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Research Article

Nutritional composition and FT-IR functional group analysis of pharaoh cuttlefish (*Sepia pharaonis*) from Puducherry coastal waters, India

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Abstract

The nutritional composition and functional groups of the edible portion of the most often utilized cuttlefish, *Sepia pharaonis* of Puducherry coastal waters, India was evaluated. Moisture is the basic component of the mantle with 82.45±0.15% followed by a good source of 14.5±0.1% protein. The ash, lipid, and carbohydrates were found less than 1%. The energetic value of the mantle of cuttlefish *S. pharaonis* is calculated as 6.06 KJ/g. Na and K were found in considerable quantities of 2772 and 3247 mg/kg respectively and the Na/K ratio (0.85) was less than 1. The amount of Zn, Mn, B, Al, Sr, and As was found to be relatively high when compared to a low or insignificant level of Cu, Mo, Se, Cr, Ni, Pb, and Hg. The fatty acid profile analysis revealed that it contains 71.67% saturated fatty acids,17.87% polyunsaturated fatty acids and 10.38% monounsaturated fatty acids. In all three types, the C16:0 palmitic acid (40.29%), C18:0 stearic acid (16.54%), and C22:6 docosahexaenoic acid (13.52%) represented as the dominant fatty acids. The FT-IR analysis disclosed the presence of various functional groups belonging to protein, lipids, and polysaccharides. Thus, the present study proved that the cuttlefish *S. pharaonis* is considered as a potential nutraceutical and the right choice of food for greater and wider human consumption.

Keywords: human consumption; nutraceutical; proximate composition; *Sepia pharaonis*

Introduction

Considering human nutrition and related health benefits, knowledge on nutritional composition of a particular food is essential for selection, utilization, and achieving nutritional security. Cuttlefish belongs to the class Cephalopoda of the phylum Mollusca, is one of the most exploited marine organisms in the world due to their increasing demand in the local, national, and international markets. Cephalopods are the second major marine fishery food resources exported from India due to the increased awareness about the nutritional quality and considered as an alternative to finfishes (Zlatanos *et al.*, 2006). In India, for the past four decades, cephalopods landing has risen steadily from 1617 t in 1970 to 220844 t in 2018 (FRAD, CMFRI, 2019).

Cephalopods are exclusively marine and distributed in all the seas and oceans of the world except the black sea (Jereb *et al.*, 2005). Cephalopods are active predators and feed on small fishes and crustaceans exhibiting phenomenal diversity in size and lifestyle patterns. They are a commercially important food item

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and a source of bioactive compounds. There are about 700 well-classified cuttlefish species available in tropical and polar seas (Chakraborthy *et al.*, 2016). About 80% of cephalopod body mass comprises the edible portion when compared to shellfish and fin fishes favouring a significant effect on its marketability. It is evident that cephalopods are rich in protein, PUFA and minerals (Adeniyi *et al.*, 2012). Since cuttlefishes offer a reasonable price in the national and international market and form unavoidable seafood of global trade, information regarding the nutritional quality, mineral composition, and functional groups are essential to ensure that they meet the requirements of food regulations (Watermann, 2000).

Union Territory of Puducherry is situated on the Coromandel Coast and it lies between North Latitudes 11° 46' and 120 03' and East Latitudes 790 52' bounded by Bay of Bengal on the East and holds its identity mark in marine fishery resources. Puducherry coastal water is sustained by five commercially important cuttlefish viz. *Sepia pharaonis, Sepia aculeata, Sepia brevimana, Sepia prashadi,* and *Sepiella inermis.* Among the five commercially important cephalopods, the *Sepia pharaonis* is a predominant fishery resource, available almost throughout the year (except for the mechanized fishing ban period) and most commonly consumed by the local population of the Puducherry region. Considering the availability and large-scale utilization of *Sepia pharaonis*, the present study was undertaken to investigate the nutritional parameters such as protein, lipid, carbohydrate, moisture, ash content, energetic value, mineral composition, fatty acid profile and functional groups of the edible portion of *S. pharaonis* from Puducherry coastal waters.

