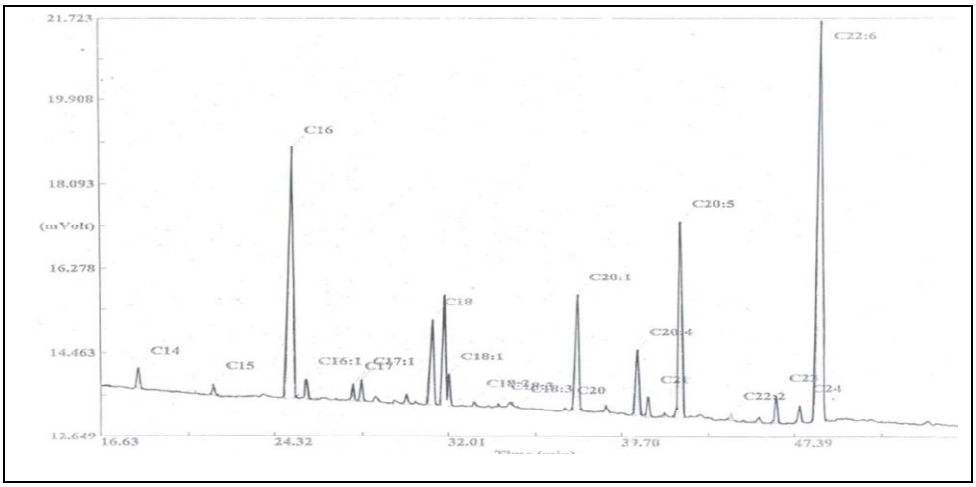

Fig 3: GC chromatogram of *M. flammea*

Table 2: Fatty acid composition of *M. flammea*

Fatty acid name	% of fatty acids in terms of total fatty acid
Saturated	
C12	0.03±0.0
C13	0.03±0.0
C14	0.85±0.0
C15	0.55±0.0
C16	20.31±1.1
C17	1.05±0.0
C18	5.37±0.5
C20	0.08±0.0
C21	1.11±0.1
C23	1.50±0.1
C24	1.08±0.0
Mono unsaturated	
C16:1	0.91±0.0
C17:1	0.03±0.0
C18:1	6.27±0.5
C20:1	7.37±0.6
Poly unsaturated	
C18:2	0.28±0.0
C18:3	0.07±0.0
C20:4	3.42±0.1
C20:5	12.02±0.9
C22:2	0.31±0.0
C22:6	35.85±2.6
TOTAL	98.18

*Mean values ± S.D of determinations for triplicate sample

3.3 Amino acid profile of *M. flammea*

The amino acid profiling of *M. flammea* shows that it is a potential source of most of the amino acids which are essential for the development of brain health and development of immune system as well. Most of the studies on squid show that squids are normally deficient in amino acids. In such case this squid species is an ideal source of amino acid. All over the world the studies on squid is going on to explore its health benefits and bring it handy for the human population. PCSIR scientists have recently directed their attention to exploit deep sea squid as a possible source of animal protein and a promising healthy commercial species for the future [14]. Amino acids also help to modulate adiposity and enhance antioxidant activity in the body. All the essential amino acids are present in considerable amount in the studied squid species *M. flammea*. Valine, Alanine, Glycine and Isoleucine are in good amount and are biologically very important and each one imparts each function in our body. Alanine is the primary amino acid in sugar metabolism and important source of energy for muscle as well. Isoleucine is an energy source for muscle tissue and required in the formation of hemoglobin and Lysine helps in the formation of collagen, the connective tissue present in bones, ligaments, tendons, and joints. The noticeable presence of all these important amino acids makes this squid species a health potential food for human race.

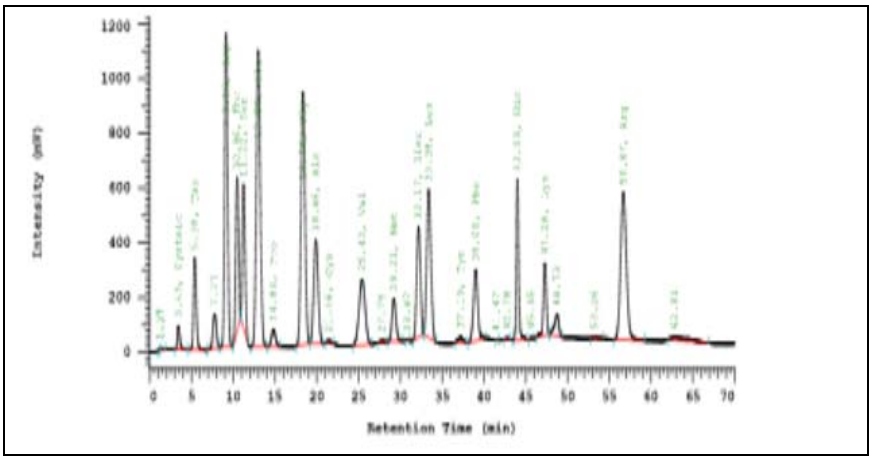

Fig 4: HPLC chromatogram of amino acid analysis of *M. flammea*

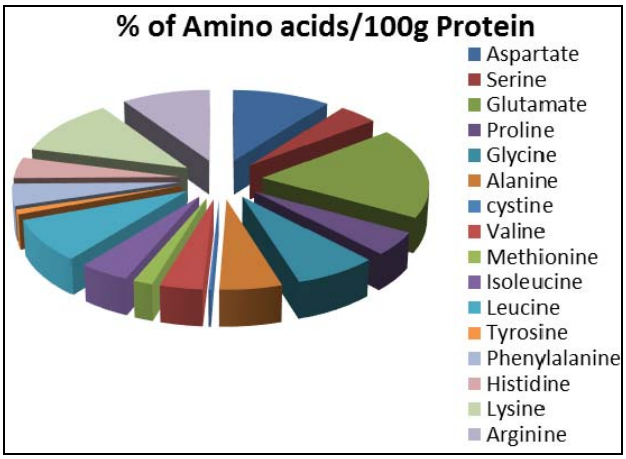

Fig 5: Amino acid composition of *M. flammea*

Mostly deep sea squid are not in prominent use for food purpose. But the present study reveals that it can be a great marine nutrient rich food source for human consumption.

4. Conclusion

This study fills the gap of knowledge on proximate and biochemical composition of selected squid species *M. flammea*. It is accomplished that the generation of proper awareness on the biochemical composition of Indian deep-sea squid among Indian population may be helpful in the utilization of deep-sea squid species for human healthcare benefits especially in pregnant women, young children and aged population. However, a greater understanding of the individual ω -3 polyunsaturated fatty acids available in squid and their regulatory roles in human health, protection and repair is needed to make appropriate dietary recommendations for therapeutic interventions in targeted population.

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