Materials and Methods

The research was carried out between July and August 2019 at the Department of Zoology, Kanchi Mamunivar Government Institute for Postgraduate Studies and Research, Puducherry, India.

Sample collection

After ascertaining the species identity, the fresh and mature individuals of *Sepia pharaonis* were procured directly from the fish landing centre of Puducherry.

Processing of sample

The cuttlefish were iced immediately in an insulated box and transported to the laboratory within 30 m. It was allowed to thaw gradually and dissected to remove the mantle which is the main edible portion. About 500 g (mean weight) of the mantle was cleaned with water and blotted to remove the surface water and then homogenized to form a mince and stored at 0 °C for further analysis.

Nutritional composition

The nutritional composition like moisture, protein, lipid, ash, carbohydrate and fatty acid was analysed by the conventional method of AOAC (Association of Official Analytical Chemistry, 2000). Mineral content was determined by the method of AOAC, (2011 and 2015).

Energetic value

The energetic value of the cuttlefish *S. pharaonis* was determined indirectly using Rubner's coefficients for aquatic organisms: 9.5 kcal g^{-1} for lipids, 5.65 kcal g^{-1} for proteins and 4.1 kcal g^{-1} for carbohydrate (Winberg, 1971) and expressed in kJ g^{-1} wet mass by multiplying the energy value by 4.184.

Analysis of minerals (AOAC, 2011 and AOAC, 2015)

The minerals were analysed by inductively coupled plasma mass spectrometry (ICP-MS model Agilent, 7700 series) after microwave-assisted acid digestion. 1.0 g of each sample was digested with 4.0 mL of 65% (v/v)

HNO3 and 0.5 mL of 35% (v/v) H2O2. The mineral content of the digested samples was determined by ICP-MS and the minerals were expressed in mg/kg.

FT-IR analysis

5 mg of fine power of mantle tissue was made into a pellet and placed in an IR Golden cell cavity. FT-IR (Nicolet iS5, Thermo Scientific, US) spectrum was recorded between 4000-400 cm^{-1} for all the samples under study.

Results and Discussion

The nutritional composition of mantle, the main edible portion of cuttlefish *S. pharaonis* is shown in Table 1. Results are presented as mean ± standard deviation. The level of moisture content was 82.45± 0.15% in the edible portion of *S. pharaonis*. The result revealed that water is the main constituent of the mantle of cuttlefish than all other biochemical constituents. The percentage composition of protein was found to be 14.5±0.1. The crude protein content of edible cephalopods varied from 12 to 19.50%. Chakraborthy *et al.* (2016) were reported 19.50% protein in the edible portion of *Ureteuthis duvauceli* and 12.02% in *Amphioctopus neglectus* Considering the various levels of protein content in different commercially important cephalopods, the amount of protein (14.5±0.1) obtained from this study was following the report of (Thanonkaew *et al.*, 2006) therefore the cuttlefish can be substituted for the animal protein source.

Table 1. Proximate composition of *S. pharaonis* (n=3)

Parameter	Content (%)
Moisture	82.45±0.15
Protein	14.5± 0.1
Lipid	0.85± 0.05
Carbohydrate	0.66±0.2
Ash	0.89±0.2
Energetic Value (kJ/g)	6.06

Level of the carbohydrate content of *S. pharaonis* edible portion was 0.66±0.2 percent. Cephalopods showed relatively low carbohydrate content when compared to other molluscs. The lipid content was very low with the percentage composition of 0.85±0.05 in the mantle portion of *S. pharaonis*. Lipid content in cephalopod mantle is less than 1g of its wet weight since mantle exhibits poor absorption and storage of any lipid (O'Dor *et al.*, 1984) rather it is stored in nidamental glands, which help for the maturation of gonad and development of the eggs.

The ash content of *S. pharaonis* was 0.89±0.2%. In cephalopods the level of ash content of head and mantle found between 0.7-0.9% indicating that S. *pharaonis* is a good source of minerals (Brita Nicy *et al.*, 2016). The energetic value of the mantle of cuttlefish *S. pharaonis* is calculated as 6.06 KJ/g.

The mineral contents (Table 2) of the mantle of *S. pharaonis* are presented as a macro (Na, K) and micro minerals (Zn, Cu, Mn, Mo, Se, Cr, B, Al, Ni, As, Sr, Pb, Hg) and expressed in mg/kg body weight. The edible part of the cuttlefish was found to be rich in both macro and micro minerals. Na and K were found in considerable quantities of 2772 and 3247 mg/kg respectively and the Na/K ratio (0.85) remains in less than 1. Na being an extracellular and K being intracellular ions are playing key roles in maintaining the physiological balance of the body. Less than 1Na/K ratio of the present study indicates that the mantle is a potential source of food for human health and able to protect from strokes, kidney stones and reduce blood pressure (Hibino *et al.,* 2010). More than 1g/ kg of K in the body fluid is vital to regulate the osmotic pressure and pH of the body (Ensminger *et al.*, 1995).

Table 2. The mineral composition of *S. pharaonis*

Parameter	Content (mg/kg)	MAC by FSG	Reference
Macrominerals			
Na	2772 ± 0.36	2000 mg/day	WHO
K	3247 ± 0.57	3510 mg/day	WHO
Microminerals			
Zn	62.24 ± 0.55	30 mg/day	FAD, 1983
Cu	10 ± 0.5	30 mg/day	WHO, 1995
Mn	32.22 ± 0.53	1mg/day	FAD, 1983
Mo	<0.03	NR	
Se	1.34 ± 0.54	1mg/day	MHSAC, 2005
Cr	2.97 ± 0.54	12-13 mg/day	USFDA, 1993
B	15.40 ± 0.5	1-13 mg/day	WHO 1996
Al	286.80± 0.37	60mg/day	WHO, 1989
Ni	0.85± 0.35	0.5–0.6 mg/kg	WHO, 1985
As	4.56± 0.41	2.0 mg/kg	WHO, 1985
Sr	162.93± 0.33	130 mg/kg	IAEA (2003)
Pb	0.48± 0.55	114 µg/day	WHO, 1995
Hg	0.27± 0.53	0.1 µg/kg/day	WHO, 2011

The micro minerals *viz.* Zn (62.24 ± 0.55 mg/kg), Mn (32.22 ± 0.53 mg/kg), B (15.40 ± 0.5 mg/kg), Al (286.80± 0.37mg/kg), Sr (162.93± 0.33 mg/kg) and As (4.56± 0.41 mg/kg) were found significantly greater values in the mantle of *S. pharaonis*. The minerals do not have any energy value in the biological system; they play a vital role in the metabolic functions since these are called essential minerals. The mineral composition of a particular organism is based on the habitat and its nourishment (Lall, 2002; Chakraborty and Joseph, 2015). The Zn and Mn act as a cofactor for over 200 enzymes involved in immunity, new cell growth, acid-base regulation, etc. Zn is directly involved in the carbonic anhydrase synthesis Deficiency of Zn in the diet may be more dangerous to living organisms including humans than its high concentration in the diet (Ogunlesi *et al.*, 2010). The *Sepia pharaonic* is efficiently absorbed and retained Zn both from diet and seawater (Villanueva and Bustamante, 2006). Chromium is present in human tissues in variable concentrations and its deficiency is characterized by disturbance in glucose, lipid, and protein metabolism (Schumacher *et al.*, 1993) and it is considered as an essential trace element involved in various metabolic activities. Selenium played a vital role in the synthesis of thyroid hormone and combat against oxygen reactive species and cancer (Jackson *et al.,* 2008). The micromineral Mo functions as a cofactor for at least 4 enzymes: sulfite oxidase, xanthine oxidase, aldehyde oxidase, and mitochondrial amidoxime reducing component (Novotny, 2011). Copper is incumbent for enzymes needed for aerobic metabolisms such as cytochrome c oxidase, lysyl oxidase, dopamine monooxygenase, and ceruloplasmin (Percival, 1991).

Strontium is naturally found in seawater and seafood. Strontium increases bone mineral density, improves bone microarchitecture, and decreases the risk of fracture in postmenopausal women with osteoporosis (Ikeda *et al.*, 2002). The amount of Sr (162.93±0.33 mg/kg) reported in this study indicates that the cuttlefish *Sepia pharaonis* is a good source of Strontium and promoted as a "natural" way "to maintain strong bones. The *S. pharaonis* was demonstrated to provide the highest value of Al of about 286.80± 0.37mg/kg in the mantle might be considered a good source of this mineral and recommended to treat antiperspirants, hyperhidrosis. In the present investigation all these minerals were found considerably more than the advocated level of FAO/WHO. Apart from these dominant minerals, the cephalopod was also contained a low or insignificant level of Cu, Mo, Se, Cr, Ni, Pb, and Hg. Though the result of the present study was almost similar with the findings of Thanonkaew *et al.* (2006) and Chakraborty and Joseph (2015) on commercially important cephalopods, but the quantity of Zn, Mn, B, Al, Sr and As were found to be relatively high in *S. pharaonis* which might be due to the genetic and environmental factors.

Thirteen selected fatty acids of the *S. pharaonis* mantle were shown in Table 3. The fatty acid compositions of the present study fall under saturated, monounsaturated, and polyunsaturated fatty acid types. In the present investigation, the saturated fatty acids were found higher with 71.67% followed by polyunsaturated fatty acids (17.87 %) and monounsaturated fatty acids (10.38%). In all the three types, the C16:0 palmitic acid (40.29%), C18:0 stearic acid (16.54%) and C22:6 docosahexaenoic acid (13.52%) represented as the dominant fatty acids.

Table 3. Fatty acid composition of *S. pharaonis*

S. no.	Fatty acids	% composition
Saturated fatty acids		
1	C12:0 Lauric acid	0.40
2	C14:0 Myristic acid	2.51
3	C15:0 Pentadecanoic acid	2.53
4	C16:0 palmitic acid	40.29
5	C17:0 Heptadecanoic acid	2.79
6	C18:0 Stearic acid	16.54
7	C20:0 Arachidic acid	2.12
8	C22:0 Behenic acid	4.49
ΣSFA		**71.67%**
Monounsaturated fatty acids		
9	C18:1 Oleic acid	9.06
10	C20:1 Eicosenoic acid	1.32
ΣMUFA		**10.38%**
Polyunsaturated fatty acids		
11	C18:2 Linoleic acid	0.93
12	C20:3 Eicosatrienoic acid	3.42
13	C22:6 Docosahexaenoic acid	13.52
ΣPUFA		**17.87%**

The fatty acid profile of all the edible cephalopods demonstrated that the palmitic and stearic acids were the predominant fatty acids found in the mantle (Chakraborty and Joseph, 2015), which was consistent with the present investigation as well. The role of palmitic acid has been focused negatively for a long period as an agent detrimental to health, shadowing its remarkable physiological functions. Palmitic acid guarantees cell membrane physical properties, biosynthesis of palmitoylethanolamide, surfactant activity in the alveoli of the lungs (Gianfranca Carta *et al.*, 2017) and stearic acid is probably increasing the low-density lipoprotein levels. Based on this, the mantle of *S. pharaonis* not only considered as edible but also a healthy diet. Among the two monounsaturated fatty acids recorded in the present study, the omega -9 Oleic acid found in the mantle for about 9.06% was following the report of Zlatanos *et al.* (2006) on three Mediterranean cephalopods. Omega - 9 Oleic acid has beneficial effects on insulin sensitivity and type II diabetes, agents to treat Alzheimer's disease (Carrillo *et al.*, 2012), anti-inflammatory, wound healing, and antioxidant properties (Tzu-Kai Lin, 2018) therefore the cuttlefish *S. pharaonis* might be substituted to vegetable oil.

The polyunsaturated fatty acids reported in the present investigation were found to be an appreciable quantity of 17.87%. Three polyunsaturated fatty acids were identified as C18:2 linoleic acid (omega-6), C20:3 eicosatrienoic acid (omega-6), and C22: 6 docosahexaenoic acid (omega- 3). The percentage of n-3PUFA (13.52%) was found to be high as compared with both n-6 PUFAs, which represented 75% of the total PUFA content of the present investigation. When compared to other cephalopods,the n-3 docosahexaenoic acid (DHA) content of *S. pharaonis* was high,(Chakraborty and Joseph, 2015). The long-chain omega-3 polyunsaturated fatty acids especially DHA play an important role in the prevention and management of cardiovascular diseases, hypertension, inflammation, diabetes (Finley and Shahidi, 2001), and the improvement of learning ability (Suzuki *et al.*, 1998). Therefore, the cuttlefish *S. pharaonis* can be treated as a significant natural source for n-3 PUFAs and the best alternative to the finfishes.

The absorption spectra of edible portion *S. pharaonis* in the medium infrared region between 500 and 4000 cm^{-1} were shown in Figure 1. The observed wave number, intensity, and the probable vibrational assignments of the functional groups of various compounds present in the mantle were shown in table: 4. FTIR spectra of a compound have two general areas: 4000-1500 cm^{-1} and 1500-500 cm^{-1}. The region between 4000-1500 cm^{-1} named the functional group region, which is characteristic of specific kinds of bonds. The region between 1500 and 500 cm^{-1} called the fingerprint region. Peaks in this region arise from complex deformations of the various molecules specific to particular species. Therefore, the FT-IR spectrum can be used as fingerprints for identification of specific functional groups belonging to protein, lipids, and polysaccharides of a particular food (Venkataraman *et al.,* 2010). It is also used to determine if there are any unsaturation and/or aromatic rings in the structure. The main stretching band in liquid water is shifted to a lower frequency of 3418 cm^{-1} due to the hydrogen bonding of the type H-O···H and/or N-H···O types. This band is also assigned to the N-H stretching vibration of the peptide bonds. The N-H band of protein present in the mantle overlaps with the water stretching band. The bending deformation frequency of water is increased to 1652 with a very strong intensity. The C=O stretching band is also observed at this high intense frequency. For carboxyl groups in the absence of hydrogen bonding, the C=O stretching band is observed above 1740 cm^{-1}. The redshift clearly shows the presence of hydrogen bonding. The medium bands observed at 2958 and 2928 cm^{-1} is attributed to the methylene asymmetric stretching vibrations of protein. The band observed at 2871 cm^{-1} is assigned to the methylene symmetric stretching vibration.

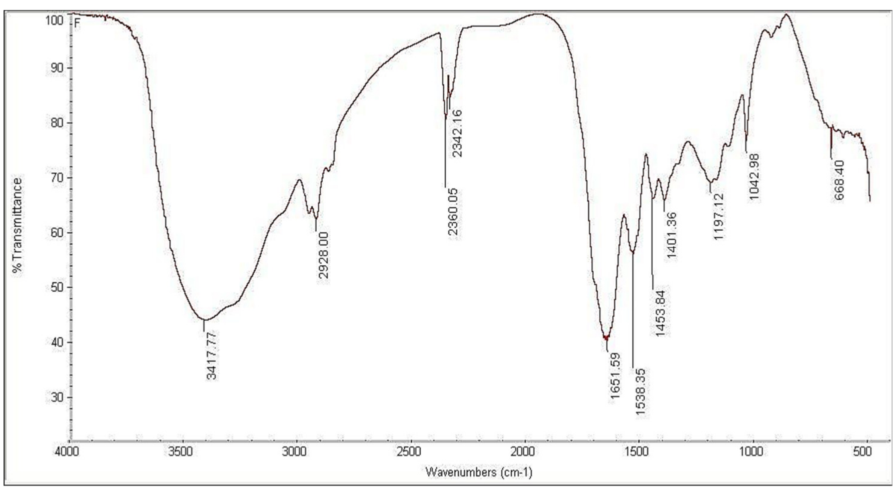

Figure 1. FT-IR Spectra of mantle of *S. pharaonis*

The IR spectrum of the protein is characterized by a set of absorption regions known as the amide (I, II, and III) region and the C–H region. The amide I region detected from C=O stretching vibration of the peptide group with a characteristic absorption at 1640 to 1690 cm^{-1}. Amide II band is primarily N–H bending with a characteristic absorption at 1550 to 1640 cm^{-1}. The amide III absorption is normally weak and arises from N–H stretching vibration with a characteristic absorption at 3100 - 3500 cm^{-1}. In the present study, the peaks at 1651 cm^{-1} and 3417 cm^{-1} were in good agreement with the presence of amide I and amide II respectively. The peaks observed at 2342 and 2360 cm^{-1} is weak but very specific to fin fishes and shellfishes (Nuno Sousa *et al.,* 2018). These are the frequencies of the two stretching vibrations of C=O formed from amide groups. The two C-O stretching vibrations in the COO- group are coupled and thus the frequencies of the two stretching modes are normally observed at near 1400 and 1570 cm^{-1}.

Table 4. The observed wave number, intensity and the probable vibrational assignments of the various compounds present in the mantle of *S. pharaonis*

Wave number with intensity	Assignments
3418 s	H_2O asymmetric stretching / N-H stretching
3276 m	H_2O symmetric stretching
2958 m	CH_2 asymmetric stretching
2928 m	CH_2 asymmetric stretching
2871 m	CH_2 symmetric stretching
2360 w	CO_2 symmetric axial deformation
2342 w	CO_2 symmetric axial deformation
1652 vs	H_2O deformation / C=O stretching
1538 m	C-O stretching in COO^- group.
1454 m	C-H stretching
1401 m	C-O stretching in COO^- group.
1328 m	N-H in-plane bending
1197 m	CH_2 wagging (out of plane bending)
1169 m	O-H in-plane bending
1118 m	C-N stretching
1043 m	C-H in-plane bending
919 vw	C-O in-plane bending
884 vw	C-O in-plane bending
668 m	C-Cl rocking
614 m	CH_2 rocking

In the present investigation, the vibrational modes observed at 1401 and 1538 cm^{-1} arise from the carboxylate group. The medium band observed at 668 cm^{-1} is due to the C-Cl vibration of the acyl halide group. The presence of lipid is characterized by hydrocarbon bonding mainly alkane and alkenes with an absorption maximum at 1450 cm^{-1} for alkane which is due to C-H bending and 2840 - 3000 cm^{-1} due to C-H stretching frequencies. The peaks obtained in the present study at 1453, 1538, and 2928 cm^{-1} ascertain the presence of lipids in the mantle of *S. pharaonis*. Therefore, all this information obtained in this study may be valuable for quality assurance and selection of this *S. pharaonis* as a potential food for human consumption.

Conclusions

Reliable up-to-date information on the nutritional profile of foods ensures the choice available to the consumer when selecting any particular type of food. The present study revealed that the edible portion of *S. pharaonis* is a good source of protein and fatty acids with an appreciable quantity of palmitic acid, stearic acid, linoleic acid, eicosatrienoic acid, and docosahexaenoic acid. It also contains all the macro and micro minerals significantly rich in Zn Mn, B, Al, Sr, and As. The FT-IR analysis disclosed the presence of various functional groups belonging to protein, lipids, and polysaccharides. Therefore, it can be concluded that the cuttlefish, *S. pharaonis* is considered as a potential nutraceutical and the right choice of food for greater and wider human consumption.

Authors' Contributions

KJ: Has done sample collection, processing and laboratory works including handling of instruments, preparation of reagents, chemical analysis, characterization of samples, review of literature, manuscript writing and statistical analysis. RV: conceptualization, supervision and review of the manuscript.

Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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